RESEARCH ARTICLE Legume Research- An International Journal, Volume 49 Issue 3: 458-463 (March 2026)

Limited Irrigation Effects on Biomass, Growth Characteristics, Nutritive Value and Hyperspectral Wavelength in Alfalfa

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10.18805/LRF-890

ABSTRACT

Background: Soil moisture affects alfalfa biomass, nutritive value and physiological processes. Implementing precision agriculture for moisture management during alfalfa cultivation can help sustain high-quality forage production. This study aimed to assess limited irrigation effects on biomass, growth characteristics, nutritive value and hyperspectral wavelength in alfalfa (*Medicago sativa* L.).

Methods: The experiment was conducted in a greenhouse at Kansas State University from May to November 2024. Irrigation was applied based on field capacity (FC) at four levels namely 55% (FC55), 70% (FC70), 85% (FC85) and 100% (FC100).

Result: The findings indicated that above- and below-ground biomass and growth characteristics, including growth stage, leaf area index, number of nodules, crown size and taproot diameter as well as normalized difference vegetation index (NDVI) improved with increasing FC. FC85 appeared to be the optimal growth condition related to roots and nodules. The leaf to stem ratio was highest in FC55, which showed the same trend as nutritive values, including crude protein (CP), acid detergent fiber (ADF) and relative feed value (RFV). The hyperspectral reflectance was high in FC55, indicating drought stress. The optimal field capacity for balanced above- and below-ground growth in alfalfa appeared to be 85% while showing signs of water stress in FC55.

Key words: Alfalfa, Biomass, Field capacity, Nutritive value, Precision agriculture, Water limiting.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a perennial forage legume its high biomass and superior nutritive value (Putnam *et al.,* 2007). In 2024, Kansas ranked 14th in U.S. hay production, with 1.5 million tons of alfalfa from 580,000 acres and over 84% of this was rainfed (USDA-NASS, 2024, 2025). However, rising temperatures and declining summer precipitation have increased drought frequency in Kansas (NOAA, 2022).

Drought is a major constraint to plant survival and production, often reducing yield up to 70% (Farooq *et al.,* 2012; Baral *et al.,* 2022). Under the drought, alfalfa allocates more resources to root development while reducing leaf size and area, stem number and length with delaying maturity (Diatta *et al.,* 2021). These shifts lower photo-synthesis and biomass, but may enhance nutritive vale by increasing the leaf to stem ratio and reducing fiber content, which in followed by improving alfalfa quality (Diatta *et al.,* 2021).

The Ogallala Aquifer, spanning several Great Plains states including Kansas, is under threat of depletion by 2100 (Haacker *et al.,* 2016). As its availability declines, precise water management strategies based on soil moisture content will be critical for sustaining high alfalfa production.

Precision agricultural technologies offer solution to maximize both forage biomass and quality (Dey *et al.,* 2024, 2025). The normalized difference vegetation index (NDVI) is widely used to detect moisture stress (Zhang *et al.,* 2019) and biomass (Ehlert, 2002), though responses vary by species (Hatfield *et al.,* 2008). Soil moisture-based irrigation scheduling is important to optimize water use (Dey *et al.,* 2024). Thus, assessing optimal water supply thresholds based on soil moisture levels is crucial for alfalfa cultivation. Hyperspectral imaging offers potential to monitoring biomass (Feng *et al.,* 2020), cutting time (Cevoli *et al.,* 2022), morphology and biochemical composition (Garriga *et al.,* 2020) and nutritive value (Gámez *et al.,* 2024; Dey *et al.,* 2025). While commonly applied in crops like corn and wheat under drought (Okyere *et al.,* 2024), its application in alfalfa remains limited. Previous studies have focused mainly on morphology or forage quality, there is a need to integrate physiological measures with hyperspectral imaging to more precisely identify water-stress thresholds in alfalfa.

Most greenhouse studies on alfalfa have focused on factors such as drought influences on root and leaf

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How to cite this article: Kim, J., Dey, S., Jha, G. and Min, D. (2026). Limited Irrigation Effects on Biomass, Growth Characteristics, Nutritive Value and Hyperspectral Wavelength in Alfalfa. *Legume Research*. **49(3):** 458-463. doi: 10.18805/LRF-890.

Submitted: 23-07-2025 **Accepted:** 25-10-2025 **Online:** 08-11-2025

morphology (Pembleton *et al.,* 2009; Prince *et al.,* 2022) and forage quality (Halim *et al.,* 1989). There is a lack of research on how alfalfa biomass and growth characteristics respond to real-time soil moisture monitoring.

It is hypothesized that monitoring drought stress in alfalfa can be used to determine the soil moisture threshold that achieves an optimal balance between production and forage quality. This study aimed to evaluate the effect of water limitation on alfalfa biomass, growth characteristics, nutritive value and spectral responses under controlled greenhouse environment and identify critical water stress thresholds using hyperspectral image.

MATERIALS AND METHODS

This experiment was conducted in a greenhouse at Kansas State University, Manhattan, Kansas, from May to November 2024 for seven months. The alfalfa variety used was L442RR. The seeds were planted in the North Agronomy Farm at Manhattan, KS on April 17, 2024. Later, at the early vegetative growth stage four alfalfa plants were transplanted into pots.

A loam textured soil was used for pH of 7.95, phosphorus (P) of 31 ppm and potassium (K) of 182 ppm. The pots were made up of PVC pipes with a length of 50 cm and diameter of 20 cm. The temperature was set at 24/18°C in the day/night cycle and the lights were continuously turned on. To ensure uniform growth before treatment, all plants were cutted at 10% bloom. Subsequent cuttings were performed at approximately 30 days interval. Alfalfa samples and data were collected on July 23, August 22, September 26 and October 29.

The experiment followed a randomized complete block design (RCBD) with five replications. Four irrigation treatments were applied based on soil field capacity (FC) 55% (FC55), 70% (FC70), 85% (FC85) and 100% (FC100), with average daily water application rates of 176, 248, 307 and 386 mm, respectively. Soil moisture levels were continuously monitored using a soil moisture sensor (TDR-315H, Acclima, USA).

Aboveground biomass was calculated by multiplying the dry matter (DM) percentage by fresh weight, with DM determined after drying samples at 65°C for 72 hours. Belowground biomass was measured after the fourth cut by weighing the roots, using the same method as aboveground biomass. Dried samples used for nutritive value analysis were grounded with a Wiley mill to pass through a 1 mm mesh.

Growth stages were assessed following Fick and Mueller (1989). Plant height was measured from the soil surface to the top of the plant. The leaf to stem ratio was determined by manually separating leaves and stems calculating its respective dry weights. Leaf area was measured using an LI-3100C area meter (LI-COR, USA) and leaf area index (LAI) was calculated by multiplying leaf area by the number of leaves and dividing by the pot area. Taproot diameter was measured at a 5 cm depth below the soil surface. Nodules were counted by activating it on the roots and crown size was measured by determining the crown diameter. The root/shoot ratio was calculated by comparing below-and above-ground biomass after the fourth cut.

Forage nutritive value was analyzed using a near-infrared reflectance spectroscopy (NIRS) following Marten *et al.* (1985), including crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrients (TDN), *in vitro* dry matter digestibility (IVDMD), lignin, relative feed value (RFV) and relative forage quality (RFQ).

Chlorophyll content was measured using a SPAD-502 Plus meter (Konica Minolta, Japan). NDVI was assessed using a green seeker sensor (Trimble, USA). Hyperspectral reflectance was recorded using an ASD FieldSpec 3 spectroradiometer (ASD Inc., USA) within a wavelength range of 350-2,000 nm. Hyperspectral reflectance was calibrated and processed with RS3 and ViewSpec Pro software (ASD Inc., USA).

Data analysis was analyzed using SPSS 24 (IBM Inc., 2019). The significance of treatments on the above-and below-ground biomass, growth characteristics, nutritive value and sensing data were tested using analysis of variance (ANOVA). Mean differences among treatments were compared using the least significant difference (LSD) test at a 5% significance level.

RESULTS AND DISCUSSION

Above-and below-ground biomass

Aboveground biomass significantly increased with higher FC levels ($P<0.05$, Fig 1a). Compared to FC100, above-ground biomass in FC55, 70 and 85 were decreased by 77.7, 51.9 and 32.2%, respectively. Total aboveground biomass followed a similar trend in 8.6, 18.6, 28.1 and 38.6 g pot^{-1} with FC55, 70, 85 and 100, respectively ($P<0.05$, Fig 1b). Belowground biomass of FC55, 70, 85 and 100 was 6.2, 9.1, 13.1 and 12.6 g pot^{-1}, respectively, with significantly higher values in FC85 and 100 than in FC55 and 70 ($P<0.05$, Fig 1c).

Reduced aboveground biomass under drought through delaying the alfalfa growth is consistent with previous studies (Farooq *et al.,* 2012; Diatta *et al.,* 2021). Also, alfalfa appeared to have a more pronounced decrease in aboveground biomass than in belowground biomass during drought consistent with findings by Li *et al.* (2011).

Growth characteristics

Plant height increased significantly with higher FC levels ($P<0.05$, Table 1). Growth stage was significantly greater in FC85 and 100 than in FC55 and 70 ($P<0.05$, Fig 2a), with FC55 showing the youngest maturity. As stem height increases, alfalfa tends to mature, leading to an increase in stem weight (Ray *et al.,* 1999). In water stress, delayed growth led to lower alfalfa maturity, as reported by Diatta *et al.* (2021).

Taproot diameter was the highest in FC85 (P<0.05, Table 1), while crown size and nodule number were significantly higher in FC85 and 100 (P<0.05). Previous study has also reported that drought reduces nodule formation in alfalfa (Diatta *et al.,* 2021). In FC55, the reduced nodule count suggests that it is not an optimal condition.

Morphological chanage

Leaf to stem ratio was significantly higher in FC55 than in other treatments (P<0.05, Fig 2b). Drought increased leaf to stem ratio by 20% due to reduced stem development (Petit *et al.,* 1992), which enhanced forage quality especially CP content as increasing leaf ratio (Avci *et al.,* 2018; Diatta *et al.,* 2021).

Leaf area of alfalfa increased with higher FC levels (P<0.05, Table 1). Similarly, LAI was significantly higher in FC85 and 100 than in FC55 and 70 (P<0.05, Fig 2c). LAI of FC55 was reduced by 82.4% compared to FC100. The root/shoot ratio was significantly higher in FC55 than in other treatments (P<0.05, Fig 2d).

These are indicating a shift toward root development under stress. This aligns with Carter and Sheaffer (1983), who observed a 39% reduction in leaf size under drought. Alfalfa reduces leaf expansion and enhances root growth to conserve water (Aranjuelo *et al.,* 2011; Erice *et al.,* 2010). Smaller leaves under stress improve drought tolerance by reducing transpiration (De Micco and Aronne, 2007). Based on these results, FC85 appeared to be the optimal threshold for alfalfa growth.

Nutritive value

CP content with FC55 was significantly higher than FC100 (P<0.05, Table 2, while ADF showed the opposite trend (P<0.05). Notably, CP content decreased by 11.5% from FC100 compared to FC55. RFV was significantly higher in FC55 at 266.3 compared to FC100 at 209.9 (P<0.05, Table 2). There were no significant differences these from FC50 to FC85 treatments (P>0.05, Table 2). Other nutritive contents did not differ by the treatments (P>0.05, Table 2).

Drought conditions typically raise CP and lower fiber content due to increased leaf proportion (Fiasconaro *et al.,* 2012). Although Staniak and Harasim (2018) found no difference in CP between FC70 and FC40 in pots, other

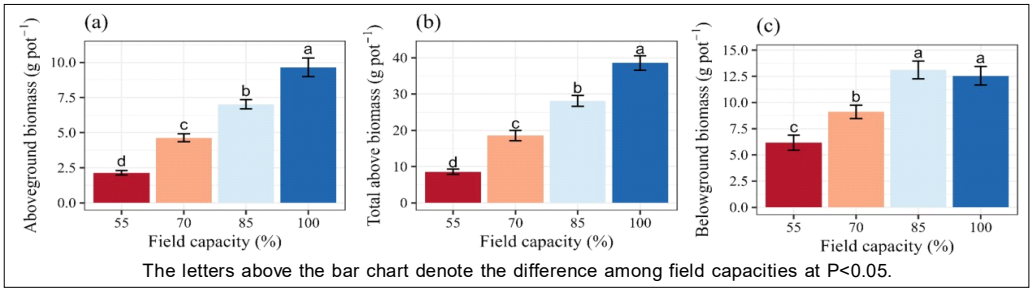


Fig 1: Aboveground (a), total aboveground (b) and belowground (c) biomass of alfalfa by water-limiting treatments.

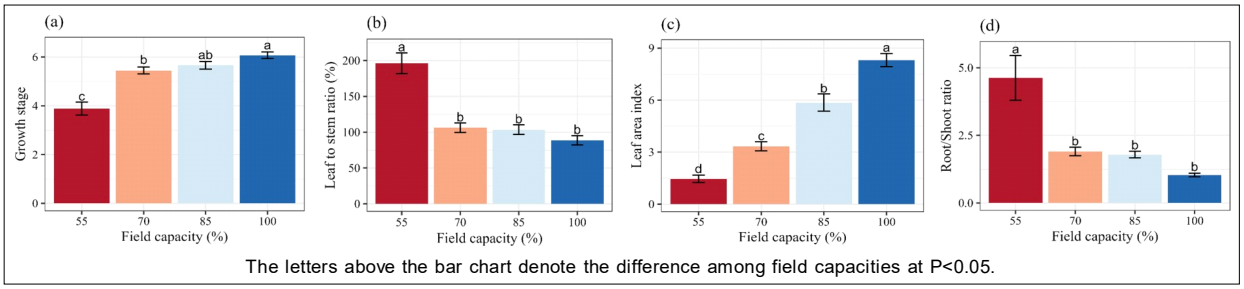


Fig 2: Growth stage (a), leaf to stem ratio (b), leaf area index (c) and root/shoot ratio (d) of alfalfa by water-limiting treatments.

Table 1: Above- and below-ground morphology and sensing data of alfalfa by water-limiting treatments.

Items (Units)	FC55	FC70	FC85	FC100
Plant height (cm)	28.2±12.4d	37.4±13.6c	45.9±10.2b	52.4±12.5a
Leaf area (cm^2)	32.0±18.1c	42.2±10.5bc	53.6±29.9ab	64.4±19.6a
Tap root diameter (mm)	2.2±0.9b	2.8±0.9ab	3.6±1.1a	3.3±1.1ab
Crown size (mm)	16.7±4.2b	20.1±5.2ab	22.7±3.0a	23.4±3.2a
Number of nodules	35.2±18.1b	59.6±15.0a	71.4±12.2a	64.4±26.7a
Normalized difference vegetative index	0.43±0.10c	0.52±0.11b	0.63±0.08a	0.64±0.14a
SPAD unit	70.7±9.2ns	73.5±9.5ns	70.6±9.4ns	68.6±10.9ns

Note: FC: Field capacity; Data are represented as mean ± SD. Different letters indicate significant differences within the same row at P<0.05. ns- indicates no significant difference of each row.

studies reported improved quality under stress (Abid *et al.,* 2016; Holman *et al.,* 2016). Delayed growth under drought conditions enhanced the nutritive value of alfalfa (Diatta *et al.,* 2021; Fiasconaro *et al.,* 2012; Karayilanli and Ayhan, 2016; Marković *et al.,* 2022). FC55 appeared to produce high quality alfalfa and its nutritive value was not different from that of the FC85 treatment.

NDVI, chlorophyll content and hyperspectral reflectance

NDVI values of FC55, 70, 85 and 100 were 0.43, 0.52, 0.63 and 0.64, which were significantly higher in FC85 and 100 than in FC55 and 70 (P<0.05, Table 1). These values were lower than field values (0.55-0.80; Masialeti *et al.,* 2010), likely due to lower plant density, which was four plants per pot compared to 20 alfalfa plants ft² recommended in the field (Mueller *et al.,* 2007). NDVI was lowest in FC55, reflecting greater stress and unhealthy. NDVI values in FC85 and 100 were similar, possibly due to saturation in biomass and LAI (Del Pozo *et al.,* 2023), suggesting growth was optimized at FC85. The SPAD values did not significantly differ among treatments (P>0.05, Table 1).

Alfalfa exhibits a typical hyperspectral reflectance pattern, in which strong chlorophyll absorption in the blue (450-500 nm) and red (600-700 nm) bands results in lower reflectance, while higher reflectance in the near-infrared (NIR; 800-900 nm) band is caused by scattering in the spongy mesophyll (Feng *et al.,* 2020). FC55, which was the highest reflectance, showed reduced chlorophyll absorption and lower leaf pigment concentration, as indicated by its higher overall reflectance values (Okyere *et al.,* 2024). Notably, this study observed higher NIR reflectance in FC55, which was the youngest growth stage, compared to the other treatments, which were at older growth stages, consistent with Zhao *et al.* (2023). Furthermore, FC55 exhibited a smaller decline in reflectance at the water absorption bands of 1450 and 1950 nm compared to other treatments, indicating severe drought stress. In contrast, FC70 to FC100 displayed similar absorption features at these wavelengths [Fig 3 (a); (b)], indicating adequate water content. Consequently, hyperspectral wavelength suggests that FC55 experienced significant drought stress, potentially representing the water-stress as the drought threshold for alfalfa. Using hyperspectral reflectance as a real-time forecasting tool could enhance alfalfa production and maintaining FC85 would optimize biomass, growth charateristics and forage quality simultaneously.

Table 2: Nutritive value of alfalfa by water-limiting treatments.

Items (Units)	FC55	FC70	FC85	FC100
Crude protein (%)	26.0±2.0a	25.0±2.0ab	23.5±1.9ab	23.0±1.7b
Acid detergent fiber (%)	20.9±3.8b	22.2±4.8ab	24.4±4.6ab	25.9±4.0a
Neutral detergent fiber (%)	26.2±4.9ns	27.7±5.4ns	30.0±5.5ns	31.2±4.9ns
Relative feed value	266.3±59.3a	249.5±61.0ab	224.3±52.7ab	209.9±42.0b
Relative forage quality	279.2±66.7ns	269.3±79.8ns	244.4±67.9ns	226.4±57.7ns
Total digestible nutrients (%)	68.8±2.8ns	69.1±4.3ns	67.9±4.2ns	66.8± 4.0ns
In vitro dry matter digestibility (%)	86.3±3.4ns	85.5±4.2ns	84.5±3.8ns	83.2±4.2ns
Lignin (%)	4.5±0.9ns	4.6±1.3ns	4.8±1.3ns	5.1±1.2ns

Note: FC: Field capacity; Data are represented as mean ± SD. Different letters indicate significant differences within the same row at P<0.05, ns- indicates no significant difference of each row.

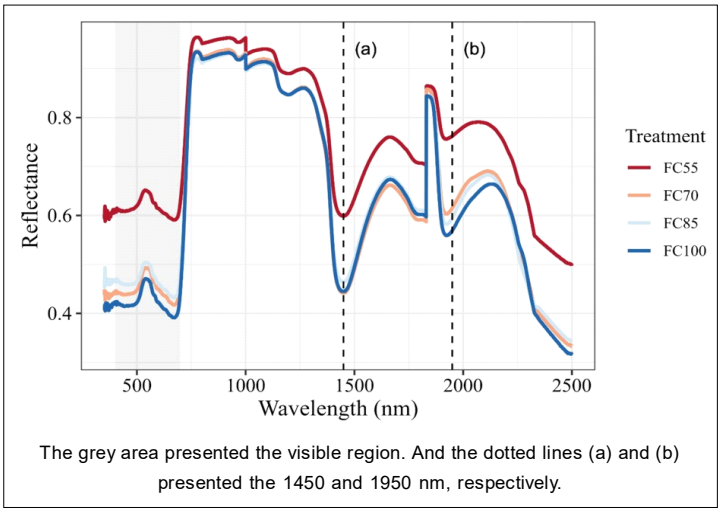


Fig 3: Hyperspectral reflectance curve by water-limiting treatments.

CONCLUSION

This study examined the effects of water-limited conditions on alfalfa biomass, growth characteristics and nutritive value, while also using hyperspectral reflectance to identify the threshold of water stress. Both aboveground biomass and growth characteristics increased with higher FC, whereas belowground biomass and growth improved at FC85. FC55 showed higher leaf-to-stem and root/shoot ratios, along with improved nutritive values, which partially offset its reduced biomass. Hyperspectral reflectance was the higested at FC55, indicating that this treatment experienced the most severe water stress. Overall, FC85 represented the optimal balance, supporting both above- and below- ground biomass while maintaining forage quality comparable to FC55. Since this experiment was conducted as a one-year greenhouse trial, further multi-year studies on cropland are necessary to validate these findings, including the production and economic benefits of alfalfa.

ACKNOWLEDGEMENT

The present study was supported by the USDA National Institute of Food and Agriculture, Alfalfa Seed and Alfalfa Forage System Program (ASAFS) (Grant no: 2023-70005-41080).

Disclaimers

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Informed consent

This research did not involve any animal experiments.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

REFERENCES

Abid, M., Mansour, E., Yahia, L.B., Bachar, K., Ben Khaled, A. and Ferchichi, A. (2016). Alfalfa nutritive quality as influenced by drought in South-Eastern Oasis of Tunisia. *Italian Journal of Animal Science*. **15:** 334-342. doi: 10.1080/1828051X.2016.1175916.

Aranjuelo, I., Molero, G., Erice, G., Avice, J.C. and Nogues, S. (2011). Plant physiology and proteomics reveals the leaf response to drought in alfalfa (*Medicago sativa* L.). *Journal of Experimental Botany*. **62(1):** 111-123. doi: 10.1093/jxb/erq249.

Avci, M., Hatipoglu, R., Çinar, S. and Kiliçalp, N. (2018). Assessment of yield and quality characteristics of alfalfa (*Medicago sativa* L.) cultivars with different fall dormancy rating. *Legume Research*. **41(3):** 369-373. doi: 10.18805/LR-364.

Baral, R., Lollato, R.P., Bhandari, K. and Min, D. (2022). Yield gap analysis of rainfed alfalfa in the United States. *Frontiers in Plant Science*. **13:** 931403. doi: 10.3389/fpls.2022. 931403.

Carter, P.R. and Sheaffer, C.C. (1983). Alfalfa response to soil water deficits: I. Growth, forage quality, yield, water use and water-use efficiency. *Crop Science*. **23(4):** 669-675. doi: 10.2135/cropsci1983.0011183X002300040016x.

Cevoli, C., Di Cecilia, L., Ferrari, L., Fabbri, A. and Molari, G. (2022). Evaluation of cut alfalfa moisture content and operative conditions by hyperspectral imaging combined with chemometric tools: In-field application. *Biosystems Engineering*. **222:** 132-141. doi: 10.1016/j.biosystemseng.2022.08.004.

De Micco, V. and Aronne, G. (2007). Anatomical features, monomer lignin composition and accumulation of phenolics in 1-year-old branches of the Mediterranean *Cistus ladanifer* L. *Botanical Journal of the Linnean Society*. **155(3):** 361-37. doi: 10.1111/j.1095-8339.2007.00705.x.

Del Pozo, A., Espinoza, S., Barahona, V., Inostroza, L., Gerding, M., Humphries, A., Lobos, G., Cares, J. and Ovalle, C. (2023). Aerial and ground-based phenotyping of an alfalfa diversity panel to assess adaptation to a prolonged drought period in a Mediterranean environment of central Chile. *European Journal of Agronomy*. **144:** 126751. doi: 10.1016/j.eja.2023.126751.

Dey, S., Jha, G. and Min, D. (2024). Precision irrigation technologies for water wise and climate resilient alfalfa production. *Crops and Soils*. **57(4):** 4-11. doi: 10.1002/crso.20383.

Dey, S., Widanagamage, N., Achar, S., Debangshi, U., Palla, S., Kim, J. and Jha, G. (2025). Precision Agriculture Tools, Techniques and Future directions for climate resilience. In: Climate-Smart Agricultural Technologies: Approaches for Field Crops Production Systems. *Springer Nature Singapore*. pp. 89-115. doi: 10.1007/978-981-96-7699-6_5.

Diatta, A.A., Min, D. and Jagadish, S.K. (2021). Drought stress responses in non-transgenic and transgenic alfalfa-current status and future research directions. *Advances in Agronomy*. **170:** 35-100. doi: 10.1016/bs.agron.2021.06.002.

Ehlert, D. (2002). PA-precision agriculture: Advanced throughput measurement in forage harvesters. *Biosystems Engineering*. **83(1):** 47-53. DOI: 10.1006/bioe.2002.0101.

Erice, G., Louahlia, S., Irigoyen, J.J., Sanchez-Diaz, M. and Avice, J.C. (2010). Biomass partitioning, morphology and water status of four alfalfa genotypes submitted to progressive drought and subsequent recovery. *Journal of Plant Physiology*. **167(2):** 114-120. DOI: 10.1016/j.jplph.2009. 07.016.

Farooq, M., Hussain, M., Wahid, A. and Siddique, K. (2012). Drought Stress in Plants: An Overview. In: Aroca, R. (Eds.), Plant Responses to Drought Stress. Springer, Berlin, Heidelberg. pp. 1-33. doi: 10.1007/978-3-642-32653-0_1.

Feng, L., Zhang, Z., Ma, Y., Du, Q., Williams, P., Drewry, J. and Luck, B. (2020). Alfalfa yield prediction using UAV-based hyperspectral imagery and ensemble learning. *Remote Sensing*. **12(12):** 2028. doi: 10.3390/rs12122028.

Fiasconaro, M.L., Gogorcena, Y., Muñoz, F., Andueza, D., Sánchez-Díaz, M. and Antolín, M.C. (2012). Effects of nitrogen source and water availability on stem carbohydrates and cellulosic bioethanol traits of alfalfa plants. *Plant Science*. **191-192:** 16-23. doi: 10.1016/j.plantsci.2012.04.007.

Fick, G.W. and Mueller, S.C. (1989). Alfalfa: Quality, maturity and mean stage of development. Department of Agronomy, College of Agricultural and Life Sciences. Cornell University, Information Bulletin 217. Ithaca, NY, USA.

Gámez, A.L., Vatter, T., Santesteban, L.G., Araus, J.L. and Aranjuelo, I. (2024). Onfield estimation of quality parameters in alfalfa through hyperspectral spectrometer data. *Computers and Electronics in Agriculture*. **216:** 108463. doi: 10.1016/j.compag.2023.108463.

Garriga, M., Ovalle, C., Espinoza, S., Lobos, G.A. and Del Pozo, A. (2020). Use of Vis-NIR reflectance data and regression models to estimate physiological and productivity traits in lucerne (*Medicago sativa*). *Crop and Pasture Science*. **71(1):** 90-100. doi: 10.1071/CP19182.

Haacker, E.M., Kendall, A.D. and Hyndman, D.W. (2016). Water level declines in the high plains aquifer: Predevelopment to resource senescence. *Groundwater*. **54(2):** 231-242. doi: 10.1111/gwat.12350.

Hatfield, J.L., Gitelson, A.A., Schepers, J.S. and Walthall, C.L. (2008). Application of spectral remote sensing for agronomic decisions. *Agronomy Journal*. **100(S3):** S117-S131. doi: 10.2134/agronj2006.0370c.

Halim, R.A., Buxton, D.R., Hattendorf, M.J. and Carlson, R.E. (1989). Water stress effects on alfalfa forage quality after adjustment for maturity differences. *Agronomy Journal*. **81(2):** 189-194. doi: 10.2134/agronj1989.00021962008100020010x.

Holman, J., Min, D.-H., Klocke, N., Kisekka, I. and Currie, R. (2016). Effects of irrigation amount and timing on alfalfa nutritive value. *Transactions of the ASABE*. **59(4):** 849-860. doi: 10.13031/trans.59.11456.

Karayilanli, E. and Ayhan, V. (2016). Investigation of feed value of alfalfa (*Medicago sativa* L.) harvested at different maturity stages. *Legume Research*. **39(2):** 237-247. doi: 10.18805/lr.v0iOF.9292.

Li, W., Zhang, S., Shan, L. and Eneji, A.E. (2011). Changes in root characteristics, gas exchange and water use efficiency following water stress and rehydration of alfalfa and sorghum. *Australian Journal of Crop Science*. **5(12):** 1521-1532.

Masialeti, I., Egbert, S. and Wardlow, B.D. (2010). A comparative analysis of phenological curves for major crops in Kansas. *GIScience and Remote Sensing*. **47(2):** 241-259. doi: 10.2747/1548-1603.47.2.241.

Marković, J., Petrović, M., Terzić, D., Vasić, T., Kostć, I., Štrbanović, R. and Grubić, G. (2022). Protein fractions as influenced by cultivars, stage of maturity and cutting dates in alfalfa (*Medicago sativa* L.). *Legume Research*. **45(1):** 46-51. doi: 10.18805/LR-479.

Marten, G.C., Shenk, J. and Barton, F. (1985). Near infrared reflectance spectroscopy (NIRS): Analysis of forage quality. US Department of Agriculture, Agricultural Research Service.

Mueller, S.C., Frate, C.A. and Mathews, M.C. (2007). Alfalfa Stand Establishment. In: Irrigated Alfalfa Management for Mediterranean and Desert Zones. [C.G. Summers and D.H. Putnam (Eds.)], Chapter 4. University of California, Agriculture and Natural Resources Publication 8290, Davis, CA, USA. pp. 1-21.

NOAA. (2022). State Climate Summaries 2022-KANSAS. National Centers for Environmental Information. Washington, DC, USA.

Okyere, F.G., Cudjoe, D.K., Virlet, N., Castle, M., Riche, A.B., Greche, L., Mohareb, F., Simms, D., Mhada, M. and Hawkesford, M.J. (2024). Hyperspectral imaging for phenotyping plant drought stress and nitrogen interactions using multivariate modeling and machine learning techniques in wheat. *Remote Sensing*. **16(18):** 3446. doi: 10.3390/rs16183446.

Pembleton, K.G., Rawnsley, R.P., Donaghy, D.J. and Volenec, J.J. (2009). Water deficit alters canopy structure but not photosynthes is during the regrowth of alfalfa. *Crop Science*. **49(2):** 722-731. doi: 10.2135/cropsci2008.08.0456.

Petit, H.V., Pesant, A., Barnett, G., Mason, W. and Dionne, J. (1992). Quality and morphological characteristics of alfalfa as affected by soil moisture, pH and phosphorus fertilization. *Canadian Journal of Plant Science*. **72(1):** 147-162. doi: 10.4141/cjps92-015.

Prince, S., Anower, M.R., Motes, C.M., Hernandez, T.D., Liao, F., Putman, L., Mattson, R., Seethepalli, A., Shah, K., Komp, M., Mehta, P., York, L.M., Young, C. and Monteros, M.J. (2022). Intraspecific variation for leaf physiological and root morphological adaptation to drought stress in alfalfa (*Medicago sativa* L.). *Frontiers in Plant Science*. **13:** 795011. doi: 10.3389/fpls.2022.795011.

Putnam, D.H., Summers, C.G. and Orloff, S.B. (2007). Alfalfa Production Systems in California. In: Irrigated Alfalfa Management for Mediterranean and Desert Zones. [C.G. Summers and D.H. Putnam (Eds.)], Chapter 1. University of California, Division of Agriculture and Natural Resource Publication 8287, Davis, CA, USA. pp: 1-19.

Ray, I.M., Townsend, M.S., Muncy, C.M. and Henning, J.A. (1999). Heritabilities of water-use efficiency traits and correlations with agronomic traits in water-stressed alfalfa. *Crop Science*. **39(2):** 494-498. doi: 10.2135/cropsci1999.0011183X0039000200032x.

SPSS 24. (2019). IBM SPSS Statistics ver. 24.0. IBM Corporation. Somers, NY, USA. in front of the Staniak and Harasim.

Staniak, M. and Harasim, E. (2018). Changes in nutritive value of alfalfa (*Medicago* × *varia* T. Martyn) and *Festulolium* [*Festulolium braunii* (K. Richt) A. Camus] under drought stress. *Journal of Agronomy and Crop Science*. **204(5):** 456-466. doi: 10.1111/jac.12271.

USDA-NASS. (2025). Crop Production 2024 Summary. United State Department of Agriculture, National Agricultural Statistics Service. Harrisburg, PA, USA.

USDA-NASS. (2024). 2022 Census of Agriculture-2023 Irrigation and Water Management Survey. United State Department of Agriculture, National Agricultural Statistics Service. Harrisburg, PA, USA.

Zhang, L., Zhang, H., Niu, Y. and Han, W. (2019). Mapping maize water stress based on UAV multispectral remote sensing. *Remote Sensing*. **11(6):** 1-24. doi: 10.3390/rs11060605.

Zhao, X., Zhang, S., Shi, R., Yan, W. and Pan, X. (2023). Multi-temporal hyperspectral classification of grassland using transformer network. *Sensors*. **23(14):** 6642. doi: 10.3390/s23146642.