

EVALUATION OF GREEN, RED AND NEAR  
INFRARED BANDS FOR PREDICTING  
WINTER WHEAT BIOMASS AND  
NITROGEN UPTAKE

By

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# EVALUATION OF GREEN, RED AND NEAR INFRARED BANDS FOR PREDICTING WINTER WHEAT BIOMASS AND NITROGEN UPTAKE

## ABSTRACT

Presently, normalized difference vegetative indexes (NDVI) based on red (RNDVI) or green (GNDVI) reflectance are commonly used to evaluate plant health, biomass, and nutrient content. This study was conducted to determine which of these two indexes is better correlated with biomass, forage N uptake, and final grain yield of winter wheat. Three experimental sites were established in Oklahoma in the fall of 2001 at Stillwater. Spectral reflectance measurements were taken at Feekes growth stage 4, 5, 6, and 10 followed by winter wheat forage harvest. Across all growth stages and locations GNDVI was a better predictor of above ground biomass when compared to RNDVI ( $R^2=0.45$  and  $0.37$ , respectively). However, when evaluated by specific stages of growth, RNDVI was consistently better. GNDVI and RNDVI were more highly correlated with forage N uptake than with dry biomass, with GNDVI being the better index across all growth stages ( $R^2=0.82$  and  $0.75$ , respectively). Across all locations and growth stages RNDVI was a slightly better predictor of final grain yield than GNDVI ( $R^2=0.76$  and  $0.74$ , respectively). At individual locations and growth stages, neither index appeared to have a sizeable advantage over the other, suggesting that both perform equally well at predicting biomass, forage N uptake, and grain yield in winter wheat.

## INTRODUCTION

Soil nutrient availability has traditionally been determined by soil sampling from crop fields. However, use of coarse resolutions encompassing large areas of land cannot provide reliable information about the inherent spatial nutrient variability in the field. According to Raun et al. (1998) large composite samples do not address the variability encountered in the field. Wibawa et al. (1993) in his evaluation of soil fertility variation using grid sampling for field maps indicated that variation in soil nitrate-N ( $\text{NO}_3\text{-N}$ ) occurred over very short distances within a  $15\text{m}^2$  grid. Thus it is important to identify the optimum area of land where there is uniform nutrient distribution in the soil for precise management of agricultural inputs. Solie et al. (1996) showed that the optimum field element size could be less than  $1\text{m}^2$ , and variable rates should be adjusted to a resolution of  $1\text{m}^2$  in order to optimize fertilizer inputs. They further defined field element size as the area which provides the most precise measure of the available nutrients where the level of that nutrient changes with distance. Cahn et al. (1994) analyzed spatial variability of soil properties and nutrient concentrations for site specific crop management and concluded that reducing sampling intervals from 50 to 1m would reduce variability of soil water content, soil organic carbon (C),  $\text{NO}_3\text{-N}$ , phosphate-P ( $\text{PO}_4\text{-P}$ ), and potassium (K) estimates by 74, 95, 25, 64, and 58%, respectively. Thus in order to treat nutrient availability in the field, soil sampling should be carried out for each  $1\text{m}^2$  area, recognizing that it would be virtually impossible for large sized fields. The use of indirect and non-destructive sensor based technology could be a way to avoid time-consuming on-the-go wet chemistry and costs associated with soil sampling (Raun et al., 1998). Bauer (1975) and Walburg et al. (1982) reported that the use of canopy



reflectance measurements and remote sensing techniques offer the potential for monitoring crop growth conditions over large areas. Sawyer (1994) stated that determination of crop yield variability with on-the-go sensors was still futuristic. The work of Stone et al. (1996) showed that it would be possible to use on-the-go sensing methods to monitor soil and yield variability.

The potential replacement of wet chemical methods with non-destructive spectral analysis was first applied over 20 years ago (Thomas and Oenther, 1972). Previous research has shown that near infrared (NIR) diffuse reflectance spectrometry was used to measure protein, moisture, fat, and oil in agricultural products. Bausch et al. (1994) also reported that canopy reflectance measurements might be an alternative tool for estimation of N status.

Research on light spectrometry has indicated that there is a strong relationship between the amount of chlorophyll and nitrogen content of a crop. Chlorophyll, as a plant tissue, is composed of different organic molecules of which nitrogen is the major component. Morta et al. (1991) reported that organic molecules have unique light absorption features at different wavelengths of light due to stretching and bending vibrations of molecular bonds between elements. The degree of sensitivity of specific organic compounds to a specific wavelength of light may quantify the constituents of that molecule (e.g. amino-acid (R-NH<sub>2</sub>)-nitrogen). Reflectance measurements on sweet pepper (*Capcicum annum* L. var. 'Yolo wonder') in the red and green region were used to determine the relationship between nitrogen and chlorophyll concentration in the leaf tissue (Thomas and Oerther, 1972). Filella et al. (1995) indicated that leaf chlorophyll-*a* content is primarily determined by N availability. They found improved correlation

between measured chlorophyll-*a* and leaf reflectance at 550 nm and 680 nm, compared to NDVI. Blackmer et al. (1994) studied light reflectance of corn leaves from plots with different N treatments within the 400-700 nm range. They found that reflectance near 550 nm measured on detached maize leaves was able to separate N treatments and it was also strongly related with both leaf N concentration and chlorophyll meter readings. Ma et al. (1996) also found that reflectance at 550 or 650 nm, differentiated between N treatments. Thomas and Gaussman (1977) found better correlation between reflectance at 550 nm and chlorophyll concentration than when using reflectance at 675 nm. Buschman and Nagel (1993) for intact bean leaves and Gitelson and Merzlyak (1994a, b) for maple and chestnut leaves, found that reflectance over a wide range near 550 nm is more sensitive to chlorophyll concentration than in the main absorption bands of photosynthetic pigments, including 675 nm. Work by Carter (1993, 1994) found that reflectance was most sensitive to plant stress in the 535-640 nm and 685-700 nm ranges. Gitelson and Merzlyak (1994a,b) and Gitelson et al. (1996) observed high sensitivity of reflectance both in the green and the red (near 700) regions for chlorophyll and found that the relationship between chlorophyll-*a* and 550nm, as well as 700nm, is hyperbolic with a high degree of accuracy. Gitelson et al. (1996) found that the inverse of the reflectance in the green band was proportional to Chl-*a* concentration with correlation  $r^2 > 0.95$ . Varvel et al. (1997) reported that there are significant differences among hybrid and N treatments for chlorophyll meter readings and canopy reflectance values measured on different growth stages, expressed as GNDVI. Yoder and Waring (1994) also used the green band (500-600 nm or 565-575 nm in a vegetation index) and

found better correlation ( $r^2=0.83$ ) with photosynthesis activity of miniature Douglas-fir trees than when using the red (680nm) band.

Reflectance measurements for predicting of forage biomass and N uptake are dependent on the growth stage, whether it is wheat (*Triticum durum* Desf. Var produra) (Jackwon et al., 1983), grasses (Hagger et al., 1984), and/or barley (*Hordeum vulgare*, L) (Kleman and Fagerlund, 1987). Research in barley showed that the relationship between fresh biomass and NIR/red changed with time, indicating the ratio was dependent on growth stage (Kleman and Fagerlund, 1987). Gausman et al. (1971) reasoned that the impact of growth stage on reflectance reading resulted from young plant tissue, having less air spaces within the mesophyll than older leaves, and thus, showed decreased NIR spectral radiance. Sembiring et al. (2000) reported that the amount of variability in total N uptake as explained by NDVI increased with advancing growth stages, which can be further evidence that NDVI is a reliable predictor of biomass and in-season N uptake. Taylor et al. (1998) showed that red and green NDVI readings were significantly correlated with forage yield and N uptake of bermudagrass.

Previous work at Oklahoma State University has shown that final grain yield can be predicted using an in-season estimate of yield (INSEY) which is calculated using NDVI and number of days where growing degree days are greater than zero (Raun et al., 2001). The objectives of this experiment were to determine the relationship between the RNDVI, GNDVI, forage biomass and forage N uptake at various stages of growth winter wheat, and to determine if GNDVI is a better predictor of wheat forage biomass and/or forage N uptake than RNDVI, when vegetative coverage of the soil exceeds 50%.

## MATERIALS AND METHODS

This experiment was conducted on four long-term fertility trials (222-Stillwater, 301-Efaw, AA-Efaw and AA-Hennessey). Data were collected beginning in December 2001 and extending through June, 2002. Pre-plant soil test results are listed in Table 2. Winter wheat cultivar 'Custer' was planted at a seeding rate of 78 kg/ha at all sites. Planting dates ranged between October and November and sown with a spacing of 19 cm at Stillwater and 15 cm at Hennessey and Efaw. Treatments were laid out in a randomized complete block design with four replications at 222-Stillwater and three at 301-Efaw, AA-Efaw and AA-Hennessey sites. A complete list of treatments for each experiment is listed in Table 1.

Spectral reflectance was measured using an Oklahoma State University designed optical sensor that consisted of three upward directed photodiode sensors that received incident light through cosine corrected Teflon<sup>®</sup> coated windows fitted with red ( $671\pm6$  nm), near-infrared (NIR) ( $780\pm6$  nm) and green ( $550\pm12.5$ nm) interference filters. The sensor also measures light irradiance from target plants with three down-looking photodiode sensors that received collimated light at the same wavelength as three upward looking sensors. The instrument used a 16bit A/D converter to simultaneously capture and convert the signals from the four photodiode sensors. Collimation was used to constrain the view of the down looking sensors to a  $0.80\text{ m}^2$  circular area at the plant surface. Stability of the sensor was maintained across time by dividing spectral readings by day and time-specific barium sulfate white plate 100% reflectance reading. The reflectance of the barium sulfate coated was assumed to be 1.0 for all three spectral bands investigated. Reflectance values (the ratio of incident and reflected values) were used in

the calculation of NDVI to minimize the error associated with cloud cover shadows and sun angle. Reflectance based average NDVI was calculated using the following equations:

$$NDVI_{red} = \left[ \frac{NIR_{ref}}{NIR_{inc}} - \frac{RED_{ref}}{RED_{inc}} \right] / \left[ \frac{NIR_{ref}}{NIR_{inc}} + \frac{RED_{ref}}{RED_{inc}} \right]$$

$$NDVI_{green} = \left[ \frac{NIR_{ref}}{NIR_{inc}} - \frac{GREEN_{ref}}{GREEN_{inc}} \right] / \left[ \frac{NIR_{ref}}{NIR_{inc}} + \frac{GREEN_{ref}}{GREEN_{inc}} \right]$$

Where,  $NIR_{ref}$ ,  $RED_{ref}$  and  $GREEN_{ref}$  = magnitude of reflected light,

and  $NIR_{inc}$ ,  $RED_{inc}$  and  $GREEN_{inc}$  = magnitude of incident light.

The dates where readings were collected ranged between Feekes growth stage 4 (leaf sheaths beginning to lengthen), 5 (pseudo-stem strongly erected), 6 (first node of stem visible), and 10 (flowering)(Large, 1954). For all experiments, individual 1m<sup>2</sup> plots were hand clipped (immediately following sensor readings) and weighed prior to being dried in a forced air oven at 60°C. Once dry, samples were ground to pass a 0.125mm (120-mesh) sieve and analyzed for total N using a Carlo-Erba (Milan, Italy) NA-1500 dry combustion analyzer (Schepers et al., 1989). Early-season plant N uptake was determined by multiplying dry matter yield by the total N concentration determined from dry combustion. Nitrogen uptake and forage biomass were correlated with green and red NDVI values to determine their relationship at different wheat growth stages. Statistical analyses were performed using Excel and SAS statistical packages (SAS institute, 1988).

## RESULTS AND DISCUSSION

### Forage biomass

At the first sensing date (December, 2001, Feekes growth stage 4), both GNDVI ( $R^2=0.62$ ) and RNDVI ( $R^2=0.78$ ) readings were highly correlated with biomass of winter wheat (Figures 1 and 2). At the second sensing date (March, 2002, Feekes 6), both GNDVI ( $R^2=0.69$ ) and RNDVI ( $R^2=0.55$ ) were still highly correlated with biomass (Figures 1 and 2, APPENDIX). For both of the Feekes 4 and Feekes 6 sampling dates, there was a tendency for the RNDVI readings to have a broader range than the GNDVI, evidenced in the smaller slope components. At the final sensing date (April, 2002), Feekes 10, both indexes showed decreased correlation with biomass (Figures 3 and 4, APPENDIX). The ability to predict biomass using either RNDVI or GNDVI decreased with increasing stage of growth. The by growth stage RNDVI readings tended to show improved correlation with biomass when compared to GNDVI.

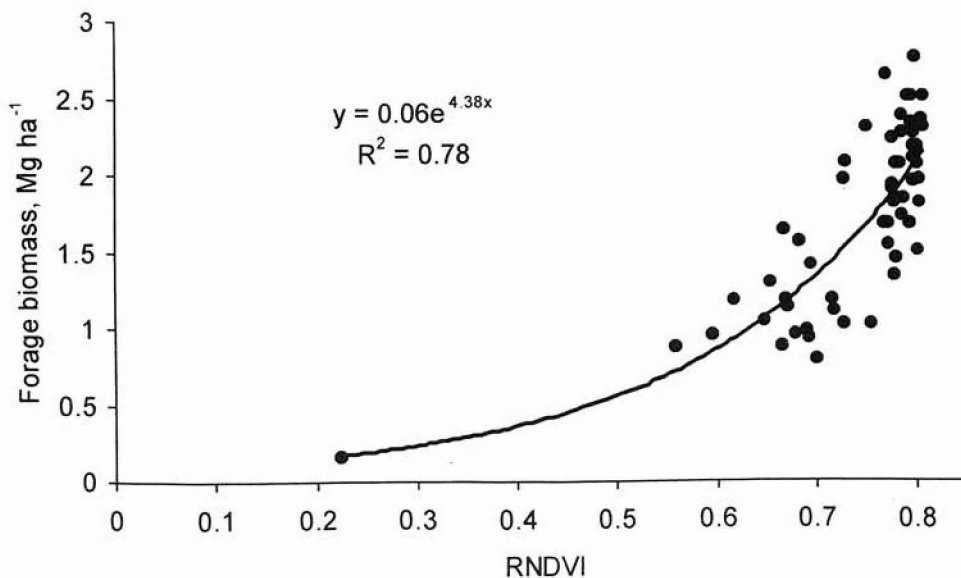


FIGURE 1. Relationship between RNDVI and forage biomass at Feekes growth stage 4 across all locations, 2001-2002.

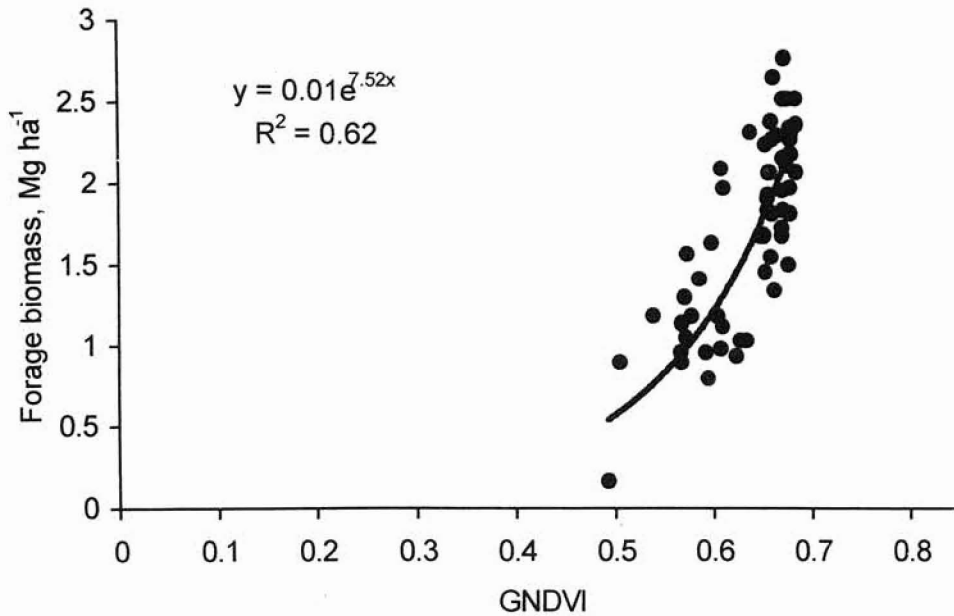


FIGURE 2. Relationship between GNDVI and forage biomass at Feekes growth stage 4 across all locations, 2001-2002.

### Forage N uptake

At Feekes 4, RNDVI and GNDVI were highly correlated with forage N uptake ( $R^2=0.78$  and  $0.77$ , respectively) (Figures 3 and 4). Similarly, at Feekes 6 RNDVI and GNDVI were highly correlated with forage N uptake ( $R^2=0.91$  and  $0.90$ , respectively) (Figures 5 and 6). Red and green NDVI were still highly correlated with forage N uptake at Feekes 10 ( $R^2=0.86$  and  $0.88$ , respectively) (Figures 7 and 8). Red and green NDVI were more highly correlated with forage N uptake than biomass, which is consistent with previous studies (Stone et al., 1996). There appeared to be only small differences between red and green NDVI in the ability to predict forage N uptake at the various stages of growth evaluated here.

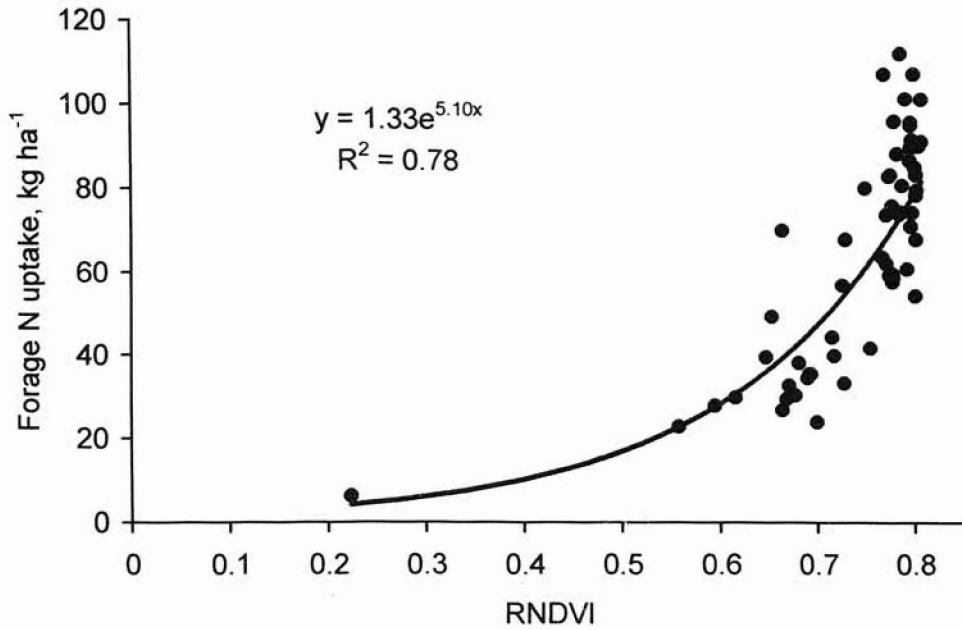


FIGURE 3. Relationship between RNDVI and forage N uptake at Feekes growth stage 4 across all locations, 2001-2002.

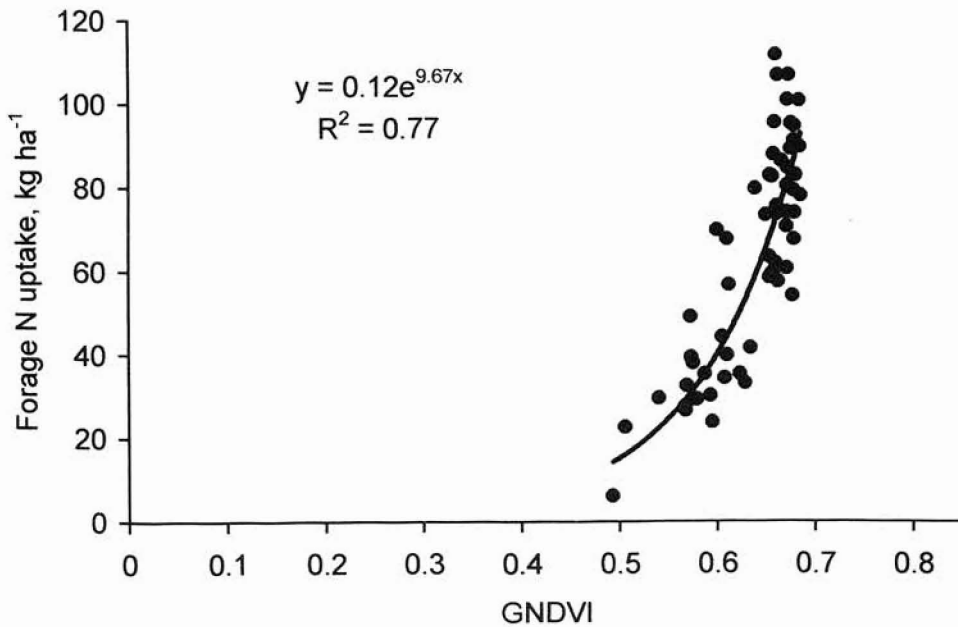


FIGURE 4. Relationship between GNDVI and forage N uptake at Feekes growth stage 4 across all locations, 2001-2002.



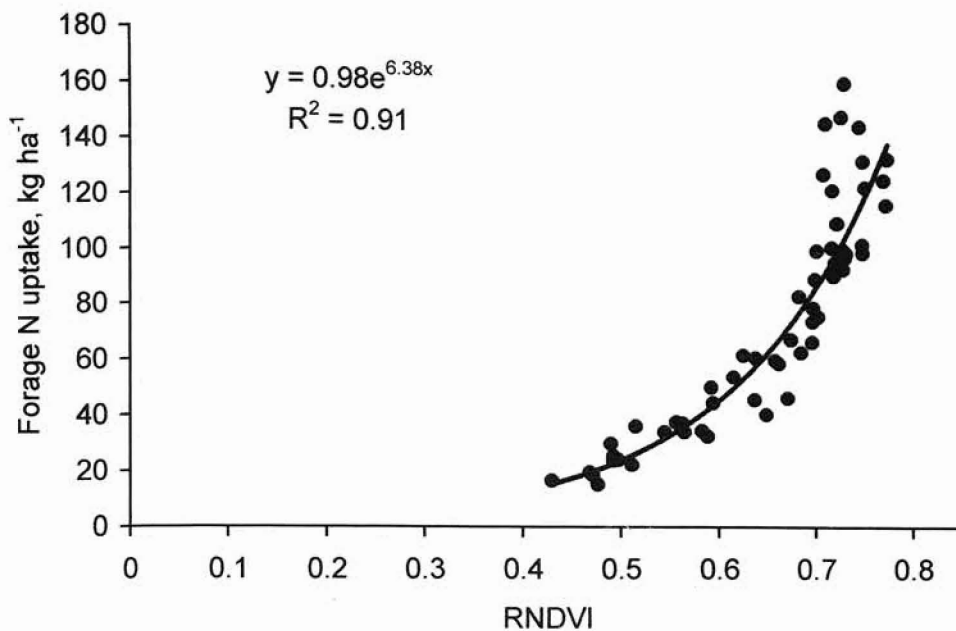


FIGURE 5. Relationship between RNDVI and forage N uptake at Feekes growth stage 6 across all locations, 2001-2002.

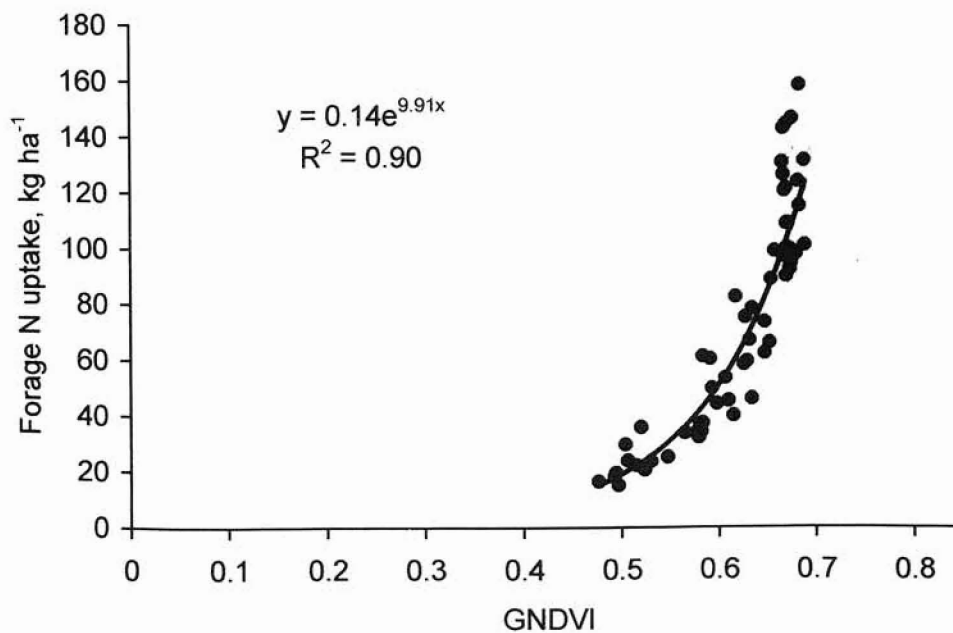


FIGURE 6. Relationship between GNDVI and forage N uptake at Feekes growth stage 6 across all locations, 2001-2002.

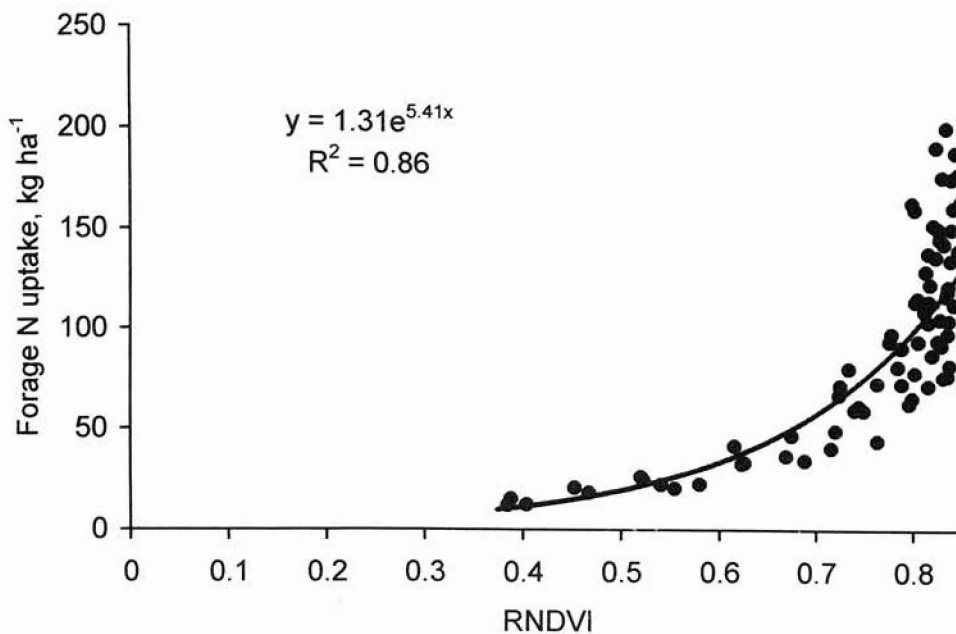


FIGURE 7. Relationship between RNDVI and forage N uptake at Feekes growth stage 10 across all locations, 2001-2002.

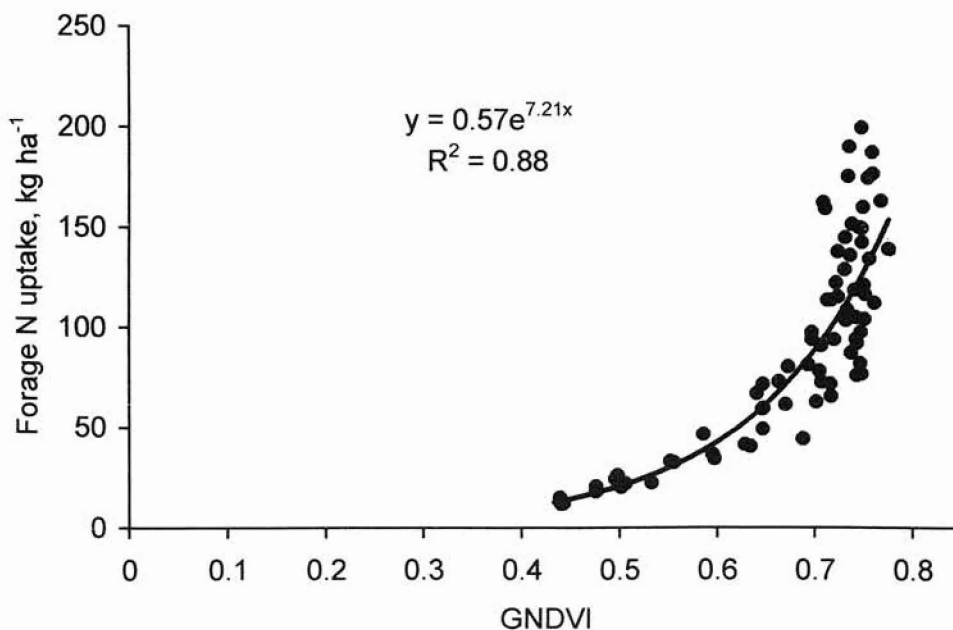


FIGURE 8. Relationship between GNDVI and forage N uptake at Feekes growth stage 10 across all locations, 2001-2002.

## Grain yield

Spectral measurements taken at Feekes 5 were used to determine whether RNDVI or GNDVI was a better predictor of final grain yield. Both indexes were highly correlated with final grain yield across all locations (RNDVI,  $R^2=0.76$  and GNDVI,  $R^2=0.74$ ) (Figures 9 and 10). When these same four locations were evaluated using INSEY (in-season estimated yield) as a predictor of wheat grain yield, correlation was not significantly improved versus the use of NDVI alone.

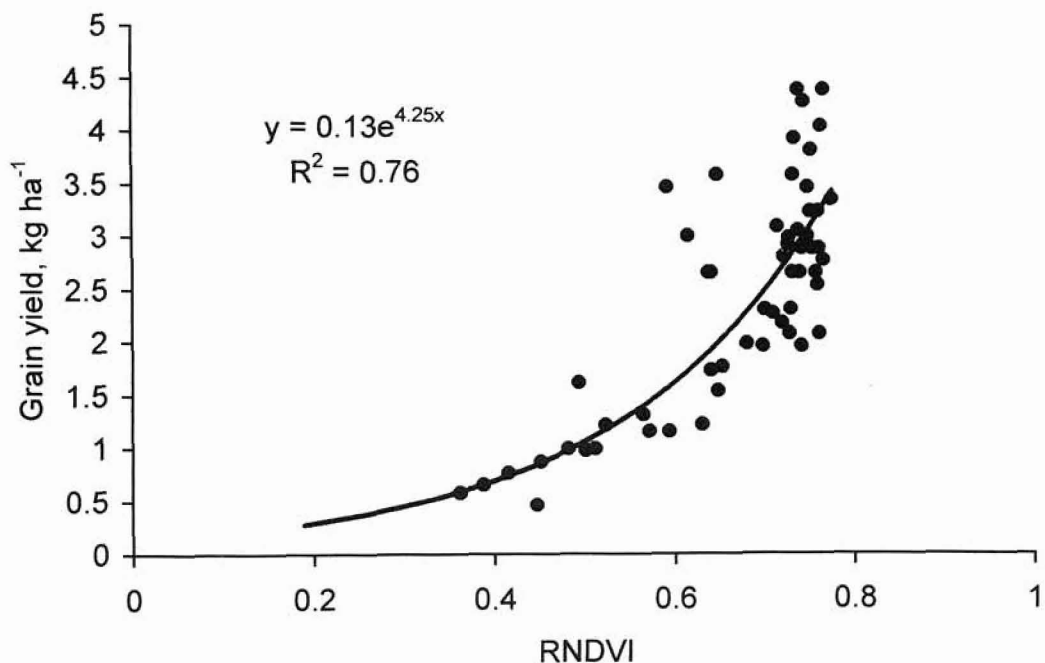


FIGURE 9. Relationship between RNDVI readings taken at Feekes growth stage 5 and grain yield across all locations, 2001-2002.

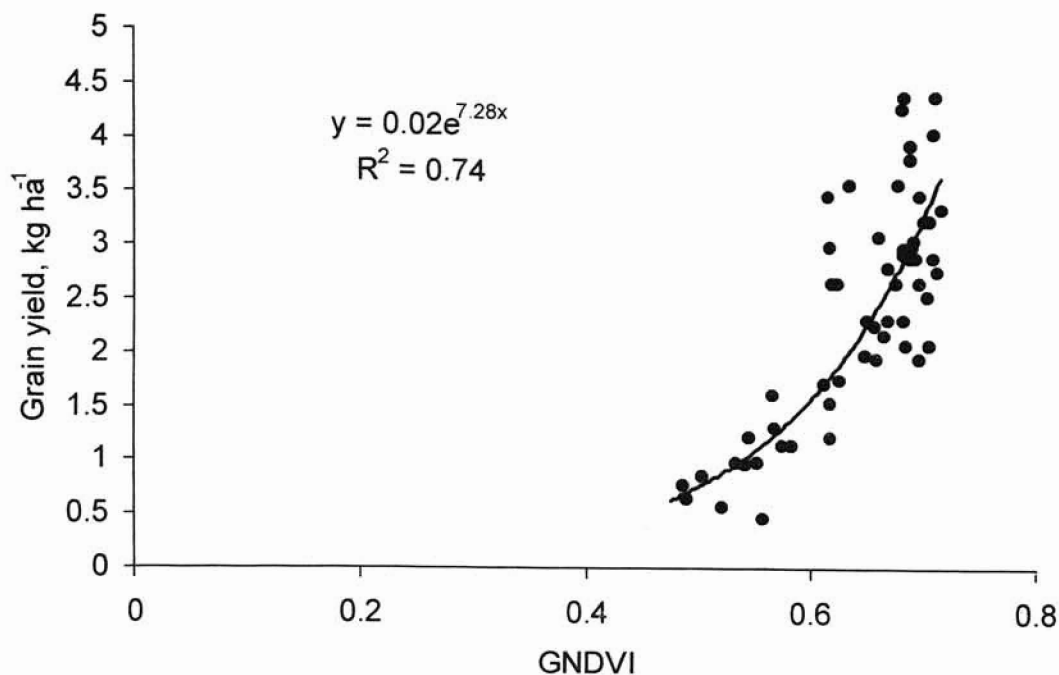


FIGURE 10. Relationship between GNDVI readings taken at Feekes growth stage 5 and grain yield across all locations, 2001-2002.

### Grain N uptake

As with grain yield, spectral measurements for prediction of grain N uptake were taken at Feekes 5. Both indexes were highly correlated with final grain N uptake across all locations (RNDVI,  $R^2=0.75$  and GNDVI,  $R^2=0.75$ ) (Figures 11 and 12). Thus prediction of grain N uptake was just as good as prediction of grain yield.

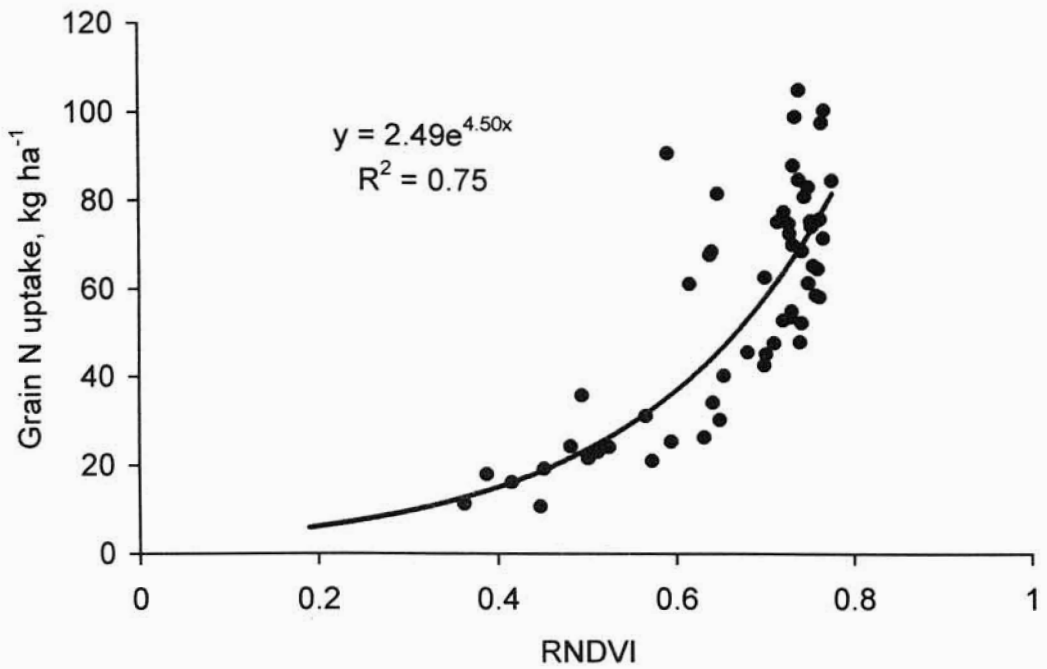


FIGURE 11. Relationship between RNDVI readings taken at Feekes growth stage 5 and grain N uptake across all locations, 2001-2002.

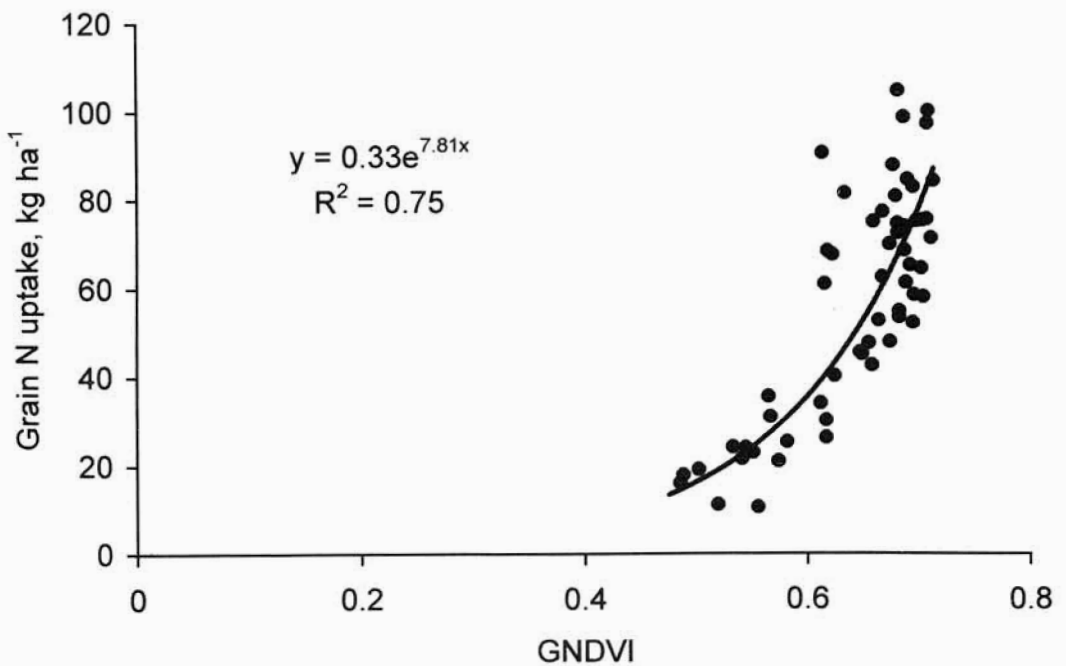


FIGURE 12. Relationship between GNDVI readings taken at Feekes growth stage 5 and grain N uptake across all locations, 2001-2002.

## CONCLUSION

There does not appear an advantage of either index over the other. Thus for the growth stages where data was collected in this work, the use of either RNDVI or GNDVI should be a reliable predictor of forage biomass, forage N uptake, grain yield, or grain N uptake. There was a tendency for the RNDVI readings to provided a wider range (compared to GNDVI) in values where differences could be more easily partitioned.

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TABLE 1. Fertilizer rates of N, P, and K at Hennessey and Stillwater, OK.

Efaw AA and Hennessey AA*	Stillwater 301*	Stillwater 222*
-----N-P-K (kg ha <sup>-1</sup> )-----		
0-0-0	0-0-0**	0-0-0**
56-0-0 <sup>†</sup>	45-0-0	0-29-37
90-0-0 <sup>†</sup>	90-0-0	45-29-37
123-0-0 <sup>†</sup>	179-0-0	90-29-37
56-0-0 <sup>‡</sup>		
90-0-0 <sup>‡</sup>		
123-0-0 <sup>‡</sup>		

\*-Blanket application of P and K to 100% sufficiency

\*\*- No P and K application

<sup>†</sup> - N applied as anhydrous ammonia

<sup>‡</sup> - N applied as ammonium nitrate

TABLE 2. Field activities and soil characteristics for each experiment, 2001-2002.

Plot activity	Stillwater 222	Efaw 301	Efaw AA	Hennessey AA
Planting date	10/01/01	10/12/01	10/04/01	10/30/01
Variety	Custer	Custer	Custer	Custer
Seeding rate, kg/ha	78	78	78	78
Sensing date	12/20/01	12/20/01	12/20/01	04/09/02
	03/26/02	03/12/02	03/12/02	04/25/02
	04/25/02	04/25/02	04/25/02	
Preplant fertilization date	09/25/01	09/27/01	09/11/01	10/18/01
Harvest date	06/03/02	06/28/02	06/28/02	06/12/02
Soil characteristics				
Soil pH	5.7	5.9	6.1	5.6
Organic C, g/kg	9.6	9.4	10.0	11.9
Total N, g/kg	0.8	0.7	0.8	1.05
P, mg/kg	42	18	13	95.6
K, mg/kg	199	125	117	558
NH <sub>4</sub> -N, mg/kg	9	10	7	19.3
NO <sub>3</sub> -N, mg/kg	17	17	23	14.5

Organic C and total N – dry combustion, P and K – Mehlich III extraction, NH<sub>4</sub>-N and NO<sub>3</sub>-N – 2 M KCl extra

APPENDIX

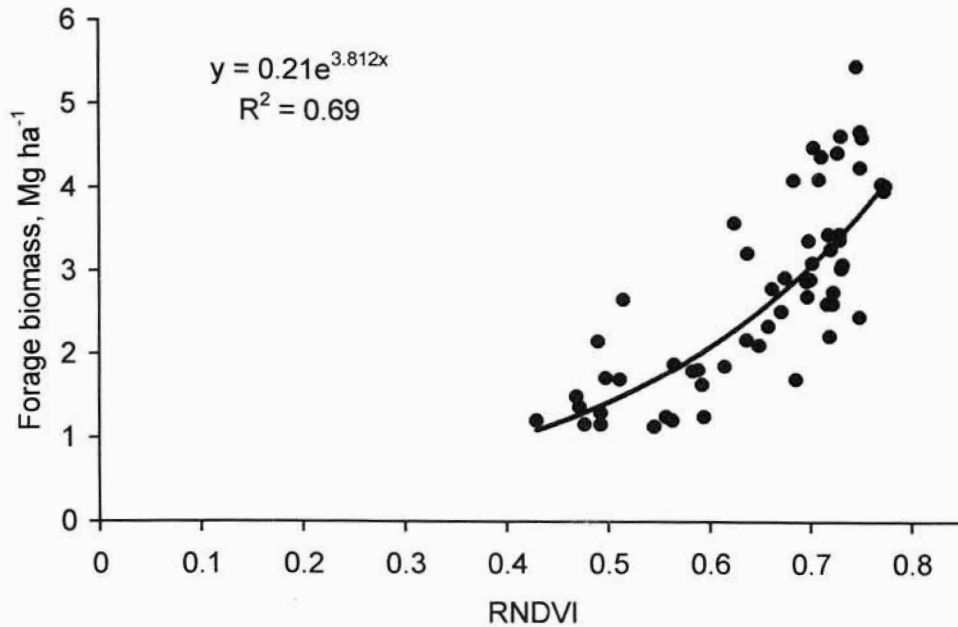


FIGURE 1. Relationship between RNDVI and forage biomass at Feekes growth stage 6 across all locations, 2001-2002.

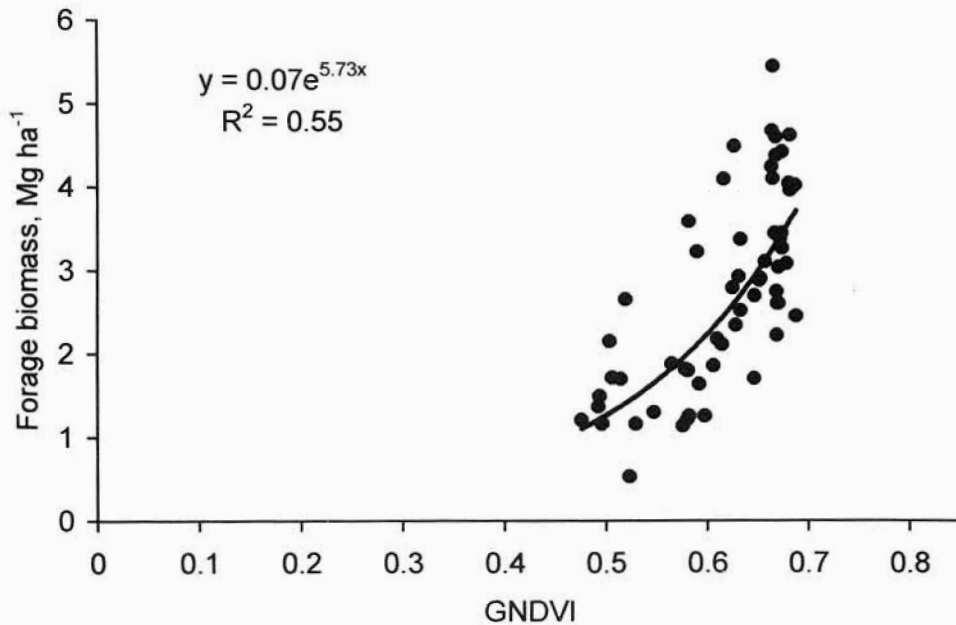


FIGURE 2. Relationship between GNDVI and forage biomass at Feekes growth stage 6 across all locations, 2001-2002.

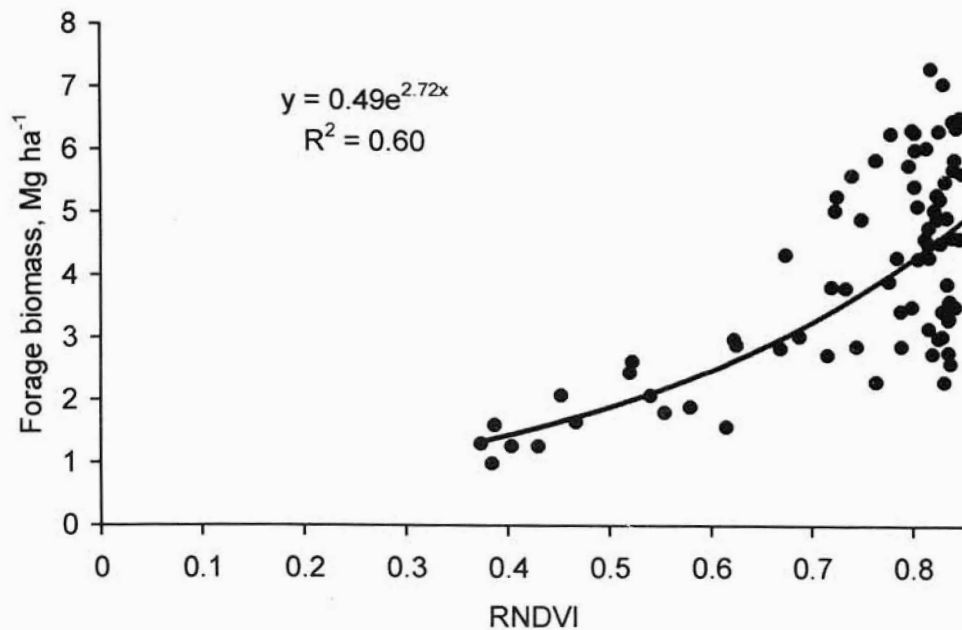


FIGURE 3. Relationship between RNDVI and forage biomass at Feekes growth stage 10 across all locations, 2001-2002.

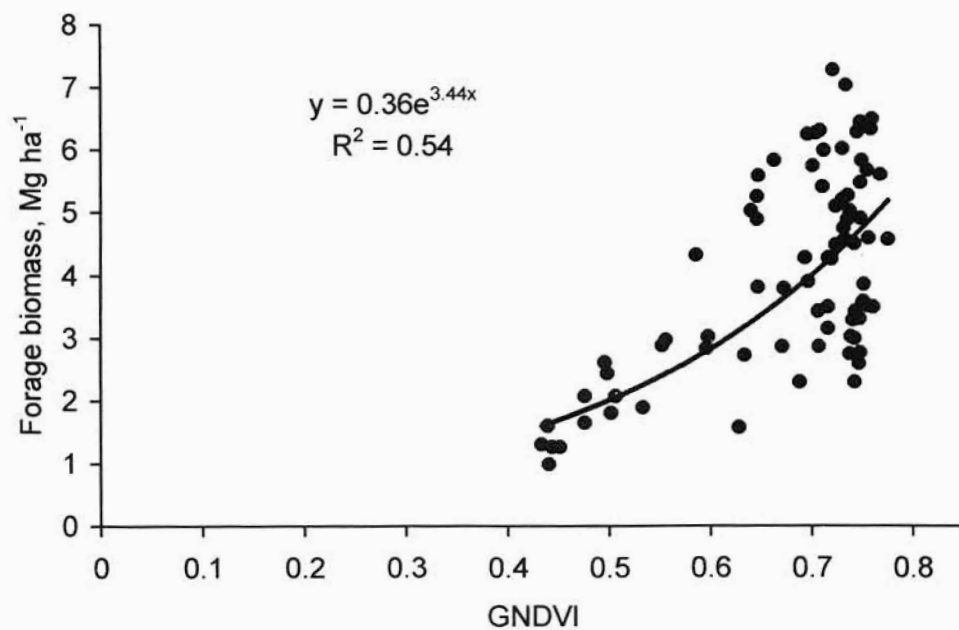


FIGURE 4. Relationship between GNDVI and forage biomass at Feekes growth stage 10 across all locations, 2001-2002.

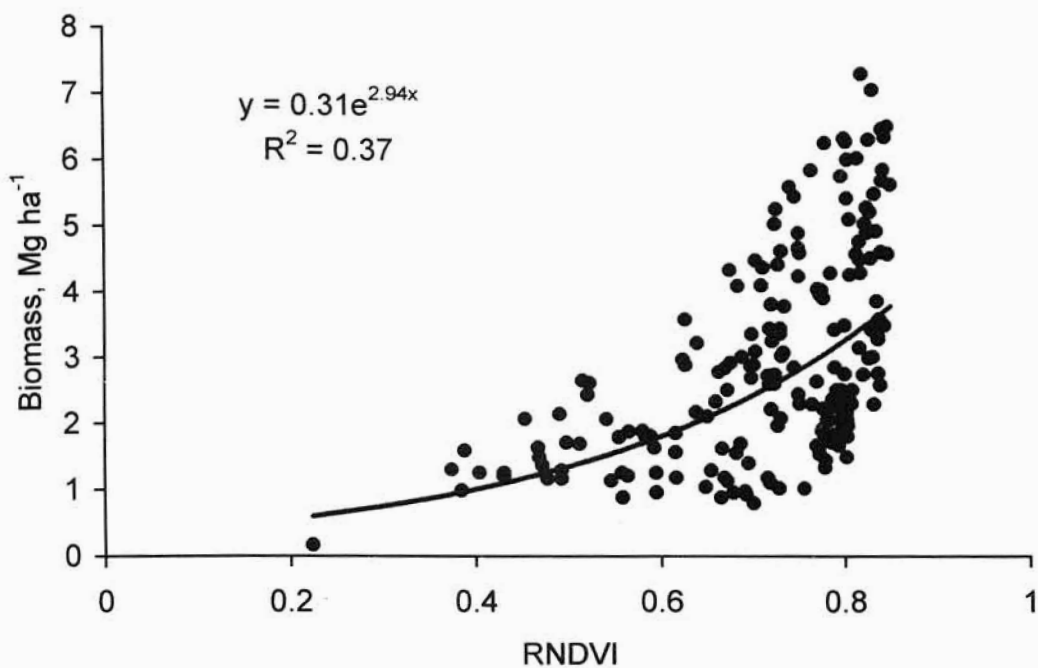


FIGURE 5. Relationship between RNDVI and biomass across all growth stages and locations, 2001-2002.

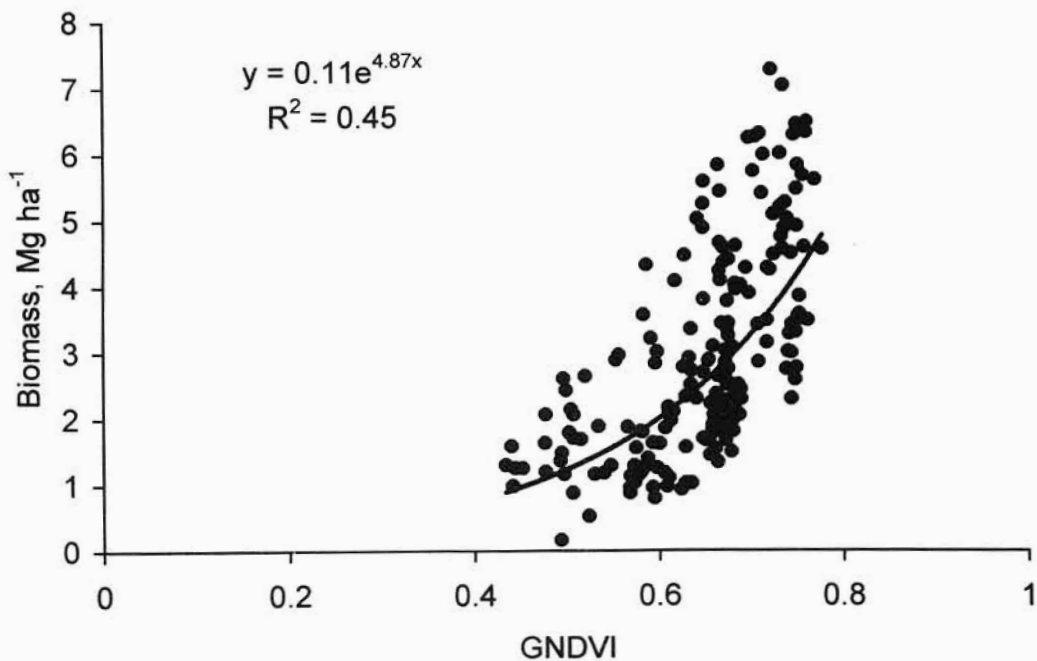


FIGURE 6. Relationship between GNDVI and biomass across all growth stages and locations, 2001-2002.

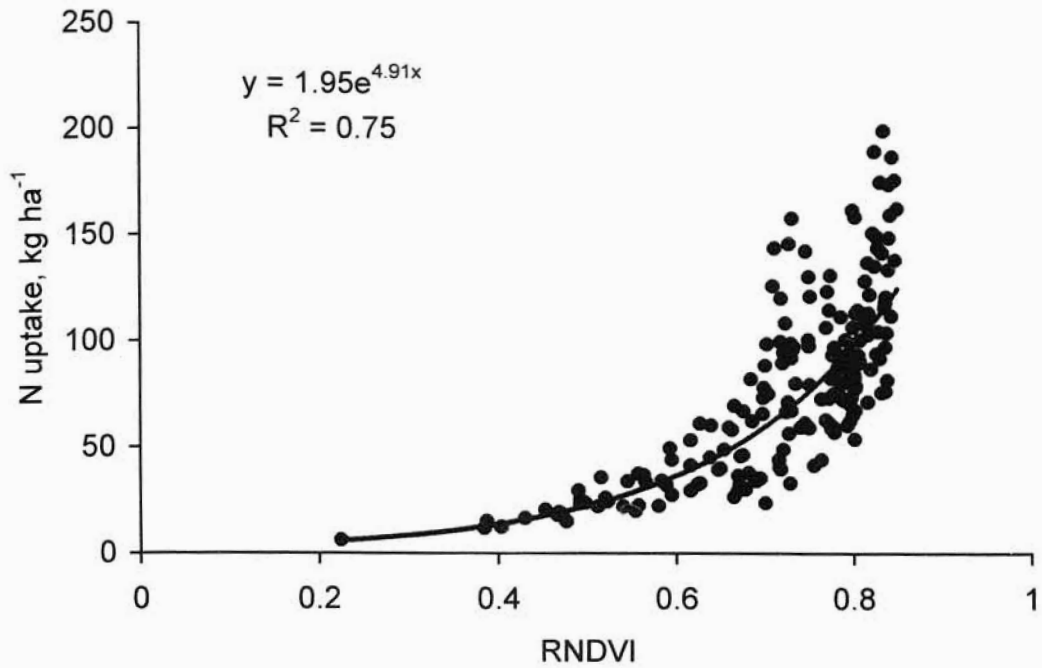


FIGURE 7. Relationship between RNDVI and forage N uptake across all growth stages and locations, 2001-2002.

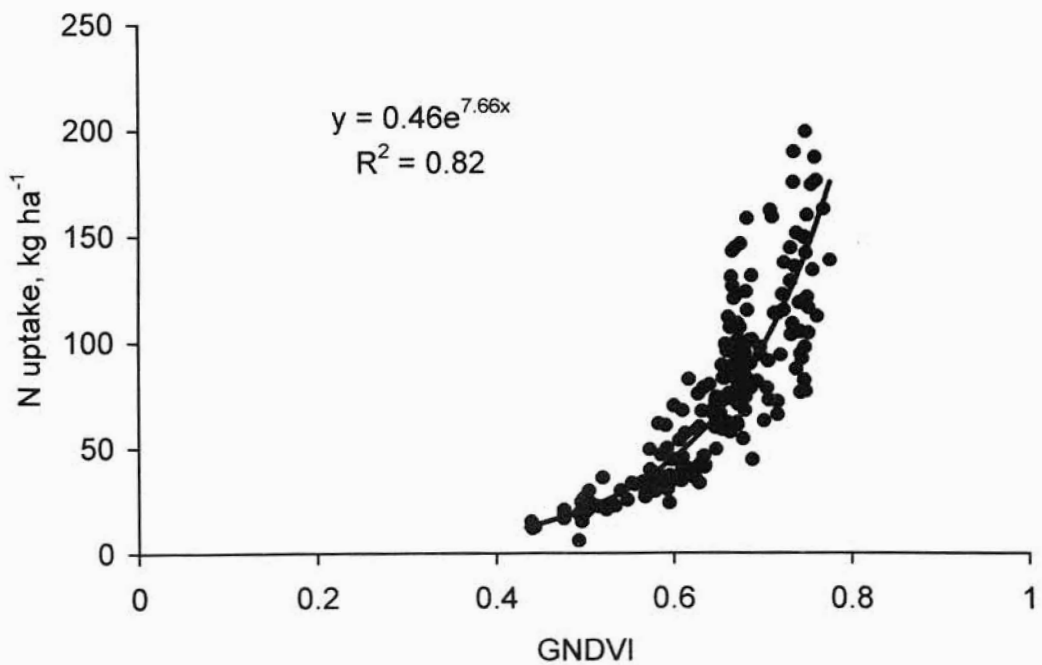


FIGURE 8. Relationship between GNDVI and forage N uptake across all growth stages and locations, 2001-2002.

2.

VITA

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Master of Science

Thesis: EVALUATION OF GREEN, RED AND NEAR INFRARED BANDS FOR  
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