# Low lignin alfalfa cutting management study

by

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B.S., China Agricultural University, 2008
M.S., China Agricultural University, 2010

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

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# Abstract

Alfalfa (*Medicago sativa* L.) is the fourth most valuable cash crop in the U.S., following corn, wheat, and soybean, and its production is vital to sustain the dairy, beef, and hay industries. As the world population increases alongside environmental degradation and water scarcities, identifying monthly climate variables related to alfalfa production can provide growers and researchers more time to deal with the effects of climate change. The purpose of Chapter 1 was to propose a statistical learning method to investigate, access, and predict the relationship between the alfalfa yield and monthly climate information with much larger numbers of variables in the state scale. To address the high-dimensionality of monthly climate variables, we employed a penalized regression model, called Lasso. Less than six relevant climate variables (i.e. monthly maximum and minimum temperature, and monthly precipitation) were identified as important predictors for alfalfa yields in each state. It was also shown that at least one of climate variables in June, July, and August was highly correlated to alfalfa yields.

Reduction of lignin level in the forage legume alfalfa by the conventional breeding technology results in increased digestibility and may extend peak harvest dates by up to 10 days. This could benefit alfalfa growers by avoiding undesirable weather conditions, such as heavy rain, and increasing dry matter yield and nutritive values. The objective of Chapter 2 was to evaluate field performance and cash value of low lignin and reference variety under a four-cut system. The experiment was a split-plot design with a randomized complete block, the whole plot factor with two levels of variety and the subplot factor with six levels of harvest schedule. Forage yield differences among harvest schedules were more pronounced than yield differences between two varieties. Harvest interval had a significant effect on the nutritive value of alfalfa

and a more substantial effect than variety selection. The nutritive value of low lignin alfalfa variety was significantly greater than the conventional variety.

The purpose of Chapter 3 was to compare forage yield and nutritive value of low lignin alfalfa and two reference varieties subjecting to two harvest intervals and three seeding rates. The experimental design was in a split-split plot arrangement with four replicates, where harvest intervals (28-day and 35-day) were assigned to whole plots, seeding rates were subplots, and varieties were sub-subplots. The weighted mean nutritive value was applied to two production years of 2016 and 2017. Hi-Gest 360 (low lignin alfalfa) provided similar yield potential and increased nutritive value compared to two reference varieties. Harvest interval had a large effect on nutritive value and a more significant effect on alfalfa dry matter yield than variety selection. Seeding rate did not affect alfalfa yield and nutritive value.

Based on two production year research in Manhattan, KS, the low lignin alfalfa variety under a shorter harvesting interval (every 28-day) appears be profitable management practice regardless of seeding rate.

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# Chapter 1 - Using the Lasso method to study the effects of climate variation on alfalfa yields in the midwestern United States


## Abstract

Alfalfa (*Medicago sativa* L.), a cool-season perennial legume, is vital to sustaining the dairy, beef, and hay industries. As the world population increases alongside environmental degradation and water scarcity, it becomes essential to investigate the relationship between alfalfa production and climate factors. Identifying monthly climate variables related to alfalfa production can provide growers and researchers more time to deal with the effects of climate change. The objectives of this study were to: (1) propose the Lasso method to study the relationship between the alfalfa yield and monthly climate information with much larger numbers of variables in the state scale; (2) investigate the impacts of climate variables returned by the Lasso on the alfalfa yield for each state separately; and (3) assess the validity of the new prediction model by comparing yield observations to estimated yields of each state. To address the high-dimensionality of monthly climate variables, we employed a penalized regression model, called Lasso. As a result, less than six relevant climate variables were identified as important predictors for alfalfa yields in each state. All regression models with the selected climate variables achieved relatively high $R^2$ values (0.64 to 0.82 except for Ohio). It was also shown that at least one of climate factors in June, July, and August was highly correlated to alfalfa yields. In addition, the result clearly showed that the prediction performance of the Lasso models was remarkably better than that of the average yield prediction method.



Keywords: alfalfa yield, temperature, precipitation, Lasso, midwestern United States

# Introduction

Alfalfa is the fourth most valuable cash crop in the U.S., following corn, wheat, and soybean, and its production is vital to sustain the dairy, beef, and hay industries. As the world population increases alongside environmental degradation and water scarcities, identifying monthly climate variables related to alfalfa production can provide growers and researchers more time to deal with the effects of climate change. Grain yields have increased remarkably with the development of genetic technology and fertility management (Thompson, 1986). However, alfalfa breeders often pay more attention to quality over yield. As such, alfalfa has had fewer advancements in biotechnology over the years when compared to corn or soybean (USDA-ARS, 2013). Tannura et al. (2008) conducted an economic analysis of corn and soybean crops using a model that included a term to account for the technological advances that have attributed to the increase in yield over the years. Since alfalfa has had fewer advancements in biotechnology, it is rational to exclude the technological effect. Since alfalfa is a perennial legume that can grow for four to five years, annual climate patterns can have a significant impact on overall alfalfa production. Research on the impact of temperature and precipitation without influence of technology is more reasonable for alfalfa. Unfortunately, few studies focus on revealing such a climate-yield relationship in perennial crops (Lobell and Field, 2011).

Many studies have investigated the risks to agricultural production due to climate change, especially for annual crops such as corn, wheat, and soybean (Andresen et al., 2001; Bannayan and Sanjani, 2011; Lobell and Burke, 2010; Tannura et al., 2008; Thompson, 1986). Few studies have assessed how climate variation impacts alfalfa yields over aggregate areas in terms of using statistical models. Especially in the last three decades, warmer global temperatures have altered regional climates, and the effect of climate on alfalfa yields is not comprehensively understood. Whether climate change will boost or restrain alfalfa yields remains a subject of debate.

In statistical modeling, "scaling-up" refers to process-based models, while "scaling-down" refers to the statistical models (Hansen and Jones, 2000; Everingham et al., 2009; Lobell and Burke, 2010). The process-based models require more work at the soil and plant-level for a relatively small area, which can have difficulties when applied to a large area (Hansen and Jones, 2000). However, statistical models overcome the limitation of heterogeneity of growing environments (Everingham et al., 2009) by omitting plant, soil, and management data (Lobell and Burke, 2010). The application of statistical models over statewide scales are often more reliable. Lobell et al. (2007) demonstrated that certain large-scale variables can explain how winter climate affects alfalfa dormancy and annual crop yield. White (1985) used a stepwise multiple regression to demonstrate a relationship between four selected forage grass species and precipitation in April and May in the northern Great Plains with an average correlation coefficient of 0.69. Bannayan and Sanjani (2011) demonstrated how the variance of growing season mean temperature decreased alfalfa yields in Birjand, Iran, and they also found that number of hot days with maximum temperature were uncorrelated with alfalfa yields. Lobell et al. (2007) also examined effects of temperature and precipitation of 12 major California crops from 1980 to 2003, including alfalfa hay. His evaluation shows that high precipitation and minimum temperature in February reduced hay yields. A crop simulation model was conducted to study the impact of climate on alfalfa yields in the Great Lakes region for 102 years, but the impact of climate on alfalfa yields was inconsistent (Andresen et al., 2001).

Although linear regression models have been commonly used to estimate yield trends associated with the effects of temperature and precipitation over a statewide scale, there are severe limitations in either variable selection or model interpretation. On one hand it might be arbitrary to draw conclusions based on fewer variables, while on the other hand involving too many variables in the model could be difficult to interpret. The addition of too many climate variables without the addition of supporting yield data can lead to a high-dimensional issue (Donoho, 2000) in which traditional linear regression methods often struggle (e.g. the ordinary least squares method cannot generate a unique result of parameters). Moreover, multicollinearity will cause the estimators to have a large variance (Akkol, 2014). It is impossible for analysts to extract crucial information from an enormous dataset when the number of observations is less than the number of predictors. In the era of big data, the least absolute shrinkage and selection operator, often referred to as the Lasso, plays a key in high-dimensional data analysis. (Tibshirani, 1996; Faraway, 2014). By imposing zero coefficients on unimportant predictors, the Lasso effectively eliminates the irrelevant predictors from the regression model avoiding overfitting, so a subset of the original variables is included in the final model.

In this study, the Lasso method was employed to identify important climate variables related to the alfalfa yields. Compared to the other common shrinkage methods, the Lasso has a unique advantage for this study. In ridge regression, for example, while the estimated coefficients of all the variables shrink to a smaller value, they still remain in the model. Thus, the ridge regression does not perform variable selection. However, using the least absolute shrinkage, the Lasso forces small coefficients to be set to zero. Therefore, some redundant variables can be eliminated from the model (Hastie et al., 2002). Moreover, regularizing results from the Lasso lead to a sparse solution, which is more interpretable and preferred (Tibshirani, 1996).

The objectives of this study were to: (1) propose the Lasso method to study the relationship between the alfalfa yield and monthly climate information with much larger numbers of variables in the state scale; (2) investigate the impacts of climate variables returned by the Lasso on the alfalfa yield for each state separately; and (3) assess the validity of the new prediction model by comparing yield observations to estimated yields of each state.

## Materials and Methods

Monthly temperature and precipitation data from 1985 to 2014 for eight states, including monthly average temperature (*tave*), maximum temperature (*tmax*), minimum temperature (*tmin*), and monthly precipitation (*ppt*), were obtained from the National Oceanic and Atmospheric Administration (NOAA). Statewide data on alfalfa yield from 1985 to 2014 were obtained from the National Agricultural Statistics Service of the United States Department of Agriculture (NASS).

The Lasso regression model was developed with linear and quadratic terms for each of eight states, Indiana, Minnesota, Nebraska, North Dakota, Ohio, Oklahoma, South Dakota, and Wisconsin (Fig. 1.1), where all monthly climate variables were included as linear terms and monthly climate variables from April to September were also included as quadratic terms. Months in the model included October in the previous year to September. Note that the relationship between alfalfa yield and climate variations can be explained by the coefficients in the regression model. The total number of predictors was 72, which caused the high dimensional statistical problems.

The Lasso method was implemented with all climate variables as the predictors and alfalfa annual yield as the response. Data from 1985 to 2009 were defined as the training set to perform the analysis, whereas the data from 2010 to 2014 were defined as the test set to assess the validity of the developed model. All predictors were standardized in the analysis. In order to find the optimal tuning parameter λ, which controls the degrees of sparsity in the coefficient estimates, a five-fold cross-validation was used on the training data set. Specifically, the training data set were partitioned into five sets of equal size, fitted the model each four selected sets excluding the fifth fold, after that λ and cross-validation error for the fifth fold were computed.

$$\underset{\boldsymbol{\beta}}{\operatorname{argmin}}(y - \boldsymbol{X\beta})' \, (y - \boldsymbol{X\beta}) + \lambda \sum_{j=1}^{p} |\beta_j|$$

To measure the goodness of fit of the Lasso model, correlations and the mean squared prediction error (MSPE) were calculated for both the training set and the test set. The Lasso method and cross-validation were realized using the R package *glmnet*.

## Results and Discussion

### Climate variable selection

The dotted vertical lines in Fig. 1.2 in the plot represent the minimum mean square error (MSE) for a set λ (on the left) and the one standard error from the minimum (on the right). The number of non-zero variables coefficients selected were on the top of the plot. Except for Ohio, the tuning parameters λ were selected in a way such that six or less variables were identified (Table 1.1). Since the intercept-only model worked better than the Lasso model in Ohio, a model with five variables were used in order to examine the effects of monthly climate (Table 1.1).

The non-zero coefficients estimated by the Lasso are summarized in Table 1.1. The fitted Lasso on the training set produced models with relatively large coefficients of determination, denoted by $R^2$, as follows: $R^2 = 0.82$ in Indiana, $R^2 = 0.80$ in Oklahoma, $R^2 = 0.79$ in Minnesota,

$R^2$ = 0.74 in South Dakota, $R^2$ = 0.72 in Wisconsin, $R^2$ = 0.71 in Nebraska, $R^2$ = 0.64 in North Dakota, and $R^2$ = 0.41 in Ohio (Fig. 1.1). Lobell and Burke (2010) reported eight Lasso models for perennial crops including hay which can explain more than 46% of the variability in yields in California. Prior to that, Lobell et al. (2007) provided three climate variables in an ordinary least squares model with $R^2$ approximately 0.72 for alfalfa hay in California. White (1985) analyzed four perennial forage yields over six years in the northern Great Plains, the $R^2$ of model for crested wheatgrass and April and May precipitation was 0.59. Fick (1984) explored a simulation model by using physiological and environmental factors, which explained 76% of the variation of field data. In this study, we achieved a high $R^2$ value not relying on field data from either soil or plant. Only data from two different categories (temperature and precipitation) could be adequate for building an interpretable model. Our result demonstrated that the Lasso was a powerful tool to better understand alfalfa yields in response to variable climate conditions on large scales.

### Climate-yield relationships

All models in eight states included climate variables in June, July and August, which are highly related with alfalfa yields due to its C3 physiology (Barnes and Nelson, 2003). Except in Wisconsin, at least one variable in the summer included precipitation. All the temperature variables in the summer showed a negative effect on the alfalfa yield (Fig. 1.1), while the precipitation variable had a positive relationship with alfalfa yields, suggesting that higher precipitation in the summer would be expected to produce more yield, and rain damage in the summer was not a problem in the Midwest during research period. The omission of the precipitation variable tends to be rational for the perennial crops which are irrigated (Lobell and Field, 2011). Since most of the alfalfa fields in the Midwest are non-irrigated, the alfalfa production system was assumed homogenous statewide. Everingham et al. (2009) indicated that crops in the non-irrigated system would be easier to fit a model than crops in irrigation system.

Alfalfa was defined to start fall dormancy from October to March in this study. Climate variables in dormancy period existed in all eight models. Associations between alfalfa yields and climate variables varied across eight states. The inconsistency of model structure in eight states was due to their climate and geographical differences. Bannayan and Sanjani (2011) analyzed the correlation of crop yields and climate indicators in Iran; their results showed alfalfa yields were highly correlated with growing season mean temperature, maximum temperature and the number of days with maximum temperature higher than 30 °C within a year. However, the different climate conditions across the domain area in this study led to different model structures. Models were built separately in each state to confront geographic and meteorological variations.

Models in Minnesota and Wisconsin involved April *tmin* which had a positive relationship with the alfalfa yield. However, April *tave* in the model for Oklahoma showed a negative impact on the alfalfa yield. The heterogeneity of the growing conditions between northern and southern portion of the Great Plains led to the contrary effect of temperature variable in April.

All models contained at least one quadratic term, which represented a strongly negative relationship to alfalfa yields (Lobell and Burke, 2010). There were no quadratic variables during dormancy. Both Oklahoma and Indiana had one variable in the early growing season. Models involving variables of the dormancy period could account for the yield variability in the early season (Sharratt et al., 1986). Tannura et al. (2008) refined Thompson's original model (Thompson, 1963) by eliminating quadratic terms in the early and later seasons with poor correlations. Similarly, quadratic terms only included the growing season variables and represented the relationship of alfalfa yields and climate variables in downward facing parabola (Table 1.1). However, excessive quadratic terms reduced the probability of obtaining the highest degrees of freedom (Tannura et al., 2008).

## Analysis of states

### Wisconsin and Minnesota

Higher October *tave* improved alfalfa yields in Wisconsin, which benefits the storage of carbohydrates before dormancy. Similarly, higher February *tmin* in Minnesota also increased alfalfa yield. As a result of the increase of temperature during the dormancy, alfalfa plant could save more nonstructural carbohydrates for boosting regrowth in the following months (Al-Hamdani, 1988). All the summer temperature variables in two states showed negative relationships to alfalfa yields, which means the impact of excessively high temperatures would be lower alfalfa yields. Slow growth encourages the closure of leaf stomata, reducing the production of carbohydrates during the summer slump. Quadratic terms of June *tmax* and July *tmax* indicated the severe impacts of extremely high monthly maximum temperature on alfalfa yields. In Minnesota, the last harvest was delayed due to excessive *ppt* in August, however, moderate damage was observed. In Wisconsin, the variation in *ppt* was not dramatic over 30 years. Absence of precipitation related variables in the Wisconsin model provides evidences that moderate precipitation deficiencies would not be a problem for alfalfa production. Temperature variables in the early season determined the increase of alfalfa yields. Inversely, the summer maximum temperature determined the declines of alfalfa yields.

### Indiana and Ohio

October *tmax* had a positive impact on alfalfa yields as well as June *ppt* in Indiana. Inversely December and May *ppt* had negative impacts. Larger amounts of June *ppt* might alleviate the damage of excessive May *ppt* on alfalfa yields. For Ohio, the intercept only model provided the lowest MSPE. In order to see the impact of monthly climate variables on alfalfa yields, lambda was decreased to get five non-zero coefficients (Table 1.1). Alfalfa yields and *ppt* in October, December, and June were negatively correlated. Decreases in alfalfa yields due to low October *tmax* could be explained by avoiding low temperature injury and insufficient regrowth after last harvest (Al-Hamdani, 1988), and higher December *ppt* caused extra soil moisture leading to alfalfa root diseases. The absences of variables in April and May were observed in Ohio, which suggests that moisture in the early growing season was adequate and alfalfa demonstrates a good adaption due to its genetic diversity (Barnes and Nelson, 2003).

**North Dakota and South Dakota**

Scales of annual precipitation in North Dakota and South Dakota were lower than of the other states (Fig. 1.1). More than 63% of the variation in alfalfa annual yield could be explained by four climate variables in these two states (Fig. 1.1). Alfalfa yields were found closely associated with precipitation variables from all seasons. The importance of May *ppt* agrees with the finding of White (1985) that May *ppt* was highly related with crested wheatgrass yields in the northern Great Plains. June *tmin* was retained in quadratic form, which suggests that excessive high June *tmin* could negatively affect alfalfa yields. Furthermore, similar to Wisconsin, both temperature variables in June (e.g. Jun *tmax* and Jun *tmin*) showed negative relationship to alfalfa yields, and precipitation variables leading to rain damage were not included as predictors in either state. Moreover, no climate variables embodied the winter injury or early dormancy breaks.

**Nebraska and Oklahoma**

The impact of climate variables on alfalfa yields in Nebraska was similar to variables in Minnesota, even though they are not neighboring states. June *ppt* would lighten the summer slump in Nebraska, as well as July *ppt* in Minnesota. In Oklahoma, November and June *ppt* showed a positive linear relationship with alfalfa yields. Previous studies found that rain damage might cause 11.2% hay yield loss (Rotz and Abrams, 1988). However, the presences of precipitation variables in the model did not suggest any saturation or negative effect on alfalfa yields. The early season soil moisture and the growing season drought stress were the two aspects related to precipitation in Oklahoma. Extremely high April *tave* could have more negative impacts on alfalfa yields due to the quadratic relationships. The negative relationship agrees with the finding of Al-Hamdani (1988) that high temperature in early season might boost alfalfa regrowth abnormally. Storage and usage of nonstructural carbohydrates might be disturbed by unusual climate condition. Overall, the effects of temperature and precipitation variables on alfalfa yields were complicated; the impact of more precipitation alleviated the alfalfa loss, while high temperature in the early and growing season decreased alfalfa yields.

## Prediction

To assess the prediction performance of the Lasso models, MSPE, which represents the difference between observed values and predicted values, was computed using the test set (the data from 2010 to 2014). In order to verify the importance of the climate variables identified by the Lasso, we compared the MSPE of the Lasso ($MSPE_{Lasso}$) with the MSPE of the average yield prediction model ($MSPE_{avg}$), where the average yield prediction model was obtained by fitting a linear regression model only with the intercept for the training data (the data from 1985 to 2009). Since $MSPE_{Lasso}$ was always smaller than $MSPE_{avg}$ in each state, the result clearly showed that the Lasso model with the important climate variables indeed improved the alfalfa yield prediction on test set (Table 1.2).

To measure the accuracy of the prediction, the correlation coefficient between the predicted yields and the observed yields was also computed. The result suggested that the Lasso method could capture the trend of the future yields in six out of eight states (i.e., Indiana, North Dakota, South Dakota, Minnesota, Nebraska, and Oklahoma) (Fig. 1.1).

## Conclusions

This study utilized a statistical procedure to demonstrate how climate variation affects state-level alfalfa yields in the Midwestern United States. The advantage of using the Lasso regression model is that a large number of predictors can be eliminated from the regression when some variables are irrelevant to the response. One common characteristics of models across different states was the impact of summer temperatures on alfalfa yields, which confirms the summer slump trait of the cool season forages.

Model construction emphasized the number of selected climate predictors. The selected variables could be the most important information to help growers and scholars adjust their management practices in order to adapt to climate changes. In addition, accumulated climate changes could be interpreted by the Lasso model, but the more extreme weather such as hail or frost, especially over long periods of time, would be hard to simulate. Some models like Ohio and Wisconsin had poor results (low value of $R^2$ and correlations), because alfalfa yields responded more strongly to severe climate than the typical climate variables.

In this study, with the minimal MSPE, the value of $\lambda$ from the cross-validation still resulted in retaining a relatively large size of variables. Even though the Lasso regression effectively reduced the number of variables down to approximately ten variables, this selection sample was still relatively large for making meaningful inferences in regard to alfalfa yields. Thus, to get the ideal number of variables that can be easily interpreted, the value of λ can be adjusted with the related knowledge of the topic. With an improved number of predictors, MSPE from the regression model increase slightly, but the model might be more interpretable.

## References

Akkol, S. (2014). SHRINKAGE REGRESSION METHODS APPLIED TO AGRICULTURE. Agriculture and Food.

Al-Hamdani, S. H. (1988). Effect of temperature on alfalfa growth, carbohydrate root reserves, photosynthesis, respiration, and photosynthate translocation (Doctoral dissertation, Oklahoma State University).

Andresen, J. A., Alagarswamy, G., Rotz, C. A., Ritchie, J. T., & LeBaron, A. W. (2001). Weather impacts on maize, soybean, and alfalfa production in the Great Lakes region, 1895–1996. Agronomy Journal, 93(5), 1059-1070.

Bannayan, M., & Sanjani, S. (2011). Weather conditions associated with irrigated crops in an arid and semi arid environment. Agricultural and forest meteorology, 151(12), 1589-1598.

Barnes, R. F., Miller, D. F., & Nelson, J. C. (1995). Forages: An introduction to grassland agriculture (Vol. 1). Ames, IA, USA: Iowa State University Press. pp. 3-25

Donoho, D. L. (2000). High-dimensional data analysis: The curses and blessings of dimensionality. AMS math challenges lecture, 1(32), 375.

Everingham, Y. L., Smyth, C. W., & Inman-Bamber, N. G. (2009). Ensemble data mining approaches to forecast regional sugarcane crop production. Agricultural and forest meteorology, 149(3-4), 689-696.

Faraway, J. J. (2016). Linear models with R. Chapman and Hall/CRC.

Fick, G. W. (1984). Simple Simulation Models for Yield Prediction Applied to Alfalfa in the Northeast 1. Agronomy Journal, 76(2), 235-239.

Hansen, J. W., & Jones, J. W. (2000). Scaling-up crop models for climate variability applications. Agricultural Systems, 65(1), 43-72.

Trevor, H., Robert, T., & JH, F. (2009). The elements of statistical learning: data mining, inference, and prediction.

Lobell, D. B., & Burke, M. B. (2010). On the use of statistical models to predict crop yield responses to climate change. Agricultural and Forest Meteorology, 150(11), 1443-1452.

Lobell, D. B., Cahill, K. N., & Field, C. B. (2007). Historical effects of temperature and precipitation on California crop yields. Climatic change, 81(2), 187-203.

Lobell, D. B., & Field, C. B. (2011). California perennial crops in a changing climate. Climatic Change, 109(1), 317-333.

Rotz, C. A., & Abrams, S. M. (1988). Losses and quality changes during alfalfa hay harvest and storage. Transactions of the ASAE, 31(2), 350-0355.

Sharratt, B. S., Baker, D. G., & Sheaffer, C. C. (1986). Climatic effect on alfalfa dry matter production. Part I. Spring harvest. Agricultural and forest meteorology, 37(2), 123-131.

Tannura, M. A., Irwin, S. H., & Good, D. L. (2008). Weather, technology, and corn and soybean yields in the US corn belt. Technology, and Corn and Soybean Yields in the US Corn Belt (February 1, 2008).

Thompson, L. M. (1963). Weather and technology in the production of corn and soybeans.

Thompson, L. M. (1986). Climatic Change, Weather Variability, and Corn Production 1. Agronomy Journal, 78(4), 649-653.

Tibshirani, R. (1996). Regression selection and shrinkage via the lasso. Journal of the Royal Statistical Society Series B, 58(1), 267-288.

USDA-ARS. (2013) Roadmap For Alfalfa. https://www.ars.usda.gov/ARSUserFiles/50901500/pdf's/ AlfalfaRoadMap.pdf.

White, L. M. (1985). Stand age, precipitation, and temperature effects on forage yield. Journal of Range Management, 39-43.

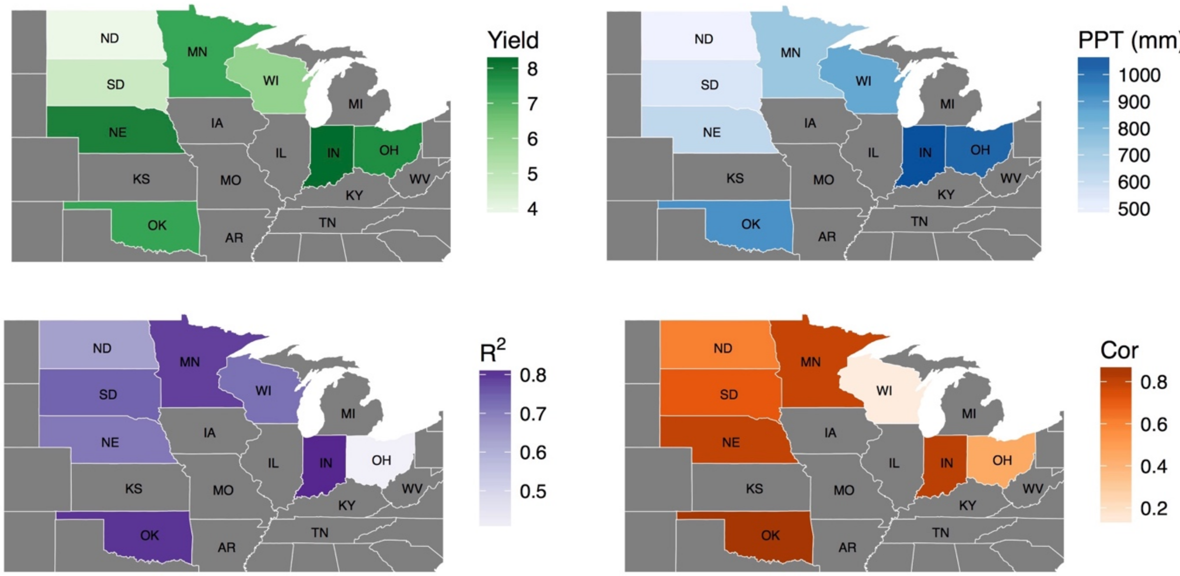
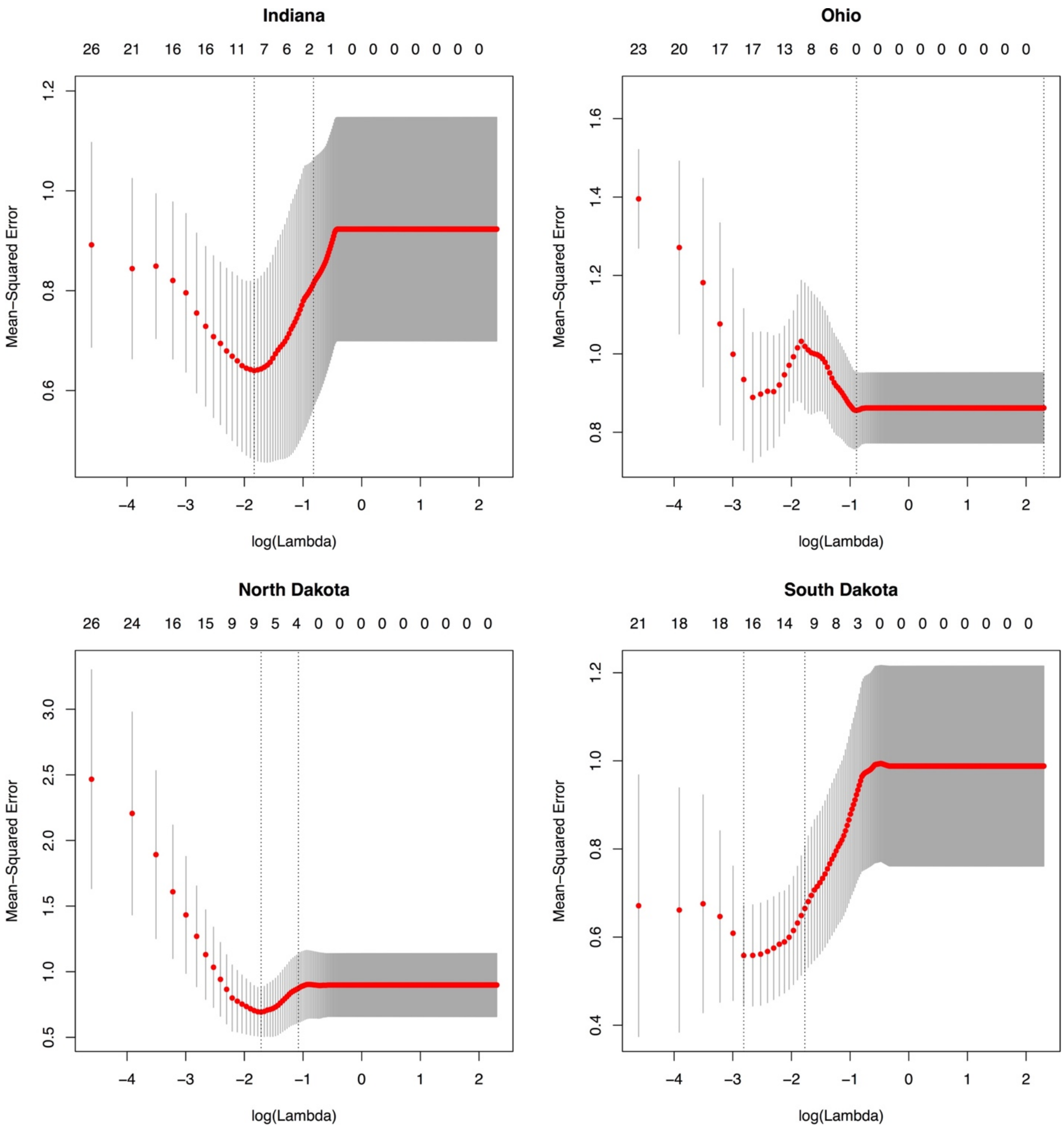



Figure 1.1 The upper two plots show average statewide alfalfa yields (Mg $ha^{-1}$) and precipitation (PPT, mm). The lower two plots show yield variation on the training set ($R^2$), and the trend between predicted and observed alfalfa yields on the test set.


Indiana
26 21 16 16 11 7 6 2 1 0 0 0 0 0 0 0
Mean−Squared Error
0.6 0.8 1.0 1.2
−4 −3 −2 −1 0 1 2
log(Lambda)
Ohio
23 20 17 17 13 8 6 0 0 0 0 0 0 0 0 0
Mean−Squared Error
0.8 1.0 1.2 1.4 1.6
−4 −3 −2 −1 0 1 2
log(Lambda)
North Dakota
26 24 16 15 9 9 5 4 0 0 0 0 0 0 0 0 0
Mean−Squared Error
0.5 1.0 1.5 2.0 2.5 3.0
−4 −3 −2 −1 0 1 2
log(Lambda)
South Dakota
21 18 18 16 14 9 8 3 0 0 0 0 0 0 0 0
Mean−Squared Error
0.4 0.6 0.8 1.0 1.2
−4 −3 −2 −1 0 1 2
log(Lambda)

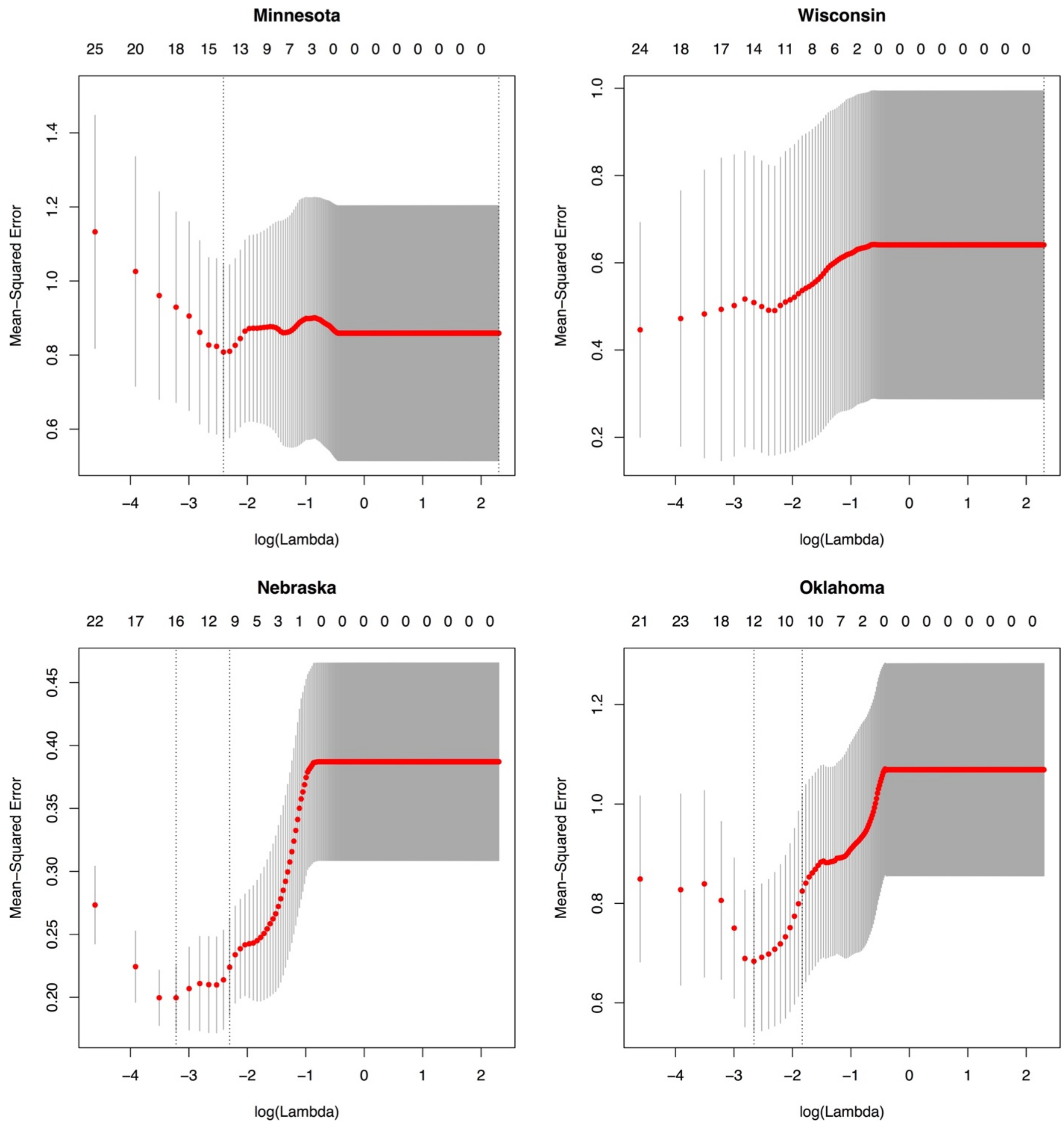



Figure 1.2 The cross-validated mean squared error as a function of λ in eight states. It includes the cross-validation curve (red dotted line), and upper and lower standard deviation curves along the λ sequence (error bars). Two selected λ's are indicated by the vertical dotted lines. Each plot shows how many non-zero variables are in the model at the top.

Table 1.1 Non-zero coefficients selected by the Lasso regression in eight states.

| **IN** | | **OH** | | **ND** | | **SD** | |
|---|---|---|---|---|---|---|---|
| Intercept | 8.3295 | Intercept | 7.8043 | Intercept | 3.7934 | Intercept | 4.6877 |
| Oct *tmax* | 0.0212 | Oct *ppt* | -0.0669 | Feb *ppt* | 0.0033 | Oct *ppt* | 0.0441 |
| Dec *ppt* | -0.0806 | Dec *ppt* | -0.0157 | May *ppt* | 0.0627 | Jun *tmax* | -0.0377 |
| Jun *tmax* | -0.1062 | Aug *tmax* | -0.0742 | Jul *ppt* | 0.0723 | Jun *ppt* | 0.1769 |
| Jun *ppt* | 0.2016 | Aug *ppt* | 0.0184 | Jun $tmin^2$ | -0.0276 | Jun $tmin^2$ | -0.0109 |
| May $ppt^2$ | -0.0723 | Jun $ppt^2$ | -0.0390 | | | | |

| **MN** | | **WI** | | **NE** | | **OK** | |
|---|---|---|---|---|---|---|---|
| Intercept | 7.5024 | Intercept | 5.9911 | Intercept | 7.8868 | Intercept | 7.6403 |
| Feb *tmin* | 0.1077 | Oct *tave* | 0.0413 | Oct *tmin* | 0.0113 | Nov *ppt* | 0.0150 |
| Apr *tmin* | 0.0159 | Oct *tmax* | 0.0557 | Oct *ppt* | 0.1652 | Jun *tmax* | -0.0393 |
| Jul *ppt* | 0.0919 | Apr *tmin* | 0.0196 | Jun *tmax* | -0.0663 | Jun *ppt* | 0.1944 |
| Jun $tmax^2$ | -0.1358 | Jun $tmax^2$ | -0.1479 | Jun *ppt* | 0.0120 | Jul *tmax* | -0.1485 |
| Aug $ppt^2$ | -0.0372 | Jul $tmax^2$ | -0.0063 | Jun $tmin^2$ | -0.0876 | Aug *tave* | -0.0426 |
| | | | | | | Apr $tave^2$ | -0.0083 |

Table 1.2 Evaluation of training set and test set.

| | IN | OH | ND | SD | MN | WI | NE | OK |
|---|---|---|---|---|---|---|---|---|
| **$MSPE_{Lasso}$** | 0.4409 | 0.3660 | 1.0339 | 0.6428 | 0.8805 | 0.5538 | 0.8736 | 3.4668 |
| **$MSPE_{avg}$** | 0.8265 | 0.4772 | 1.1969 | 0.9976 | 1.0357 | 0.6203 | 1.2460 | 6.0376 |

# Chapter 2 - The Analysis of Forage Yield and Nutritive Values of Low Lignin and Conventional Alfalfa

## Abstract

Under the four-cut system, low lignin alfalfa (*Medicago sativa* L.) may extend harvesting intervals improving harvest management flexibility and produce forage products with higher nutritive values. The objectives were to compare forage yield and nutritive values of low lignin and conventional alfalfa varieties when applied to six harvest schedules in the first and second production year. There were 12 treatments of two alfalfa varieties as whole plots and six harvest schedules as subplots. Across harvest schedules, there were four cuttings in two production years. Three harvest intervals including “Standard” (high quality, HQ), “Standard+5-day” (medium quality, MQ), and “Standard+10-day” (high yield, HY) were chosen for the first cutting, and 30-day (HQ) and 35-day (HY) for the second cuttings. The third and fourth cuttings in 2016 were timed near final harvest date and in 2017 occurred at 35-day (MQ) and 40-day (HY). Variety by harvest schedule interaction was not significant, but whole plot and subplot effects were significant. Hi-Gest 360 was consistently higher in nutritive value and with similar yield as Gunner. Harvest schedule did not consistently differ in forage yield and nutritive values. HS-1 (“Standard” + 35-day + Medium Quality + High Yield) with shorter first two cutting intervals provided lower acid detergent fiber (ADF), neutral detergent fiber (NDF), higher relative feed value (RFV), and similar forage yield compared to other schedules. HS-1 had highest economic incomes when considering RFV and yield among the six harvest schedules.

Keywords: low lignin, alfalfa yield, harvest schedule, nutritive values

# Introduction

The tradeoff between yield and quality of alfalfa production could be changed by introducing low lignin varieties. By reducing lignin content about 6 to 10%, the nutritive values of alfalfa can be expected to improve or maintain (Shadle et al., 2007; Undersander et al., 2009; Gallego-Giraldo et al., 2011). As a perennial legume, the performance of low lignin varieties needs to be evaluated, specifically in the non-irrigated areas with variable weather conditions. Decreasing lignin content could bring more nutritive value for growers, however, it could alter yield, disease resistance, and morphological and physiological developments (Guo et al., 2001; Undersander et al., 2009; Newman and Justen, 2016; Sulc et al., 2016).

Previous studies have documented that shorter harvest frequency has a yield or stand persistent advantage with aggressive harvest regimes (Kallenbach et al., 2002). Undersander et al. (2009) reported a 20 to 30% alfalfa yield increase compared 3-cut to 4-cut in a season, testing a new reduced lignin variety. Two recent studies including reduced and low lignin varieties also found benefits of reducing the number of cuts per season (Sulc et al., 2016; Grev et al., 2017). There is limited data on the performance of the new variety in the first and second production year, under a fixed harvest frequency. Evidence from other studies suggests that higher harvest frequency could account for yield loss, reduced stand density and crown mass (Rimi et al., 2014). Information is needed on the response of low lignin alfalfa subject to diverse harvest schedules.

Alfalfa is required to have a fall cutting four to six weeks before the first killing frost (Sheaffer and Marten, 1990). The flexibility of adjusting cutting intervals is limited when growers fix harvest frequency because of the first killing frost. It is essential to refine the timing of the first two harvests per season. Several studies have documented more significant effects of different cutting intervals in spring and early summer on alfalfa yield and nutritive value, compared to late summer (Kallenbach et al., 2002; Brink et al., 2010; Rimi et al., 2014). Brink and Marten (1989) reported that alfalfa quality of systems occurred earlier with the first and second cuttings, and later with the third or fourth cutting were superior, which compared to alfalfa quality of systems with fixed intervals. For alfalfa varieties with low lignin content, the first cutting according to plant height might follow the same time producers harvest conventional varieties (Newman and Justen, 2016).

Growers often take small risks with cutting schedules, but new varieties with low lignin content and competitive yields may allow growers to adjust the time of harvest (Newman and Justen, 2016). The objective of this study was to evaluate field performance and cash value of low lignin and conventional varieties under four-cut systems, with diverse harvest schedules during the first and second production year.

## Materials and Methods

Hi-Gest 360 and Gunner were seeded on August 25, 2015, at the Department of Agronomy Ashland Bottoms Research Farm (39.13° N, 96.63° W), near Manhattan, KS, on a Rossville silt loam (very rarely flooded). Hi-Gest 360 represents the low lignin variety (conventional breeding technology), while Gunner represents the conventional variety.

Soil samples were taken and submitted to the Kansas State University Soil Testing Lab to check soil fertility, with soil pH 7.9, 125 mg $kg^{-1}$ phosphorus, and 332 mg $kg^{-1}$ potassium.

Lambda-cyhalothrin was applied at 25 g $ha^{-1}$ late spring and early summer in 2016 and 2017 to control alfalfa weevil. Zeta-cypermethrin*S-Cyano was applied at 28 g $ha^{-1}$ in early May to control other insect pests.

The experimental design was a randomized complete block in a split-plot arrangement. There were 12 treatments of two alfalfa varieties as whole plots and six harvest schedules as subplots. In general, the first two cuttings in 2016 and 2017 were arranged in the same way (Fig. 2.1). Three harvest intervals including “Standard” (high quality, HQ), “Standard+5-day” (medium quality, MQ), and “Standard+10-day” (high yield, HY) were chosen in the first cutting. “Standard” represented the first cutting taken at late bud stage or when plants reached 60 cm tall approximately; two intervals including 30-day and 35-day were selected in the second cutting; the third and fourth cuttings were set to have similar last harvesting dates in 2016, and final cuts across schedules were selected within five days of each other to allow alfalfa to have enough time to restore carbohydrates before the fall dormancy. In 2017, the third and fourth cuttings were set to 35-day and 40-day, which was aiming to see the yield potential of the four-cut system. Six harvest schedules were defined as HS-1 (HQ+HQ+MQ+HY), HS-2 (HQ+HY+MQ+HY), HS-3 (MQ+HQ+MQ+HY), HS-4 (MQ+HY+MQ+HY), HS-5 (HY+HQ+MQ+HY), and HS-6 (HY+HY+MQ+HY). Data were analyzed using the GLM procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). Means separations were performed on significant effects using Fisher’s least significant difference test (LSD).

Individual plots were 1.5 by 4.5 m. Plots were harvested with a Carter small plot harvester, leaving 5-cm tall stubble. No plots were harvested in the establishment year (2015). All plots were harvested four times during the first and second production year. In order to condition harvest cost and compare six intensive schedules, four cutting times were used (Fig. 2.1).

Fresh mass data of the harvested alfalfa was collected. A subsample from each plot was weighed fresh and dry to determine dry matter yield. After that, dry samples were ground in a

Wiley mill to pass a 1-mm screen for nutritive value analysis. Crude protein (CP) were analyzed for three replications of samples collected in 2016 and 2017. ADF, and NDF were determined for all samples collected in 2016 and 2017. Wet-chemistry methods were applied in this study for analyzing CP, ADF, and NDF.

The relative feed value index was calculated by digestible dry matter (DDM) of the alfalfa from ADF and the dry matter intake (DMI) potential from NDF (Rohweder et al., 1978). The index is then calculated as follows:

$$DDM = 88.9 - (0.779 \times \%ADF)$$

$$DMI = 120/(\%NDF)$$

$$RFV = (DDM \times DMI)/1.29.$$

## Results and Discussion

For alfalfa yield, nutritive values, and economic incomes, statistical analysis showed no significant interactions between variety and harvest schedule; therefore, only the main effects were reported.

### Forage Yield

#### Variety Response

Low lignin alfalfa Hi-Gest 360 and conventional alfalfa Gunner did not show a significant difference in yield in 2016 and 2017. The low lignin variety (10.8 Mg $ha^{-1}$ and 9.5 Mg $ha^{-1}$ in 2016 and 2017, respectively) tended to have a similar dry matter yield compared to the conventional variety (11.4 Mg $ha^{-1}$ and 9.7 Mg $ha^{-1}$ in 2016 and 2017, respectively), but with no statistical difference was found (Table 2.2). The first public research comparing low lignin and reference alfalfa varieties in Minnesota has also concluded that forage yield differences were less showed in the first production year (Grev et al., 2017).

### Harvest Schedule Response

Alfalfa yields significantly ($p < 0.05$) differed among six harvest schedules and the trend was relatively consistent across two years. Yield differences were greater in the second production year (2017) than the first production year (2016). In 2016, HS-1 and HS-2 provided the highest forage yields, while HS-3 and HS-5 were significantly lowest with alfalfa yield (Table 2.2). Extended intervals were not necessary to achieve greater yields regardless of variety. Testa et al. (2011) attributed the loss of leaves to delay harvest time. Choosing 28-day or fewer days intervals for the first and second cuttings in this study result in avoiding unfavorable drought weather during the summer, especially in July 2017 (Table 2.2). The regrowth of HS-1, HS-2, and HS-3 after the second cutting obtained the marginal rainfall before the summer slump compared to the HS-4, HS-5, and HS-6.

## Crude Protein

### Variety Response

Varieties showed no differences in CP concentration. Numerically, Hi-Gest 360 produced more protein values than Gunner did in 2016 and 2017, no statistical evidence was shown. The study in California and Pennsylvania showed that varieties including Hi-Gest 360 had similar CP concentrations in the seeding year (Sulc et al., 2016). Grev et al. (2017) also reported the similar CP concentrations for reduced lignin alfalfa (54HVX41) compared to other varieties in two of three locations in both seeding year and first production year. The improved traits of new varieties hardly show advantages in CP levels (Grev et al., 2017).

### Harvest Schedule Response

Harvest schedule treatment did not influence CP concentration (Table 2.2). The significant differences in harvest schedules existed in the first and the second cuttings, and the intervals of each harvesting were controlled in a realistic way to represent alfalfa production. As ten days differences between HS-1 and HS-6 in the first and second cuttings were not enough to show a significant result in CP level. These results were expected and agree with results from previous studies indicating a fluctuated trend could be observed as delaying harvesting, but results are not statistically significant (Weir et al., 1960; Nocek and Grant, 1987; Hoffman et al., 1993).

## Fiber Content and RFV

### Variety Response

Across two years, Hi-Gest 360 contained less NDF concentrations compared to Gunner (Table 2.2). Variety did not significantly differ in ADF concentration and RFV in 2016 and 2017. Sulc et al. (2016) found reduced NDF concentrations for Hi-Gest 360 compared to one of the reference varieties (54R02). Other previous studies reported that NDF concentrations were less for reduced lignin alfalfa (HarvXtra) compared to reference varieties (Shadle et al., 2007; Getachew et al., 2011; Grev et al., 2017). Given weather variation between 2016 and 2017 in this study, our results demonstrated that low lignin alfalfa Hi-Gest 360 had higher nutritive value potential in the first and second production year than the conventional alfalfa Gunner.

### Harvest Schedule Response

The harvest schedule effects on forage nutritive value were more pronounced in the first production year (2016) than in the second production year (2017). In 2016, harvest schedules were categorized into two major parts, HS-1 and HS-2 demonstrated a premium quality, while the other four schedules fell into the low-quality category. ADF and NDF concentrations, and

RFV for all harvest schedules ranged from 361 to 395 g $kg^{-1}$, 445 to 482 g $kg^{-1}$, and 113 to 129. In 2017, the alfalfa responded to extreme weather differently. ADF concentration had no significant difference in 2017 and the LSD tests showed the limited effect of harvest schedules on NDF concentration. In contrast to results in 2016, HS-2 to HS-6 maintained a higher RFV (128 to 129) compared to HS-3 (122). The inconsistency of nutritive value between 2016 and 2017 was likely due to the drought in June and July 2017 (Table 2.1). Drought might have delayed the maturity resulting in a higher quality forage (Peterson et al., 1992).

Under the normal weather condition, especially during the growing season, the results of nutritive values were expected. Alfalfa is more likely to develop its morphological characteristics according to different schedules. The design of bringing forward the first two cuttings which happen in late spring and early summer would provide forage with high nutritive value when the precipitation is not extremely limited. Similarly, Brink et al. (2010) proposed similar suggestions in humid regions. In Fig. 2.3 and Fig. 2.4, six harvest schedules showed similar trends in 2017 and more effect of shorter intervals were more pronounced in 2016.

## Forage yield of the first and second Cuttings

Yield and nutritive value were analyzed again after removing data of the third and fourth cuttings each year since the first and second harvesting represent more than two third of the total yield (Fig. 2.2). Moreover, the major differences in the harvest intervals were assigned to the first and second cuttings for both years.

### Variety Response

In 2016, alfalfa dry matter yield of the first two cuttings were 65% and 63% of annual yield for Hi-Gest 360 and Gunner, respectively. In 2017, alfalfa yield of the first two cuttings was 50.5% of annual yield for both varieties. Hi-Gest 360 showed a slightly lower yield in the first production year (Table 2.3). In 2017, the rainfall of May and June was 168.4 mm less than the rainfall of May in 2016. The second cutting of each harvest schedules was affected by limited rainfall (Fig. 2.2).

### Harvest Schedule Response

HS-2 targeting on getting high quality and high yield behaved the greatest compared with other schedules in the first and second production year. Yield results were variable due to different weather conditions in two years. Lengthening intervals in the first and second cuttings were not the essential condition for attaining more yields, which suggests that harvesting alfalfa could rely on the morphological development other than rigidly according to a fixed number of intervals (Hall et al., 2000). In the first production year, there were no differences between HS-4 and HS-1 in forage yield. HS-3 and HS-4 produced the least forage yield when combining data in two years. Harvest schedule subjected to fixed lengths or conservative patterns were unlikely to overcome the effect of extreme weather conditions.

## Fiber Content and RFV of the first and second Cuttings

### Variety Response

Across the years, Hi-Gest 360 maintained lower NDF concentrations Gunner (Table 2.3). ADF concentrations were less and RFV were greater for Hi-Gest 360 (338 g $kg^{-1}$, 136) compared to Gunner (350 g $kg^{-1}$, 128) in 2017. Sulc et al. (2016) reported that Hi-Gest 360 was significantly different in nutritive value when compared with one of the reference varieties (54R02).

### Harvest Schedule Response

ADF, NDF concentrations and RFV differed among six harvest schedules regarding the first and second cuttings in 2016 and 2017. Across two years, HS-1 showed a good adaption to different weather pattern producing high quality alfalfa product. Bringing forward harvest dates in early growing season increased forage nutritive value and studies investigating the harvest intervals have also found similar reduced NDF for shorter intervals or more frequent harvest systems (Kallenbach et al., 2002; Brink et al., 2010; Testa et al., 2011; Sulc et al., 2016; Grev et al., 2017). However, in 2017, ADF concentrations were less for HS-4 (332 g $kg^{-1}$) compared to HS-6 (352 g $kg^{-1}$), and NDF concentrations were less for HS-1 (430 g $kg^{-1}$) and HS-4 (430 g $kg^{-1}$) compared to HS-6 (450 g $kg^{-1}$). Extreme weather conditions such as drought could alter the effects of different harvest schedules on forage nutritive value. Growers would adjust their harvest strategies integrating both morphological traits and environmental factors.

## Economic Incomes

Alfalfa was valued at $1.00 per point RFV. Economic incomes ($ $ha^{-1}$) were equal to the relative feed value times multiplied by the yield from each cutting.

### Variety Response

Two alfalfa varieties in this study were tolerant of a diversity of harvest schedules and had no difference in economic incomes throughout the first and second production year. Hi-Gest 360 showed slightly more value than Gunner when combining economic incomes in both years (Table 2.4).

### Harvest Schedule Response

HS-1 and HS-2 demonstrated a consistent higher value per hectare than other schedules in 2016 and combining data in two years, which suggests that if growers only adjust the first two cuttings, shorter intervals with 28-day for the first cutting and 30-day for the second cutting benefit their incomes regarding forage quality in the early cuttings and longer intervals bring more production in the later cuttings (Table 2.4). The loss of nutritive value in the third and fourth cuttings could be compensated by increased production, the decline rate of nutritive value in the late season was less compared to the early season. However, the delaying strategy needs to be reconsidered when alfalfa suffers drought and extremely hot summer.

## Conclusions

Forage yield differences among harvest schedule were more pronounced than yield differences between two varieties. Harvest schedules prone to higher nutritive values tend to maintain a similar forage yield compared to harvest schedules with longer intervals.

The nutritive value data suggest two things. First, harvest interval has a significant effect on nutritive value of alfalfa and a more substantial effect than cultivar selection. Second, the nutritive value of low lignin alfalfa variety was significantly greater than conventional variety, offering producers the flexibility to adjust the harvesting schedule confronting unpredictable weather.

The concept of comparing the economic incomes of different varieties and harvest schedule provides an alternative way of reviewing forage management practices more reasonably and comprehensively. Regardless of weather pattern variation between the two years, HS-2 aiming to gain higher nutritive value significantly produces more economic incomes than other schedules. It appears that the low lignin alfalfa variety also has the potential to increase producers’ incomes compared to the conventional variety.

Alfalfa producers might benefit from using low lignin varieties regards to certain nutritive values. For a four-cut system in northeast Kansas, the first two cuttings are suggested to occur at the late bud stage, and the third and fourth cuttings are suggested to have at least 35-day and 40-day intervals, respectively.

## References

Brink, G. E., & Marten, G. C. (1989). Harvest management of alfalfa—Nutrient yield vs. forage quality, and relationship to persistence. Journal of Production Agriculture, 2(1), 32-36.

Brink, G., Hall, M., Shewmaker, G., Undersander, D., Martin, N., & Walgenbach, R. (2010). Changes in alfalfa yield and nutritive value within individual harvest periods. Agronomy journal, 102(4), 1274-1282.

Gallego-Giraldo, L., Jikumaru, Y., Kamiya, Y., Tang, Y., & Dixon, R. A. (2011). Selective lignin downregulation leads to constitutive defense response expression in alfalfa (Medicago sativa L.). New Phytologist, 190(3), 627-639.

Getachew, G., Ibáñez, A. M., Pittroff, W., Dandekar, A. M., McCaslin, M., Goyal, S., ... & Putnam, D. H. (2011). A comparative study between lignin down regulated alfalfa lines and their respective unmodified controls on the nutritional characteristics of hay. Animal Feed Science and Technology, 170(3-4), 192-200.

Grev, A. M., Wells, M. S., Samac, D. A., Martinson, K. L., & Sheaffer, C. C. (2017). Forage accumulation and nutritive value of reduced lignin and reference alfalfa cultivars. Agronomy Journal, 109(6), 2749-2761.

Guo, D., Chen, F., Wheeler, J., Winder, J., Selman, S., Peterson, M., & Dixon, R. A. (2001). Improvement of in-rumen digestibility of alfalfa forage by genetic manipulation of lignin O-methyltransferases. Transgenic research, 10(5), 457-464.

Hall, M. H., Smiles, W. S., & Dickerson, R. A. (2000). Morphological development of alfalfa cultivars selected for higher quality. Agronomy Journal, 92(6), 1077-1080.

Hoffman, P. C., Sievert, S. J., Shaver, R. D., Welch, D. A., & Combs, D. K. (1993). In situ dry matter, protein, and fiber degradation of perennial forages. Journal of Dairy Science, 76(9), 2632-2643.

Kallenbach, R. L., Nelson, C. J., & Coutts, J. H. (2002). Yield, quality, and persistence of grazing-and hay-type alfalfa under three harvest frequencies. Agronomy Journal, 94(5), 1094-1103.

Newman, Y. C., & Justen, V. (2016, January). Reduced lignin alfalfa. In Proc. 2016 Wisconsin Crop Manage. Conf., Madison, WI (pp. 69-72).

Nocek, J. E., & Grant, A. L. (1987). Characterization of in situ nitrogen and fiber digestion and bacterial nitrogen contamination of hay crop forages preserved at different dry matter percentages. Journal of Animal Science, 64(2), 552-564.

Peterson, P. R., Sheaffer, C. C., & Hall, M. H. (1992). Drought effects on perennial forage legume yield and quality. Agronomy Journal, 84(5), 774-779.

Rimi, F., Macolino, S., Leinauer, B., Lauriault, L. M., & Ziliotto, U. (2014). Fall dormancy and harvest stage impact on alfalfa persistence in a subtropical climate. Agronomy Journal, 106(4), 1258-1266.

Rohweder, D. A., Barnes, R. F., & Jorgensen, N. (1978). Proposed hay grading standards based on laboratory analyses for evaluating quality. Journal of animal science, 47(3), 747-759.

Shadle, G., Chen, F., Reddy, M. S., Jackson, L., Nakashima, J., & Dixon, R. A. (2007). Down-regulation of hydroxycinnamoyl CoA: shikimate hydroxycinnamoyl transferase in transgenic alfalfa affects lignification, development and forage quality. Phytochemistry, 68(11), 1521-1529.

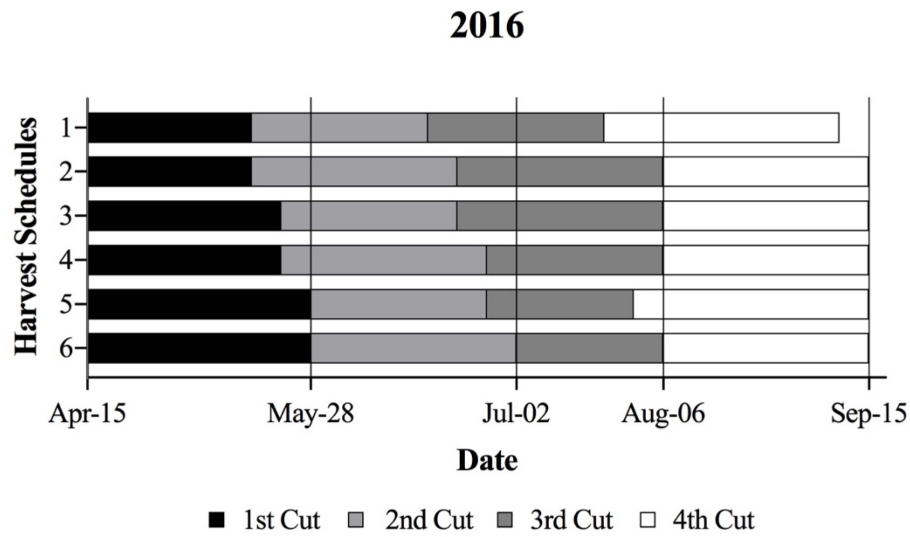
Sheaffer, C. C., & Marten, G. C. (1990). Alfalfa cutting frequency and date of fall cutting. Journal of Production Agriculture, 3(4), 486-491.

Sulc, M., Parker, A., Albrecht, K., Cassida, K., Hall, M., Min, D., Orloff, S., Undersander, D., & Xu, X. (2016). Low lignin alfalfa: Wide area field test results. In 2016 California Alfalfa and Forage Symposium.

Testa, G., Gresta, F., & Cosentino, S. L. (2011). Dry matter and qualitative characteristics of alfalfa as affected by harvest times and soil water content. European journal of agronomy, 34(3), 144-152.

Undersander, D., McCaslin, M., Sheaffer, C., Whalen, D., Miller, D., Putnam, D., & Orloff, S. (2009, December). Low lignin alfalfa: Redefining the yield/quality tradeoff. In Proc., 2009 Western Alfalfa & Forage Conference (pp. 2-4).

Weir, W. C., Jones, L. G., & Meyer, J. H. (1960). Effect of cutting interval and stage of maturity on the digestibility and yield of alfalfa. Journal of Animal Science, 19(1), 5-19.



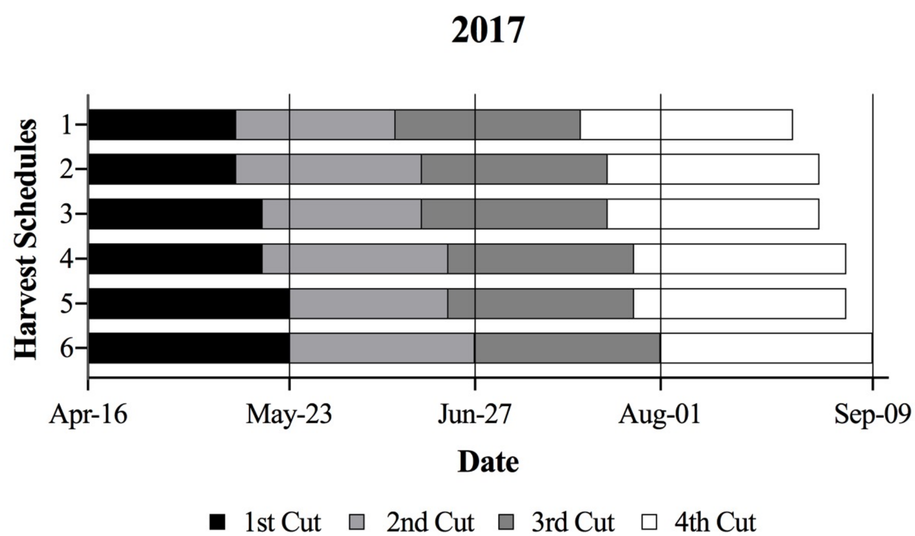




Figure 2.1 Days spent on each harvest of alfalfa plots in 2016 and 2017.

HS-1: "Standard" + 30-day + Medium Quality + High Yield, (HQ+HQ+MQ+HY)

HS-2: "Standard" + 35-day + Medium Quality + High Yield, (HQ+HY+MQ+HY)

HS-3: "Standard+5d" + 30-day + Medium Quality + High Yield, (MQ+HQ+MQ+HY)

HS-4: "Standard+5d" + 35-day + Medium Quality + High Yield, (MQ+HY+MQ+HY)

HS-5: "Standard+10d" + 30-day + Medium Quality + High Yield, (HY+HQ+MQ+HY)

HS-6: "Standard+10d" + 35-day + Medium Quality + High Yield, (HY+HY+MQ+HY)

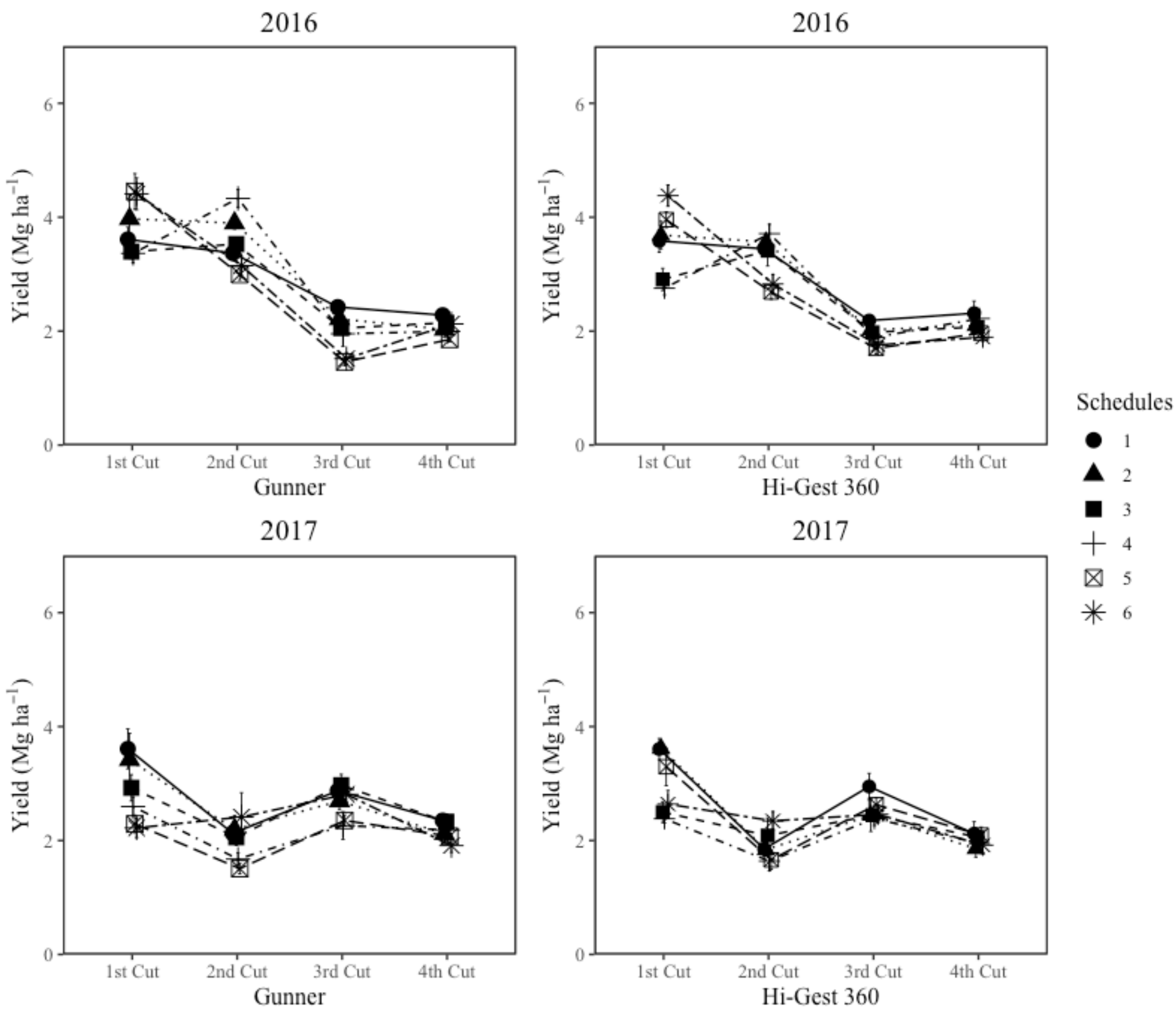



Figure 2.2 Yield at each harvest of low lignin and conventional varieties under four-cut in 2016 and 2017.

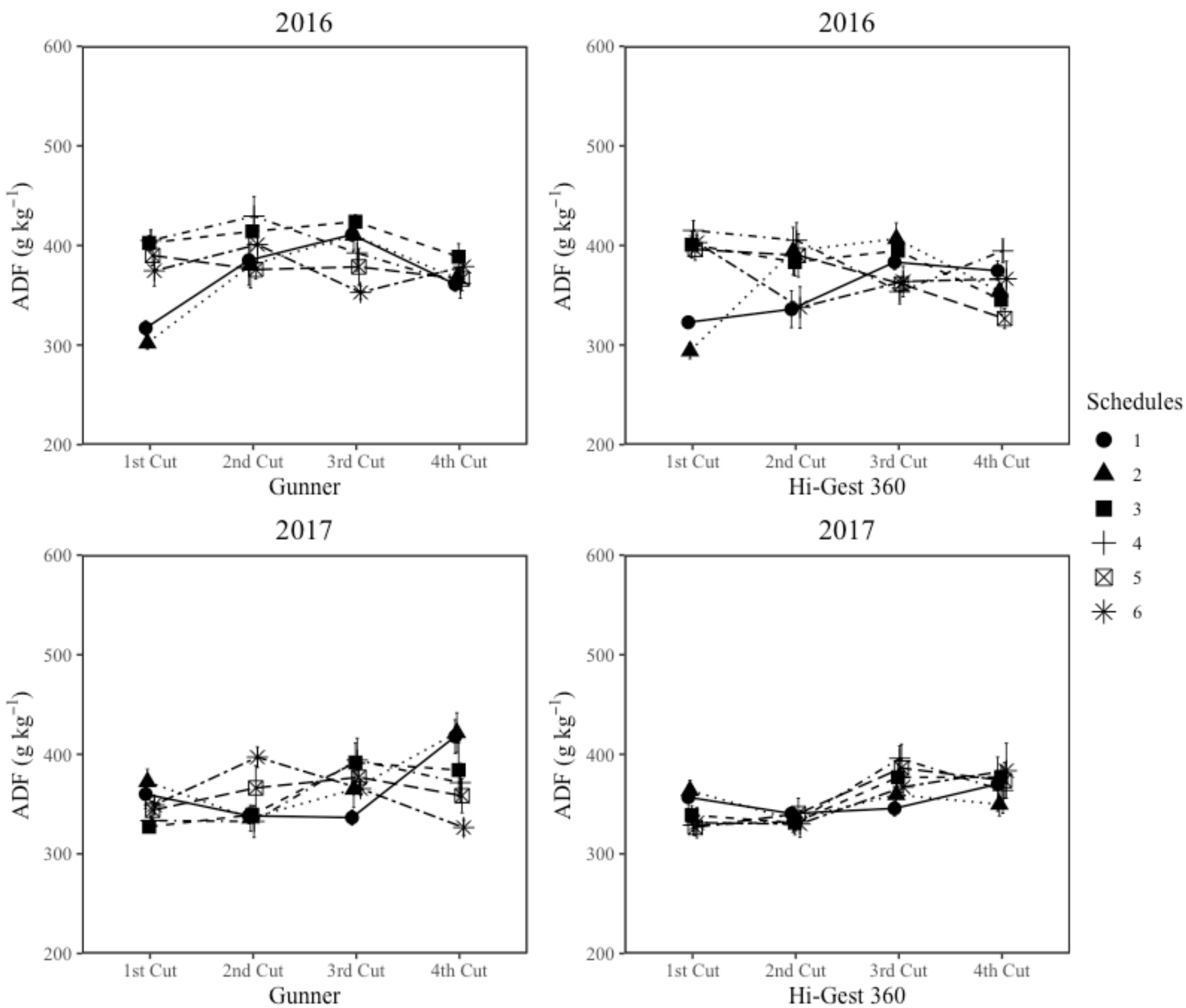



Figure 2.3 Acid detergent fiber concentration at each harvest of low lignin and conventional varieties under four-cut in 2016 and 2017.

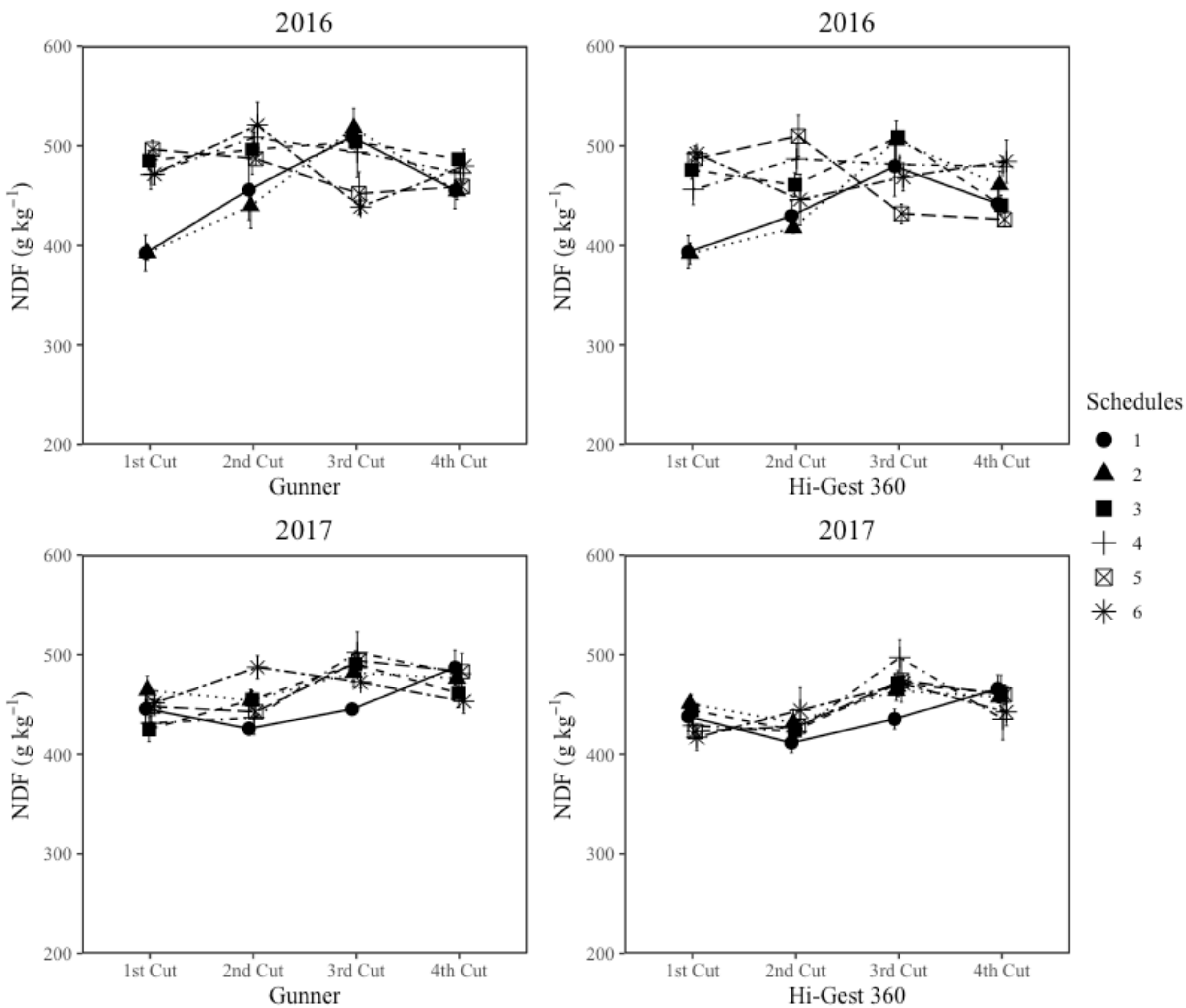



Figure 2.4 Neutral detergent fiber concentration at each harvest of low lignin and conventional varieties under four-cut in 2016 and 2017.

Table 2.1 Mean maximum and minimum air temperatures (C) and rainfall (mm).

| | 2016 | | | 2017 | | |
|---|---|---|---|---|---|---|
| **Month** | **Max Temp** | **Min Temp** | **Rainfall** | **Max Temp** | **Min Temp** | **Rainfall** |
| January | 4.5 | -6.1 | 12.7 | 6.4 | -5.1 | 24.9 |
| February | 11.8 | -3.0 | 10.2 | 13.4 | -1.1 | 11.9 |
| March | 18.2 | 2.4 | 11.2 | 15.5 | 2.6 | 106.9 |
| April | 21.3 | 7.7 | 214.6 | 19.4 | 7.6 | 126.8 |
| May | 23.7 | 11.3 | 177.3 | 24.7 | 11.2 | 96.8 |
| June | 33.5 | 19.5 | 39.4 | 31.0 | 17.2 | 71.6 |
| July | 32.4 | 21.2 | 155.0 | 33.4 | 20.5 | 33.8 |
| August | 30.4 | 19.6 | 185.7 | 28.6 | 16.0 | 154.7 |
| September | 28.5 | 16.0 | 105.7 | 29.1 | 15.0 | 20.6 |
| October | 24.3 | 9.4 | 70.4 | 21.2 | 7.2 | 93.0 |
| November | 17.4 | 3.5 | 7.6 | 14.1 | 0.7 | 2.3 |
| December | 5.6 | -7.1 | 21.1 | 6.4 | -5.8 | 2.8 |

Table 2.2 Dry matter yield and nutritive value for alfalfa grown in 2016 and 2017 as determined by variety and harvest schedule treatment.

| **Treatment** | **DM (Mg ha$^{-1}$)** | | **CP (g kg$^{-1}$)** | | **ADF (g kg$^{-1}$)** | | **NDF (g kg$^{-1}$)** | | **RFV** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| **Variety** | | | | | | | | | | |
| Gunner | 11.4 | 9.7 | 148 | 160 | 382 | 363 | 473 a | 462 a | 118 | 124 |
| Hi-Gest 360 | 10.8 | 9.5 | 157 | 172 | 371 | 354 | 461 b | 446 b | 123 | 127 |
| LSD | 0.9 | 1.7 | 24 | 29 | 12 | 16 | 12 | 13 | 5 | 4 |
| | | | | | | | | | | |
| **Harvest Schedule** | | | | | | | | | | |
| 1.HQ+HQ+MQ+HY† | 11.6 a | 10.7 a | 151 | 173 | 361 b | 358 | 445 b | 444 b | 129 a | 125 ab |
| 2.HQ+HY+MQ+HY | 11.7 a | 10.1 ab | 153 | 166 | 364 b | 363 | 448 b | 460 a | 128 a | 128 a |
| 3.MQ+HQ+MQ+HY | 10.8 b | 9.7 bc | 146 | 166 | 394 a | 358 | 482 a | 454 ab | 113 d | 122 b |
| 4.MQ+HY+MQ+HY | 11.1 ab | 8.5 d | 154 | 163 | 395 a | 357 | 481 a | 454 ab | 113 cd | 126 ab |
| 5.HY+HQ+MQ+HY | 10.5 b | 9.0 cd | 155 | 166 | 373 b | 359 | 469 a | 457 ab | 120 b | 123 ab |
| 6.HY+HY+MQ+HY | 11.0 ab | 9.4 bcd | 155 | 160 | 372 b | 356 | 475 a | 455 ab | 118 bc | 129 a |
| LSD | 0.8 | 0.9 | 12 | 12 | 13 | 17 | 16 | 14 | 7 | 6 |

* means significantly different at the 95% level of confidence.

† HQ+HQ+MQ+HY, HQ (high quality), HY (high yield), and MQ (medium quality).

Table 2.3 Dry matter yield and nutritive value of combining the first and second cuttings for alfalfa in 2016 and 2017 as determined by variety and harvest schedule treatment.

| Treatment | DM (Mg ha$^{-1}$) | | CP (g kg$^{-1}$) | | ADF (g kg$^{-1}$) | | NDF (g kg$^{-1}$) | | RFV | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| **Variety** | | | | | | | | | | |
| Gunner | 7.4 a | 4.9 | 150 | 161 | 381 | 350 a | 468 a | 447 a | 120 | 128 b |
| Hi-Gest 360 | 6.8 b | 4.8 | 157 | 174 | 373 | 338 b | 454 b | 434 b | 124 | 136 a |
| LSD | 0.6 | 1 | 16 | 22 | 21 | 8 | 11 | 10 | 6 | 2 |
| **Harvest Schedule** | | | | | | | | | | |
| 1. “Standard”+30d | 7.0 bc | 5.6 a | 160 | 172 | 340 c | 349 ab | 418 b | 430 b | 141 a | 134 ab |
| 2. “Standard”+35d | 7.6 a | 5.5 a | 160 | 171 | 343 c | 352 a | 410 b | 450 a | 142 a | 128 b |
| 3. “Standard+5d”+30d | 6.6 c | 4.8 bc | 149 | 170 | 400 ab | 334 bc | 480 a | 437 ab | 112 b | 134 ab |
| 4. “Standard+5d”+35d | 7.1 abc | 4.1 c | 150 | 165 | 414 a | 332 c | 481 a | 430 b | 110 b | 137 a |
| 5. “Standard+10d”+30d | 7.0 abc | 4.4 bc | 150 | 165 | 388 b | 344 abc | 495 a | 435 ab | 111 b | 133 ab |
| 6. “Standard+10d”+35d | 7.4 ab | 4.8 b | 152 | 162 | 379 b | 352 a | 483 a | 450 a | 116 b | 128 b |
| LSD | 0.6 | 0.9 | 15 | 14 | 24 | 16 | 20 | 17 | 12 | 7 |

Table 2.4 Economic incomes for alfalfa grown in 2016 and 2017 as determined by variety and harvest schedule treatment.

| **Treatment** | **Economic Incomes (\$ ha$^{-1}$)** | | |
|---|---|---|---|
| | 2016 | 2017 | Total |
| **Variety** | | | |
| Gunner | 1350 | 1181 | 2531 |
| Hi-Gest 360 | 1333 | 1218 | 2551 |
| LSD | 173 | 175 | 275 |
| | | | |
| **Harvest Schedule** | | | |
| 1.HQ+HQ+MQ+HY | 1529 a | 1105 | 2634 ab |
| 2.HQ+HY+MQ+HY | 1554 a | 1204 | 2759 a |
| 3.MQ+HQ+MQ+HY | 1213 b | 1263 | 2477 bc |
| 4.MQ+HY+MQ+HY | 1237 b | 1227 | 2464 bc |
| 5.HY+HQ+MQ+HY | 1230 b | 1144 | 2374 c |
| 6.HY+HY+MQ+HY | 1288 b | 1252 | 2540 bc |
| LSD | 129 | 173 | 177 |

# Chapter 3 - Effects of harvest intervals and seeding rates on dry matter yield and nutritive value of alfalfa


## Abstract

The maturation process of alfalfa (*Medicago sativa* L.) is averted by the reduction of lignin content through either conventional breeding or transgenic technology. As a result, alfalfa could exhibit increased leaf/stem ratio or declined yield. The objective of this study was to compare forage yield and nutritive value of low lignin alfalfa and two reference varieties subjecting to two harvest intervals and three seeding rates. The experimental design was a randomized complete block in a split-split plot arrangement with four replicates, where harvest intervals (28-day and 35-day) were assigned to whole plots, seeding rates were subplots, and varieties were sub-subplots. The weighted mean nutritive value was applied to two production years of 2016 and 2017. Hi-Gest 360 (low lignin alfalfa) provided similar yield potential and increased nutritive value compared to two reference varieties. Harvest interval had a large effect on nutritive value and a more significant effect on alfalfa dry matter yield than variety selection. Seeding rate did not affect alfalfa yield and nutritive value. Over a two-year production period, alfalfa harvested at every 28-day interval provided more economic incomes than those at the 35-day interval. For the seeding year and first production year, five cuts made by the 28-day interval produced more yield than four cuts by the 35-day interval; due to limited rainfall in May 2017, a sharp drop of the first cutting overturned the advantage of the five-cut system. Shorter intervals between harvests generally decreased crude protein (CP) concentrations. In 2016, neutral detergent fiber (NDF) concentrations were 12 g $kg^{-1}$ less for Hi-Gest 360 compared to Gunner; was 15 g $kg^{-1}$ less compared to Gunner and 19 g $kg^{-1}$ compared to RR Tonnica in 2017. The differences in relative feed value (RFV) between two harvest intervals tended to be great during

the first and second cuttings. Based on two production year data, selecting the 28-day interval for a stable system provided high nutritive value products. Seeding rate did not play an essential role in producing alfalfa with high yield. A low lignin variety with better management practices would be a more resilient strategy for producing alfalfa with high nutritive values.
Keywords: low lignin, alfalfa yield, seeding rate, nutritive values, economic incomes

## Introduction

Maintaining alfalfa nutritive value along with the increase of its yield becomes the focus with the release of low lignin alfalfa varieties. Lignin content accumulates while morphological development of plant (Albrecht et al., 1987; Iiyama and Wallis, 1990). The detergent fiber system built by Van Soest (1967) is the most prevalent criterion in forage-livestock industries, and lignin is defined as the least digestible portion of the fiber of the forage sample. Cell wall digestibility altered by lignin content level stands for the nutritive value of the alfalfa product (Jung and Engels, 2002). Pedersen et al. (2005) reviewed that the decrease of lignin content could increase the leaf/stem ratio. However, researchers also suspect the yield loss and lodging issue due to the decrease of lignin content. Studies involving reduce lignin alfalfa have shown their potential to maintain forage yield in the seeding and first production year (Undersander et al., 2009; Grev et al., 2017).

Lignin content in alfalfa is proportional to non-NDF fiber and acid detergent fiber (ADF) (Nocek and Grant, 1987). The correlation between ADF and acid detergent lignin (ADL) has been documented ranging from 78% to 86% (Van Soest, 1967; Casler, 1987; Jung et al., 1997). Van Soest (1967) estimated digestibility of the cell wall by a linear equation regarding ADF only. A recent study involving reduced lignin alfalfa showed that ADF, NDF and crude protein

CP concentrations for harvesting under 40-day and 45-day intervals were similar (Grev et al., 2017).

The timing of harvests plays an essential role in forage yield and quality (Brink et al., 2010). 28-day harvesting interval has been used for alfalfa production regarding yield and quality. Delaying from 28-day to 35day results in yield increase and quality decline for alfalfa varieties without low lignin trait. Weir et al. (1960) reported alfalfa harvested at 1/10 bloom stage provided high nutritive values, recorded reasonable yield when averaging data from three production years. A study Minnesota has shown that alfalfa at early flower has the highest leaf yield across three locations, and harvest intervals were 35-day and 40-day for the second and third cuttings (Sheaffer et al., 2000). The differences in forage yield and nutritive values between 30-day and 40-day harvest intervals were consistent (Grev et al., 2017). Kallenbach et al. (2002) suggested harvest alfalfa five times for high quality and four times for high yield in the lower Midwest.

The concentration of CP increased with shorter harvest intervals (Lloveras et al., 1998; Kallenbach et al., 2002). Sheaffer and Marten (1990) reported a similar negative relationship between CP concentration and harvest interval except for the final cutting due to the slower growth in the late season. However, one of the previous studies has shown that no differences in CP concentrations between alfalfa cutting at bud flower and full flower (Testa et al., 2011).

Alfalfa varieties with low lignin trait could develop a higher leaf/stem ratio or delaying plant maturity resulting in lower lignin content compared to reference varieties (Grev et al., 2017). Results from an experiment in seeding year in California and Pennsylvania showed that Hi-Gest 360 and HarvXtra-008 were similar in neutral detergent fiber concentrations (NDFD) and relative forage quality (RFQ). However, the reduced lignin alfalfa produced less ADL compared to the low lignin alfalfa (Undersander et al., 2009).

Another suggestion for cutting alfalfa is at the 50% of the bud stage, as a trade-off between quantity and quality of forage, based on using average quality data, the first three cuttings, or individual cutting. In this study, we introduce the weighted mean nutritive value regarding forage production of each cutting, and economic income index simulating the value of each cutting based on RFV and then summation.

Research from Missouri suggests that seeding rate around 17 kg ha$^{-1}$ have no effect on alfalfa stand (Hall et al., 2004). Lloveras et al. (2008) found that only using seeding rate of 10 kg ha$^{-1}$ had little effect on alfalfa total yield of the first three years in one of three experiments, while total yield and stand densities of seeding rates with 20 kg ha$^{-1}$ were similar to those of 30 kg ha$^{-1}$ and 40 kg ha$^{-1}$. Similar results were reported by Hansen (1973) with no yield increase at the seeding rate of 18 kg ha$^{-1}$ in the second year compared to 9 kg ha$^{-1}$. The fineness of stems related to forage quality could be the result of using a high seeding rate (Krueger and Hansen, 1974). Lignin, the majority polymer of cell walls, hardens and strengthens cell walls (Humphreys and Chapple, 2002). Hi-Gest 360 was reported 7-10% lower in lignin content than non-selected commercial varieties (Newman and Justen, 2016). Impact of the low seeding rate could be compensated by low lignin feature of the new varieties. However, few studies have been done on evaluating the effect of seeding rate on forage yield and quality of low lignin alfalfa variety.

Field evaluations are necessary for comprehensively understanding the performance of low lignin alfalfa under diverse treatments. The objective of this study was to compare forage yield and nutritive value of low lignin alfalfa and two reference varieties subjecting to two harvest intervals and three seeding rates during the seeding year, the first and second production years.

## Materials and Methods

Alfalfa was seeded in 2015 and harvested in 2016 and 2017 at the Department of Agronomy Ashland Bottom Research Farm (39.13° N, 96.63° W) near Manhattan, KS. The soil type was Belvue silt loam (rarely flooded). Soil samples were taken and submitted to the Kansas State University Soil Testing Lab to check soil fertility, with soil pH 7.9, 125 mg $kg^{-1}$ phosphorus, and 332 mg $kg^{-1}$ potassium. Monthly mean maximum, minimum temperature, and monthly rainfall data were collected for each year (Table 3.1) (Mesonet, 2017).

The experimental design was a randomized complete block in a split-split plot arrangement with four replicates. The whole plots were two cutting intervals, subplots were three seeding rates, the sub-subplots were three varieties. The field was divided into four blocks with enough alleyways. Each block was divided into two whole plots, and two harvest intervals were randomly assigned to the whole plots within each block. Each whole plot was divided into three subplots, and three seeding rates were randomly assigned to the subplots within each whole plot. Each subplot was divided into three sub-subplots, and three varieties were randomly assigned to each sub-subplot. Two cutting intervals were 28-day and 35-day and three seeding rates were 17 kg $ha^{-1}$, 20 kg $ha^{-1}$ and 23 kg $ha^{-1}$. Three alfalfa varieties, Hi-Gest 360 (low lignin), Gunner (conventional) and RR Tonnica (roundup ready) were planted on April 29th, 2015. Harvest regimes of on 28-day interval and 35-day interval were demonstrated with the daily weather data for three years (Fig. 3.1 to Fig. 3.5). The first cutting of alfalfa under 28-day interval in each year were determined when alfalfa plant reached the height of 60 cm or at the late bud stage, and 35-day intervals were seven days later following the early cut.

The harvesting was performed 3 cm high from soil level by a flail type forage harvester. Dried alfalfa samples were ground through a 1 mm Wiley mill and analyzed for CP, ADF, and NDF concentrations. CP concentrations for subsamples were determined by measuring total N content using the micro-Kjeldahl technique outlined by (Wall Sr and Gehrke, 1975) and then multiplying total N percentages by 6.25. ADF and NDF concentrations for subsamples were determined using the wet chemistry methods reported by (Van Soest et al., 1991).

The relative feed value index determines digestible dry matter (DDM) of the alfalfa from ADF and estimates the dry matter intake (DMI) potential from NDF (Rohweder et al., 1978). The index is then calculated as follows:

$$DDM = 88.9 - (0.779 \times \%ADF)$$

$$DMI = 120/(\%NDF)$$

$$RFV = (DDM \times DMI)/1.29.$$

In this study, average CP, ADF, NDF, and RFV were the weighted mean and calculated as follows:

$$\bar{x} = \frac{\sum_{i=1}^{n}(x_i \cdot y_i)}{\sum_{i=1}^{n} y_i}$$

$n$ represents harvesting times, $y_i$ is the dry matter production for the i$^{th}$ cutting, and $x_i$ represent the nutritive values for the i$^{th}$ cutting. The application of the weighted mean takes consideration of the yield when combining all cuttings.

Alfalfa value was \$1.00/point RFV. Economic incomes (dollar ha$^{-1}$) were equal to the relative feed value multiplied by the yield from each cutting.

Data were analyzed using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). The experimental unit is the single plot, and statistical significance was set at $p \leq 0.05$. The analysis of the seeding year (2015), first production year (2016), and the second production year (2017) was completed separately. Block was a random effect; harvest interval, seeding rate, and variety were fixed effects. For the 28-day interval, there were four cuts in 2015 and five cuts in 2016 and 2017; 35-day interval, there were three cuts in 2015 and four cuts in 2016 and 2017. Yield data were collected for three years, nutritive data only included 2016 and 2017. Multiple comparisons were performed on significant effects using the Bonferroni test.

## Results and Discussion

### Weather information

Mean monthly air temperature for the 2015 summertime tended to be cooler than the 2016 and 2017 summertime (June to August) (Table 3.1). Rainfall from April to September contributed 77%, 87% and 68% of total rainfall in 2015, 2016 and 2017, respectively. Average monthly maximum air temperature from June to Aug was 32.1°C, which was 1°C higher compared to 2015 and 2017. In 2017, daily rainfall during June, July, and August occurred less frequently compared to the previous two years.

### Forage Dry Matter Yield

There was no harvest interval × seeding rate × variety interaction for dry matter yield in 2015, 2016 and 2017, respectively (Table 3.2). Two-way interactions were observed in 2015 and 2017. Combining the highest seeding rate, RR Tonnica provided the highest dry matter yield in 2015 (Fig. 3.6). Sund and Barrington (1976) reported that dry matter yields were only observed increasing proportionally to the seeding rate (up to 32 kg $ha^{-1}$) in the seeding year. Another previous study has indicated that the seeding year dry matter yield increases as the seeding rates increased up to 17 kg ha$^{-1}$ with irrigation and 13.5 kg ha$^{-1}$ under dryland (Hansen, 1973). In the seeding year, seeding rate had an effect on dry matter yield of alfalfa with irrigation or under favorable weather condition.

Harvest interval and variety interaction differed in alfalfa yield in 2017 (Table 3.2). The yield increase rate of RR Tonnica was less than the increase rate of Hi-Gest 360 and Gunner from the 28-day to 35-day (Fig. 3.7). The results at southwest Missouri also suggest that interactions between variety and harvest frequency were not significant for any year (Hansen, 1973). This indicates that harvest management strategies should be similar among different varieties if the higher yield is the goal of a producer.

Harvest interval affected dry matter yield across three years (Table 3.2). For the seeding year and first production year, five cuts made by 28-day interval produced more yield than four cuts by the 35-day interval (Table 3.3). However, the inadequate rainfall in May 2017 affected the first cutting overturning the advantage of the five-cut system. With limited regrowth of alfalfa before the first cutting due to unfavorable weather condition, alfalfa production for the first and second cuttings was abolished by short intervals (Table 3.4).

The effect of variety only pronounced during the seeding year in 2015 with the higher yield of RR Tonnica compared with the yield of Hi-Gest 360. Three varieties did not show a significant difference in yield in the first and second production year (Table 3.3). The research comparing reduced lignin with reference varieties in Minnesota, U.S. has concluded that varieties showed little differences in forage dry matter yield in the first production year (Grev et al., 2017). Hansen (1973) showed that yield differences among varieties were observed in the first and second years. Moreover, Kallenbach et al. (2002) reported more significant effects of variety on alfalfa yield except for the third year of a five-year study.

In 2016, alfalfa yield of the 28-day interval tended to be low in the first cutting, higher for the second and the third cutting, and low again for the fourth and fifth cuttings (Table 3.4). In 2017, limited rainfall during June and July attributed to relatively low alfalfa yield under 28-day interval, and the trends of the 35-day interval (4-cut) were similar in both years (Table 3.4). Several studies have documented similar yield fluctuations at St. Paul, Minnesota and southwest Missouri (Brink and Marten, 1989; Kallenbach et al., 2002). For a shorter interval, a sharp production drop in the late cutting agrees with the results from a previous study comparing four harvest intervals of alfalfa with irrigation in California (Weir et al., 1960). Newman and Justen (2016) suggest that the first cutting of reduced lignin alfalfa might follow either when plant reaches a height of 60 cm or the same time producers harvest other conventional varieties.

## Crude Protein

Harvest interval × seeding rate × variety interaction was observed in 2016 (Table 3.2). Results showed that more differences among the three varieties were observed in two harvest intervals (Fig. 3.8). Under 28-day interval (5-cut), RR Tonnica with the lowest seeding rate provided the highest CP concentration and that was different from the other variety and seeding rate combinations. However, under the 35-day interval (4-cut), the effects of the lowest seeding rate on CP concentration of RR Tonnica tended to be decreased compared to the other two varieties.

Short harvest interval consistently increased average CP concentration from all cuttings in both production years (Table 3.5). Harvest interval increasing CP concentration was found for the third cutting in 2016 and the second and fourth cuttings in 2017. The effects of harvest interval were more consistent in 2017, and differences between harvest intervals tended to be great. That was due to limited rainfall before the growing season, and alfalfa plant was less mature due to the slow regrowth under the short harvest interval.

Our results are consistent with reports on the effect of harvest intervals on alfalfa crude proteins (Sheaffer and Marten, 1990; Lloveras et al., 1998). Shorter intervals between harvests decreased CP concentrations. Grev et al. (2017) found that harvest intervals had a greater effect on CP concentration than the effect of varieties across four locations in Minnesota. The patterns of CP concentrations trends of alfalfa under 28-day and 35-day intervals were similar except 28-day interval in 2017 (Table 3.6). This agrees with findings from the previous studies (Kallenbach et al., 2002). CP concentration for the first cutting in 2017 under 28-day harvest interval was reduced due to the extremely low rainfall before the first cutting (Fig. 3.1). Peterson et al. (1992) reported that CP level increased due to drought.

## Acid Detergent Fiber

No significant three-way and two-way interactions were observed in the average ADF concentration in 2016 and 2017. The 28-day interval (5-cut) increased average ADF concentration in both years compared to the 35-day interval (4-cut) (Table 3.5). Hi-Gest 360 was equivalent to or lower in average ADF concentration in the first and second production years than the other two varieties (Table 3.5). Seeding rates did not differ significantly for ADF concentration.

In 2016, ADF concentrations fluctuated in an 80 g $kg^{-1}$ range from the first cutting to the fifth cutting under 28-day interval and an 80 g $kg^{-1}$ range from the first cutting to the fourth cutting under 35-day interval. However, in 2017, both the ranges under two harvesting intervals fluctuated less. ADF concentrations of 28-day interval tended to be low in the first cutting, higher during summertime, and low again for the last cutting in 2016. These trends for ADF concentration by cutting among treatments were similar to the results by Kallenbach et al. (2002). In 2017, drought led to slightly higher ADF concentrations attributing a zig-zagged trend of ADF throughout cuttings.

Recent studies have reported that reduced lignin alfalfa varieties (HarvXtra technology) produced 4 to 20% less acid detergent lignin content compared with reference varieties (Sulc et al., 2016; Grev et al., 2017). Hi-Gest 360 with conventional breeding technology contains 7 to 10% less lignin content (Newman and Justen, 2016). Our results demonstrate a 4% reduction in ADF during the first and second production years.

Hall et al. (2000) reported that higher forage quality of improved varieties was more pronounced during the first two cuttings. However, comparing ADF concentrations for each cutting in this study, the one-week delay did not always account for the higher ADF concentration of short interval, due to variable weather conditions. The average ADF concentration was calculated as the weighted mean regarding alfalfa production by cutting, which showed consistent differences in 2016 and 2017.

## Neutral Detergent Fiber

Harvest interval × seeding rate × variety interaction was significant in 2016 for NDF concentration (Table 3.2). Patterns for NDF fluctuation of variety and seeding rate combinations under 28-day interval was different from those under 35-day interval (Fig. 3.9). More differences of NDF concentrations among the three varieties were observed at low and high seeding rates under the 28-day interval. Inversely, more differences of NDF concentrations among the three varieties were observed at the medium seeding rate under 35-day interval.

Harvest intervals showed significant differences in average NDF concentrations of each cutting in 2016 and 2017 (Table 3.2). Averaged over two-year data, NDF concentrations for alfalfa under 28-day interval (5-cut) were approximately 40 g $kg^{-1}$ lower than those under 35-day interval (4-cut) (Table 3.5).

More fluctuation was observed for NDF concentration under 28-day interval in 2016 and 2017. A decrease of NDF concentration under 35-day interval at the fourth cutting existed in 2016 and 2017 regardless of different weather patterns of two years. Generally, NDF differences between two harvest intervals were larger at the first or second cutting than at the fourth or fifth cutting.

There was a significant difference in NDF among varieties in two production years (Table 3.5). Hi-Gest 360 was 12 g $kg^{-1}$ lower than Gunner in 2016 and was 15 g $kg^{-1}$ lower than Gunner and 19 g $kg^{-1}$ lower than RR Tonnica in 2017. Variety effect was not consistent throughout the growing season. Data of each cutting in 2016 and 2017 suggest that low lignin variety tends to produce alfalfa with lower NDF, especially for the later cuttings (Table 3.8). Three varieties all showed the same patterns throughout all cuttings, NDF concentrations were higher in the first, second, and third cuttings, and decreased for the fourth and fifth cuttings. In table 3.8, NDF concentrations of variety section were the average of two harvest intervals for the first to the fourth cutting. NDF concentrations for the fifth cutting only represented the 28-day interval. Previous studies have documented that NDF increases more rapidly in spring than in late summer (Brink and Marten, 1989; Hall et al., 2000; Kallenbach et al., 2002).

Only the first three samples cut were used determining alfalfa forage for a four-year study in southern Italy (Testa et al., 2011). NDF concentrations in the second and third production years showed differences but no difference in NDF concentration on average of the two years.

Our results could be an alternative way of evaluating forage quality on average of each cutting within a year. Moreover, weather variations likely contributed to the differences in NDF concentration among years, also morphological development (i.e., leaf /stem ratio, the mean stage count) of alfalfa plant could be affected by those weather changes when applying the fixed length of harvest intervals (Brink and Marten, 1989; Hall et al., 2000).

Differences of NDF concentrations among varieties across three locations were less pronounced during both the seeding year and first production year (Grev et al., 2017). In a multi-location study, Undersander et al. (2009) reported that there was no difference in NDF concentrations between Hi-Gest 360 and HarvXtra-008 in the seeding year.

## Relative Feed Value

Harvest interval × seeding rate × variety interaction was significant in 2016 for RFV (Table 3.2). Patterns for RFV fluctuation of variety and seeding rate combinations under 28-day interval were different from those under 35-day interval (Fig. 3.10). More differences of RFV concentrations among the three varieties were observed at seeding rates of 17 and 23 kg $ha^{-1}$ under the 28-day interval. Inversely, more differences of RFV concentrations among the three varieties were observed at a seeding rate of 20 kg $ha^{-1}$ under 35-day interval. This result was as expected due to the similar trends found in NDF (Fig 3.9). No significant two-way interaction terms were observed in RFV.

Varieties showed small differences in RFV (Table 3.5). Average over two production years, RFV for alfalfa harvested five times (28-day) was 20 higher than those when harvesting four times (35-day) (Table 3.5). Our data suggest that Hi-Gest 360 produces alfalfa with higher RFV than the other two non-low lignin varieties. Producers aiming to make higher quality hay using low lignin alfalfa will have to choose the shorter harvest intervals in Kansas or places with similar weather conditions to Kansas.

The differences in RFV between two harvest intervals tended to be great during the first and second cuttings (Table 3.5). RFV differences between two harvest intervals ranged from 2 to 37 in 2016, fewer differences in 2017 (8 to 31). Large differences of RFV occurred in the earlier cuttings.

In 2016, the lowest RFV happened at the second cutting for the 28-day interval, and at the third cutting for the 35-day interval. However, in 2017, the lowest RFV happened at the first cutting for the 28-day interval, and at the second cutting for the 35-day interval. Changes of RFV by cutting suggest that weather had a large effect on forage nutritive value at the second or third cutting due to summer slump.

The RFV index is widely used in forage-livestock industries, representing both digestibility and intake potential of forage product (Rohweder et al., 1978; Moore and Undersander, 2002). However, the weighted RFV of each cutting takes consideration of the yield of each cutting and provides a more comprehensive way to review the quality of all cuttings.

Relative feed value approximates digestible energy intake potential of forages with some variations coming from the real intake capacity from livestock and digestibility of NDF (Sheaffer et al., 1995; Jeranyama and Garcia, 2004). The major problem of RFV is underestimated the DMI of grasses (Moore and Undersander, 2002). This study only dealt with alfalfa varieties so the comparison of RFV should be applicable.

## Economic Incomes

No significant interaction terms were observed for EI in 2016 and 2017. In 2016 and combining data from two years, harvest interval had a significant effect on EI. In 2017, harvest intervals, seeding rates, and varieties did not affect EI (Table 3.10).

For the 28-day interval, EI approximately decreased from 448 dollars/ha for the first cutting to 212 dollars/ha for the fifth cutting in 2016. Due to weather variations in 2017, RFV reached the peak at the second cutting (339 dollars/ha) and hit the bottom at the fourth cutting 237 dollars/ha. However, for the 35-day interval in two years, EI tended to increase from the first cutting to second cutting, and then generally decreased with the other two cuttings. Most valuable production under the 35-day interval was from the second cutting. Our data indicate that EI from each cutting of 4-cut system was higher than EI from each cutting of 5-cut system in 2017. Extra cutting of 5-cut system might not guarantee more incomes to surpass 4-cut system under different weather conditions. The below average rainfall during May, June and July in 2017 reduced production for the second, third and fourth cuttings in the five-cut system (28-day) and directly decreased the contribution of EI similar to the four-cut system (35-day). However, total economic incomes of the first and second production years justified the advantage of low lignin variety.

There were no differences among annual EI of the three varieties each year (Table 3.10). EI was greater for Hi-Gest 360 compared to Gunner for the fourth cutting in 2016 and was greater compared to RR Tonnica for the first cutting in 2017. Numerically, Hi-Gest 360 provided the highest incomes in both 2016 and 2017. Our results suggest that harvest interval combining weather condition play a critical role in the profitability of alfalfa production.

## Conclusions

The combination of variety, seeding rate, and harvest interval determined forage yield, nutritive value and economic incomes of certain management practice. It appears that Hi-Gest 360 (low lignin alfalfa) can provide similar yield potential and increased nutritive value compared to two reference varieties. Harvest interval had a large effect on forage quality and a greater effect on forage yield than variety selection.

Under different harvesting system (5-cut, 28-day interval vs 4-cut, 35-day interval), the seeding rate and variety combination differed in CP and NDF concentrations, and RFV in 2016.

The interactions of seeding rates and varieties had an effect on dry matter yield in the seeding year. In the second production year, under drought condition, yield differences between 28-day and 35-day interval of three varieties were not consistent.

The incidence of the rainfall played an essential role in alfalfa nutritive value. Our results suggest that adequate rainfall might not be the necessary condition for producing alfalfa of higher quality, and deficient rainfall could have a greater effect on forage yield compared to forage quality.

Based on two production year data, selecting the 28-day interval for a stable system provided high nutritive value products. Seeding rate did not play an essential role in producing alfalfa with high yield. A low lignin variety with better management practices would be a more resilient strategy for producing alfalfa with high nutritive values.

## References

Albrecht, K. A., Wedin, W. F., & Buxton, D. R. (1987). Cell-Wall Composition and Digestibility of Alfalfa Stems and Leaves 1. Crop science, 27(4), 735-741.

Brink, G. E., & Marten, G. C. (1989). Harvest management of alfalfa—Nutrient yield vs. forage quality, and relationship to persistence. Journal of Production Agriculture, 2(1), 32-36.

Brink, G., Hall, M., Shewmaker, G., Undersander, D., Martin, N., & Walgenbach, R. (2010). Changes in alfalfa yield and nutritive value within individual harvest periods. Agronomy journal, 102(4), 1274-1282.

Casler, M. D. (1987). In Vitro Digestibility of Dry Matter and Cell Wall Constituents of Smooth Bromegrass Forage 1. Crop science, 27(5), 931-934.

Grev, A. M., Wells, M. S., Samac, D. A., Martinson, K. L., & Sheaffer, C. C. (2017). Forage accumulation and nutritive value of reduced lignin and reference alfalfa cultivars. Agronomy Journal, 109(6), 2749-2761.

Hall, M. H., Nelson, C. J., Coutts, J. H., & Stout, R. C. (2004). Effect of seeding rate on alfalfa stand longevity. Agronomy Journal, 96(3), 717-722.

Hall, M. H., Smiles, W. S., & Dickerson, R. A. (2000). Morphological development of alfalfa cultivars selected for higher quality. Agronomy Journal, 92(6), 1077-1080.

Hansen, L. H., & Krueger, C. R. (1973). Effect of Establishment Method, Variety, and Seeding Rate on the Production and Quality of Alfalfa Under Dryland and Irrigation 1. Agronomy Journal, 65(5), 755-759.

Humphreys, J. M., & Chapple, C. (2002). Rewriting the lignin roadmap. Current opinion in plant biology, 5(3), 224-229.

Iiyama, K., & Wallis, A. F. (1990). Determination of lignin in herbaceous plants by an improved acetyl bromide procedure. Journal of the Science of Food and Agriculture, 51(2), 145-161.

Jeranyama, P., & Garcia, A. D. (2004). Understanding relative feed value (RFV) and relative forage quality (RFQ).

Jung, H. G., & Engels, F. M. (2002). Alfalfa stem tissues. Crop science, 42(2), 524-534.

Jung, H. G., Mertens, D. R., & Payne, A. J. (1997). Correlation of acid detergent lignin and Klason lignin with digestibility of forage dry matter and neutral detergent fiber. Journal of Dairy Science, 80(8), 1622-1628.

Kallenbach, R. L., Nelson, C. J., & Coutts, J. H. (2002). Yield, quality, and persistence of grazing-and hay-type alfalfa under three harvest frequencies. Agronomy Journal, 94(5), 1094-1103.

Krueger, C. R., & Hansen, L. H. (1974). Establishment method, variety, and seeding rate affect quality and production of alfalfa under dry land and irrigation. SD Farm Home Res, 25, 10-13.

Lloveras, J., Ferran, J., Alvarez, A., & Torres, L. (1998). Harvest management effects on alfalfa (Medicago sativa L.) production and quality in Mediterranean areas. Grass and forage science, 53(1), 88-92.

Lloveras, J., Chocarro, C., Freixes, O., Arqué, E., Moreno, A., & Santiveri, F. (2008). Yield, yield components, and forage nutritive value of alfalfa as affected by seeding rate under irrigated conditions. Agronomy journal, 100(1), 191-197.

Mesonet, Kansas. (2017). "Weather data library. Manhattan, KS: Kansas State University."

Moore, J. E., & Undersander, D. J. (2002, January). Relative forage quality: An alternative to relative feed value and quality index. In Proceedings 13th Annual Florida Ruminant Nutrition Symposium (Vol. 32, pp. 16-29).

Newman, Y. C., & Justen, V. (2016, January). Reduced lignin alfalfa. In Proc. 2016 Wisconsin Crop Manage. Conf., Madison, WI (pp. 69-72).

Nocek, J. E., & Grant, A. L. (1987). Characterization of in situ nitrogen and fiber digestion and bacterial nitrogen contamination of hay crop forages preserved at different dry matter percentages. Journal of Animal Science, 64(2), 552-564.

Pedersen, J. F., Vogel, K. P., & Funnell, D. L. (2005). Impact of reduced lignin on plant fitness. Crop Science, 45(3), 812-819.

Peterson, P. R., Sheaffer, C. C., & Hall, M. H. (1992). Drought effects on perennial forage legume yield and quality. Agronomy Journal, 84(5), 774-779.

Rohweder, D. A., Barnes, R. F., & Jorgensen, N. (1978). Proposed hay grading standards based on laboratory analyses for evaluating quality. Journal of animal science, 47(3), 747-759.

Sheaffer, C. C., Peterson, M. A., Mccalin, M., Volene, J. J., Cherney, J. H., Johnson, K. D., Woodward, W. T., & Viands, D. R. (1995, March). Acide detergent fiber, neutral detergent fiber concentration and relative feed value. In North American Alfalfa Improvement Conference, Minneapolis.

Sheaffer, C. C., & Marten, G. C. (1990). Alfalfa cutting frequency and date of fall cutting. Journal of Production Agriculture, 3(4), 486-491.

Sheaffer, C. C., Martin, N. P., Lamb, J. F., Cuomo, G. R., Jewett, J. G., & Quering, S. R. (2000). Leaf and stem properties of alfalfa entries. Agronomy Journal, 92(4), 733-739.

Sulc, M., Parker, A., Albrecht, K., Cassida, K., Hall, M., Min, D., Orloff, S., Undersander, D., & Xu, X. (2016). Low lignin alfalfa: Wide area field test results. In 2016 California Alfalfa and Forage Symposium.

Sund, J. M., & Barrington, G. P. (1976). Alfalfa seeding rates: their influence on dry matter yield, stand density and survival, root size and forage quality. Research Bulletin-Wisconsin University.

Testa, G., Gresta, F., & Cosentino, S. L. (2011). Dry matter and qualitative characteristics of alfalfa as affected by harvest times and soil water content. European journal of agronomy, 34(3), 144-152.

Undersander, D., McCaslin, M., Sheaffer, C., Whalen, D., Miller, D., Putnam, D., & Orloff, S. (2009, December). Low lignin alfalfa: Redefining the yield/quality tradeoff. In Proc., 2009 Western Alfalfa & Forage Conference (pp. 2-4).

Van Soest, P. J. (1967). Development of a comprehensive system of feed analyses and its application to forages. Journal of animal Science, 26(1), 119-128.

Van Soest, P. V., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of dairy science, 74(10), 3583-3597.

Wall Sr, L. L., & Gehrke, C. W. (1975). Automated total protein nitrogen method. J Assoc Off Anal Chem.

Weir, W. C., Jones, L. G., & Meyer, J. H. (1960). Effect of cutting interval and stage of maturity on the digestibility and yield of alfalfa. Journal of Animal Science, 19(1), 5-19.

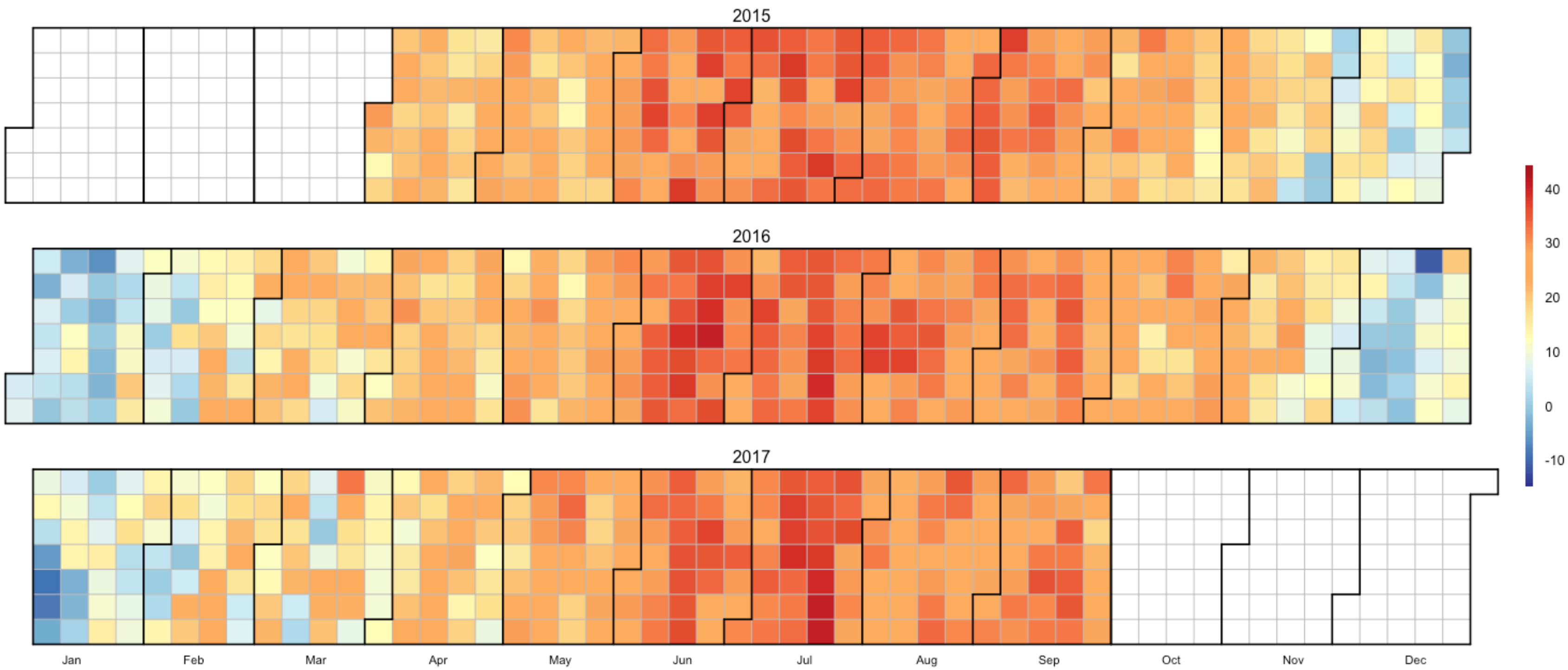



Figure 3.1 Heatmap of daily average maximum air temperature (C) in 2015, 2016 and 2017.

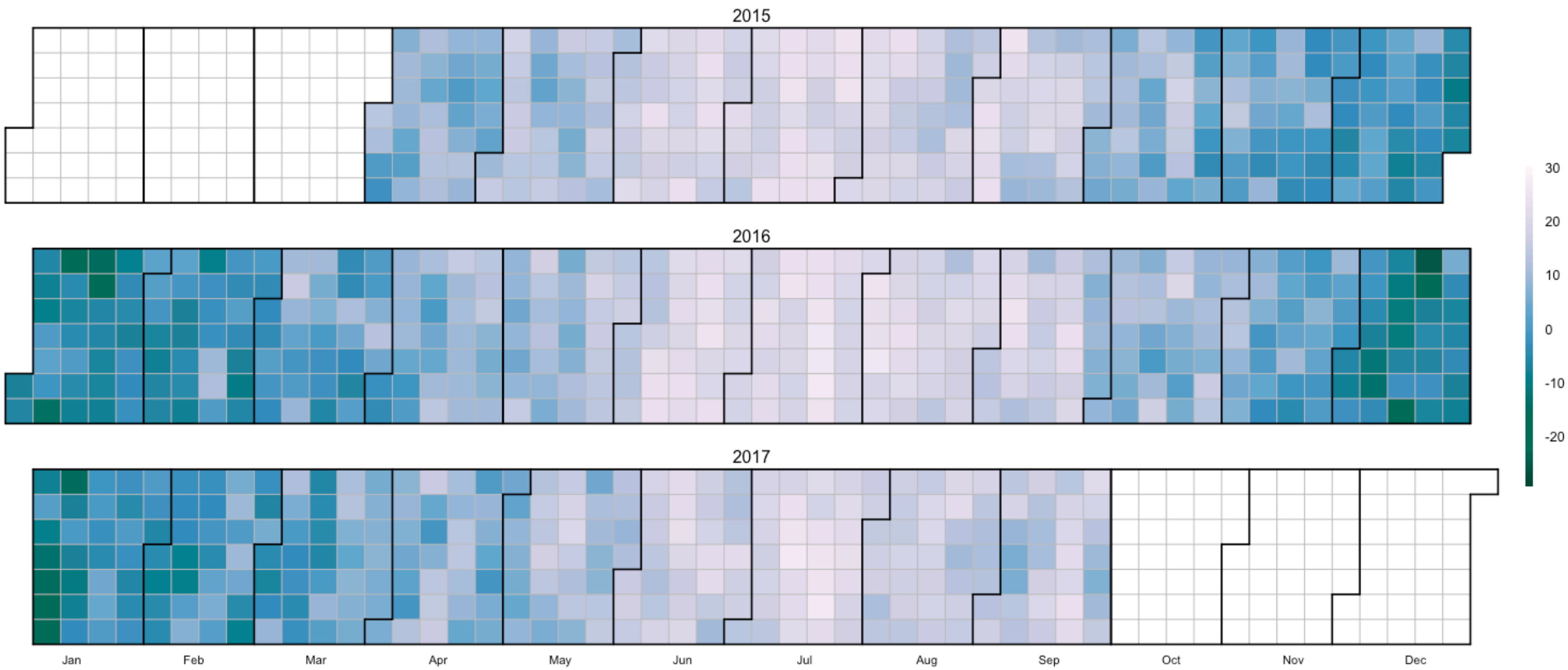



Figure 3.2 Heatmap of daily average minimum air temperature (C) in 2015, 2016 and 2017.

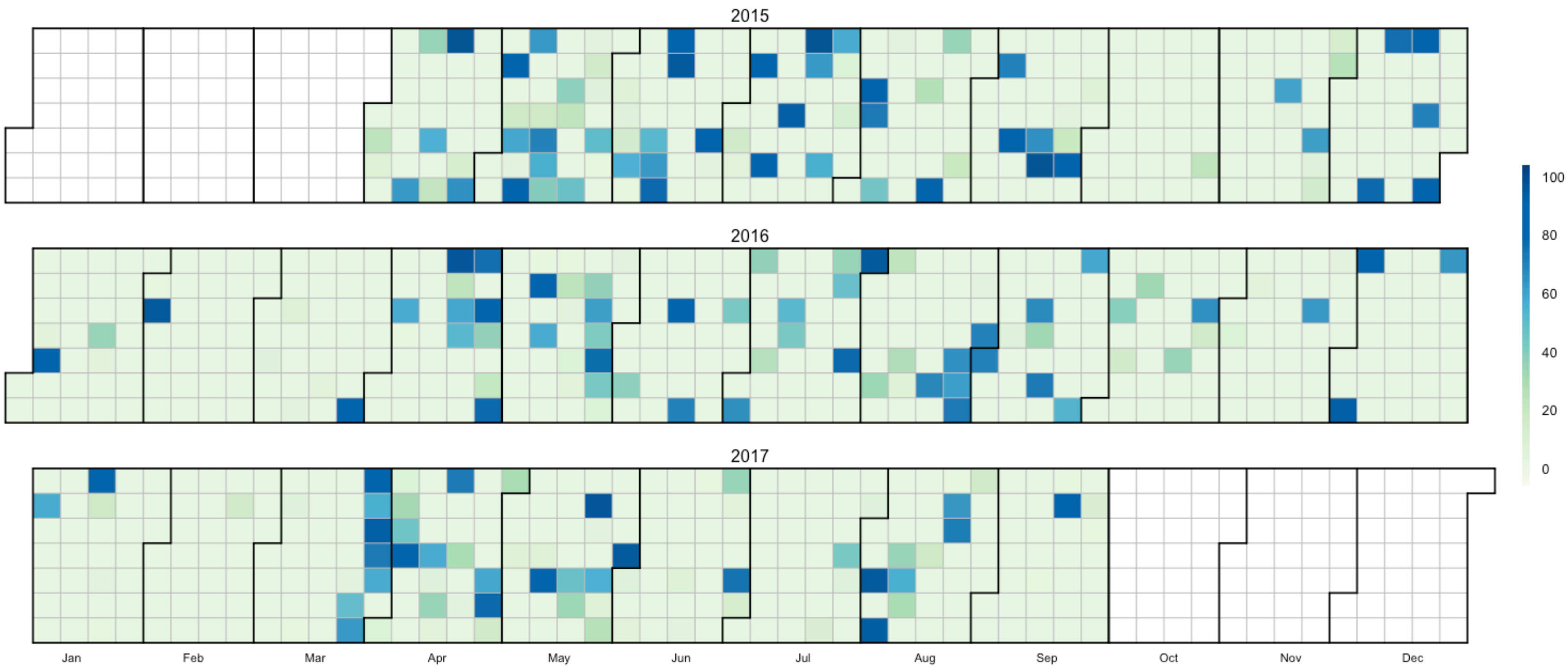


Figure 3.3 Heatmap of daily rainfall (mm) in 2015, 2016 and 2017.

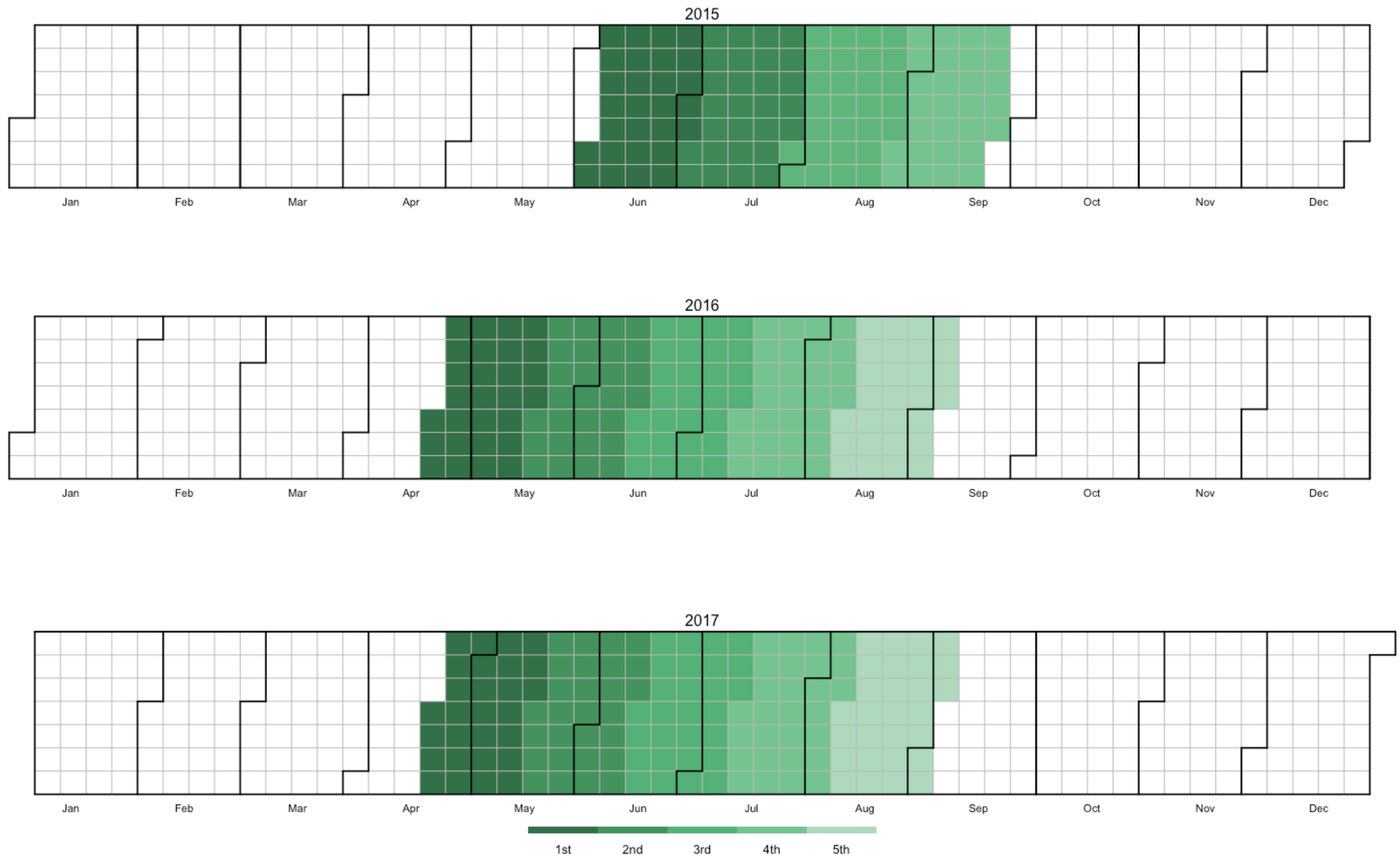



Figure 3.4 Harvest schedule of 28-day intervals in 2015, 2016 and 2017.

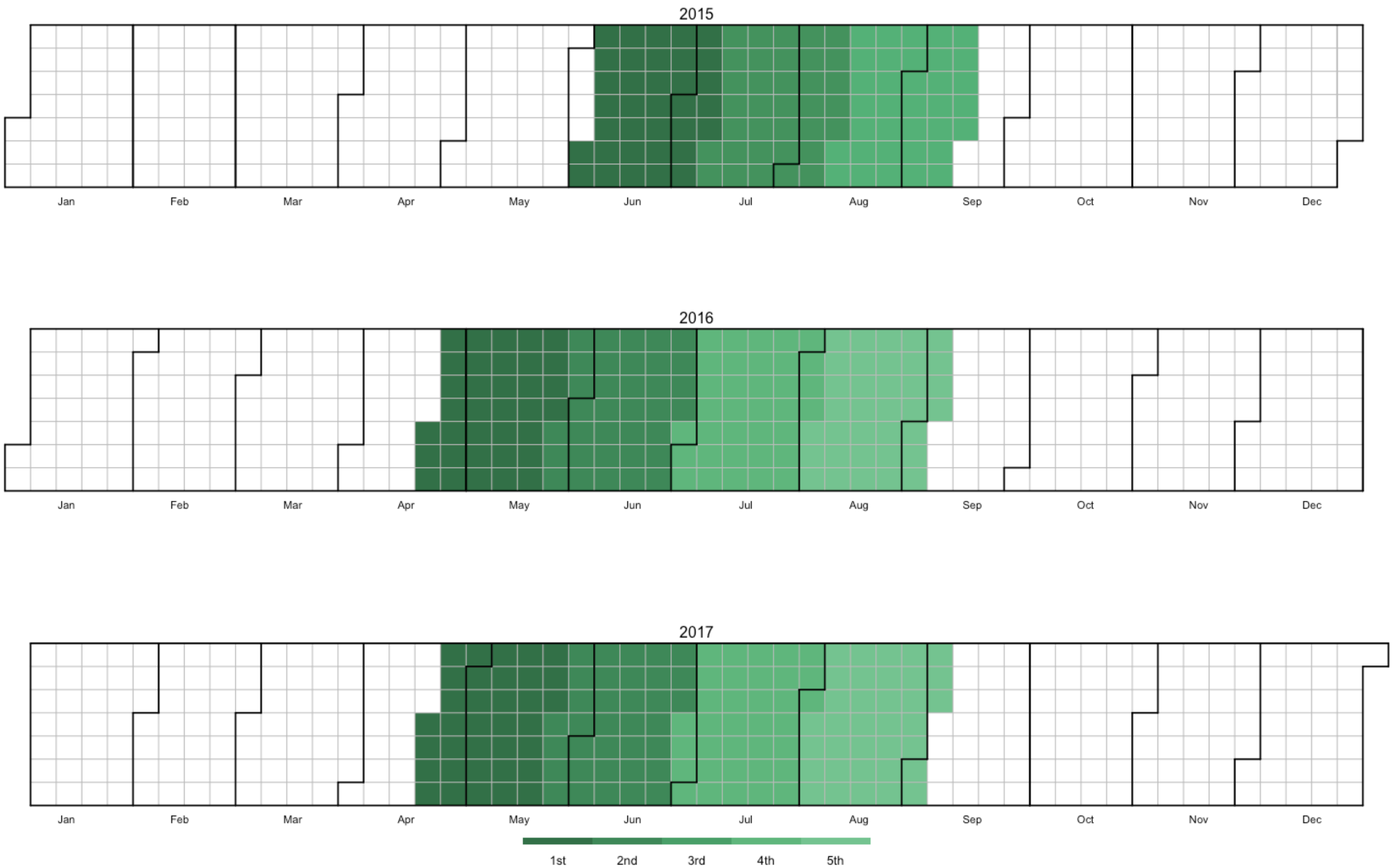



Figure 3.5 Harvest schedule of 35-day intervals in 2015, 2016 and 2017.

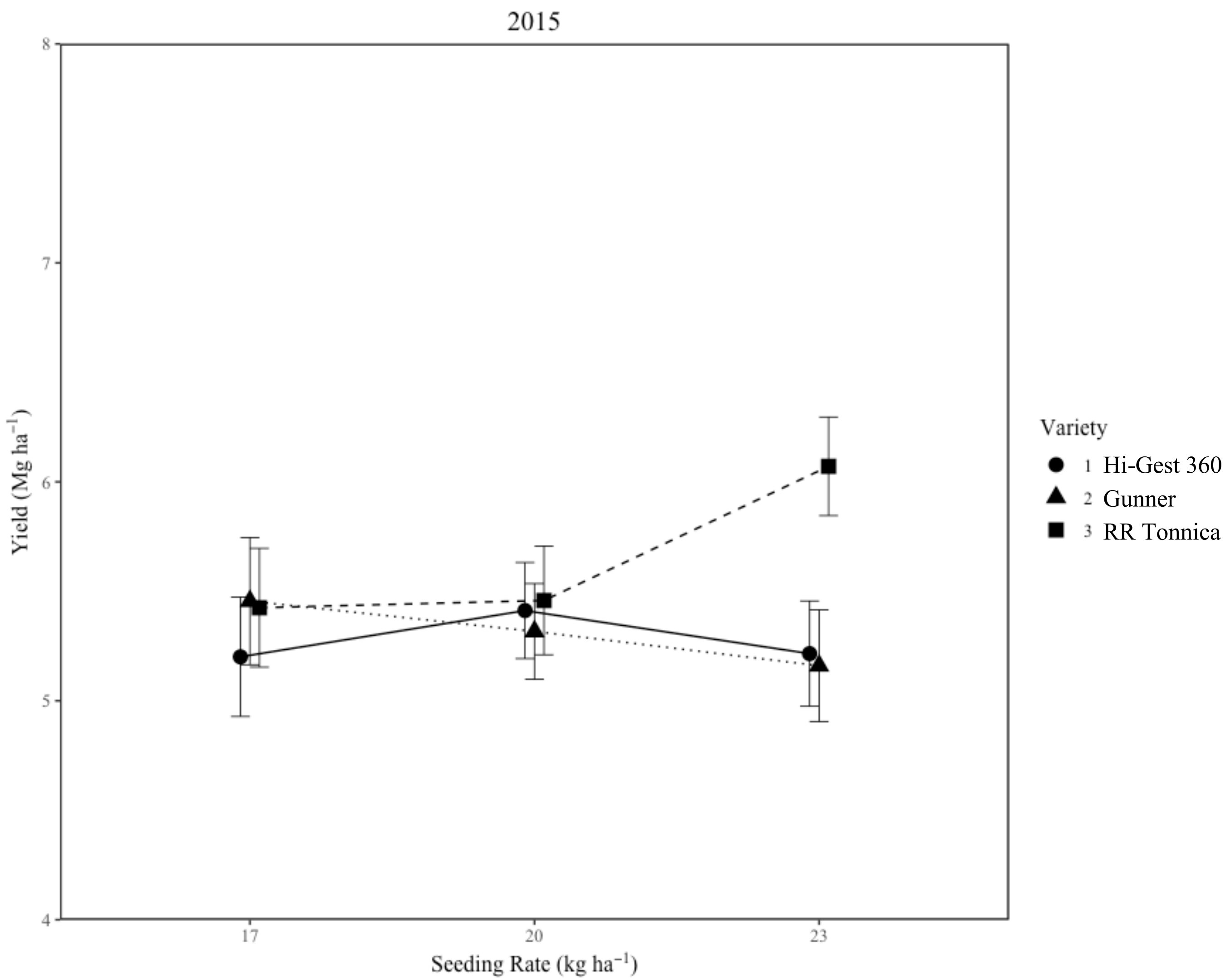



Figure 3.6 Interaction plot for dry matter yield among three varieties at three seeding rates in 2015. Variety 1 - Hi-Gest 360, Variety 2 – Gunner, Variety 3 - RR Tonnica.

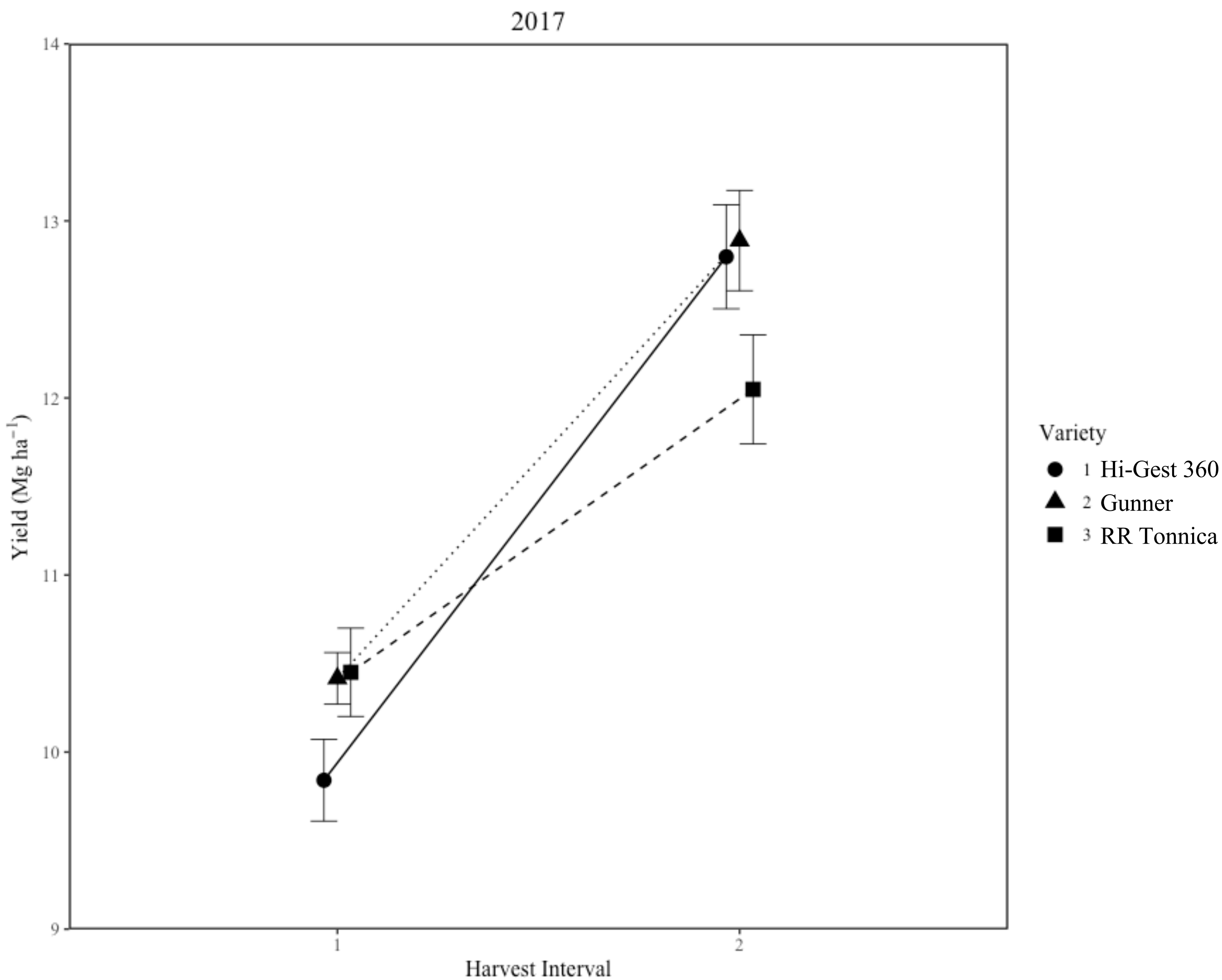


Figure 3.7 Interaction plot for dry matter yield among three varieties under two harvest intervals in 2017.

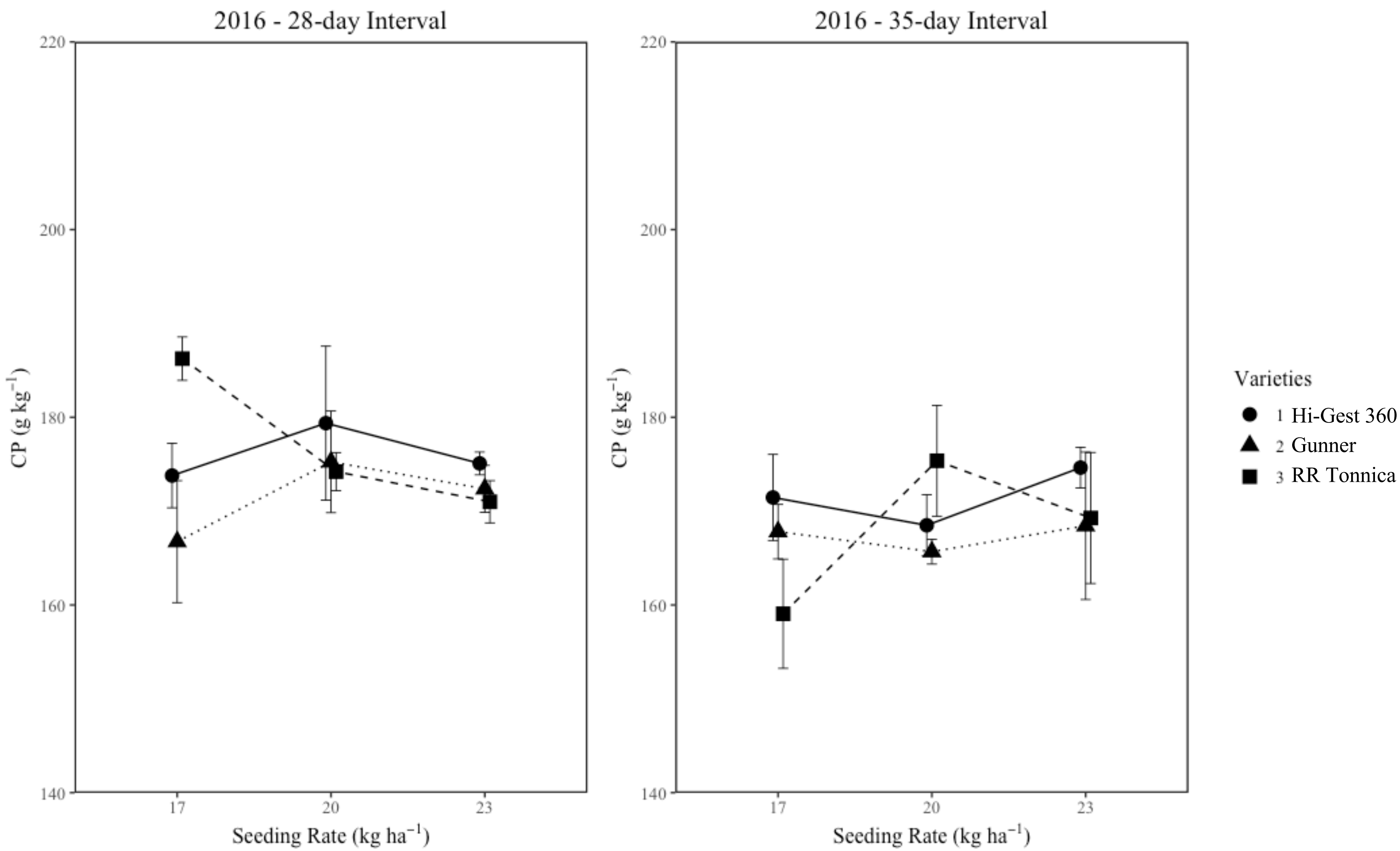



Figure 3.8 Interaction plots for CP concentrations among three varieties at three seeding rates in 2016.

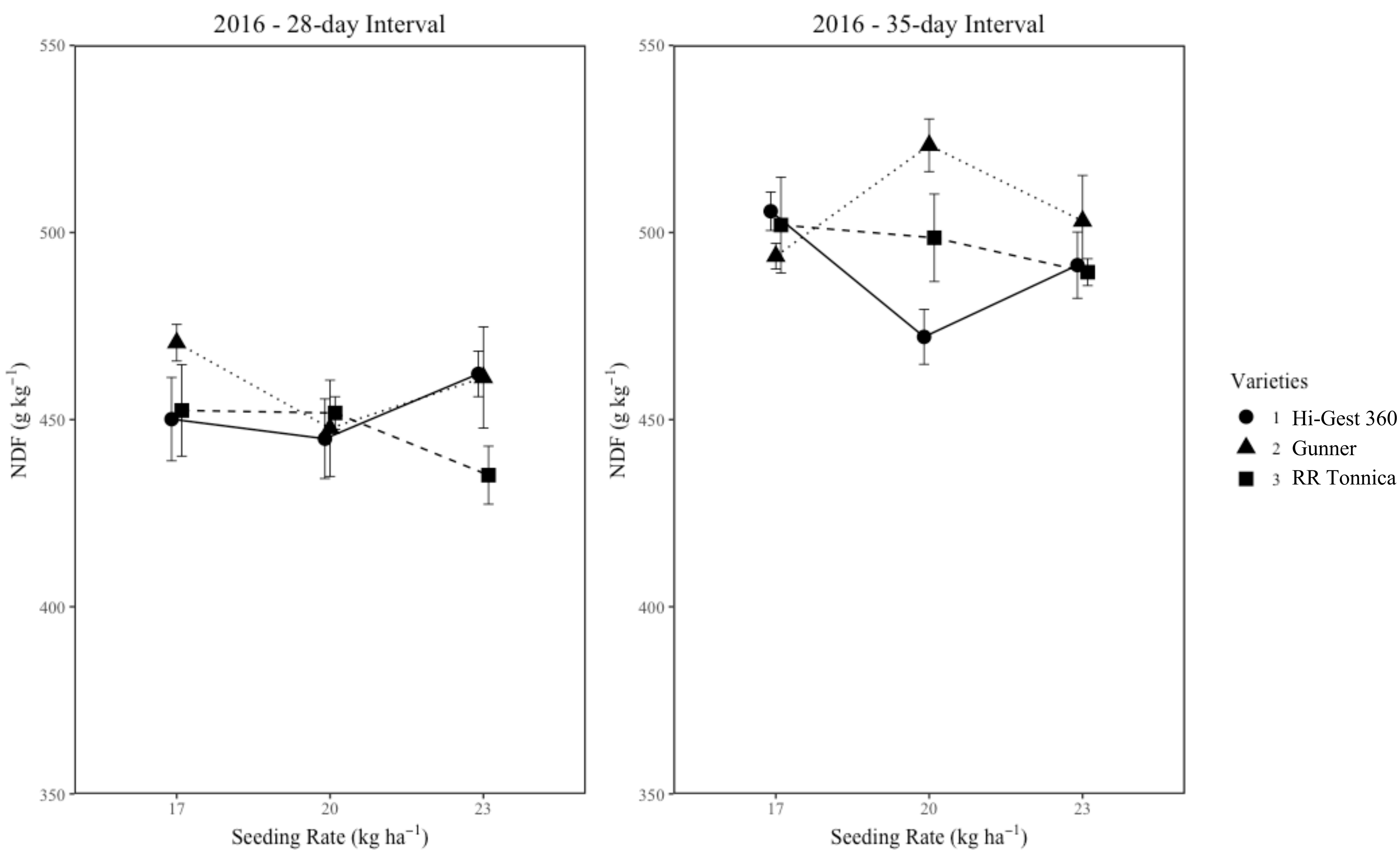



Figure 3.9 Interaction plots for NDF concentrations among three varieties at three seeding rates in 2016.

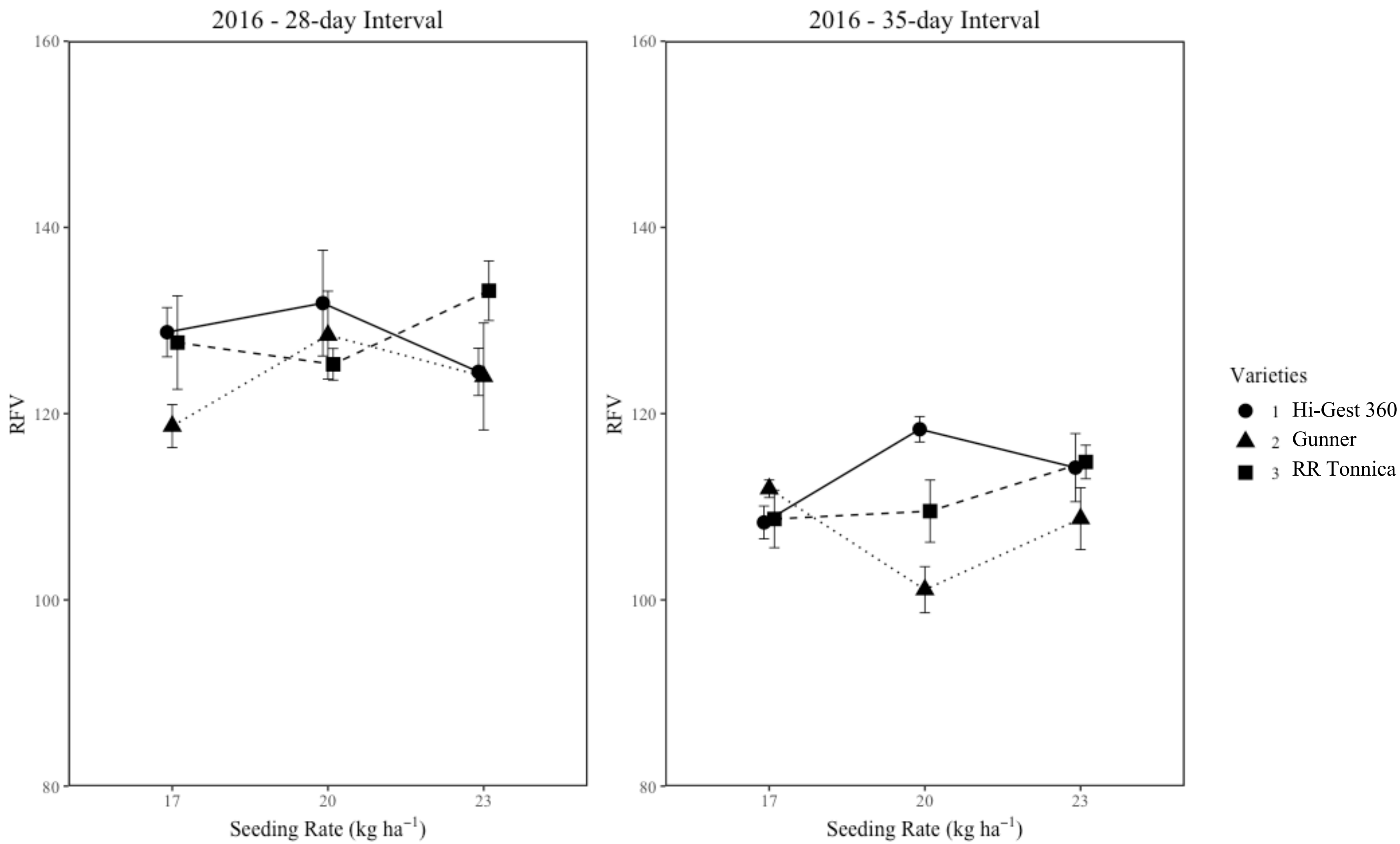



Figure 3.10 Interaction plots for RFV among three varieties at three seeding rates in 2016.

Table 3.1 Mean maximum and minimum air temperatures (C) and rainfall (mm).

| | 2015 | | | 2016 | | | 2017 | | |
|---|---|---|---|---|---|---|---|---|---|
| **Month** | **Max Temp** | **Min Temp** | **Rainfall** | **Max Temp** | **Min Temp** | **Rainfall** | **Max Temp** | **Min Temp** | **Rainfall** |
| January | 6.9 | -8.1 | 22.1 | 4.5 | -6.1 | 12.7 | 6.4 | -5.1 | 24.9 |
| February | 4.0 | -8.9 | 10.2 | 11.8 | -3.0 | 10.2 | 13.4 | -1.1 | 11.9 |
| March | 16.4 | -0.3 | 4.3 | 18.2 | 2.4 | 11.2 | 15.5 | 2.6 | 106.9 |
| April | 20.6 | 7.3 | 67.6 | 21.3 | 7.7 | 214.6 | 19.4 | 7.6 | 126.8 |
| May | 23.0 | 12.3 | 218.7 | 23.7 | 11.3 | 177.3 | 24.7 | 11.2 | 96.8 |
| June | 31.1 | 18.6 | 107.2 | 33.5 | 19.5 | 39.4 | 31.0 | 17.2 | 71.6 |
| July | 31.9 | 20.8 | 128.0 | 32.4 | 21.2 | 155.0 | 33.4 | 20.5 | 33.8 |
| August | 30.4 | 17.2 | 81.0 | 30.4 | 19.6 | 185.7 | 28.6 | 16.0 | 154.7 |
| September | 20.6 | 7.3 | 67.6 | 28.5 | 16.0 | 105.7 | 29.1 | 15.0 | 20.6 |
| October | 22.4 | 7.6 | 15.5 | 24.3 | 9.4 | 70.4 | 21.2 | 7.2 | 93.0 |
| November | 20.6 | 7.3 | 67.6 | 17.4 | 3.5 | 7.6 | 14.1 | 0.7 | 2.3 |
| December | 9.4 | -2.0 | 82.8 | 5.6 | -7.1 | 21.1 | 6.4 | -5.8 | 2.8 |

Table 3.2 P-values from mixed model analysis for the effect of harvest interval, seeding rate and variety and their interactions.

| **Effect** | **Yield** | | | **CP** | | **ADF** | | **NDF** | | **RFV** | | **EI** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2015 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| **HI** | ** | * | ** | ** | ** | * | ** | ** | ** | ** | ** | ** | NS |
| **SR** | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **HI×SR** | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **V** | * | NS | NS | NS | NS | ** | * | * | ** | ** | ** | NS | NS |
| **HI×V** | NS | NS | * | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **SR×V** | * | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **HI× SR×V** | NS | NS | NS | * | NS | NS | NS | * | NS | * | NS | NS | NS |

Significant at ** $p \leq 0.01$ and * $p \leq 0.05$; NS, not significant.

Table 3.3 Dry matter yield for alfalfa under different treatments in 2015, 2016 and 2017.

| Treatment | 2015 | 2016 | 2017 | Total |
|---|---|---|---|---|
| **Harvest Interval** | | --------Mg ha$^{-1}$-------- | | |
| **28-day** | 5.83 a (4-cut) | 12.93 a (5-cut) | 10.24 b (5-cut) | 29.00 |
| **35-day** | 4.99 b (3-cut) | 11.35 b (4-cut) | 12.58 a (4-cut) | 28.92 |
| **SE** | 0.15 | 0.33 | 0.27 | 0.53 |
| **Seeding Rate** | | | | |
| **17 kg ha$^{-1}$** | 5.36 | 12.28 | 11.21 | 28.85 |
| **20 kg ha$^{-1}$** | 5.40 | 12.15 | 11.72 | 29.26 |
| **23 kg ha$^{-1}$** | 5.48 | 12.28 | 11.29 | 28.77 |
| **SE** | 0.16 | 0.32 | 0.23 | 0.48 |
| **Variety** | | | | |
| **Hi-Gest 360** | 5.28 b | 12.23 | 11.32 | 28.83 |
| **Gunner** | 5.31 ab | 12.21 | 11.65 | 29.18 |
| **RR Tonnica** | 5.65 a | 11.98 | 11.25 | 28.88 |
| **SE** | 0.14 | 0.27 | 0.23 | 0.46 |

Different letters are significant at $p < 0.05$.

Table 3.4 Forage production at each cutting for alfalfa under different treatments in 2015, 2016, and 2017.

| **Treatment** | **2015** | | | | **2016** | | | | | **2017** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cut 1 | Cut 2 | Cut3 | Cut4 | Cut 1 | Cut 2 | Cut3 | Cut4 | Cut 5 | Cut 1 | Cut 2 | Cut3 | Cut4 | Cut 5 |
| **Harvest Interval** | | | | | | --------Mg ha$^{-1}$-------- | | | | | | | | |
| **28-day** | 0.90 | 1.86 b | 1.69 b | 1.13 | 2.72 | 3.31 b | 2.67 b | 2.26 a | 1.57 | 2.51 b | 2.25 b | 2.28 b | 1.56 b | 1.64 |
| **35-day** | 0.89 | 2.10 a | 2.00 a | - | 3.12 | 3.74 a | 2.98 a | 1.91 b | - | 3.54 a | 3.77 a | 2.79 a | 2.47 a | - |
| **SE** | 0.03 | 0.09 | 0.07 | - | 0.20 | 0.11 | 0.07 | 0.06 | - | 0.15 | 0.12 | 0.06 | 0.09 | - |
| **Seeding Rate** | | | | | | | | | | | | | | |
| **17 kg ha$^{-1}$** | 0.88 | 1.96 | 1.83 | 1.07 | 3.01 | 3.55 | 2.82 | 2.10 | 1.60 | 2.90 | 3.03 | 2.48 | 1.98 | 1.66 |
| **20 kg ha$^{-1}$** | 0.89 | 2.01 | 1.83 | 1.21 | 2.89 | 3.54 | 2.80 | 2.11 | 1.61 | 3.18 | 3.09 | 2.55 | 2.05 | 1.68 |
| **23 kg ha$^{-1}$** | 0.91 | 1.99 | 1.88 | 1.11 | 2.86 | 3.48 | 2.85 | 2.05 | 1.51 | 2.98 | 2.92 | 2.58 | 2.01 | 1.59 |
| **SE** | 0.04 | 0.10 | 0.08 | 0.06 | 0.20 | 0.11 | 0.07 | 0.05 | 0.05 | 0.14 | 0.12 | 0.06 | 0.07 | 0.08 |
| **Variety** | | | | | | | | | | | | | | |
| **Hi-Gest 360** | 0.88 | 1.88 b | 1.74 | 1.08 | 2.93 | 3.61 | 2.75 | 2.15 | 1.59 | 3.09 | 2.94 | 2.48 | 1.99 | 1.62 |
| **Gunner** | 0.86 | 1.95 ab | 1.87 | 1.16 | 3.01 | 3.49 | 2.89 | 2.06 | 1.52 | 3.15 | 3.07 | 2.59 | 2.03 | 1.63 |
| **RR Tonnica** | 0.93 | 2.11 a | 1.93 | 1.15 | 2.83 | 3.46 | 2.84 | 2.05 | 1.61 | 2.82 | 3.03 | 2.53 | 2.03 | 1.68 |
| **SE** | 0.04 | 0.09 | 0.08 | 0.06 | 0.16 | 0.11 | 0.07 | 0.05 | 0.04 | 0.15 | 0.12 | 0.06 | 0.07 | 0.07 |

Different letters are significant at $p < 0.05$.

Table 3.5 Crude protein, acid detergent fiber, neutral detergent fiber, relative feed value for alfalfa under different treatments in 2016 and 2017.

| **Treatment** | **2016** | | | | **2017** | | | |
|---|---|---|---|---|---|---|---|---|
| | CP | ADF | NDF | RFV | CP | ADF | NDF | RFV |
| **Harvest Interval** | --------g $kg^{-1}$-------- | | | | -------g $kg^{-1}$-------- | | | |
| **28-day** | 175 a | 364 b | 453 b | 127 a | 206 a | 330 b | 411 b | 145 a |
| **35-day** | 169 b | 393 a | 498 a | 111 b | 190 b | 361 a | 450 a | 128 b |
| **SE** | 2.4 | 4.3 | 3.8 | 1.4 | 1.8 | 3.4 | 4.3 | 1.7 |
| **Seeding Rate** | | | | | | | | |
| **17 kg $ha^{-1}$** | 171 | 382 | 479 | 117 | 200 | 345 | 429 | 137 |
| **20 kg $ha^{-1}$** | 173 | 379 | 473 | 119 | 197 | 346 | 431 | 136 |
| **23 kg $ha^{-1}$** | 172 | 375 | 474 | 120 | 198 | 346 | 432 | 136 |
| **SE** | 2.6 | 4.6 | 4.4 | 1.6 | 2.1 | 3.7 | 4.1 | 1.7 |
| **Variety** | | | | | | | | |
| **Hi-Gest 360** | 174 | 372 b | 471 b | 121 a | 200 | 338 b | 419 b | 141 a |
| **Gunner** | 169 | 387 a | 483 a | 115 b | 196 | 348 ab | 434 a | 134 b |
| **RR Tonnica** | 173 | 376 ab | 472 b | 120 ab | 199 | 351 a | 438 a | 133 b |
| **SE** | 2.6 | 4.2 | 4.4 | 1.5 | 2.1 | 3.7 | 4.1 | 2.1 |

Different letters are significant at $p < 0.05$.

Table 3.6 Crude protein at each cutting for alfalfa under different treatments in 2016 and 2017.

| Treatment | 2016 | | | | | 2017 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cut 1 | Cut 2 | Cut3 | Cut4 | Cut 5 | Cut 1 | Cut 2 | Cut3 | Cut4 | Cut 5 |
| **Harvest Interval** | --------g $kg^{-1}$-------- | | | | | --------g $kg^{-1}$-------- | | | | |
| **28-day** | 189 | 155 | 176 a | 172 b | 182 | 196 | 206 a | 206 | 218 a | 208 |
| **35-day** | 186 | 147 | 160 b | 183 a | - | 192 | 170 b | 196 | 199 b | - |
| **SE** | 4.4 | 4.0 | 7.9 | 3.6 | - | 4.3 | 2.7 | 2.9 | 3.2 | - |
| **Seeding Rate** | | | | | | | | | | |
| **17 kg $ha^{-1}$** | 184 | 152 | 168 | 177 | 180 | 197 | 195 | 201 | 210 | 208 |
| **20 kg $ha^{-1}$** | 188 | 151 | 169 | 179 | 186 | 190 | 182 | 206 | 208 | 204 |
| **23 kg $ha^{-1}$** | 189 | 150 | 168 | 176 | 181 | 195 | 189 | 196 | 208 | 214 |
| **SE** | 5.1 | 4.9 | 8.2 | 4.1 | 5.3 | 4.8 | 3.2 | 3.3 | 3.9 | 3.3 |
| **Variety** | | | | | | | | | | |
| **Hi-Gest 360** | 190 | 148 | 174 | 179 | 187 | 189 | 192 | 205 | 211 | 211 |
| **Gunner** | 186 | 152 | 162 | 174 | 177 | 193 | 184 | 198 | 206 | 208 |
| **RR Tonnica** | 185 | 154 | 169 | 180 | 182 | 200 | 189 | 200 | 208 | 206 |
| **SE** | 4.2 | 4.9 | 8.2 | 4.1 | 5.3 | 4.8 | 3.1 | 3.1 | 3.6 | 3.3 |

Different letters are significant at $p < 0.05$.

Table 3.7 Acid detergent fiber at each cutting for alfalfa under different treatments in 2016 and 2017.

| Treatment | 2016 | | | | | 2017 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cut 1 | Cut 2 | Cut3 | Cut4 | Cut 5 | Cut 1 | Cut 2 | Cut3 | Cut4 | Cut 5 |
| **Harvest Interval** | --------g $kg^{-1}$-------- | | | | | --------g $kg^{-1}$-------- | | | | |
| **28-day** | 334 b | 414 | 342 b | 375 | 340 | 348 | 320 b | 337 | 314 b | 321 |
| **35-day** | 405 a | 393 | 412 a | 342 | - | 360 | 376 a | 361 | 336 a | - |
| **SE** | 8.2 | 7.6 | 9.2 | 13.0 | - | 9.2 | 6.9 | 6.0 | 7.3 | - |
| **Seeding Rate** | | | | | | | | | | |
| **17 kg $ha^{-1}$** | 375 | 408 | 380 | 354 | 346 | 350 | 350 | 350 | 323 | 324 |
| **20 kg $ha^{-1}$** | 366 | 410 | 376 | 361 | 341 | 358 | 350 | 345 | 325 | 321 |
| **23 kg $ha^{-1}$** | 368 | 393 | 3.75 | 360 | 332 | 354 | 345 | 353 | 327 | 319 |
| **SE** | 7.4 | 9.3 | 10.4 | 13.3 | 9.6 | 9.5 | 7.8 | 6.2 | 8.4 | 7.4 |
| **Variety** | | | | | | | | | | |
| **Hi-Gest 360** | 367 | 410 | 369 | 332 b | 330 | 338 b | 341 | 350 | 314 | 308 |
| **Gunner** | 365 | 410 | 393 | 379 a | 343 | 353 ab | 355 | 348 | 329 | 329 |
| **RR Tonnica** | 377 | 391 | 368 | 365 ab | 346 | 370 a | 348 | 350 | 333 | 326 |
| **SE** | 7.0 | 8.2 | 10.4 | 12.6 | 9.6 | 9.4 | 7.8 | 6.2 | 7.6 | 7.4 |

Different letters are significant at $p < 0.05$.

Table 3.8 Neutral detergent fiber at each cutting for alfalfa under different treatments in 2016 and 2017.

| Treatment | 2016 | | | | | 2017 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cut 1 | Cut 2 | Cut3 | Cut4 | Cut 5 | Cut 1 | Cut 2 | Cut3 | Cut4 | Cut 5 |
| **Harvest Interval** | --------g kg$^{-1}$-------- | | | | | --------g kg$^{-1}$-------- | | | | |
| **28-day** | 407 b | 496 | 443 b | 475 | 432 | 435 | 396 b | 413 b | 395 b | 407 |
| **35-day** | 504 a | 493 | 527 a | 453 | - | 461 | 466 a | 439 a | 418 a | - |
| **SE** | 7.9 | 9.1 | 8.6 | 8.2 | - | 17.4 | 5.7 | 6.1 | 6.0 | - |
| **Seeding Rate** | | | | | | | | | | |
| **17 kg ha$^{-1}$** | 461 | 496 | 488 | 466 | 438 | 443 | 431 | 426 | 405 | 414 |
| **20 kg ha$^{-1}$** | 453 | 497 | 481 | 461 | 437 | 449 | 433 | 421 | 407 | 405 |
| **23 kg ha$^{-1}$** | 453 | 492 | 486 | 463 | 420 | 452 | 430 | 431 | 406 | 402 |
| **SE** | 8.5 | 11.1 | 10.0 | 8.8 | 10.1 | 14.4 | 6.7 | 7.1 | 7.2 | 6.3 |
| **Variety** | | | | | | | | | | |
| **Hi-Gest 360** | 456 | 503 | 476 | 441 b | 426 | 424 b | 422 | 424 | 392 b | 396 b |
| **Gunner** | 453 | 500 | 499 | 483 a | 431 | 451 ab | 439 | 425 | 410 ab | 419 a |
| **RR Tonnica** | 457 | 482 | 480 | 467 ab | 438 | 470 b | 433 | 428 | 416 a | 406 ab |
| **SE** | 8.5 | 11.1 | 9.8 | 8.8 | 10.1 | 14.4 | 6.5 | 7.0 | 6.9 | 6.3 |

Different letters are significant at $p < 0.05$.

Table 3.9 Relative feed value at each cutting for alfalfa under different treatments in 2016 and 2017.

| Treatment | 2016 | | | | | 2017 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cut 1 | Cut 2 | Cut3 | Cut4 | Cut 5 | Cut 1 | Cut 2 | Cut3 | Cut4 | Cut 5 |
| **Harvest Interval** | | | | | | | | | | |
| **28-day** | 144 a | 109 | 133 a | 118 | 135 | 134 | 151 a | 142 a | 153 a | 147 |
| **35-day** | 107 b | 111 | 101 b | 130 | - | 126 | 120 b | 130 b | 140 b | - |
| **SE** | 3.1 | 3.1 | 3.7 | 4.1 | 2.3 | 6.3 | 2.7 | 2.7 | 2.9 | 1.9 |
| **Seeding Rate** | | | | | | | | | | |
| **17 kg ha$^{-1}$** | 123 | 109 | 117 | 123 | 132 | 132 | 135 | 136 | 148 | 144 |
| **20 kg ha$^{-1}$** | 126 | 108 | 119 | 124 | 134 | 128 | 135 | 138 | 147 | 147 |
| **23 kg ha$^{-1}$** | 127 | 113 | 115 | 124 | 140 | 130 | 137 | 134 | 146 | 149 |
| **SE** | 3.2 | 3.7 | 4.3 | 4.1 | 4.7 | 5.4 | 3.1 | 3.1 | 3.5 | 3.1 |
| **Variety** | | | | | | | | | | |
| **Hi-Gest 360** | 126 | 107 | 120 | 134 a | 140 | 139 a | 140 | 137 | 154 a | 152 a |
| **Gunner** | 127 | 108 | 111 | 116 b | 135 | 129 ab | 132 | 136 | 145 ab | 142 b |
| **RR Tonnica** | 123 | 115 | 120 | 122 ab | 132 | 122 b | 134 | 135 | 142 b | 146 ab |
| **SE** | 3.0 | 3.7 | 4.1 | 4.1 | 4.7 | 5.4 | 3.1 | 3.0 | 3.4 | 3.1 |

Different letters are significant at $p < 0.05$.

Table 3.10 Economic income at each cutting and total for alfalfa under different treatments in 2016 and 2017.

| Treatment | 2016 | | | | | | 2017 | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cut 1 | Cut 2 | Cut3 | Cut4 | Cut 5 | Annual | Cut 1 | Cut 2 | Cut3 | Cut4 | Cut 5 | Annual | |
| **Harvest Interval** | --------Dollars ha $^{-1}$-------- | | | | | | --------Dollars ha$^{-1}$-------- | | | | | | |
| **28-day** | 448 a | 357 b | 354 a | 268 | 212 | 1640 a | 336 | 339 b | 324 | 237 b | 240 | 1476 | 3116 a |
| **35-day** | 290 b | 452 a | 289 b | 274 | - | 1305 b | 402 | 468 a | 333 | 336 a | - | 1540 | 2845 b |
| **SE** | 19.7 | 17.2 | 10.5 | 12.0 | 4.5 | 38.7 | 23.9 | 11.1 | 12.8 | 13.8 | 5.1 | 35.7 | 71.3 |
| **Seeding Rate** | | | | | | | | | | | | | |
| **17 kg ha$^{-1}$** | 374 | 409 | 319 | 275 | 212 | 1483 | 352 | 416 | 314 | 276 | 239 | 1478 | 2960 |
| **20 kg ha$^{-1}$** | 373 | 394 | 325 | 279 | 214 | 1479 | 385 | 404 | 338 | 298 | 247 | 1549 | 3027 |
| **23 kg ha$^{-1}$** | 361 | 412 | 319 | 259 | 211 | 1456 | 372 | 390 | 335 | 285 | 233 | 1498 | 2954 |
| **SE** | 19.8 | 21.1 | 12.5 | 14.7 | 6.8 | 44.6 | 27.4 | 13.6 | 15.6 | 14.0 | 8.5 | 34.4 | 60.4 |
| **Variety** | | | | | | | | | | | | | |
| **Hi-Gest 360** | 376 | 392 | 321 | 298 a | 221 | 1497 | 405 a | 395 | 325 | 297 | 247 | 1545 | 3042 |
| **Gunner** | 379 | 400 | 306 | 249 b | 205 | 1484 | 372 ab | 401 | 334 | 281 | 230 | 1502 | 2939 |
| **RR Tonnica** | 352 | 422 | 337 | 266 ab | 212 | 1437 | 331 b | 415 | 328 | 282 | 242 | 1477 | 2961 |
| **SE** | 18.0 | 20.3 | 11.1 | 11.6 | 6.8 | 34.0 | 21.2 | 13.6 | 12.3 | 14.0 | 8.5 | 32.1 | 59.3 |

Different letters are significant at $p < 0.05$

# Chapter 4 - Limitations and implications

## Alfalfa climate model

Given that the scale of the Lasso regression model is on the state level, constructing a more resilient model involving soil and solar radiation data is challenging. There are therefore subject biases and confounding that may have influenced our model estimates with only temperature and precipitation variables. However, soil data over a large area could be difficult to include in the state scale model. Historical solar radiation data in the selected states are not available. The contribution of irrigation to the state level alfalfa yield was not considered in the Lasso model.

To overcome these limitations in the future study of Chapter 1, one way is to scale-down to the county level and focus on one or two northern states in the Midwest. In Fig. 4.1, regarding the yield per harvested area by county, Wisconsin and Minnesota are the ideal states to carry out a similar study with the scope of county level. The associated soil and solar radiation data could be integrated into the model compared to the state level model.

Variables generated by aggregating days with temperatures higher than a threshold or lower than a threshold, or precipitation might be considered in the future study. Nighttime temperatures are often substantial, especially during the summer time.

At a minimum, the current results from Chapter 1 of one state might serve as a benchmark in efforts to assess variables selected by the new model. Since alfalfa is a perennial crop, future climate projections are relatively more critical according to the model we provided in a state. For example, the model of South Dakota only involved variables in October and June, management practices or scouting would be suggested to take place during those months.

## Alfalfa field study

In Chapter 2, we introduced diverse harvest schedules for a four-cut system in Kansas. The weather information of the experiment site was listed but not included as a covariance for analyzing two years of data together. Stand persistence is another factor in enhancing the model resilience if the year effect is included.

More varieties could be involved for the future study testing two or three harvest schedules selected or modified regarding the results of Chapter 2. The whole-plot level might be the variety with four or more levels with fewer subplot levels of harvest schedules.

No acid detergent lignin content, neutral detergent fiber digestibility, and relative feed value data were analyzed in Chapter 2 and 3. Acid detergent fiber is proportional to lignin content; however, not directly represents the digestibility of lignin content.

The analysis of economic incomes only considers the revenue of alfalfa products from a cutting system. In Chapter 2, six harvest schedules with the same cutting times were compared; in Chapter 3, the 28-day interval with five cuts was compared to the 35-day interval with four cuts. The extra labor and production might be simulated in future studies to have a more comprehensive estimation of the income.

Based on two production year research in Manhattan, KS, the low lignin alfalfa variety under a shorter harvesting interval (every 28-day) appears be profitable management practice regardless of seeding rate.

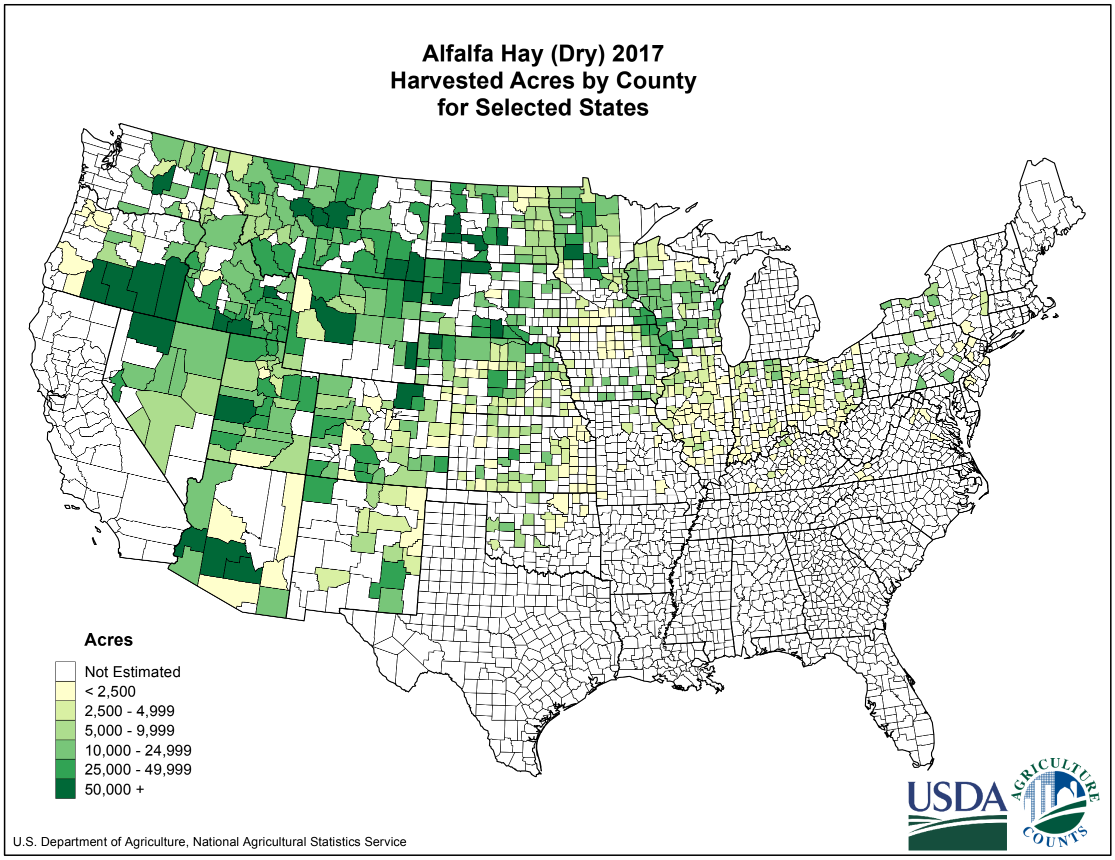



Figure 4.1 Distribution of alfalfa hay acres harvested by county in 2016 in the United States (Image and data from USDA-NASS).