POTASSIUM FERTILIZATION AND ITS IMPACT ON YIELD, NUTRITIVE VALUE, ROOT RESERVES, AND WINTER HARDINESS OF ALFALFA

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Potassium (K) is an important nutrient for winter hardiness in alfalfa (*Medicago sativa* L.). This study determined the effect of K rate and application timing, fall dormancy, and harvest stress on forage yield, nutritive value, root reserves, and winter survival in soils with different clay mineralogy. The experiments were conducted in Lisbon and Milnor, ND, in 2019 and 2020. Three fall dormancy cultivars were applied K treatments of 0, 168, and 336 kg K_2O ha^{-1} at single- and split-application. Half of the experimental units were stressed by harvesting mid-September, while the other half was non-stressed by harvesting in October. Soil K was higher with a split-application, compared with a single-application of K at the same rate. Total seasonal forage yield was significantly lower when no K was applied. Stressed alfalfa had lower root protein in both years and starch was lower in Milnor 2019 and Lisbon 2020 compared with non-stressed.

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1. INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a cool-season forage crop that provides an excellent forage for livestock. The relative forage nutritive value of alfalfa is higher than any other forage crop (Perić and Srebric, 2016). High production is attained through healthy establishment, proper harvest times, and fertilization with necessary nutrients. Potassium (K) is often an under-applied nutrient due to the cost of fertilizers containing significant amounts of K (Lloveras et al., 2012). However, inadequate K levels in the soil can contribute to winterkill of alfalfa plants (Hawkesford et al., 2011; Jungers et al., 2019). In the Upper-Midwest, growers are hesitant to harvest alfalfa in the fall due to concern of winterkill. Fall harvest, though, can increase total seasonal forage yield without depleting persistence or nutritive value (Berti et al., 2012).

There is still a lack of understanding on how management and environment allow alfalfa to survive, including the role of soil K availability (Berg et al., 2018). Studies have shown that K is important for alfalfa growth, however, the effects of K fertilization on alfalfa are inconsistent (Jungers et al., 2019). Previous studies support that soil fertility of P and K nutrients positively impact alfalfa persistence, forage yield, and winter survival, however, the impacts of P fertilization have been assessed more than K fertilizers (Berg et al., 2018). Application of K in North Dakota depends on the current soil test K values, the alfalfa tonnage removed from the previous year, and the soil's clay chemistry (Franzen and Berti, 2017). A previous study examined how K fertilization rates are related to soil clay mineralogy (Breker et al., 2019). This study found that soils with a smectite-to-illite ratio greater than 3.5 require higher amounts of K, due to the tendency of smectitic clays to 'fix' or temporarily retain K in interlayers during dry periods, rendering the K less immediately accessible plant-available K to corn (Zea mays L). This recent finding emphasizes the importance of a better understanding K fertility, and adjusting recommended rates in certain environments. Fertilization with K has shown to have a positive effect on alfalfa forage yield when soil test K levels are low (Jungers et al., 2019), however, increased K application does not always result in higher forage yield (Berg et al., 2018). Higher rates of K have shown to reduce forage nutritive value and increase forage K concentration.

Potassium aids in numerous physiological processes within a plant, however, the primary process vital for winter survival, is adequate carbohydrate and protein storage prior to dormancy (Lu et al., 2018). Plant potassium plays an important role in transportation of adenosine triphosphate (ATP), protein, starch, and other nutrients within perennial plants, moving energy sources and other compounds into root reserves. Previous findings indicate that protein reserves are important for tolerance of both defoliation and winter stresses (Volenec et al., 1996). Analysis of alfalfa taproots for total protein and starch content may help in the understanding of the role of K in autumn root compound storage. Previous analyses of sucrose reserves within alfalfa taproots have found no differences in reserves between K fertilizer rates (Berg et al., 2018).

This study focuses on the effects of K fertilization on alfalfa yield, forage nutritive value, and persistence of differing fall dormancies with K fertilizer rate and harvest timing. It evaluates how protein and starch reserves within alfalfa roots differ among K rates and application timing. Lastly, this study looks at the interaction between fall dormancy and protein and starch reserves with K fertilizer rate in soils with different clay mineralogy.

1.1. Objectives

1. To determine the effect of K fertilization on soil K availability, alfalfa forage yield, nutritive value, and persistence in alfalfa of different fall dormancy and with variable rate application and harvest stress in soils with different mineralogy.

2. To determine the changes in root reserves in alfalfa with different K rates and application timing, and fall harvest dates
3. To determine the interaction between fall dormancy and K fertilization on root storage of protein and starch.

2. LITERATURE REVIEW

2.1. History of alfalfa

Alfalfa (*Medicago sativa* L.) is a cool-season forage crop that provides an excellent feed for livestock. The origin of alfalfa was in western Asia around 8000 B.C., being first cultivated in the region that is now Iran (Brough et al., 1977; Small, 2011; Franzen and Berti, 2017). It is believed that the Medes armies introduced alfalfa to Greece, and soon afterwards alfalfa cultivation had spread across Europe (Prosperi et al., 2014). In the sixteenth century A.D., the Spanish introduced alfalfa to South America (Mexico, Peru, and Chile). At the beginning of the nineteenth century, alfalfa was introduced into California as "Chilean clover". Alfalfa reached the state of North Dakota around 1890 (Franzen and Berti, 2017). Alfalfa is currently grown throughout the United States.

2.2. Economic importance

Alfalfa hectarage ranks number three in the United States, following corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] (Heathcliffe, 2015). In the combined states of Minnesota, North Dakota, and South Dakota, 7.35 million metric tons of alfalfa and alfalfa-grass mixtures were produced in 2020 (USDA-NASS, 2020). For these three states in 2020, that production had a value of $8.76 million. North Dakota produced 1.99 million metric tons of alfalfa in 2020, with a 7% increase in alfalfa plantings, ranking as the eleventh highest alfalfa-producing state in the country. Historically, alfalfa has been produced for livestock near the location grown, though, with improved technology of mechanical handling and shipment methods, exporting of U.S. alfalfa has tripled from $200 million to $600 million in the past two decades (Putnam et al., 2019). Therefore, approximately 6% of alfalfa produced in the U.S. is exported. The highest importers of US-alfalfa are China, Japan, and Korea (Putnam et al., 2019). Arizona, California, Idaho, Nevada, Oregon, Utah, and Washington are the primary exporters of alfalfa.

2.3. Biology and physiology

Alfalfa is categorized as a perennial plant in the family Fabaceae. As a legume, alfalfa is able to fix atmospheric dinitrogen (N_2) in the soil via the symbiotic relationship with *Sinorhizobium* spp. bacteria. This, however, is only able to occur sufficiently at pH levels above 6.5 (Undersander et al., 2011). If alfalfa has not been previously planted in an area, an inoculant may be added onto the seed before planting to introduce the bacteria into the soil.

Alfalfa has optimum germination when temperatures range from 18.3-25.0°C (Undersander et al., 2011). When alfalfa emerges, it has a high tolerance to temperatures below 0°C, which gives flexibility to seeding times in the spring. Sowing rate of alfalfa seed is recommended at 10-kg pure live seed (PLS) ha^{-1}, because a higher rate may result in increased detrimental competition among alfalfa plants (Berti and Samarappuli, 2018). Increased competition weakens plants due to intra-species allelopathy, increasing susceptibility to winterkill (Dhont et al., 2004; Berti and Samarappuli, 2018).

The root structure of alfalfa includes one main taproot with fibrous roots branching off. In alfalfa, most nodule formation occurs on the fibrous roots (Undersander et al., 2011). These nodules can form as early as four weeks after germination. If the nodules are a pinkish/red flesh inside, the *Sinorhizobium* spp. are actively fixing N_2. The alfalfa taproot stores carbohydrates and protein during the growing season, and these stored compounds are important for regrowth after harvest and in the spring after dormancy is broken.

As a perennial, alfalfa is able to survive winter while in dormancy, being able to tolerate temperatures as low as -15°C at the crown level (Undersander et al., 2011). Prior to becoming dormant, alfalfa plants must go through a hardening or acclimation stage. This is essential in order to increase tolerance to winter conditions that can increase risk of injury or winterkill when a combination of sub-ideal temperatures and soil water content are present. The hardening stage continues under snow cover, with maximum freezing tolerance achieved after the soil surface has frozen (Bula and Smith, 1954; Castonguay et al., 1995). Castonguay et al. (1995) researched freezing tolerance across cultivars that are cold-sensitive (non-hardy) and cold-tolerant (very hardy). Alfalfa was planted in pots and grown under ideal conditions (16-h photoperiod; light temperature, 21°C; dark temperature 17°C) for five weeks in a growth chamber, followed by two weeks of either treatment of low-temperature acclimation (8-h photoperiod, 2°C) or inducing hardening conditions (no light; -2°C). They found that two weeks of 2°C aided in the accumulation of sucrose, raffinose, and stachyose, leading to a decreased value of 50% lethal temperature (LT_{50}). Plants went through a freezing test that lowered the initial temperature of -2°C by 2°C every 30-min. period, with a 90-min. plateau at each temperature. Plants were tested between -2 and -10°C for non-acclimated plants, without previous induced hardening conditions, while acclimated plants were tested between -4°C and -27°C. After three weeks, both acclimated and non-acclimated plants were counted to calculate the LT_{50}. The cold-tolerant cultivar and the cold-sensitive cultivar had LT_{50} values of -14.5°C and -9.5°C respectively.

Environmental changes in the fall stimulate physiological changes of alfalfa to induce hardening prior to dormancy. In the fall, as day length gets shorter and temperatures decrease, significant changes in mobilization and allocation of N to roots of alfalfa occur (Noquet et al., 2001). The Noquet et al. (2001) study compared long day (LD, 16-h day/8-h night), short day (SD, 8-h day/16-h night) and temperatures (20°C and 5°C) on plant growth and shoot/root ratios.

Findings concluded that a shorter photoperiod significantly reduced regrowth of shoots, while temperature and photoperiod significantly impacted shoot/root ratio. Plants exposed to 20°C and LD conditions had a higher growth (3-g dry weight (DW) $plant^{-1}$ of growth) than plants exposed to 5°C and SD conditions (2-g DW $plant^{-1}$ of growth). Plants exposed to 20°C and LD conditions had a shoot/root ratio of 2.6, which was significantly higher than plants exposed to 5°C and SD conditions, having a ratio of 1.8. Short photoperiod (8-h day/16-h night) exposure impacted regrowth more than shoot/root ratios. At 5°C, SD treatments produced 2.3-g DW $plant^{-1}$ and LD treatments produced 2.7-g DW $plant^{-1}$, while the shoot/root ratio was insignificant. At both temperatures, the SD treatments had a decrease in N uptake compared with LD treatments.

2.4. Winterkill: conditions, winter hardiness, and management

Winterkill is a term used to describe the failure of plants to overwinter, being evident in spring when plants fail to come out of dormancy (Leep et al., 2001). Winterkill increases when certain weather conditions occur, such as: lack of snow cover, daily temperature fluctuations, and persistent ice sheeting (Durling et al., 1995). Majority of winterkill occurs when there is waterlogging in the fall (decreased photoperiod and temperature, ~21 Sept. to ~21 Dec.), minimal (<10-cm) snow coverage, prolonged periods of temperature below -15°C (7-days) and/or long-term (>14-days) ice sheeting (Berti and Samarappuli, 2018). Ouellet (1977) investigated which months had the highest climatic contribution to winter injury, finding that April contributed to 33% and 22% of winter injury at La Pocatière (Lower St. Laurence, Quebec) and Swift Current (South-West Saskatchewan) respectively. Excess rain and waterlogging conditions going into winter decrease the ability of alfalfa to acclimate properly (Berti and Samarappuli, 2018). Lack of snow cover increases heat loss from the soil to the atmosphere. Snow has insulating properties due to the entrapment of air between snow crystals, reducing heat transfer, hindering colder air from getting into soil and retaining warmer air from leaving soil (Leep et al., 2001). A study comparing temperature differences of snow cover depths found that the extreme minimum temperature at crown level for 10- and 20-cm snow depth treatments was 12.1°C and 13.6°C warmer than the 0-cm treatment, respectively (Leep et al., 2001). The study concluded that a snow coverage of 10-cm keeps temperature above the freezing threshold for alfalfa (-15°C). Ice sheeting is when a layer of ice forms at the surface level of the plant (Leep et al., 2001). Plants encased by ice can be 'suffocated' by gaseous metabolic byproducts such as carbon dioxide (CO_2), methane, or suffer phytotoxic effects from accumulation of ethanol (Durling, 1995; Leep et al., 2001). Ice may also prevent oxygen from being accessible to the root zone. Heaving is when the crown is pushed upward out of the ground by frost during thawing and freezing periods (Durling et al., 1995). Alternating temperatures also causes expansion and contraction of smectitic 2:1 clay minerals, increasing the forces required for pushing root and crown upwards. Alfalfa taproots that are smaller in diameter, have fewer lateral roots, and lateral placement lower on the taproot are more prone to breakage from heaving (Perfect et al., 1987).

Many strategies are possible to manage alfalfa to reduce winterkill. Winter hardiness of the cultivar is a complex trait that includes cold tolerance, disease resistance, flooding tolerance, and freezing tolerance (Schwab et al., 1996). Alfalfa cultivars for northern climates have been bred to tolerate winter conditions based on fall dormancy (FD) and winter survival (WS) scoring. Dormancy ranges are rated on a scale of 1-10 by breeders, with 1 most dormant and 10 non-dormant when temperatures decline and day length shortens in the fall (northern hemisphere). Lower dormancy values tend to result in lower yields in the fall due to the cultivars higher sensitivity to dormancy signaling. Studies have shown that starch concentration in the taproot are negatively correlated with fall dormancy rating (Bula et al., 1956; Volenec, 1985; Castonguay, 1995; Cunningham and Volenec, 1996; Haagenson et al., 2003). Dormancy rating is therefore an important consideration when deciding if a fall harvest would be acceptable. The WS scoring is based on a scale of 1-6, with 1 being most tolerant and 6 being most susceptible to winter conditions. There is evidence that indicates a weaker relationship between WS and FD scores, among cultivars of the same FD score (Schwab et al., 1996). Findings suggest that alfalfa cultivars with intermediate fall dormancy ratings can be successfully grown in areas with prolonged seasonal snow cover, such as winters tend to have in the northern Great Plains.

Overall, a healthier soil produces for healthier plants. Diseases such as anthracnose (*Colletotrichum trifolii*), *Fusarium* wilt, and *Phytophthora* root rot can weaken plants, making them more susceptible to winterkill (Undersander et al., 2011). Using cultivars resistant to diseases, managing disease pressure with fungicides, and having a fertile soil assist in increasing alfalfa winter hardiness. With the final harvest of alfalfa, leaving the plant stubble between 10- to 15-cm tall will aid in catching snow that will result in an insulative environment (Undersander et al., 2011).

Another management strategy for alfalfa production in the northern Great Plains is harvest timing. The final harvest should occur before 1 September or after 30 September, before first frost in October. It is not recommended to harvest alfalfa in September in the northern Great Plains and Upper Midwest, in order to ensure storage of nutrient reserves within the taproot (Undersander et al., 2011).

2.5. Root reserves and fall harvest management

Alfalfa stores carbohydrates and protein (reserves) in the taproot throughout the season, and then uses these reserves for regrowth of shoots after harvest and for new shoot growth in the spring (Cunningham and Volenec, 1996). Growers in the Northern Great Plains of the USA avoid harvesting alfalfa in the fall to minimize the risk of winterkill. If harvested in the fall, alfalfa plants use carbon (C) and nitrogen (N) reserves to support shoot regrowth if temperatures allow it, which may later result in stress during over-wintering due to the depleted reserves. Alfalfa plant roots grow and store organic N between September and November (Justes et al., 2002; Dhont et al., 2003). When the regrowth period after a harvest (defoliation) is longer, accumulation of organic-N and starch content in the roots increase (Avice, 1997). Starch and N concentrations were significantly higher in alfalfa after 45-day regrowth compared with plants with 30-day regrowth. Other studies found that when delaying the last harvest in the fall until October, the accumulation of starch in alfalfa roots was similar to that of plants only harvested twice in the season (Gervais and Bilodeau, 1985; Sheaffer et al., 1986; Gervais, 1987; Brink et al., 1989; Dhont et al., 2003). Fall harvest, though, has potential to increase total forage yield for a season without depleting persistence or nutritive value (Dhont et al., 2003; Dhont et al., 2004; Berti et al., 2012).

Numerous studies support that N reserves play an important role in winter stress tolerance and spring regrowth in the subsequent year (Ourry et al., 1994; Volenec et al., 1996; Dhont et al., 2003). In alfalfa, N in the roots is mainly in the organic form (Volenec, 1996; Avice, 1997). Protein-N is the largest pool, but amino-N is the most easily mobilized form. Specific soluble proteins aid in N storage, cold acclimation, and freezing tolerance (Cyr and Bewley, 1990; Cunningham and Volenec, 1996). Vegetative storage proteins (VSPs), protein reserves found in numerous plants including alfalfa, are located only in the taproot and aid in N storage (Avice, 1997). The VSPs are highly correlated with soluble proteins and can show a cycling of N synthesis into protein-N (organic forms) and amino-N. Amino-N is readily mobilized from root nodules (NH_2 form in conjunction with a ‘carbon skeleton’) to growing tissue, where it is synthesized into proteins, a process that is induced by defoliation (Ourry et al., 1989; Hendershot and Volenec, 1993a,b; Volenec et al., 1996; Avice et al., 1997). There are three VSPs present exclusively in the taproot, aiding in initial shoot regrowth after a harvest, and then accumulate in the taproot after shoots are greater than 20-cm tall. They are 15, 19, and 32-ku (kilodalton) polypeptides, which together make up about 20% of soluble proteins drawn from within the taproot (Cunningham and Volenec, 1996). These VSPs have not been found in annual species of *Medicago sativa* L., supporting its importance in winter hardiness and shoot regrowth in perennial alfalfa. The most common amino acids in roots of alfalfa are aspartic acid and asparagine (Cunningham and Volenec, 1996; Avice 1997; and Dhont et al., 2003). Vegetative storage proteins are primarily located in cell vacuoles of the taproot (Avice et al., 1996b; Avice et al., 1997).

Previous studies have explored the effect of defoliation on alfalfa physiological processes (Castonguay et al., 1995; Cunningham and Volenec, 1996; Volenec et al., 1996; Avice, 1997; Berg et al., 2018). Utilizing the isotope ^{15}N researchers found that 10-d after defoliation, about 90% of N within re-growing tissues was derived from N stored in the taproot (Barber et al., 1993; Cunningham and Volenec, 1996). This indicates that after harvest N reserves are essential for shoot regrowth. Approximately six-weeks are required to fully replenish root N content after defoliation (Lemaire et al., 1992; Dhont et al., 2003). Researchers using the ^{13}C isotope found that the majority (61%) of stored C is used for root respiration during the first 30-days of regrowth and only 5% is recovered in re-growing shoots (Avice et al., 1996b, Avice et al., 1997). Even when root starch reserves are low, greater yield of alfalfa has been associated with greater N reserves prior to defoliation (Ourry et al., 1994; Avice et al., 1997). This is because with higher N content, the regrowth rate of leaves is faster. Harvest also impacts the rate of N_2 fixation in leguminous plants (Vance et al., 1978). Vance et al. (1978) found that when alfalfa is defoliated, the N_2 fixation capacity declined by 88% within 24-h after harvest when compared with unharvested alfalfa. Thirteen to 18-days after harvest, the experiment reported a slow recovery of N_2 fixation.

After alfalfa is harvested, the starch reserves in the taproot decline for two to three weeks (Boyce and Volenec, 1992). On day 14 of regrowth, starch concentrations had declined by 71% and 74% from the initial starch concentrations. When shoot regrowth reaches a threshold, excess photosynthates are then stored in the taproot. It is believed that higher starch concentrations can increase winter tolerance of alfalfa. This is because taproot starch converts to soluble sugars, which are needed to enhance cold tolerance by supplying energy and increasing intracellular solute concentration (Bula et al., 1954; Ruelke and Smith, 1956; Boyce and Volenec, 1992). In late fall, alfalfa with higher taproot starch concentration had less electrolyte leakage and developed higher cold tolerance than those with lower starch concentrations (Bula and Smith, 1954; Ruelke and Smith, 1956; Boyce and Volenec, 1992). Castonguay et al. (1995) found that during alfalfa hardening soluble sugars concentration increased significantly, while starch concentration decreased.

Evidence supports that higher sugar concentrations are associated with lower LT_{50} (Levitt, 1980; Castonguay et al., 1995). Higher sugar concentration acts as an osmoticum, increasing solute concentration in vacuoles and preventing intracellular ice formation, as well as stabilizing proteins and membranes (Levitt, 1980; Hoekstra et al., 1989; Castonguay et al., 1995). In addition, sugars are believed to aid in protecting against dehydration induced by freezing. There is evidence to support the assertion that sugar accumulation at low temperatures are a mechanism to stabilize membranes during desiccation caused by freezing. In higher concentrations, disaccharides help stabilize membranes and prevent membrane fusion during desiccation (Hoekstra et al., 1989; Castonguay et al., 1995). However, there was not significant effects found between sucrose levels and freezing tolerance when alfalfa was sampled in the field (Saint-David near Québec City, Canada) in February (Castonguay et al., 1995).

Root C reserves in the fall and winter did not decrease with a fall harvest in some studies. Correlation between fall harvest and forage yield in the subsequent spring is variable, with some authors not finding a strong correlation (Edmisten et al., 1988; Sheaffer et al., 1988; Brink and Marten, 1989; Dhont et al., 2003) while Berti et al. (2012) reported that forage yield in the first harvest in the spring consistently decreased after a fall harvest. A more recent study found that sugar levels did not correlate with winter survival of alfalfa (Berg et al., 2018). Further investigation of C reserves and its impact on winter hardiness is necessary.

In summary, total reserve concentrations of carbohydrates and proteins are determined by many abiotic factors (harvest frequency, fall harvest, nutrient availability, soil water) and biotic factors (cultivar, competition effects, root disease) (Avice, 1997). Any treatment that modifies storage of N in root reserves will affect vigor of shoot regrowth, specifically focusing on the effect of K application on storage of root protein and starch in this study.

2.6. Potassium in plants

Potassium is often an under-applied nutrient due to the cost of K fertilizers when compared to K removed in alfalfa hay harvests (Lloveras et al., 2012). Potassium is considered a macronutrient for both plants and animals, being needed in relatively large amounts in plants, and K is the only macronutrient dominated by inorganic forms within both plant and soil (Khan et al., 2013). Being dominantly in inorganic forms means that K does not depend on microbial activity in order to be available to plants. Due to plant demand for K and lack of replacement application due to cost, K is often a nutrient that has a negative balance within an alfalfa field when K removed is compared with K supplied from external sources (Lloveras et al., 2012).

Potassium is needed in large amounts because of its importance in numerous physiological reactions in plants. Enzyme activation in photosynthesis is affected by K levels (Hawkesford et al., 2012; Jungers et al., 2019). Potassium has a role in protein, ATP, starch, and sugar synthesis and translocation. The transportation of ATP, protein, water, starch, and other sugars is facilitated by K in the phloem. It is also known that K aids in stomatal function, regulating water and gas exchange necessary for photosynthesis, as well as leaf cooling and water transpiration from the plant, which governs xylem flow (Collins et al., 1986; Jungers et al., 2019). Due to K having a positive charge, its chemical property allows for water and nutrients to move into plant tissues through a diffusion concentration gradient. Potassium aids in processes vital for winter survival, such as mobilizing photosynthates to the taproot prior to dormancy (Lu et al., 2018). Starch synthetase, the enzyme needed for starch synthesis, is positively correlated with K concentration (Berg et al., 2007).

High K fertilization can reduce the forage nutritive value of alfalfa (Berg et al., 2018; Junger et al., 2019). Higher K rates have been correlated with a decreased percentage of total digestible nutrient (TDN). This is because K increases fiber content and ash, decreasing digestibility and crude protein. Lissbrant (2009) found that as K fertilization increased from 0 to 400 kg K_2O ha^{-1}, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) increased, while in vitro total dry matter digestibility (IVDMD) and crude protein (CP) decreased. There is still a lack of understanding on how management and the environment allow alfalfa to survive, including soil potassium value interpretation (Berg et al., 2018).

2.7. Potassium in soil

Soil K can be categorized into four different forms: K in soil solution, exchangeable K, fixed K, and lattice K (Syers, 1998; Ashley et al., 2006). Potassium in soil solution the K^+ ions dissolved in soil water exchangeable K is the amount of K ions adsorbed to a soil cation exchange sites, fixed K is K trapped between smectitic clay layers, and lattice K is the K still located in usually a primary mineral, such as potassium feldspar or mica. Because of the different forms, available K^+ content depends strongly on the nutrient dynamics of the soil, as well as other environmental conditions (Ashley et al., 2006).

Plants are solely able to take up K^+ when in solution form, through its roots and into the plant via the xylem. Approximately 96% of K^+ delivery to a plant root occurs via mass flow or diffusion (Oliviera et al., 2004; Ashley et al., 2006). A plant is more rapidly able to take up K^+ than other cations (Ca^{+2}, Mg^{+2}, $Na^{+)}$ (Khan et al., 2013). Potassium has a greater membrane permeability than other cations, but K is also actively taken up through ion-selective channels in root cell membranes, regulated by enzyme proteins. This allows K^+ to more easily travel across the plant cell membrane, which is why plants more readily absorb it into the system. However, there is competition between cations, such as Na^+ or NH_4^+ , $Ca2^+$ and Mg^{2+} ,for exchange sites. In soils with high soluble salts or free lime, K^+ ions may be in a minority, leading to (Spalding et al., 1999; Qi and Spalding, 2004; Russ et al., 2004; Ashley et al., 2006). An increased possibility for K^+ loss from the soil through leaching.

Arbuscular mycorrhizae fungi (AMF) are *Glomeromycota* fungal phylum that form a symbiosis with a fungi and many families of land plants (Harrison, 2012; Smith and Read, 2008; Gutjahr and Parniske, 2013). The AMF can aid in nutrient transfer mechanisms by colonizing plant roots and root-like structures extending their external hyphae into the soil, increasing that increase nutrient uptake and can transfer these nutrients to their hosts (Gutjahr and Parniske, 2013). Liu et al. (2020) found that inoculating alfalfa with AMF increased biomass P content by 4.53 times and K content by 2.06 times more than without AMF. However, this was only the case when P fertilizer and/or biochar was not applied. The study also found that AMF amendment increased the shoot biomass by 1.39 times of plants without. This indicates that AMF can increase nutrient availability when nutrient concentrations are lower in the soil. Baslam et al. (2014) found that AMF can increase forage nutritive value. In environments of elevated CO_2, AMF–colonized alfalfa had lower levels of lignin and higher levels of hemicellulose in the leaves, and higher levels of sugars in the stem.

Although a highly mobile nutrient, K^+ can be more or less available to plants in certain environments, requiring higher soil test values in more restrictive soils and environments (Breker et al., 2019). Soil type is a strong influencer on the amount of K that should be applied. For instance, sandy soils where sands are mostly quartz particles require more K inputs when compared with other soil textures over time because of leaching potential and weaker attraction of K^+ ions within organic matter (Holmqvist et al., 2003; Kayser and Isselstein, 2005). Potassium is considered the most easily leached cation , especially in sandy soils, due to its movement through the soil pores (Moraes and Dynia, 1992; Mendes et al., 2016). However, in Delaware, sands consisting mostly of potassium feldspar particles required no additional K fertilizer for maximum yield (Sparks, 1987).

The process of decaying of plant residues during the winter, as well as higher soil moisture content at beginning of growing season, is often able to supply K demands to alfalfa early in the season (Kayser and Isselstein, 2005). Because of this, K fertilizers are not recommended to be applied to alfalfa early in the spring, because of available K^+ from weathering over winter. After the first harvest of alfalfa, however, available K^+ supply to the soil solution from *in situ* sources cannot keep up with the demand from regrowth. When a K fertilizer is added into a system, a portion will be added to soluble K^+, a portion will be adsorbed onto exchange sites, and a portion may be fixed into non-exchangeable K forms indicated in Fig. 13 (Bertsch and Thomas, 1985; Goli-Kalanpa et al., 2008).

Potassium can also be “slowly available” when K^+ becomes fixed within a soil’s clay minerals (Kaiser and Rosen, 2018). The amount of K^+ that is fixed is determined by the type of clay that is within the soil (Breker et al., 2019). Due to K^+ having a positive charge and clays having a negative charge, this causes K^+ to adhere strongly to clay particles. When smectitic clay ratio soils dry up, K^+ is fixed in-between clay layers. Once the soil moisture increases, clay layers expand, releasing K^+ into the soil solution. Considering that soil tests only measure the solution and exchangeable forms of soil K^+, soil water content will influence results (Vitko et al., 2010). A study conducted in the top 2.5-cm of soil within Iowa soils found that soil water content is directly related with soil K^+ (Leubs et al., 1956; Vitko et al., 2010). With this variability, it is advised to sample soil K^+ at the same time of year (Vitko et al., 2010).

In soils with K-fixing clays, fertilizer management can be adjusted depending on a field’s clay chemistry. The ratio of smectite-to-illite clay mineralogy can availability of K^+ to plants. In the spring of 2017, all of North Dakota’s two to three major soil groups within each county were mapped for the smectite-to-illite ratio, to aid farmers in identifying the most meaningful soil test K critical value with which to base their K fertilizer application (Breker et al., 2019).

There are numerous factors that should determine the rate of K application in alfalfa such as current K soil test values, cation exchange capacity, previous year’s removal of forage, and a soil’s clay chemistry (Franzen and Berti, 2017). The goal of this study was to further investigate factors that determine rate of K application. This was done by applying varying rates and timings of K fertilizer. We also conducted different fall harvest, one in middle of September and the other in October. We hypothesize for this study that a split-application of K is the most effective treatment for supplying K demand by the plant. We also expect that the final harvest time in the middle of September will have reduced starch and protein reserves in the taproot when compared with the final harvest time in October.

3. MATERIALS AND METHODS

3.1. Field establishment and experimental design

Two separate sites were established for this experiment, east of Lisbon, ND (46°26'N, -97°11'W, 325-m elevation) and north of Milnor, ND (46°16'N, -97°28'W, 337-m elevation). The soil series in Lisbon is an Ulen fine sandy loam soil and the soil series in Milnor is a Hecla fine sandy loam soil, (Ulen: sandy, mixed, frigid Aeric Calciaquoll; Hecla: sandy, mixed, frigid Oxyaquic Hapludoll) (USDA, 2009). The experimental sites were chosen for their very low-to-low soil K^+ levels, Milnor ranging between 87-100 mg kg^{-1} and Lisbon ranging between 76-79 mg kg^{-1} in spring 2019. The two locations were also chosen for differences in their smectite-to-illite ratio of clay mineralogy. We hypothesized that the Milnor site would immobilize more K^+, due to a smectite-to-illite ratio greater than 3.5, while in Lisbon, we hypothesized that K^+ would be seasonally resistant to fixation, due to a smectite-to-illite ratio less than 3.5.

The experimental design was a factorial with a split-plot arrangement and four replicates. Each replicate contained fifteen experimental units, along with two borders areas, one on each end of the replicate. The cultivar of the border areas was the same in the experimental unit adjacent to it. The main plot treatments were alfalfa cultivars and the subplots, or experimental units, were a factorial combination of K rates and application timings.

The previous crops for Lisbon and Milnor were soybean [*Glycine max* (L.) Merr.] and corn (*Zea mays* L.), respectively. The Milnor site had winter rye (*Secale cereale* L.) as a cover crop previous to this experiment’s establishment. For the Milnor site, mowing and raking was required in order to remove the corn stalk residue and allow for the disks on the planter to properly sow the alfalfa seed . Both research sites were located within previously-established no-till managed fields. No-tillage was continued as the management strategy for both fields.

Prior to planting, 1.7-cm in dimeter soil probes were used to collect soil cores for each of the four blocks (reps) at 0-15 cm to give a baseline of current soil conditions (Table 1). The baseline soil samples were sent to the North Dakota State University Soil and Water Laboratory to analyze NO_3-N, P, K, pH, and organic matter (OM). The following methods were used: NO_3-N, calorimetric determination by trans-nitration of salicylic acid method (Vendrell and Zupancic, 2008; Nathan et al., 2012); phosphorus, Olsen procedure using Brinkmann PC 910 colorimeter (Olsen et al., 1954; Nathan et al., 2012); K, ammonium acetate method using Buck Scientific Model 210 VGP atomic absorption spectrophotometer (Carson, 1980, Nathan et al., 2012); pH, calcium chloride method; OM, loss on ignition adapted from Combs (1998) (Nathan et al., 2012).

Table 1. Soil sample analysis of 0-15 cm deep cores obtained from the two experimental locations in the spring and fall, 2019.

Location	NO_3-N	P	pH	OM†	Date	K level in each treatment‡				
						0	168	168S	336	336S
	kg ha^{-1}	mg kg^{-1}		g kg^{-1}		------------------------mg kg^{-1}---------------------				
Milnor§	8.96††	6.0	7.28	27.5	15 May	91.2	96.5	100.0	90.2	86.9
					8 Oct.	74.2	81.6	90.2	90.8	123.0
Lisbon¶	8.68	24.3	5.70	24.7	10 May	76.3	78.1	78.8	80.1	78.9
					9 Oct.	63.9	87.5	86.1	121.0	128.0

†OM=organic matter. ‡For treatments, 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. §Milnor, high smectite-to-illite ratio (>3.5). ¶Lisbon, low smectite-to-illite ratio (<3.5). ††Values are averages, where NO_3-N, P, pH, and organic matter (OM) (n=4), and K levels (n=12).

The soil pH in Lisbon prior to beet lime application was 5.7. In fall 2019, the pH ranged between 5.3-6.7. The ranges of pH within each K_2O treatment are shown below (Table 2).

Table 2. Soil pH range at each potassium treatment at two growing seasons in Lisbon, ND.

Rate K_2O	2019
kg ha^{-1}	---pH---
0[†]	5.3-6.7
168	5.4-6.2
168S	5.4-6.5
336	5.5-6.7
336S	5.4-6.6
	2020
0	5.8-7.0
168	5.7-6.7
168S	5.7-6.7
336	5.7-7.2
336S	5.8-6.7

[†]Treatments 0=0 K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September.

In 2019, the alfalfa was seeded on 10 May in Lisbon and on 15 May in Milnor. The alfalfa was seeded at a depth of 0.95-cm with an 8-row continuous plot drill XL (Wintersteiger, Salt Lake City, UT). Experimental units were 6.1-m long with eight rows spaced 15.2-cm apart. Three alfalfa cultivars were used in this study, all being glyphosate-tolerant cultivars. Each of the cultivars had a different fall dormancy score (Table 3).

Table 3. Cultivar characteristics and the seeding rates for planting at both Milnor and Lisbon sites in 2019.

Cultivar	FD[†]	WS[§]	Germination	Purity	Adjusted seeding rate
			%	%	kg ha^{-1}
RR Presteez	3.2 (low FD)	1.2	88	66	19.3
RR Stratica	4.3 (medium FD)	2.0	88	66	19.3
L-450 RR	5.0 (high FD)	1.4	80	66	21.2

[†]FD: fall dormancy. [§]WS: winter survival.

The desired seeding rate for all three alfalfa cultivars was 11.2 kg ha^{-1} pure live seed (PLS) of alfalfa. The RR Presteez (FD3) bag of seed was purchased in 2017, therefore inoculant was added to Presteez seed to ensure healthy inoculation. The rate of inoculant was 0.22 kg ha^{-1} of Pre Vail-inoculant (*Sinorhizobium meliloti, Rhizobium leguminosarum biovar trifolii, Azospirillum brasilense* - growth promoter as indicated by the company). After alfalfa was established in 2019, plastic pipes (10.2-cm) were inserted in each subplot experimental unit to mark a representative 0.10-m^2 area for measuring plant density. These pipes marked the area that was used to perform stand counts prior to each harvest of alfalfa.

In 2019 at Milnor, the experiment was fertilized with 100 kg P_2O_5 ha^{-1} as monoammonium phosphate (11-52-0) to increase soil P levels and ensure that P deficiency would not confound the experiment (Table 4). In 2019 at Lisbon, sugar beet waste lime, with pH 8.5 and a calcium carbonate equivalent (CCE) of 67%, was applied at a rate of 4.48 Mg ha^{-1} to raise pH by 0.5 units. For weed control, glyphosate [N-(phosphonomethyl) glycine] was applied at 1.1 kg acid equivalent (a.e.) ha^{-1} as needed, along with hand weeding. Insecticide (lambda-cyhalothrin) was applied at a rate of 0.09 kg a.e. ha^{-1} to control potato leafhoppers (*Empoasca fabae* Harris) when threshold was reached during the 2019 and 2020 growing season (Table 4).

Table 4. Rates and application dates of fertilizers and pesticides at two locations throughout two growing seasons 2019 and 2020.

Year	Location	Glyphosate a.e. †		P_2O_5 (11-52-0)		Beet lime		Insecticide (lambda-cyhalothrin) a.e.	
2019		kg ha^{-1}	date	kg ha^{-1}	date	Mg ha^{-1}	date	kg ha^{-1}	date
	Milnor	1.4	29 May, 17 Jul.	100	3 Jun.			0.09	17 Jul., 14 Aug.
	Lisbon	1.4	29 May, 17 Jul.			4.48	18 Jun.	0.09	17 Jul., 14 Aug.
2020									
	Milnor	1.4	27 May, 15 Jul.					0.09	7 Jul.
	Lisbon	1.4	27 May, 15 Jul.					0.09	7 Jul.

†a.e.: acid equivalent

Five treatments of rate and timing of K combinations were used in this study and are indicated in Table 5. Treatments of K_2O were applied as fertilizer grade potassium chloride (0-0-60), and were hand-broadcasted throughout the entirety of each experimental unit.

Table 5. Potassium treatments applied as fertilizer grade potassium chloride (0-0-60) and application dates at two location sites throughout two growing seasons.

Rate K_2O	Milnor		Lisbon	
kg ha^{-1}	2019			
0				
168	15 May		13 May	
168, split-application	29 Jul.	19 Sept.	30 July	19 Sept.
336	15 May		13 May	
336, split-application	29 Jul.	19 Sept.	30 Jul.	19 Sept.
	2020			
0				
168	1 Jun.		3 Jun.	
168, split-application	1 Jun.	15 Sept.	3 Jun.	15 Sept.
336	1 Jun.		3 Jun.	
336, split-application	1 Jun.	15 Sept.	3 Jun.	15 Sept.

3.2. Weather and GDD calculation

Rainfall and daily temperature were recorded by the North Dakota Agricultural Weather Network (NDAWN, 2020) from the Lisbon NDAWN station, which was used for estimating conditions at both locations, because the Lisbon NDAWN station was close in proximity for both locations. For calculating growing degree days (GDD) the following formula was utilized and the general base temperature for alfalfa was 5°C (Bélanger et al., 1992). This measured GDD on a daily basis.

$$\text{GDD} = \sum \left[\frac{(\text{maximum temperature} \ + \ \text{minimum temperature})}{2} - \text{Base temperature} \right]$$

3.3. Soil K tests and available K

Soil K^+ was measured in each plot by collecting 0-15 cm cores in the spring and fall of both years (Table 6).

Table 6. Soil sampling dates at both locations for both growing seasons.

Location	Spring 2019	Fall 2019	Spring 2020	Fall 2020
Milnor	15 May	8 Oct.	1 Jun.	9 Oct.
Lisbon	10 May	9 Oct.	1 Jun.	9 Oct.

All soil samples were brought to the soil testing laboratory at North Dakota State University and measured soil available K^+ (SAK) by the ammonium acetate method extraction from dry soil, and detected using a Buck Scientific Model 210 VGP atomic absorption spectrophotometer in emission mode (Carson, 1980; Nathan et al., 2012)

Total available K for alfalfa (SAK + fertilizer) was calculated for each growing season. The bulk density (BD) was calculated at each field by pounding a ring into the soil and collecting the enclosed soil sample, which was dried. From the dried soil weight and volume of the bulk density ring the Lisbon and Milnor location calculated as 1.30- and 1.48-g cm^{-3}, respectively. In order to convert the K_2O treatments applied in K, all K_2O rates were divided by 0.8301. The soil available K values from 10 May sampling in 2019 and 1 June sampling in 2020 were utilized as spring SAK.

$$Total\ available\ K\ for\ alfalfa = \frac{spring\ SAK\ x\ BD\ x\ soil\ sample\ depth}{10} + K\ rate\ applied$$

Total available soil K, SAK, and K rate applied were in kg ha^{-1} and soil sample depth was 15-cm.

3.4. Alfalfa yield and plant height

There were two harvests conducted in the seeding year of 2019 and four harvests in the 2020 growing season (Table 7). In 2019, the first harvest was done with alfalfa at 20% bloom stage and >61-cm. The first harvest of 2020, the growth stage was at early bud. At harvest, plant height and growth stage were noted. Plant height was measured from the ground level to the top leaf before each harvest from two shoots within each sub-plot. The alfalfa was harvested using a six-row flail forage harvester (Carter, Brookston, IN). Whole plot fresh weight was recorded, and a fresh sample was taken and weighed. The harvester cut the alfalfa leaving 7.6-cm stubble. Using hand sickles, all missed plants were cut down leaving 7.6-cm stubble to improve uniformity. Harvested samples of wet forage were dried at 37.8°C for 72-h to determine dry matter forage yield.

For the first harvest in 2019 and the first three harvests in 2020, each experimental unit was harvested entirely. For the final harvest of both years, experimental units were randomly split in half, with one-half of the experimental unit harvested in the middle of September and the remaining half harvested in October.

Table 7. Harvest dates at two locations throughout two growing seasons, in 2019 and 2020.

Year	Location	Harvest				
2019		First		Second (S†)		Second (NS)
	Milnor	29 Jul.		19 Sept.		16 Oct.
	Lisbon	30 Jul.		19 Sept.		16 Oct.
2020		First	Second	Third	Fourth (S†)	Fourth (NS)
	Milnor	1 Jun.	29 Jul.	11 Aug.	15 Sept.	12 Oct.
	Lisbon	2 Jun.	30 Jul.	11 Aug.	15 Sept.	12 Oct.

†Harvest stress treatment: S=stressed, fall harvest occurred in mid-September.; NS=non-stressed, fall harvest occurred in October prior to first frost.

The area harvested per half experimental unit was 4.6-m^2. Harvesting half the experimental unit in September stressed the alfalfa because there was sufficient GDD for shoot regrowth, drawing energy from root reserves. Harvesting the other half in October did not stress the alfalfa since minimal GDD prevented regrowth to deplete root reserves prior to total dormancy.

3.5. Biomass K removal and K balance

The K removed by the biomass was calculated for each harvest in both 2019 and 2020 using the K concentration value from the near infrared spectrometer analysis (NIR, XDS Analyzer, Foss XDS, Minneapolis, MN, USA), between wavelengths 400-2495 nm, and biomass yield. Since biomass yield was in Mg ha^{-1} and biomass K was estimated by kg K removed ha^{-1} a factor of 10 was multiplied into the equation below.

$$\%K\ from\ NIR\ x\ biomass\ yield\ x\ 10 = Biomass\ K\ removal\ (kg\ ha^{-1})$$

Potassium balance, defined as total available K (SAK + fertilizer), greater or lower than the sum of K removed by the alfalfa biomass and that in the fall soil available K, was calculated for 2019 and 2020.

$$K\ balance = Total\ \text{available K} - (Total\ \ K\ removed\ by\ biomass + fall\ SAK)$$

K balance, total available K, total K removed by the biomass, and fall SAK were in kg ha^{-1}.

3.6. Forage nutritive value analysis and biomass K

Harvested and dried samples were ground to a 1-mm size. The ground samples were analyzed using near-infrared spectroscopy, with NIR between wavelengths 400-2495 nm (XDS Analyzer, Foss XDS, Minneapolis, MN, USA). The NIR was calibrated for measuring pure alfalfa. The ground samples were also analyzed for livestock nutritive value: acid detergent fiber (ADF), ash, K, neutral detergent fiber (NDF), P, crude protein (CP), neutral detergent fiber digestibility (NDFD), fatty acids (FA), and ether extract (EE). For NDFD parameters, a 48-h time of digestion was utilized. Analyses followed the methodology of Abrams et al. (1987). Biomass K was calculated by summing measured K values at each harvest for both 2019 and 2020. Using the values of the parameters mentioned, the non-fiber carbohydrate (NFC), dry matter intake (DMI), total digestible nutrients (TDN) and relative forage quality (RFQ) were calculated (Schroeder, 2013). The formulas used in these calculations are stated below, with parameters calculated as percentages.

$$NFC = 100 - Ash - CP - EE - NDF$$

$$TDN = (NFC\ x\ 0.98) + (CP\ x\ 0.93) + (FA\ x\ 0.97\ x\ 2.25) + \frac{NDF\ x\ NDFD}{100} - 7$$

$$DMI = \left(\frac{120}{NDF}\right) + (NDFD - 45)\ x \left(\frac{0.374}{1350}\right) x\ 100$$

$$RFQ = \frac{DMI\ x\ TDN}{1.23}$$

3.7. Alfalfa plant density and persistence

Alfalfa persistence, the survival of alfalfa population over time, was indicated by stand counts taken at the first harvest and at the fall harvest in both 2019 and 2020. Stand counts were taken from the pre-marked 0.10-m^2 area previously mentioned in the field establishment methodology.

3.8. Root sampling and analysis

Root samples were collected at the last harvest of 2019 and 2020, during the non-stressed final harvest prior to first frost. Each main experimental unit was sampled from both the stressed and non-stressed sub-plots. Root samples were obtained from a depth of 15.2-cm. Samples were cut above the crown, washed, dried, and then stored in a freezer (2015 SU780UE, Stirling Ultracold, Athens, Ohio) at -80°C.

The stored root samples were analyzed for protein and starch reserve content. Root samples were separated into 50-mL flasks and covered with a Kimwipe. All samples were freeze-dried using the Virtis SP Scientific Sentry 2.0 (SP, Warminster, PA) at the USDA-ARS, Edward T. Schafer Agricultural Research Center in Fargo. Freeze-drying occurred at -70°C and

200-mTorr for 48-h. All root samples were ground and passed through 1-mm screen and separated into 5-mL tubes. Samples were then stored in -80°C freezer until analysis of protein and starch.

Protein content was determined using the method of Bradford (1976). Analyzing N reserves required 10-mg of freeze-dried, ground root sample to be suspended in 2-mL of 100-mM sodium phosphate buffer (pH 6.8). The samples were then microcentrifuged at 13,000 × g for 5-min. One part of dye reagent (Bio-Rad protein assay kit II, Bio-Rad Laboratory, Hercules, CA) was diluted using four parts of distilled water. A volume of 5-mL of diluted dye reagent was added to 50-μL of the root extract. The solution was vortexed at 2300 rotations per minute (RPM) and then incubated in the dark for 5-min. The absorbance of the mixture was measured at 595-nm against a blank (50-μL distilled water) with an ultraviolet-visible (UV-VIS) Genesys spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

Starch content was determined by the method of Smith and Zeeman (2006). For analyzing starch, 20-mg of root sample were placed in 5-mL 80% aq. vol vol^{-1} ethanol and incubated in a 100°C water bath for 3-min. Samples were centrifuged at 8500 x g for 5-min. Supernatant was discarded and the ethanol extraction was repeated two more times. The final pellet was homogenized with 4-mL distilled water. Each sample had four subsamples of 0.5-mL of the homogenate that were transferred to 2-mL microcentrifuge tubes. Tubes were incubated in a 100°C water bath for 10-min in order to gelatinize the starch particles. After cooling, 0.5-mL of 200-mM Na acetate (pH 5.5) was added to all subsamples. Starch was digested by adding 0.1-mL of α-amyloglucosidase and 0.1-mL of α-amylase to two of the subsamples, while the other two subsamples had 0.1-mL of Tris buffer (pH 7.5) and 0.1-mL of α-amylase solution without enzyme added for the controls. All tubes were incubated at 37°C for 4-h.

After incubation, the samples were microcentrifuged at 13,000 × g for 5-min. The samples of the supernatant were assayed for glucose using glucose oxidase. A measured 200-µL of supernatant sample and 890-µL distilled water were combined in a 1-cm-wide crystal cuvette. The optical density (OD) reading was recorded at 340-nm. An enzymatic assay of 5-µL hexokinase and 5-µL glucose 6-phosphate dehydrogenase was added to the cuvette, converting glucose to 6-phosphogluconate with accompanying reduction of nicotinamide adenine dinucleotide (NAD) to NADH[6]. The optical density (OD) was recorded again at 340-nm, monitoring the NADH[6] production.

In order to calculate starch content of tissue samples, the amount of glucose in the cuvette (µmol) was calculated by taking the change in OD divided by 6.22, which is the millimolar extinction coefficient of NADH at 340-nm (Smith and Zeeman, 2006). This mean value for the control samples (A_c) was subtracted from the mean value of the enzyme samples (A_e). The net value (A_s) was then carried out in the equation shown below. The starch content of the tissue in µmol glucose equivalents per gram of dry weight was then multiplied by 162, the mass of anhydroglucose, converting the value to µg starch per gram of dry weight root tissue.

$$\frac{As}{0.2\ ml\ (vol.assayed)}\ \ x\ \ 2\ \ x\ \frac{5}{wt\ of\ tissue\ (.20\ g)} = \mu mol\ glucose\ eq/g\ dry\ wt$$

3.9. Statistical analysis

The data collected was analyzed using the MIXED procedure of SAS 9.4 (SAS Systems Inc., Cary, NC) with the repeated measures function for the different harvests, for plant height, and nutritive values within a same year. Soil available K, total available K for alfalfa, biomass K removal, K balance, stand persistence, root protein, and root starch were analyzed according to the experimental design. Year, fertilization treatments, and harvest times were fixed effects. Location was also a fixed effect because of the differing smectite-to-illite clay ratios. Replicate was a random effect. Analysis of all measured parameters were based on a split-plot design. For means separation treatment an LSD at 95% confidence of interval was used.

4. RESULTS AND DISCUSSION

4.1. Rainfall, temperature, GDD

The 2019 and 2020 growing season acquired similar total rainfall amounts; 43.4-cm and 41.8-cm respectively. The temperatures were above the 5°C base tempeature for alfalfa GDD from end of May until end of September in 2019 and from mid-May to mid-September in 2020 (Fig. 1). The total GDD for 2019 from middle of May through October was 1,851 GDD. The total GDD for 2020 from beginning of April through October was 2,003 GDD.

The winter of 2019-2020 was mild and only had one occurrence of eight consecutive days that were <-15˚C, which occurred in January. Average air temperatures from November-April ranged from -11˚C to 4˚C. Conditions of the winter months were unlikely for winter injury or winterkill of alfalfa to occur.

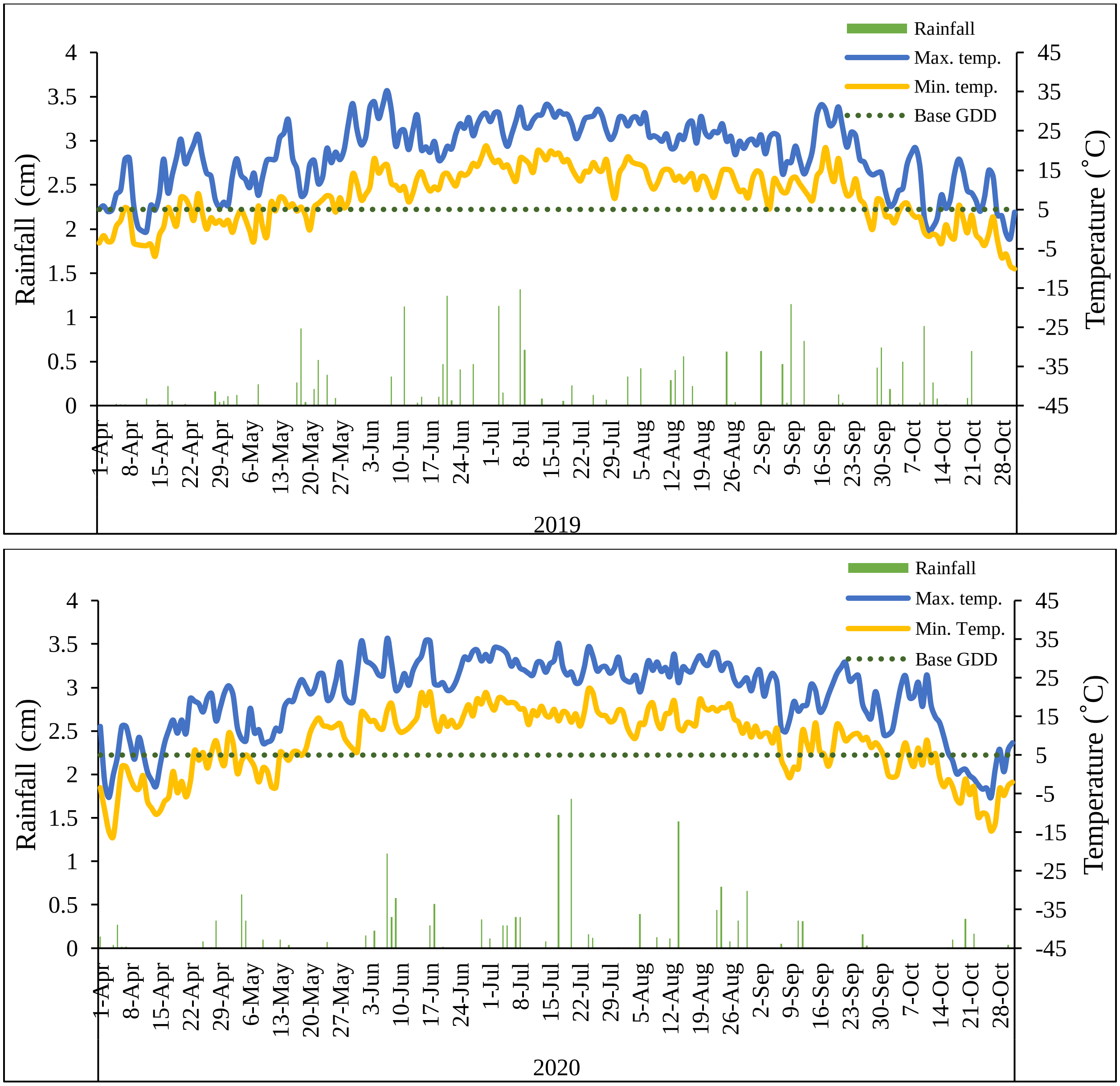

Fig. 1. Daily rainfall, maximum temperature, and minimum temperatures of two growing seasons representing conditions in both Milnor and Lisbon, ND.

Due to K availability depending on soil moisture for its releasing of K^+ fixed between clay layers, it is vital to acknowledge rainfall prior to soil K^+ samplings in both years (Vitko et al., 2010). The accumulated rainfall one week prior the soil samples were taken is shown in Table 8. At the time fall soil samples were taken, in both years the sites received similar rainfall in 2019 and 2020, reducing the possibility that soil moisture would have affected available K^+, especially considering both sites have sandy-loam texture. In spring 2019, 6.3-mm of rainfall occurred within the week prior to soil sampling, while in the spring of 2020 no rainfall was observed the week prior to sampling. This, however, was before any K treatments were applied, in 2019, but soil moisture could have affected the release of initial soil K^+ trapped in clay layers (Vitko et al., 2010). The soil moisture would have also aided in moving K from lysed residue cells into the soil (Kayser and Isselstein, 2005).

Table 8. Accumulated rainfall one week prior to soil K sampling in the spring and fall for both Lisbon and Milnor, ND, in 2019 and 2020.

Year	Sampling date	Rainfall	Sampling date	Rainfall
		--cm--		--cm--
2019	5-12 May	0.63	1-8 Oct.	1.83
2020	25 May-1 June	0.00	2-9 Oct.	1.90

4.2. Soil available K

In both 2019 and 2020, the K treatment, sampling time, sampling time by location, and sampling time by K treatment significantly affected soil available K^+ (Table 9). In 2019 alone, there was an effect between location by K treatment and an effect between cultivar by sampling time. Location or K treatment by cultivar did not affect soil available K in either year, correlating with findings in Junger et al. (2019). In 2020, location by K treatment and cultivar by sampling time did not affect soil available K^+.

Table 9. Analysis of variance and mean squares of soil available K^+ for two locations (Loc), five K treatments (Trt) two sampling times (Stimes) and three cultivars (Var) in two locations (Loc), in 2019 and 2020.

SOV	df	2019	2020
Rep	3	11590	31279
Loc	1	1302	306
Loc(rep)	3	4434	10521
Var	2	279	2619
Loc x var	2	313	1361
Loc x var x rep	12	568	1139
Trt	4	5144*	49749*
Loc x trt	4	1054*	1326
Var x trt	8	374	1350
Loc x var x trt	8	248	891
Stime	1	4603*	19171*
Loc x stime	1	5930*	9263*
Var x stime	2	928*	375
Trt x stime	4	6153*	9950*
Loc x var x stime	2	28	3851
Loc x trt x stime	4	523	2451
Var x trt x stime	8	102	805
Loc x var x trt x stime	8	206	1611
Residual	162	274	1831

* Significant at $P \leq 0.05$, level of probability

Soil available K^+ was significantly affected by K treatment in both 2019 and 2020, however, and interaction of location by K treatment was only significant in 2019. In Milnor alone, K treatment of 336 kg K_2O ha^{-1} had significantly higher soil available K^+ when in split-application timing than in full (Fig. 2). Whereas Lisbon did not have significantly different soil available K^+ between K treatments of the same rate and different application times. When split-application occurs, less K is added at once to the soil nutrient pool, decreasingthe possibility of exchange site saturation and subsequent K^+ leaching. (Bertsch and Thomas, 1985; Goli-Kalanpa et al., 2008). In 2020, there was a significant increase of soil available K^+ at Milnor for both 168 and 336 kg K_2O ha^{-1} in split-application than in full, which was not the case for Lisbon. This indicates that with a higher smectite-to-illite ratio, a split-application allows for higher available soil K^+ for the plant than an application at once. The longer the K^+ is available in the soil solution the higher probability of being lost to leaching or fixed into the clays.

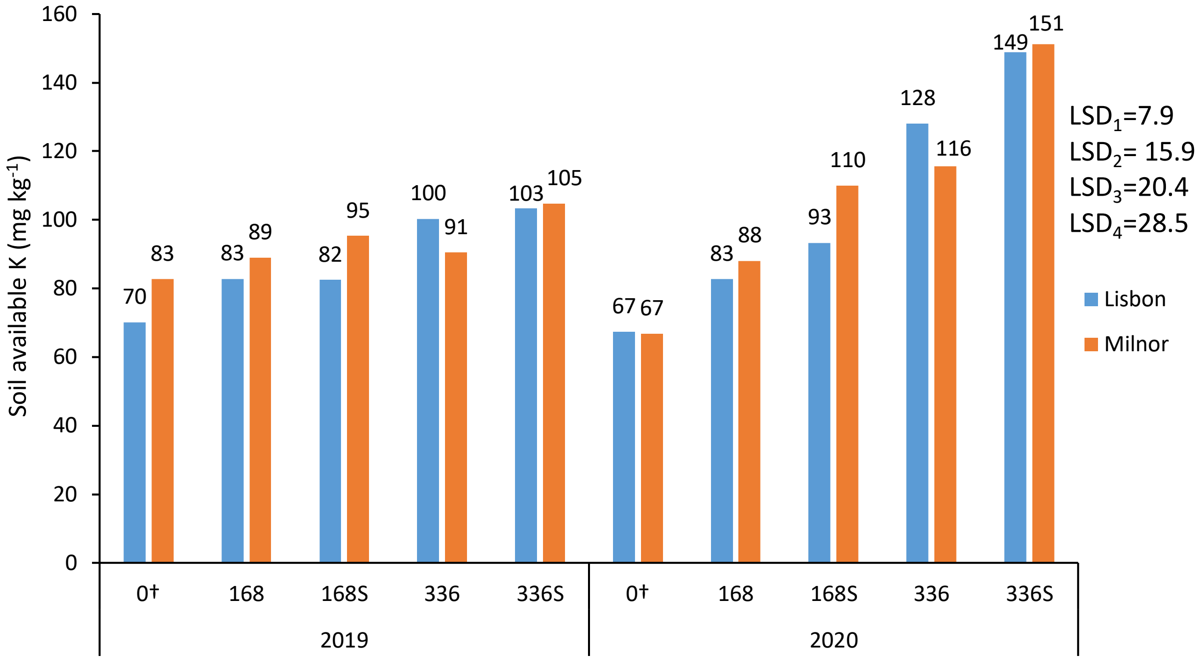

Fig. 2. Average soil available K^+ (SAK) levels at each K treatment in two locations averaged across four sampling times and three cultivars. †Treatments 0=0 K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020; 336S=split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD_1= to compare SAK between K treatment means at same location in 2019. LSD_2= to compare SAK between K treatment means across locations 2019. LSD_3= to compare SAK between K treatment means at same location in 2020. LSD_4= to compare SAK between K treatment means across locations in 2020. $P \leq 0.05$.

When analyzing the two different locations, it would be expected that Milnor would have lower soil available K^+ levels due to its higher smectite-to-illite ratio. But, the two locations were not significantly different, however, it can be seen that Lisbon had steady increase in soil K^+ availability over the four sampling times, while Milnor soil levels did not increase until the final sampling on 9 October, 2020 (Fig. 3). In spring of 2020, Lisbon soil levels averaged approximately at 10 mg kg^{-1} higher soil available K^+ than in Milnor soil. This could have been due by the clay mineralogy of Milnor, for the available soil K^+ may have been fixed before the spring sampling if soils were saturated during thawing. As mentioned previously, there was no rainfall recorded the week prior to 1 June sample date, which would prevent fixed K^+ from being released with clay layer expansion.

In the fall of 2020, however, the soil in Milnor averaged approximately 15 mg kg^{-1} higher soil available K^+ than the soil in Lisbon, when averaged across all K treatments (Fig. 3). Though the soil in Milnor was expected to have lower soil available K^+, K^+ can only be fixed until the exchange sites are saturated, which would be more likely with increased rates of K. The week prior to the 9 October sampling, there was 1.9-cm of rainfall, which would increase the amount of K^+ in solution and expand clay layers in order to release fixed K^+ (Vitko et al., 2010). The Milnor soil likely had higher fixed K^+, which may have increased K^+ in the soil solution after the rainfall.

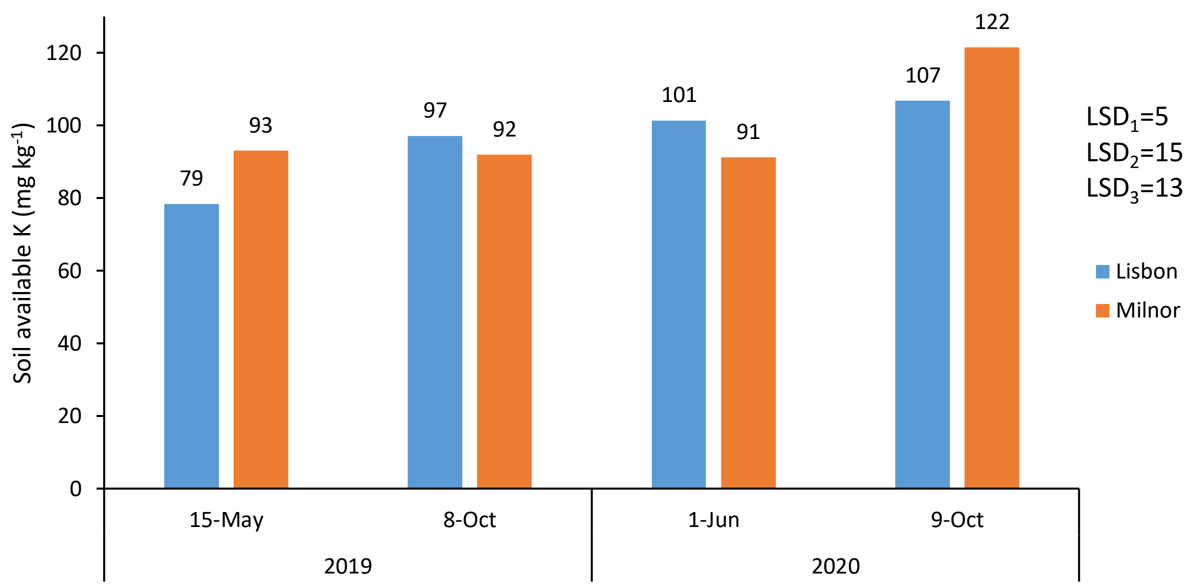

Fig. 3. Average soil available K^+ (SAK) levels in 2019 and 2020 for four samplings at two locations averaged across three cultivars and five K treatments. LSD_1= to compare SAK between sampling date means at same location in 2019; LSD_2= to compare SAK between sampling date means across locations in 2019; LSD_3= to compare SAK between sampling date means at same location in 2020; LSD_4= to compare SAK between sampling date means across locations in 2020. $P \leq 0.05$.

In 2019, soil available K^+ for the experimental units with L-450 RR (FD5) cultivar did not significantly change from May to October, but in experimental units planted with RR Presteez (FD3) and RR Stratica (FD4) soil available K^+ increased by 14 and 11 mg kg^{-1}, respectively (Fig. 4). This implies that FD5 had the highest usage of K, which is expected with its higher biomass production in the fall. The more dormant the cultivar, the less biomass production in the fall and therefore the less demand of nutrients (Lyons et al., 2016; Jungers et al., 2019). This effect only occurred in 2019, which may be because 2020 had higher total seasonal yield, making the impact of fall biomass production less significant, and therefore making the removal of the soil available K^+ less significant across cultivars.

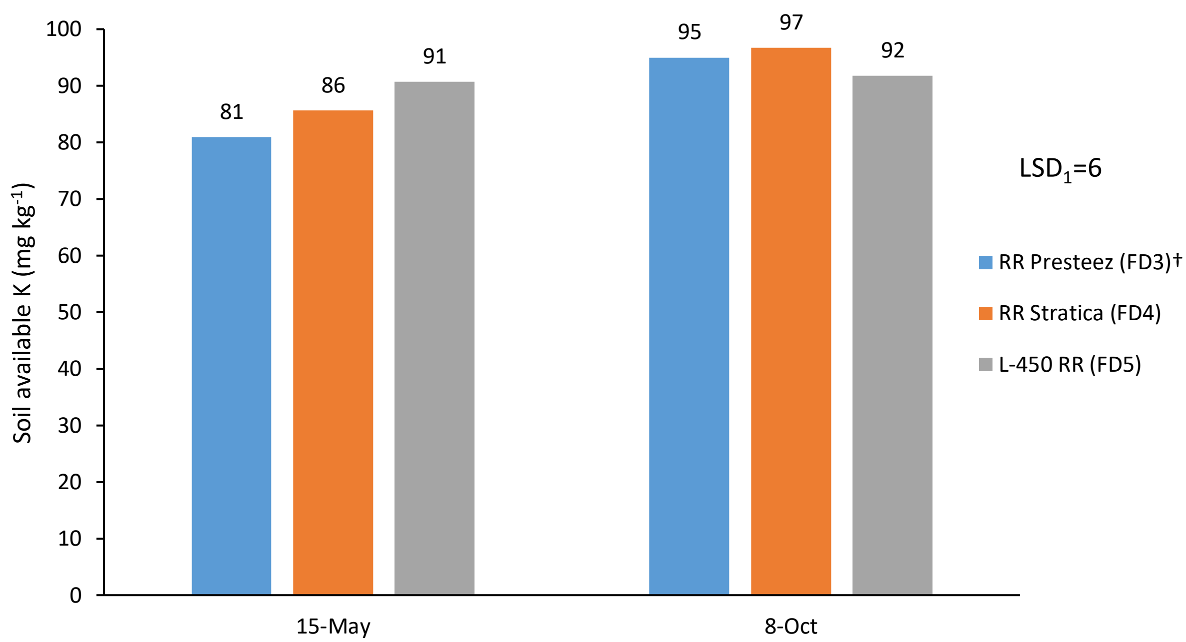

Fig. 4. Average soil available K^+ (SAK) at two sampling dates in 2019 and three cultivars averaged across locations and five K treatments. †FD=fall dormancy. LSD_1= to compare SAK between sampling date means within same cultivar; LSD_2= to compare SAK between sampling date means across cultivars. $P \leq 0.05$.

As expected, the highest rates of K applied caused the highest levels of soil available K^+ for all soil samples following the initial test in 2019 (Fig. 5). The split-application treatments had higher soil available K^+ than their corresponding single-application rates. In 2019, the soil with

168 kg K_2O ha^{-1} in split-application had approximately 9.5 mg kg^{-1} higher soil available K^+ than the 168 kg K_2O ha^{-1} rate applied in full-application (Fig. 5). The soil with 336 kg K_2O ha^{-1} in split-application had approximately 19 mg kg^{-1} higher soil available K^+ than the 336 kg K_2O ha^{-1} rate applied in full (Fig. 5). In 2020, the same response was observed only for the highest rate of 336 kg K_2O ha^{-1} (Fig. 5). This could be explained by potential leaching of K^+ below 15-cm in depth when the soil's cation exchange sites are saturated (Rosolem et al., 2018), which would more likely occur when K is applied at a higher rate in a full-application, than with a split-application.

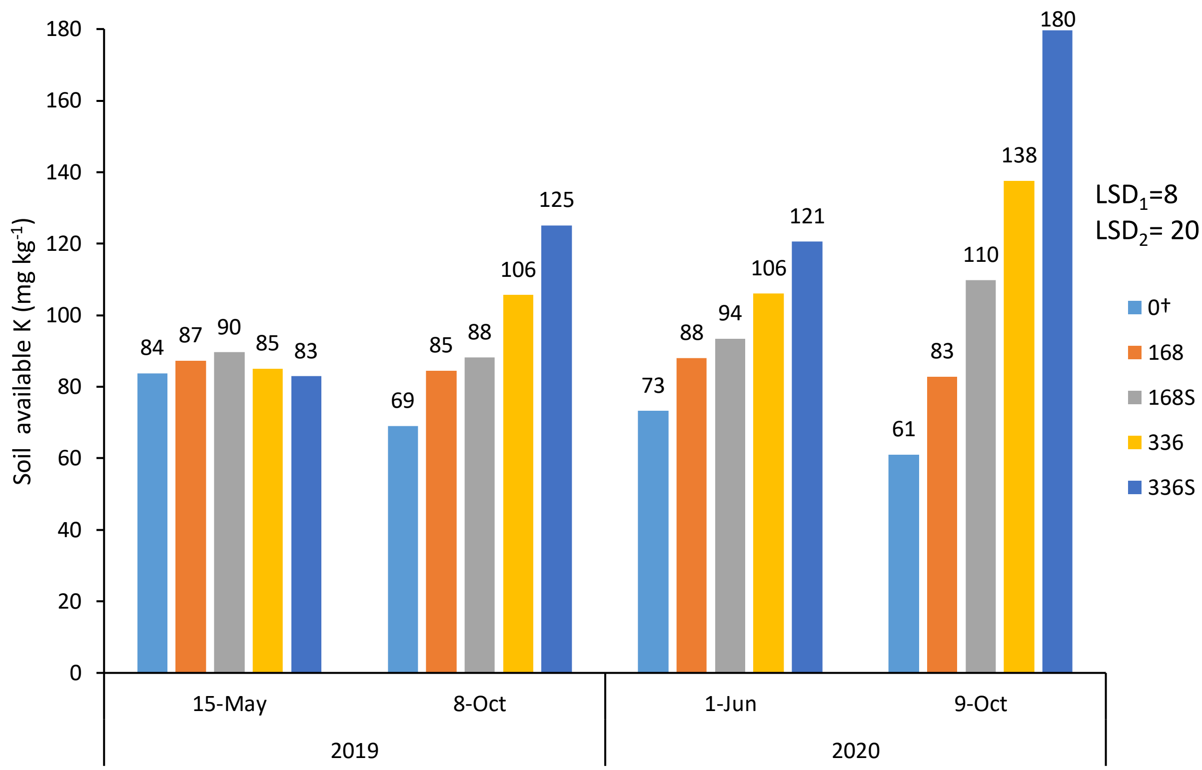

Fig. 5. Average soil available K^+ (SAK) at five K treatments and four sampling dates in 2019 and 2020 averaged across locations and three cultivars. †Treatments 0=0 K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020; 336S=split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD_1= to compare SAK between means of K treatments in both sampling dates in 2019; LSD_2= to compare SAK between means of K treatments in both sampling dates in 2020. $P \leq 0.05$.

The interactions of calculated available K for alfalfa [((soil available K from soil tests (kg ha^{-1}) x soil bulk density x 15-cm soil sample depth)/10) + K added as fertilizer] for the two locations, K rates, cultivars, and harvest stress are shown below (Table 10). In both 2019 and 2020, K treatment, and location by K treatment were significant for available K for alfalfa. In 2019, K treatment by cultivar, as well as K treatment by location by cultivar were significant for available K for alfalfa.

Table 10. Analysis of variance of available K for alfalfa† for two locations (Loc), five K treatments (Trt), fall harvest stress (Stress), and three cultivars (Var) in 2019 and 2020.

		2019	2020
SOV	df	MS	MS
Rep	3	30737	24689
Loc	1	172217	1384
Loc(rep)	3	31031	9247
Var	2	9547	5484
Loc x var	2	2221	2761
Loc x var x rep	12	3945	3365
Trt	4	621177*	1076072*
Loc x trt	4	1849*	20279*
Var x trt	8	1432*	1415
Loc x var x trt	8	1611*	1279
Stress	1	3.53E-24	8.82E-25
Loc x stress	1	8.82E-25	8.82E-25
Var x stress	2	6.63E-24	1.74E-24
Trt x stress	4	5.94E-24	5.03E-25
Loc x var x stress	2	1.64E-24	1.71E-24
Loc x trt x stress	4	1.85E-24	5.01E-25
Var x trt x stress	8	8.66E-24	1.14E-24
Loc x var x trt x stress	8	2.91E-24	1.88E-24
Residual	162	594	1301

* Significant at $P \leq 0.05$, level of probability. †[((soil available K from soil tests (kg ha^{-1}) x bulk density x 15-cm soil sample depth)/10) + K added as fertilizer].

The available K for alfalfa was higher in 2020 for experimental units that had 168 kg K_2O ha^{-1} in split-application, 336 kg K_2O ha^{-1} in a full-application, and 336 kg K_2O ha^{-1} in split-application (Fig. 6). In 2020, the 168 kg K_2O ha^{-1} rate in a full-application had the same total available K for alfalfa as 2019. This implied that the fertilizer applied did not increase the K available for alfalfa and merely sustained the amount of K removed by alfalfa in a production year, with the recommended K rate after establishment being 269 kg K_2O ha^{-1} for Milnor and 156 kg K_2O ha^{-1} for Lisbon (Franzen and Berti, 2017).

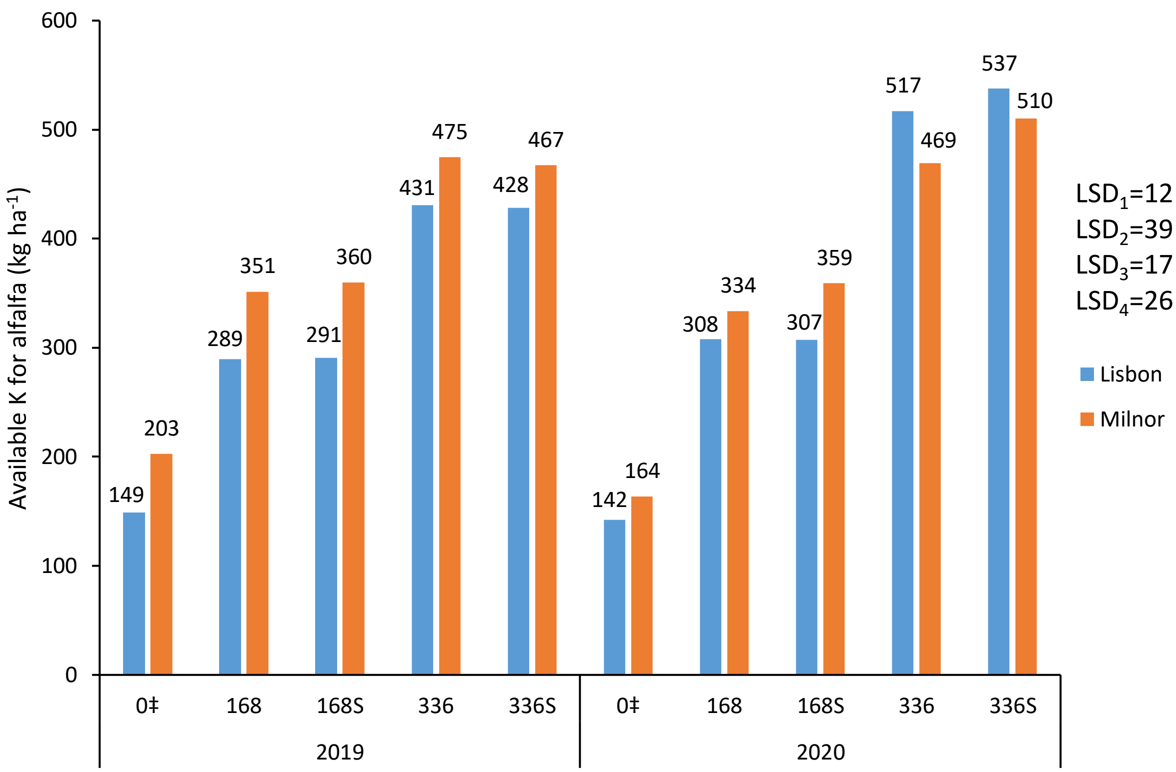

Fig. 6. Average available K^+ for alfalfa† for five K treatments and at two locations in 2019 and 2020 averaged across three cultivars and four sampling times. †[((soil available K from soil tests (kg ha^{-1}) x bulk density x15-cm soil sample depth)/10) + K added as fertilizer]. ‡Treatments 0=0 K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD_1= to compare available K for alfalfa between K treatment means at same location in 2019; LSD_2= to compare available K for alfalfa between K treatment means across locations in 2019. LSD_3=to compare available K for alfalfa between K treatment means at same location in 2020; LSD_4= to compare available K for alfalfa between K treatment means across locations in 2020. $P \leq 0.05$.

The significant interaction of available K for alfalfa for K treatment by cultivar by location was due mainly to a difference in ranking of available K for alfalfa for a particular cultivar at both rates between full- and split-application. For example, Stratica (FD4) had greater available K for alfalfa at the full-application rate of 336 kg K_2O ha^{-1} than the split-application for the same rate at both locations, but that was not observed for the other two cultivars (Table 11). At the 168 kg K_2O ha^{-1}, RR Presteez (FD3) in Milnor and RR Stratica (FD4) in Lisbon, the full rate had higher value than the split-rate, while the other cultivars remained the same or increased as RR Stratica (FD4) in Milnor. The higher the rate of K applied, the more K that is added to the soil solution exchange sites and non-exchangeable sites (Bertsch and Thomas, 1985; Goli-Kalanpa et al., 2008), therefore the available K for alfalfa is likely to increase.

Table 11. Average available K for alfalfa† for K treatment across cultivars with different fall dormancy (FD) in two locations in 2019 averaged across sampling time.

K_2O rate	Lisbon			Milnor		
(kg ha^{-1})	RR Presteez (FD3)	RR Stratica (FD4)	L-450 RR (FD5)	RR Presteez (FD3)	RR Stratica (FD4)	L-450 RR (FD5)
0†	142	147	157	208	203	198
168	270	303	295	353	321	381
168S	273	295	305	351	338	391
336	412	442	438	457	485	481
336S	424	422	439	456	469	477
LSD_1(0.05)	20.1					
LSD_2(0.05)	29.4					

†[((soil available K from soil tests (kg ha^{-1}) x bulk density x 15-cm soil sample depth)/10) + K added as fertilizer]. ‡Treatments 0=0 K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD_1= to compare available K for alfalfa between K treatment and cultivar means at the same location; LSD_2= to compare K treatment and cultivar means across locations.

4.3. Seasonal forage yield and plant height

There were significant effects found from K fertilization treatments for total seasonal forage yield in both 2019 and 2020 (Table 12). In 2019, location, stress, and location by K treatment by cultivar were significant. The only other interaction in 2020 aside from K treatment was K treatment by cultivar. In 2020, seasonal forage yield between locations was the same

($P \leq 0.05$). It was likely that the applied lime at Lisbon, increased the pH in 2020, which likely was the limiting factor for plant growth in 2019.

Table 12. Analysis of variance of alfalfa yield totals for two locations (Loc), three fall dormancies (Var), two harvest stress treatments and five K treatments (Trt) in 2019 and 2020.

		2019	2020
SOV	df	Mean square	Mean square
Rep	3	3.20	154.60
Loc	1	530.90*	154.60*
Rep(loc)	6	2.45	38.37
Var	2	11.18	15.93
Loc x var	2	5.46	12.75
Loc x var x rep	12	2.57	8.53
Trt	4	2.44*	12.12*
Loc x trt	4	1.48	1.67
Trt x var	8	3.62	5.02*
Loc x var x trt	8	6.13*	2.91
Stress	1	4.08*	0.73
Loc x stress	1	0.16	0.32
Var x stress	2	0.06	0.33
Trt x stress	4	0.23	0.70
Loc x var x stress	2	0.07	0.72
Loc x trt x stress	4	0.11	0.31
Trt x stress x var	8	0.09	0.61
Loc x var x trt x stress	8	0.06	1.01
Residual	162	0.24	2.33

* Significant at $P \leq 0.05$, level of probability

In 2019, the seasonal forage yield was greater than the control at 336 kg K_2O ha^{-1} (Fig. 7). It was expected that the K application would not increase forage yield in the establishment year since growth in the seeding year is limited. In 2020, the control with no K applied had the least seasonal forage yield.

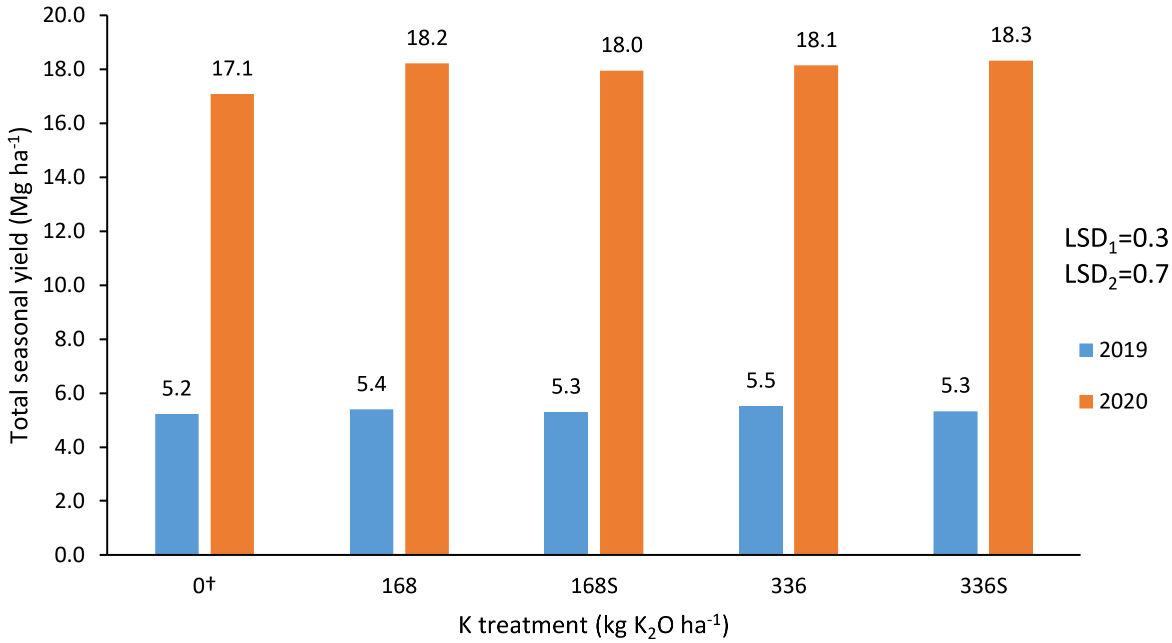

Fig. 7. Seasonal forage yield in 2019 and 2020 for five K treatments averaged across two locations, three cultivars, two harvest stress treatments, and harvest dates in 2019 and 2020. † Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest 2020, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD_1= to compare between K treatment means in 2019; LSD_2= to compare between K treatment means in 2020. $P \leq 0.05$.

In 2019, alfalfa seasonal forage yield was higher in Milnor than in Lisbon (Table 13). This could be explained by the lower pH at the Lisbon location. In spring of 2019, Milnor had an average pH of 6.82, where Lisbon had an average of 5.85. Though lime was applied in spring 2019, the pH was only increased to an average of 6.16 by spring 2020, which is still lower than ideal conditions for alfalfa (Undersander et al., 2011). A study by Popovic et al. (2008) found that dry matter yield was almost double in neutral soil (10 Mg ha^{-1} in pH 6.41) compared with acidic soils (5.4 Mg ha^{-1} in pH 3.88). Though the pH of the acidic soil in Popovic et al. (2008) was lower than this study, it supports the trend seen in the location difference for 2019. The highest forage seasonal yield at Lisbon was in cultivar L-450 RR (FD5) when applied 336 kg K_2O ha^{-1} at seeding (Table 13). A cultivar with FD5 would be expected to have the higher seasonal forage yield, due to its delayed to become dormant and higher biomass production in the fall compared with cultivars with FD4 and FD3.

Table 13. Seasonal forage yield total for two locations, five K treatments, and three cultivars with different fall dormancy (FD) in 2019 averaged across stress treatments.

	Lisbon			Milnor		
K_2O rate ($kg\ ha^{-1}$)	RR Presteez (FD3)	RR Stratica (FD4)	L-450 RR (FD5)	RR Presteez (FD3)	RR Stratica (FD4)	L-450 RR (FD5)
	--------------------------------Forage yield ($Mg\ ha^{-1}$)------------------------------					
0†	3.4	3.6	4.4	6.4	6.9	6.7
168	3.2	4.2	4.0	6.7	7.2	7.1
168S	3.7	3.7	4.3	6.3	7.4	6.5
336	3.8	4.0	4.5	7.1	7.0	6.7
336S	3.7	3.9	3.8	6.4	7.3	6.9
Location mean		3.9			6.9	
LSD_1(0.05)		0.6				
LSD_2(0.05)		0.7				

†Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD_1=to compare between K treatment and cultivar means within the same location; LSD_2=to compare between K treatment and cultivar means across different locations.

In 2020, location was not significant for seasonal forage yield, but there was an interaction between treatment and cultivar (Fig. 8). Cultivar RR Stratica (FD4) had the highest seasonal forage yield in all treatments, except for the 168 kg K_2O ha^{-1} applied in full. This cultivar is a new release from CROPLAN® and it likely has a greater forage yield potential than the other two cultivars. CROPLAN® description of this cultivar indicates *"RR Stratica – One of the highest yielding FD 4 varieties in the marketplace. Provides an excellent choice for haylage or aggressive hay production in the Upper Midwest and outstanding disease resistance for the Midwest and East."*

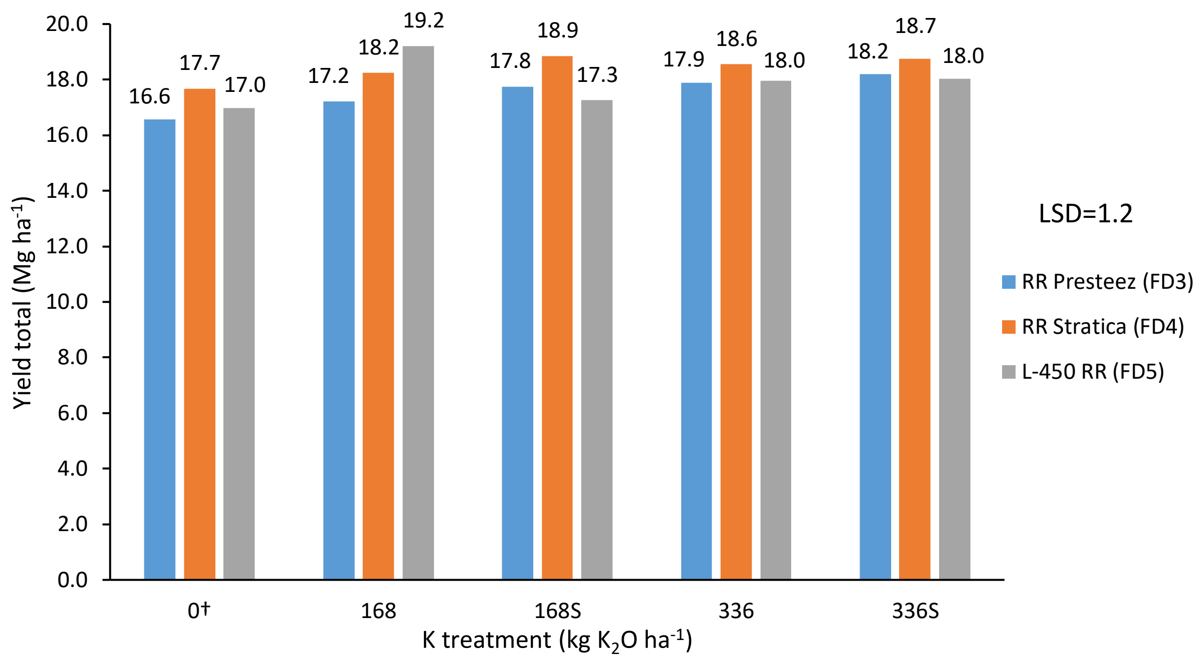

Fig. 8. Seasonal forage yield in 2020 for five K treatments and three cultivars of different fall dormancy (FD),averaged across two location and two stress treatments. †Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at first harvest, 168S=split-application, 84 kg K_2O ha^{-1} applied at first cut, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at first harvest; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD= to compare seasonal forage yield between K treatment means across cultivars. $P \leq 0.05$.

In 2019, the seasonal forage yield for the stressed treatments was lower than for the non-stressed treatments, 5.2 and 5.5 Mg ha^{-1}, respectively. This, however, was probably due to stressed treatments being harvested about one month earlier. When postponing the fall harvest until October, alfalfa plants kept growing, especially the L-450 RR (FD5) cultivar.

For further analysis, forage yield and plant height were analyzed at each harvest in 2019 and 2020. In 2019, location, cut, and location by cut were significant for forage yield, while in 2020, treatment, cut, and location by cut were significant (Table 14). For plant height significant effects were the same as for forage yield effects in both years with the exception of 2020, where other significant interactions were detected.

Table 14. Mean square values for alfalfa forage yield and plant height in each cut for 2019 and 2020 growing season for five K treatments, three cultivars, and two locations, Milnor and Lisbon, ND.

SOV	df	2019 Yield	2019 Height	df	2020 Yield	2020 Height
Rep	3	0.9	39.6	3	5.2	51.0
Loc	1	144.2*	12191.0*	1	23.8	226.6*
Rep(loc)	6	0.8	22.5	6	4.3	36.5
Var	2	2.2	22.1	2	3.2	72.2
Loc x var	2	0.7	7.0	2	1.6	5.4
Loc x var x rep	12	0.7	16.6	12	1.3	19.3
Trt	4	0.2	20.1*	4	2.0*	122.7*
Loc x trt	4	0.1	3.2	4	0.2	1.8
Trt x var	8	0.1	1.8	8	0.7	4.1
Loc x var x trt	8	0.2	2.1	8	0.3	3.8
Cut	2	113.2*	36.6*	4	107.6*	1337.9*
Loc x cut	2	16.5*	187.9*	4	5.9*	24.2*
Var x cut	4	0.1	5.9	8	0.8	8.7*
Trt x cut	8	0.2	5.0	16	0.6	4.2
Loc x var x cut	4	0.2	4.3	8	0.8	6.1*
Loc x trt x cut	8	0.1	2.1	16	0.6	2.6
Trt x cut x var	16	0.1	1.1	32	0.5	1.0
Loc x trt x cut x var	16	0.2	1.8	32	0.7	2.5
Residual	252	0.1	2.9	432	0.8	2.8

* Significant at $P \leq 0.05$, level of probability.

The first harvest of 2019 had the highest seasonal forage yield at both locations (Fig. 9), which is expected for the first harvest of alfalfa in the seeding year. The stressed harvest treatment, had significantly higher forage yield than the non-stressed harvest treatment at both locations.

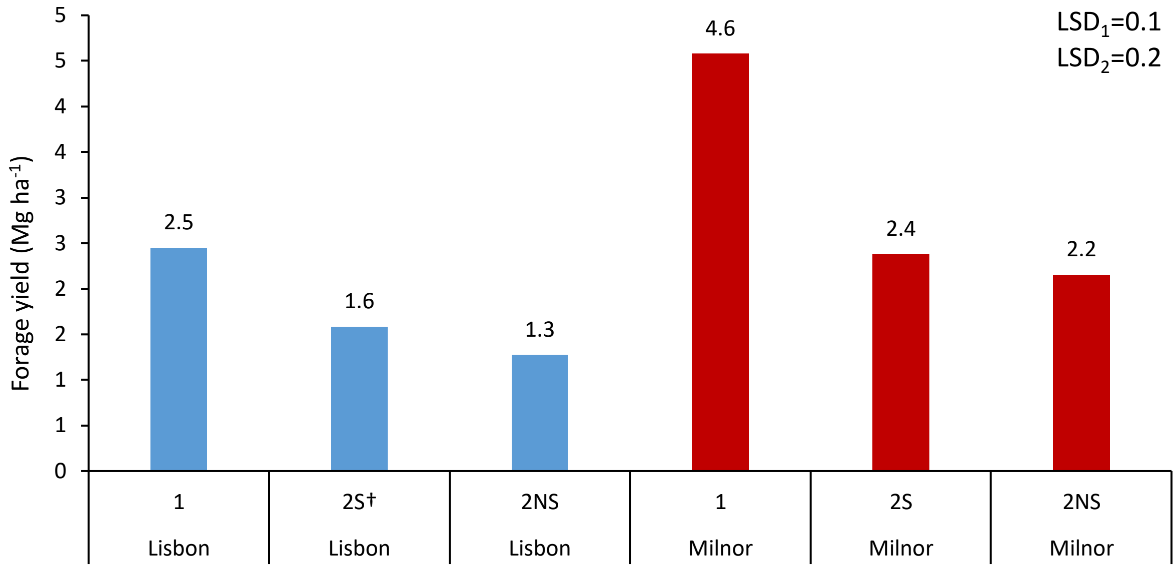

Fig. 9. Forage yield at each harvest (cut) for 2019 growing season at two locations averaged across five K treatments and three cultivars. †S indicates fall harvest as stressed treatment, NS indicates fall harvest as non-stressed treatment. LSD_1= to compare forage yield between cut means within the same location; LSD_2= to compare forage yield between cut means across locations. $P \leq 0.05$.

In 2020, the control with no K application had significantly lower forage yield that all K rates in full or in split-application (Fig. 10). These are similar to results found by Berg et al. (2018) where application of K alone significantly increased forage yield from the control, but forage yield was not significant among K rates. In 2020, K fertilization increased seasonal forage yield across locations and cultivars only to a rate of 168 kg K_2O ha^{-1}. Split application or higher rates did not have a yield response. Both locations had very low-to-low K soil levels of 76-79 mg kg^{-1} in Lisbon and 87-100 mg kg^{-1} in Milnor, in which a response to K application was expected. A rate of 168 kg K_2O ha^{-1} is the recommended rate for soil tests of 51-100 mg kg^{-1} and a soil with > 3.5 smectite to-illite (Franzen and Berti, 2017). Thus, the results of this research confirm the recommended rate increases alfalfa forage yield in the first production year.

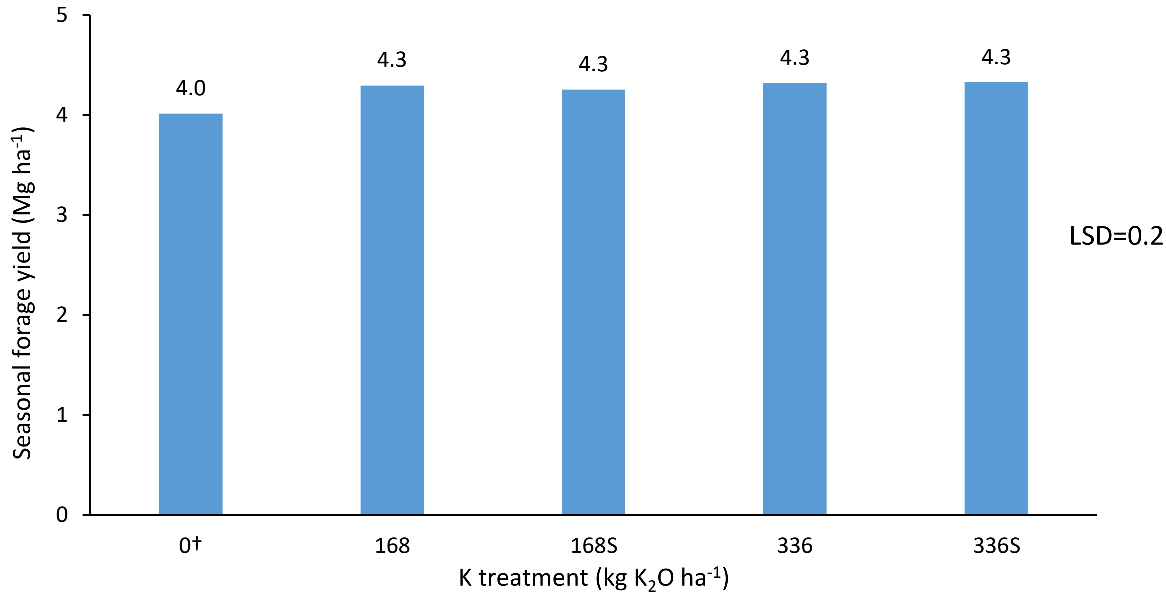

Fig. 10. Seasonal alfalfa forage yield in 2020 for five K treatments averaged across two locations, three cultivars, and five harvest times. †Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at first harvest, 168S=split-application, 84 kg K_2O ha^{-1} applied at first cut, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at first harvest; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD= to compare between K treatment means in 2020. $P \leq 0.05$.

As in 2019, the first harvest of 2020 had the highest forage yield at both locations (Fig. 11), which is similar with other studies finding highest alfalfa forage yield in the first harvest (Lissbrant et al., 2009; Kallenbach et al., 2002). Unlike the seeding year, the differences in forage yield between the stressed and non-stressed harvests were not significant. This is important to note, because it supports the opportunity for harvesting the final harvest of alfalfa until October, without losing significant forage yield.

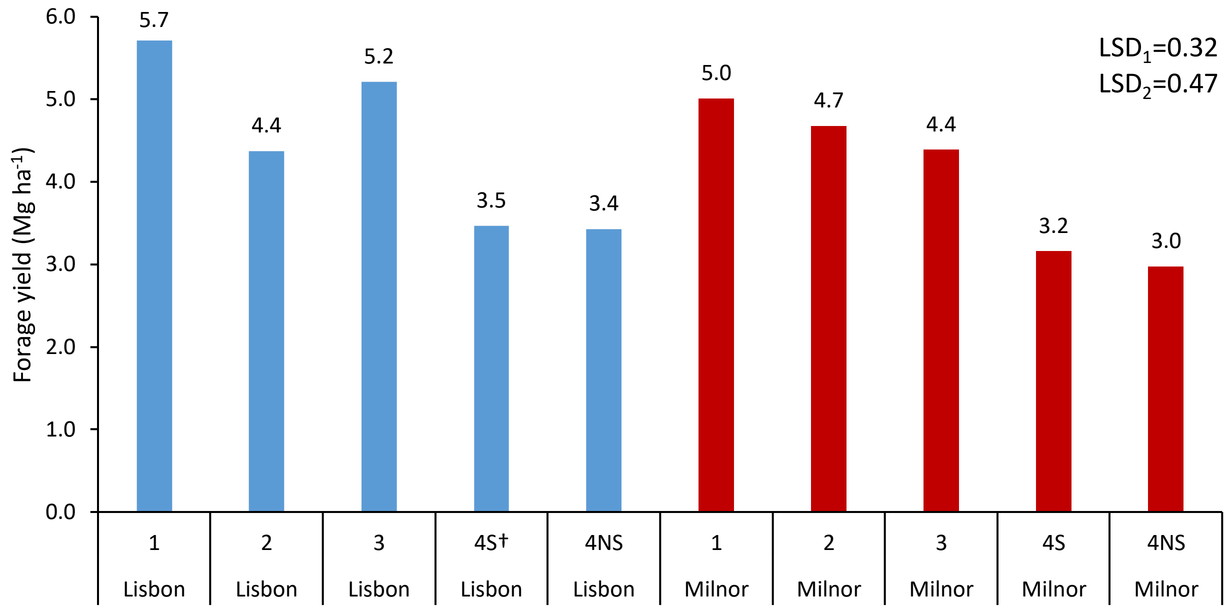

Fig. 11. Forage yield at each harvest (cut) in 2020 at two locations averaged across three cultivars and five K treatments. †S indicates fall harvest as stressed treatment. NS indicates fall harvest as non-stressed treatment. LSD_1=to compare between cut means within the same location; LSD_2=to compare between cut means across locations. $P \leq 0.05$.

In 2019, the treatments with the highest plant height were the split-application timing, at rates of 168 kg K_2O ha^{-1} and 336 kg K_2O ha^{-1}. The alfalfa with the shortest plant height was alfalfa in the control with no K applied. This suggests that supplying a more consistent rate of K throughout the seeding year can increase alfalfa plant height. In 2020, the alfalfa with the lowest plant height was again in the control with no K applied (Fig. 12). The highest average alfalfa plant height was in the treatments with the highest rates of K applied, both split-application and in total at first harvest. This is supported by findings of Kitchen et al. (1990) that also found that an increase in K fertilization increases plant height in alfalfa.

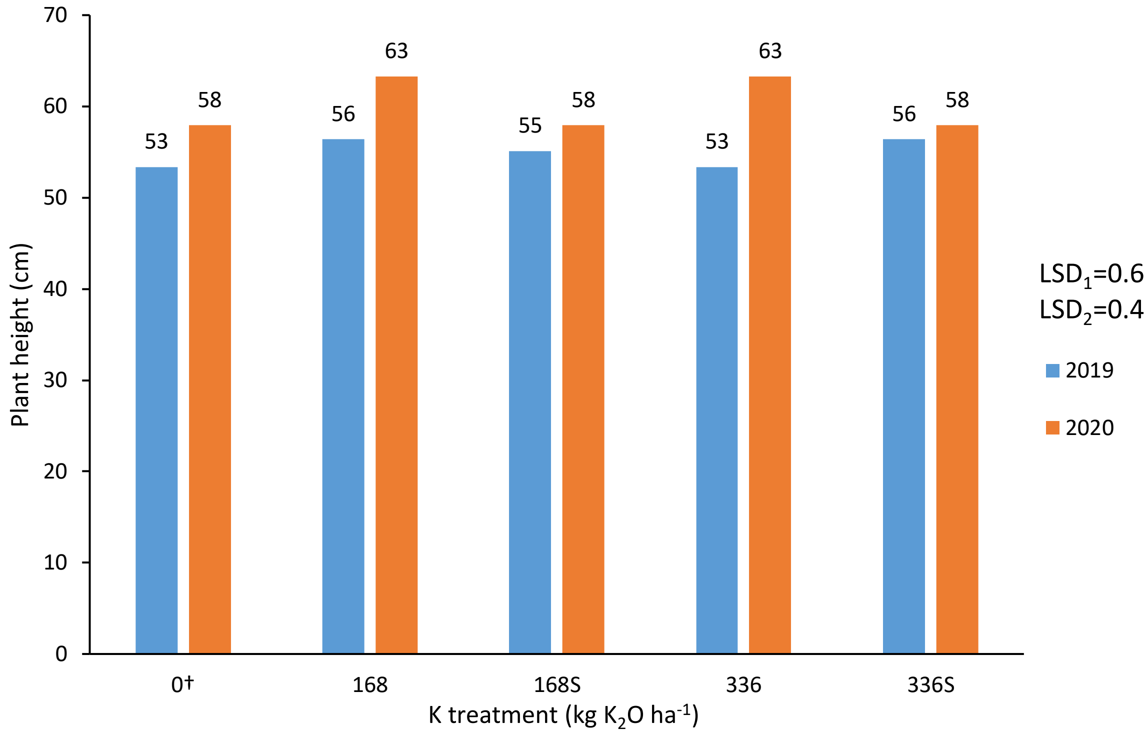

Fig. 12. Plant height and K treatments averaged across two locations, three cultivars, and two harvests in 2019 and four harvests in 2020. †Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD_1= to compare between K treatment means in 2019. LSD_2= to compare between K treatment means in 2020. $P \leq 0.05$.

Plant height of cultivar L-450 RR (FD5) cultivar was significantly higher than RR Presteez (FD3) and RR Stratica (FD4) in Lisbon on the harvest date of 12 October 2020 (Table 15). This was an expected result. Cultivar L-450 RR is rated with FD5, which is the least dormant cultivar of the ones tested in this study. Fall dormancy 5 cultivars produce more biomass in the fall as dormancy is delayed to October instead of September. Jungers et al. (2019) that also found higher FD scores had more fall growth. Cultivar RR Presteez is a FD3 cultivar and was the shortest of all cultivars at all harvest times in Lisbon, and on three of the harvest dates in Milnor. Volenec (1985) reported that more dormant cultivars have reduced shoot elongation after a harvest. What was surprising at Milnor was that RR Presteez (FD3) was not the shortest height on 12 October, when the cooler temperatures and shorter day length would signal less growth for lower FD ratings (Jungers et al., 2019).

Table 15. Alfalfa plant height at each harvest in 2020 for three cultivars of different fall dormancy (FD) at two locations averaged across five K treatments and two harvest stress treatments.

Location	Harvest date	RR Presteez (FD3)	RR Stratica (FD4)	L-450 RR (FD5)
Lisbon		---------------------------	cm	---------------------------
	2 Jun.	59.3	59.7	61.5
	30 Jul.	60.5	62.1	61.1
	11 Aug.	74.7	75.3	77.5
	15 Sept.	51.3	55.9	56.4
	12 Oct.	50.6	52.9	56.0
Milnor		---------------------------	cm	---------------------------
	1 Jun.	64.5	66.6	62.6
	29 Jul.	58.3	61.8	62.3
	11 Aug.	75.3	78.2	80.3
	15 Sept.	55.8	61.6	60.0
	16 Oct.	57.9	57.7	58.9
	$LSD_1(0.05)$	0.9		
	$LSD_2(0.05)$	1.3		
	$LSD_3(0.05)$	1.4		

LSD_1= to compare between harvest date means within same cultivar and at same location; LSD_2= to compare heights between harvest date means across cultivars and at same location; LSD_3= to compare between harvest date means within same or different cultivars across locations. $P \leq 0.05$.

4.4. Biomass K removal and K balance

Aboveground biomass K removal was significantly affected by location, K treatment, and harvest stress in 2019 and 2020 (Table 16). In 2019, biomass K removal was significant for location by cultivar by K treatment. In 2020, biomass K removal was significant for K treatment by cultivar. The K balance, calculated as the available K from SAK and fertilizer, greater or lower than the sum of K taken up by the alfalfa biomass and K in the fall soil test was significantly affected by K treatment, K treatment by location, and K treatment by cultivar by location, in both 2019 and 2020. It was hypothesized that location would have an effect on the biomass K removal and soil K due to Milnor having the greater smectite-to-illite ratio and expecting higher fixation of K, therefore less biomass K removal and higher K levels with unknown fate in the fall probably masked the clay mineralogy expected effect (Fig. 13).

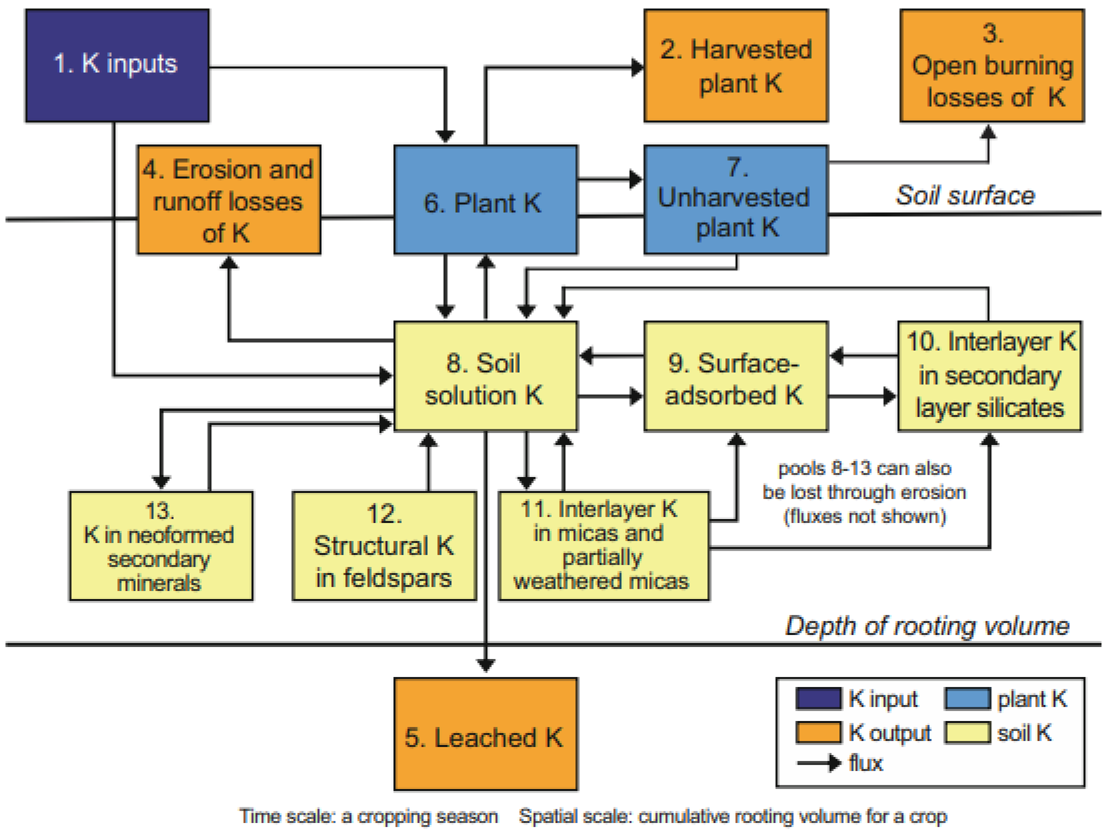

Fig. 13. Sources of soil K and interactions between sources from Franzen et al. (2021).

Table 16. Analysis of variance of aboveground biomass K removal (Bio K) and K balance for two locations (Loc), five K treatments (Trt), fall harvest stress (Stress), and three cultivars (Var) in 2019 and 2020.

SOV	df	2019		2020	
		Bio K	K balance	Bio K	K balance
Rep	3	69	15660	22037	228159
Loc	1	165039*	10689	214443*	594
Loc(rep)	3	1756	20687	13322	455981
Var	2	2788	12548	10800	1185
Loc x var	2	878	2196	5481	62285
Loc x var x rep	12	1330	7545	4541	44432
Trt	4	3552*	226743*	67070*	59063*
Loc x trt	4	289	7093*	1133	51505*
Var x trt	8	241	3048	2772*	21834
Loc x var x trt	8	428*	4601*	2488	29390*
Stress	1	5421*	5420	15191*	15177
Loc x stress	1	1	1	16	16
Var x stress	2	30	30	180	180
Trt x stress	4	144	145	351	351
Loc x var x stress	2	22	22	337	337
Loc x trt x stress	4	67	67	118	119
Var x trt stress	8	53	53	215	216
Loc x var x trt x stress	8	32	32	421	421
Residual	162	129	1610	1368	12191

* Significant at $P \leq 0.05$, level of probability

Biomass K removal by alfalfa was significantly higher in Milnor than in Lisbon in 2019 (Fig. 14). The higher biomass K removal is connected to the higher forage yield, causing higher nutrient demand (Franzen and Berti, 2017). In 2019, alfalfa in Lisbon had lower forage yield, likely caused by the low pH of 5.85, compared with alfalfa in Milnor with a pH at 6.82, which is lower than ideal conditions for alfalfa (Undersander et al., 2011). In 2020, alfalfa in Lisbon had higher forage yield than in Milnor and therefore removed higher amounts of K in the biomass. This supports the change in biomass K removal across locations from 2019 to 2020.

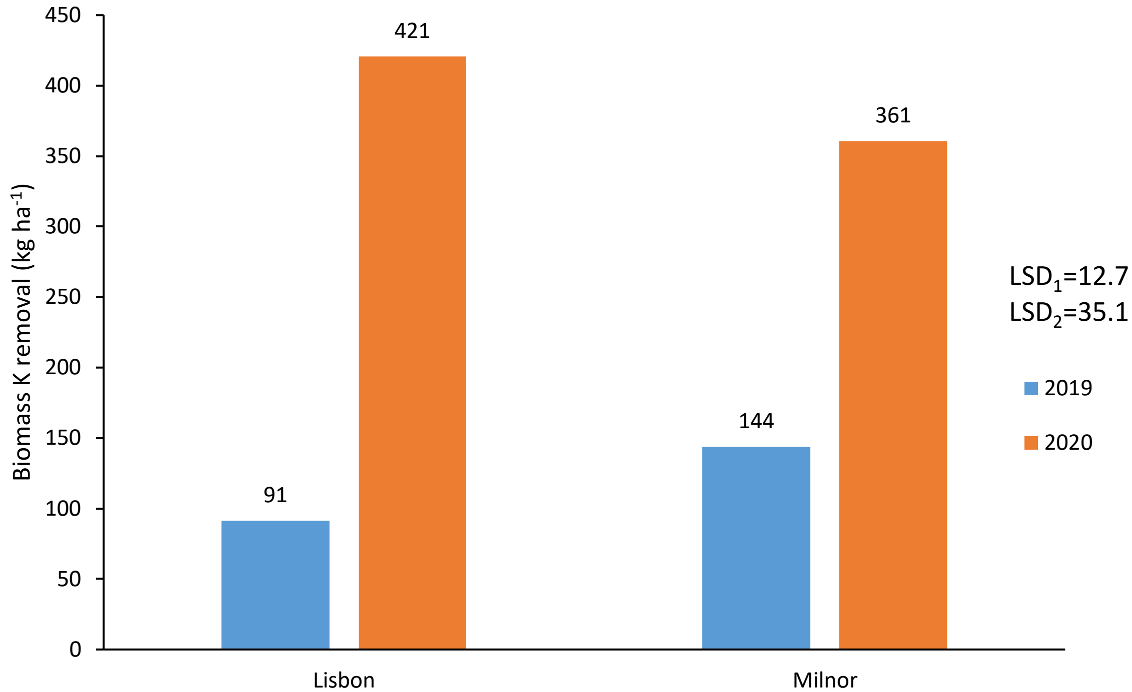

Fig. 14. Aboveground biomass K removal in 2019 and 2020 at two locations averaged across five K treatments, three cultivars, and two harvest stress treatments. LSD_1= to compare between location means in 2019; LSD_2= to compare between location means in 2020. $P \leq 0.05$.

In 2019, the alfalfa with applied 336 kg K_2O ha^{-1} at seeding had the highest amount of K removed from the biomass (Fig. 15). The split-application treatments did not significantly remove more K in the biomass than the control. The 168 kg K_2O ha^{-1} rate in full at seeding had higher biomass K removal than all other K treatments including the control. In 2020, the timing of application did not affect the biomass K removal.

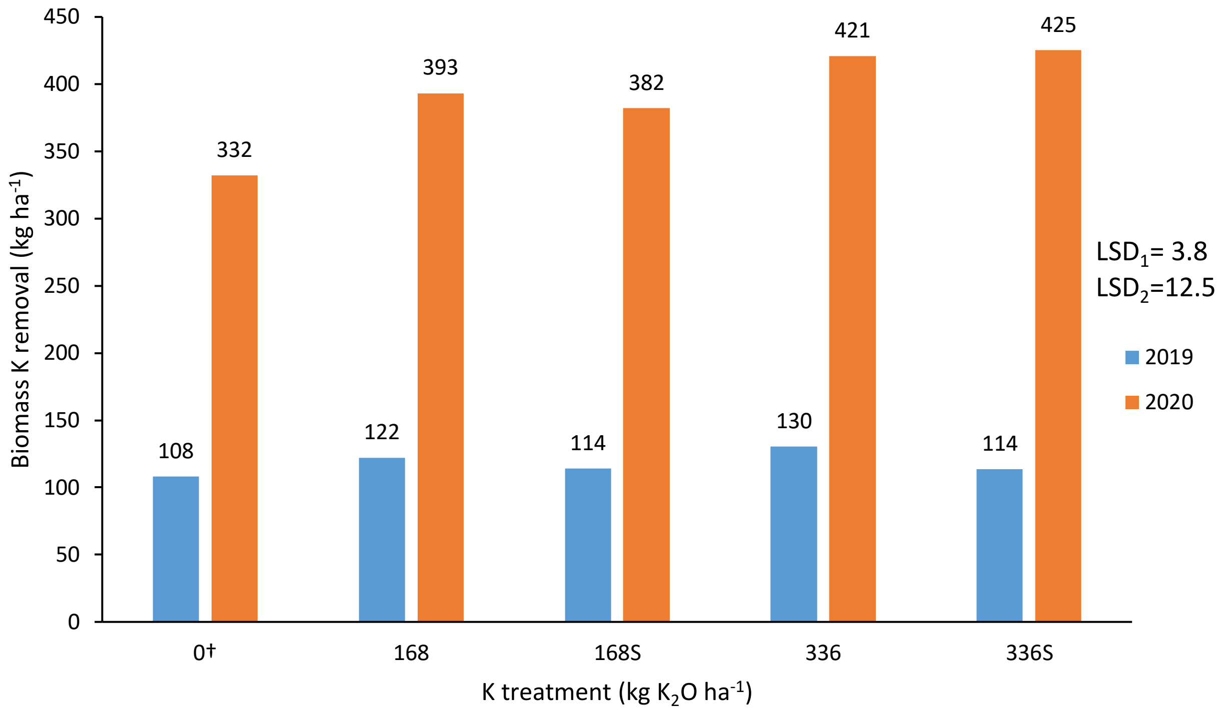

Fig. 15. Aboveground biomass K removal for five K treatments in 2019 and 2020 averaged across two locations, three cultivars, two harvest stress treatments, and sampling times. †Treatments, 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD_1= to compare between K treatment means in 2019; LSD_2= to compare between K treatment means in 2020. $P \leq 0.05$.

Alfalfa that was stressed by harvesting mid-September had significantly higher aboveground biomass removal of K than the non-stressed in both 2019 and 2020 (Fig. 16). This could be attributed to the higher forage yield in the harvest stress treatment compared with non-stressed. The later the fall harvest, the more mature the alfalfa. As alfalfa matures, lower leaves fall off due to senescence from shading and disease within the canopy (Albrecht et al., 1987; Buxton et al., 1985; Sheaffer et al., 1988; Grev et al., 2020). The harvest in mid-September also occurs prior to the acclimation for dormancy, before plants are able to translocate K from aboveground biomass to the taproot (Lu et al., 2018).

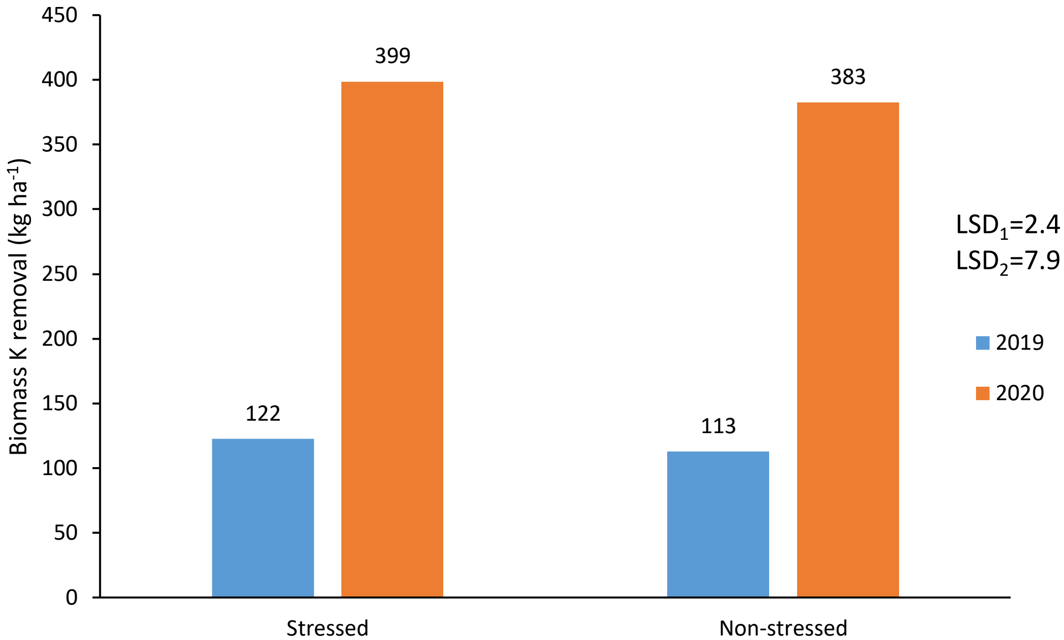

Fig. 16. Aboveground biomass K removal in 2019 and 2020 for two harvest stress treatments averaged across two locations, five K treatments, sampling times, and three cultivars. LSD_1= to compare between stress treatment means in 2019; LSD_2= to compare between stress treatment means in 2020. $P \leq 0.05$.

In 2019, aboveground biomass K removal had a significant interaction with K treatment across cultivars and locations (Table 17). The cultivar RR Presteez (FD3) had the lowest biomass K removal with no K fertilization at both locations. Lower fall dormancy rating means alfalfa regrowth in the fall is less, which explains the lower K removal. The full rate of 336 kg K_2O ha^{-1} was significantly higher than the control with no fertilization in all cultivars at both locations (Table 17). This response was expected since the full rate of 336 kg K_2O ha^{-1} had significantly higher total seasonal forage yield from the control in 2019. Since the aboveground biomass K removal calculation entails forage yield, higher yield increases biomass K removal. Also, higher K rates increase available K^+, giving plants potential for luxury consumption.

Table 17. Aboveground biomass K removal for five K treatments, three cultivars of different fall dormancy (FD) at two locations averaged across harvest stress treatments in 2019.

	Lisbon			Milnor		
K_2O rate (kg ha^{-1})	RR Presteez (FD3)	RR Stratica (FD4)	L-450 RR (FD5)	RR Presteez (FD3)	RR Stratica (FD4)	L-450 RR (FD5)
	---------------------------Biomass K removed kg ha^{-1}-------------------------					
0†	77	81	96	126	135	135
168	75	104	98	146	154	155
168S	84	85	101	130	151	134
336	95	104	112	162	157	152
336S	84	90	87	131	150	141
LSD_1 (0.05)	9					
LSD_2 (0.05)	17					
LSD_3 (0.05)	16					

†Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD_1=to compare between K treatment means within same cultivar and location; LSD_2= to compare between K treatment means within same cultivar and across locations. LSD_3= to compare between K treatment means for different cultivars and within same or different locations.

In 2020, K treatment by cultivar interaction was significant for biomass K removal (Fig. 17). Biomass K removal was the same ($P \leq 0.05$) for the 336 kg K_2O ha^{-1} rate in full or split-application for all cultivars. The cultivar L-450 RR (FD5) with 168 kg K_2O ha^{-1} in split-application had significantly lower biomass K removal than when applied in full. Since L-450 RR cultivar (FD5) would be expected to have more growth in the fall (Jungers et al., 2019), therefore the 168 kg K_2O ha^{-1} in full at first cut allowed for more K to be removed throughout the 2020 season.

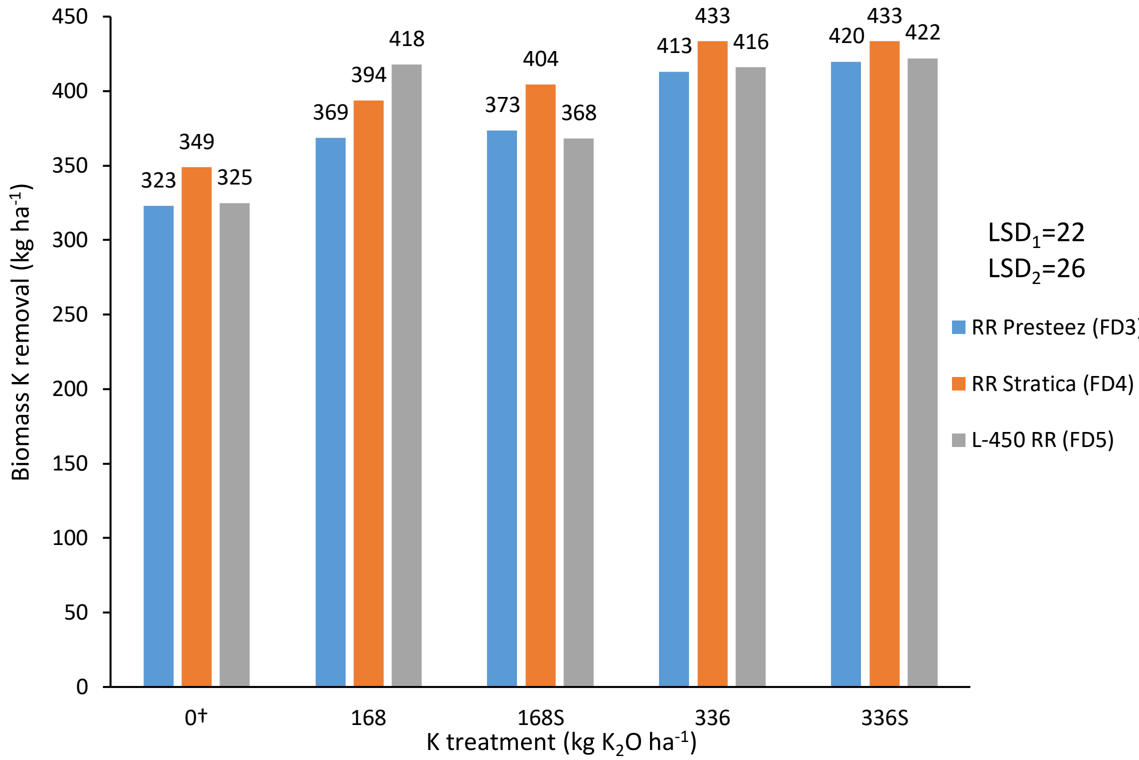

Fig. 17. Aboveground biomass K removal in 2020 for five K treatments and three cultivars with different fall dormancy (FD), averaged across two locations and harvest stress treatments. †Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD_1= to compare between K treatment means within same cultivar; LSD_2= to compare between K treatment means across cultivars. $P \leq 0.05$.

The further investigate the amount of soil K+ being removed via aboveground biomass throughout the growing season, the aboveground biomass removal was analyzed at each harvest (Table 18). There was significant interaction between aboveground biomass K with location, K treatment, cut, and location by cut in both 2019 and 2020, as well as an interaction of K treatment by cut in 2019 alone.

Table 18. Analysis of variance of aboveground biomass K removed (Bio K) at each harvest (Cut) for two locations (Loc), five K treatments (Trt), and three cultivars (Var) in 2019 and 2020.

	2019		2020	
SOV	df	MS	df	MS
Rep	3	49	3	2210
Loc	1	40088*	1	27095*
Loc(rep)	3	630	3	1302
Var	2	1035	2	1920
Loc x var	2	174	2	602
Loc x var x rep	12	351	12	652
Trt	4	923*	4	9799*
Loc x trt	4	45	4	196
Var x trt	8	58	8	341
Loc x var x trt	8	94	8	244
Cut	2	54139*	4	89759*
Loc x cut	2	8777*	4	3200*
Var x cut	4	36	8	331
Trt x cut	8	298*	16	405
Loc x var x cut	16	73	8	394
Loc x trt x cut	8	85	16	385
Var x trt x cut	16	48	32	317
Loc x var x trt x cut	16	73	32	403
Residual	251	71	432	459

* Significant at $P \leq 0.05$, level of probability

In 2019, Milnor removed higher biomass K than Lisbon at each harvest time (Fig. 18). This was likely caused by the lower yield potential at Lisbon with the acidic soil (Popovic et al., 2008). At both locations, the biomass K removal was highest at the first harvest in July. The beginning of the growing season also has higher yield potential (Lissbrant et al., 2009; Kallenbach et al., 2002).

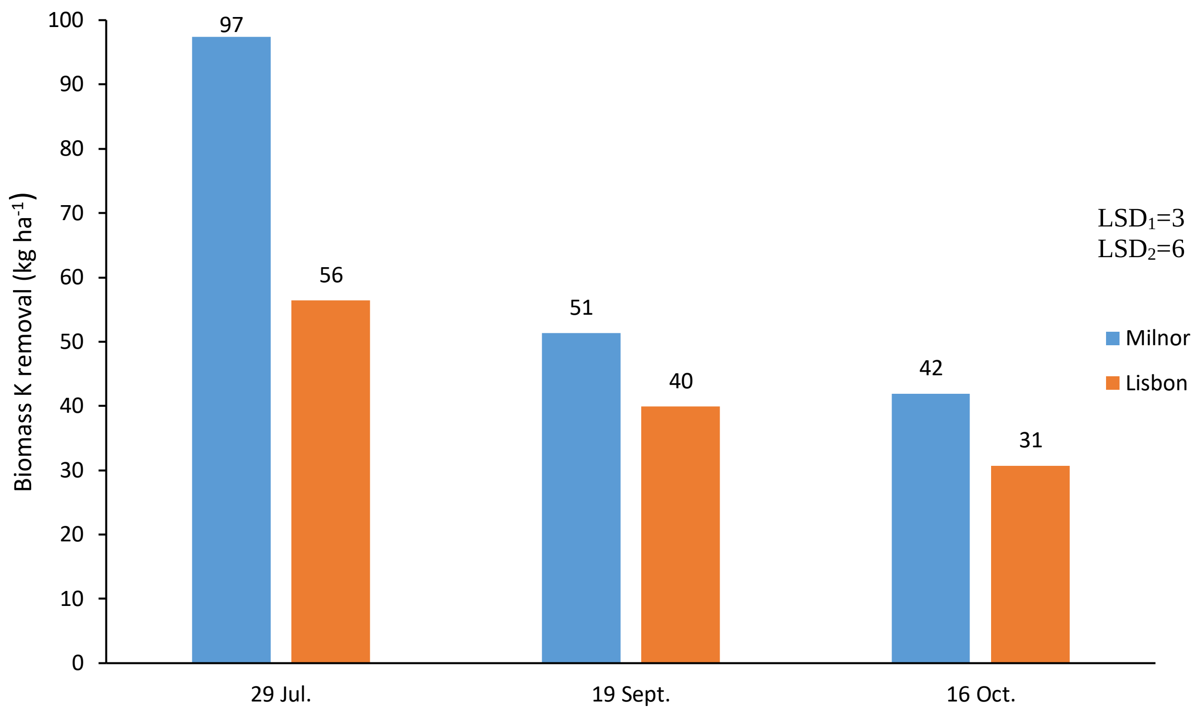

Fig. 18. Aboveground biomass K removal in 2019 for three harvest dates at each location averaged across five K treatments and three varieties of different fall dormancy. LSD_1= to compare between harvest date means within same location; LSD_2= to compare between harvest date means across cultivars. $P \leq 0.05$.

In 2020, the K_2O treatment and cut were both significant, however, K_2O treatment by cut was not. As in 2019, the first cut in 2020 had the highest yield, and therefore the highest biomass K removal (Fig.19). The trend of highest K removed from biomass at first cut supports the split-application timing of applying at that time, in order to replenish the higher amounts removed from the soil.

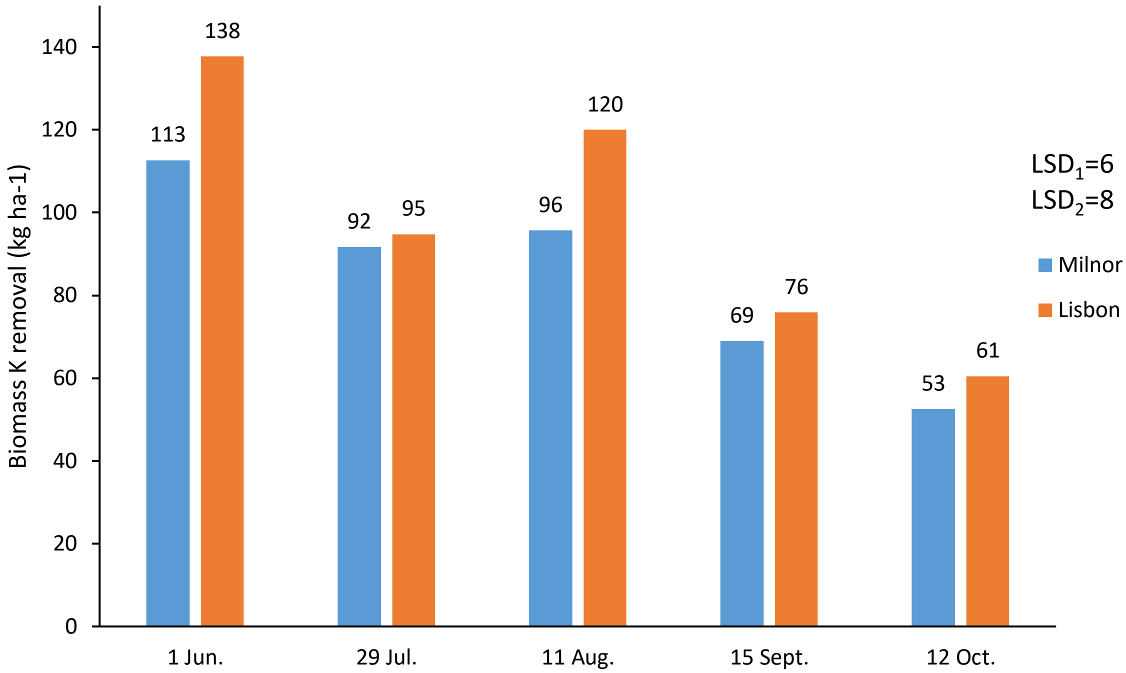

Fig. 19. Aboveground biomass K removal in 2020 for five harvest dates at each location averaged across five K treatments and three varieties of different fall dormancy. LSD_1= to compare between harvest date means within same location; LSD_2= to compare between harvest date means across cultivars. $P \leq 0.05$

Higher rates of K_2O applied in the growing season had higher biomass K removal for each harvest in 2019 (Fig. 20). Also, rates in full had higher removal than split-application, except for 16 October harvest, where 336 kg K_2O ha^{-1} split-application was higher than in full, though, the difference was not significant. This implies that the plant will take up more soil K for luxury consumption, when available.

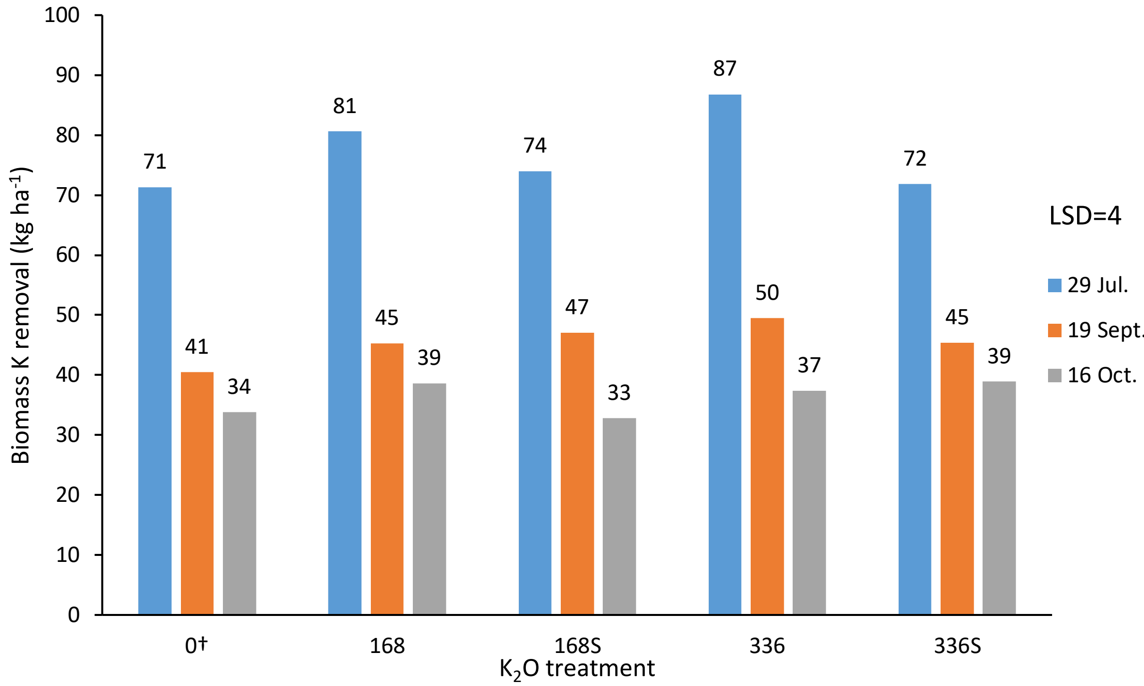

Fig. 20. Aboveground biomass K removal in 2019 for five K treatments at three harvest dates averaged across two locations and three varieties of different fall dormancy. LSD= to compare between K treatment means within same and different harvest dates. $P \leq 0.05$.

In 2020, similar trends were seen where higher rates of K_2O applied had higher biomass K removal (Fig. 21). This continues to support that when there's more available K^+ in the soil, the plant will take up more K into the biomass. The timing of split-application compared to in full was not significantly different within the same treatment.

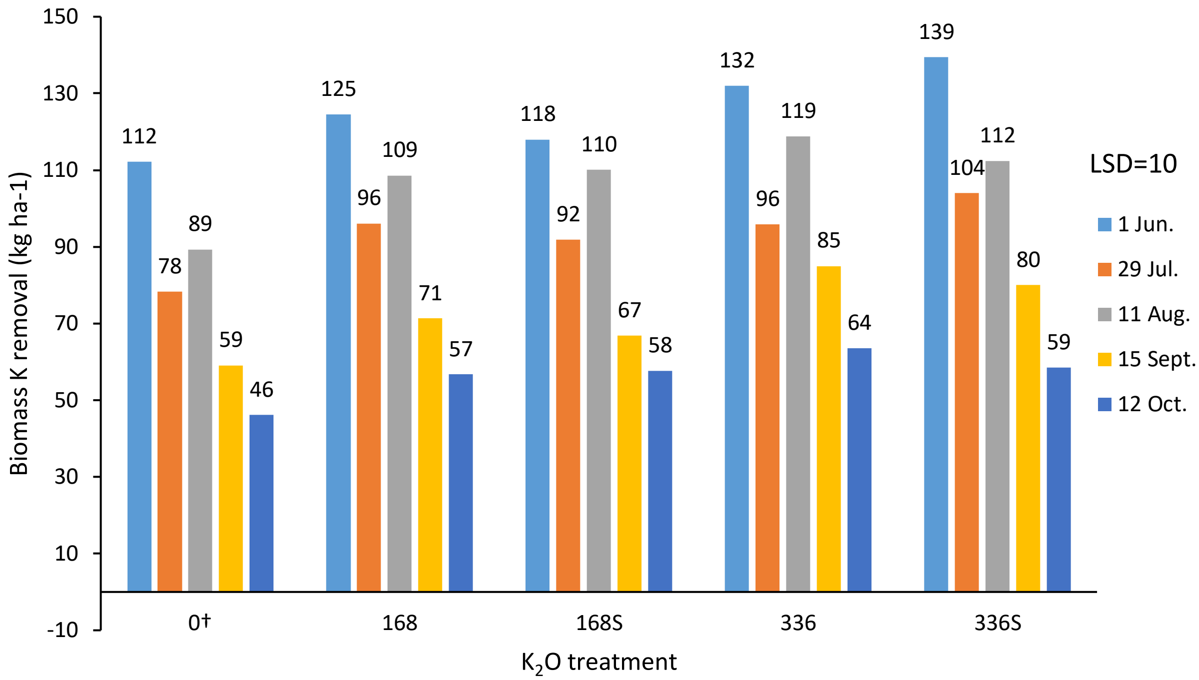

Fig. 21. Aboveground biomass K removal in 2020 for five K treatments at three harvest dates averaged across two locations and three varieties of different fall dormancy. LSD= to compare between K treatment means within same and different harvest dates. $P \leq 0.05$.

In 2019, all fertilized treatments at both locations showed that alfalfa growth in the first year was not able to take up all the K^+ available in the soil and the K added as fertilizer (Fig. 22). In Lisbon, the highest fertilizer rates, in split- or full-application, had the most K that was not removed by the biomass or in the soil test in the fall. This can be explained for some movement of K^+ below the 15-cm layer or by the clays fixing K^+ (Fig. 13). The first would be more likely since fall of 2019 was extremely wet, receiving 18-cm of rainfall in September and October, and leaching of K^+ below 15-cm would have been likely since the soil has a sandy-loam texture. In addition, K^+ is more likely to be fixed when the soil is drier trapping K^+ between layers.

The spring soil K^+ plus the K added from fertilizer, was lower than the sum of the K removed by alfalfa plus the fall soil K levels, in the control treatment in 2019 in Lisbon and all K treatments in both locations in 2020 (Fig. 22). These treatments had a negative K balance, indicating that there was more K^+ available for alfalfa than that shown in the soil tests plus the K added as fertilizer. This negative value is not surprising, though, because the soil K^+ was sampled at a depth of 0-15 cm and it would be expected that alfalfa taproots were longer than 15-cm and able to extract K^+ from deeper in the soil, especially in 2020. In addition, it is likely that part of the K fertilizer applied in 2019 moved below the 15-cm layer and it might account for some of the K in the negative balance of 2020 for fertilized treatments. It was expected that the Milnor location soil would have higher amounts of unaccounted K due to its high smectite-to-illite clay ratio. Though the interaction of K treatment by location was significant, there was no significant difference between locations within the same treatment (Fig. 22). This suggests that Milnor did not significantly have more K^+ being fixed than at the Lisbon location, at least not in the 15-cm of top soil tested.

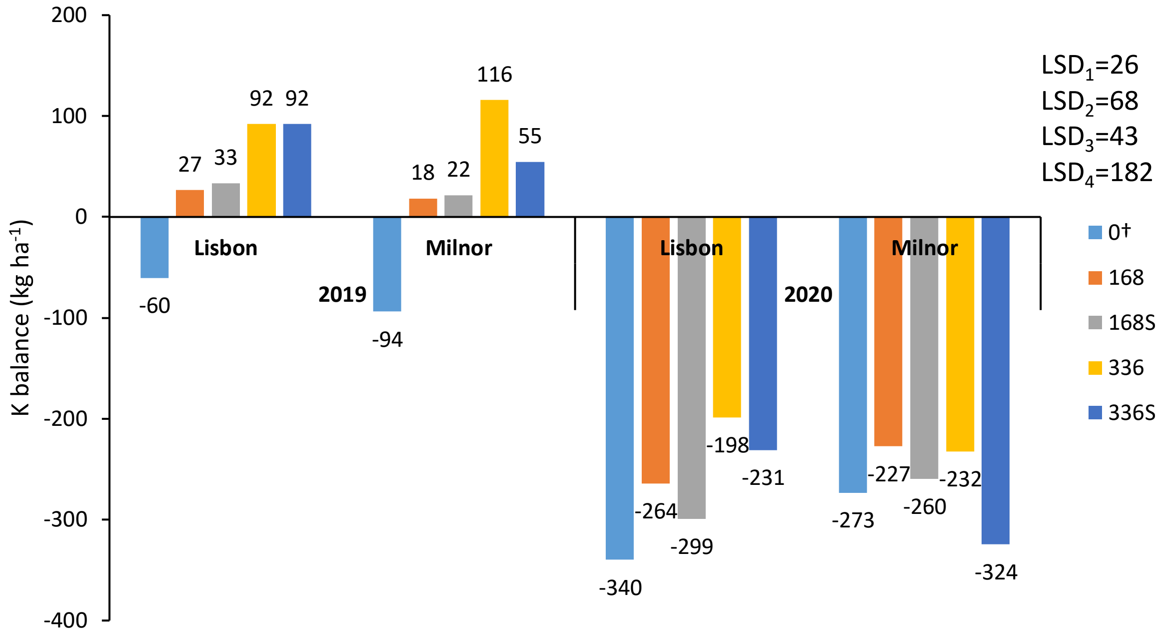

Fig. 22. K balance at the end of 2019 and 2020 growing season for five K treatments in two locations averaged across three cultivars and two harvest stress treatments. †Treatments, 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding in 2019 and first harvest in 2020; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD_1= to compare between K treatment means within same location in 2019; LSD_2= to compare between K treatments means across locations in 2019; LSD_3= to compare between K treatment means within same location in 2020; LSD_4= to compare between K treatment means across locations in 2020. $P \leq 0.05$.

4.5. Forage nutritive value

Location and cut had significant interactions with CP, ADF, NDF, NDFD, TDN, and RFQ in 2019 and 2020 (Table 19 and 20). In both years, the K treatment effect was significant for all mentioned parameters, except for CP in 2020 and NDFD in both years. Cultivar was significant across all nutritive value parameters in 2019, and significant for ADF, NDF, and RFQ in 2020.

Table 19. Analysis of variance for alfalfa forage nutritive value analysis for two locations (Loc), three cultivars (Var), five K treatments (Trt) and three harvest times (Cut) in 2019 at Lisbon and Milnor, ND.

SOV	df	CP†	ADF	NDF	NDFD	TDN	RFQ
Rep	3	4.6	13.4	13.0	5.7	13.7	419.4
Loc	1	766.9*	3682.6*	3015.4*	908.7*	3161.9*	103510.0*
Rep(loc)	6	5.3	18.7*	22.5*	7.0	13.4	584.9*
Var	2	21.1*	125.9*	157.8*	20.2*	87.7*	4248.7*
Loc x var	2	3.3	4.7	5.1	0.7	2.2	310.6
Loc x var x rep	12	2.5*	4.6	3.9	3.9*	5.0*	137.3
Trt	4	12.9*	36.2*	47.0*	3.0	44.2*	1316.1*
Loc x trt	4	0.8	1.4	1.2	2.3	4.0	29.7
Var x trt	8	0.7	2.5	2.6	1.7	2.5	110.1
Loc x var x trt	8	1.0	2.1	2.4	1.3	1.6	77.1
Cut	2	240.7*	2960.0*	2440.9*	3328.4*	2404.7*	120863.0*
Loc x cut	2	4.5*	100.0*	43.0*	77.4*	83.7*	1367.9*
Var x cut	4	0.5	2.0	2.0	4.5*	4.7	102.1
Trt x cut	8	0.9	2.7	4.3	2.1	6.9*	243.6*
Loc x var x cut	4	1.3	1.8	1.9	0.1	0.9	35.2
Loc x trt x cut	8	1.2	2.4	2.2	0.3	1.1	52.9
Trt x cut x var	16	0.4	1.2	1.3	0.7	0.9	36.6
Loc x trt x cut x var	16	1.0	4.1	4.3	1.1	3.0	124.4
Residual	252	1.2	3.2	3.0	1.3	2.6	88.5

* Significant at $P \leq 0.05$, level of probability †Quality parameters: crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF),), neutral detergent fiber digestibility (NDFD), total digestible nutrients (TDN), relative forage quality (RFQ).

Table 20. Analysis of variance and mean squares for alfalfa quality analysis for two locations (Loc), three cultivars (Var), five K treatments (Trt) and five harvest times (Cut) in 2020 at Lisbon and Milnor, ND.

SOV	df	CP†	ADF	NDF	NDFD	TDN	RFQ
Rep	3	1.3	21.0	31.3	8.5	7.5	585.1
Loc	1	335.8	1570.5*	1026.2*	273.1*	1156.2*	40758.0*
Rep(loc)	6	3.8	11.7	19.0	10.4	6.1	365.3
Var	2	5.8	86.6*	127.8*	23.3	45.4	3483.5*
Loc x var	2	5.5	0.7	0.5	1.2	0.2	4.8
Loc x var x rep	12	10.5	12.3	13.6	6.5	12.8	385.6
Trt	4	31.2	48.3*	112.7*	3.3	64.0*	2683.5*
Loc x trt	4	1.6	7.7	7.0	4.2	5.9	233.3
Var x trt	8	2.2	10.6	10.4	3.2	6.3	249.9
Loc x var x trt	8	1.9	7.5	6.8	2.5	5.1	227.0
Cut	4	159.0	171.7*	302.7*	1162.3*	482.4*	10448.0*
Loc x cut	4	102.1	178.1*	149.4*	55.5*	121.6*	5118.0*
Cut x var	8	2.6	7.9	9.2	0.8	3.1	230.5
Trt x cut	16	1.8	2.6	4.5	2.8	4.1	164.4
Loc x var x cut	8	1.9	6.2	5.9	1.6	3.1	173.5
Loc x trt x cut	16	0.6	3.8	5.1	1.7	2.5	138.4
Trt x var x cut	32	0.9	3.8	3.8	1.7	2.9	120.6
Loc x trt x cut x var	32	1.8	5.4	6.0	2.1	3.3	160.0
Residual	432	1.7	6.1	6.5	2.5	4.0	193.4

* Significant at $P \leq 0.05$, level of probability †Crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), neutral detergent fiber digestibility (NDFD), total digestible nutrients (TDN), relative forage quality (RFQ).

The cultivar RR Presteez (FD3) had significantly higher, CP, TDN and RFQ in 2019 across two harvests, and significantly higher RFQ in 2020, than RR Stratica (FD4) and L-450 RR (FD5) across four harvests (Table 21). The cultivar RR Presteez is rated FD3 and has a multifoliolate trait (>3 leaflets per leaf). Most of the protein and digestible fiber of alfalfa hay are in the alfalfa leaflets, this explain the higher CP in RR Presteez. It is also known, more dormant cultivars have higher CP (Rimi et al., 2012). This is because of the lower stem-to-leaf ratio, decreasing fiber, which indirectly correlates with CP. The was possibly due to the minimal variation between dormant and semi-dormant cultivars producing more or less forage biomass in the fall after the establishment year.

Also, RR Presteez (FD3) goes into dormancy earlier which explained the shorter plant height of RR Presteez from other cultivars. Cultivars with more dormancy have less shoot elongation after harvest (Volenec et al., 1985). The cultivar RR Presteez (FD3) had the lowest ADF and NDF compared with the other cultivars, giving it the highest TDN and RFQ as well, in both 2019 and 2020. The multifoliolate trait likely decreased the stem-to-leaf ratio and increased the crude protein content (Table 19). Juan et al. (1993) found that the multifoliolate trait can increase the plant's leaf percentage by 3% on average. The lower the stem-to-leaf ratio, the less fiber accumulation, which would increase crude protein, fiber digestibility, and overall nutritive value. Similar to 2019, RFQ was highest in the RR Presteez (FD3) in 2020 although CP was not (Table 21). This is likely because of the earlier dormancy and multifoliolate trait.

Table 21. Forage nutritive value analysis for three cultivars with different fall dormancy (FD) averaged across cuts, K treatments, and locations in 2019 and 2020.

Cultivar	CP†	ADF	NDF	NDFD	TDN	RFQ
	--------------------------------------(%)--------------------------------------					
			2019			
RR Presteez (FD3)	23.8	33.7	45.4	41.5	66.9	142
RR Stratica (FD4)	23.2	35.4	47.3	40.9	65.7	132
L-450 RR (FD5)	23.0	35.5	47.3	40.7	65.3	131
LSD(0.05)	0.5	0.6	0.7	0.4	0.6	3
			2020			
RR Presteez (FD3)	21.1	31.0	43.9	42.7	67.7	148
RR Stratica (FD4)	21.2	31.9	45.9	42.2	67.2	142
L-450 RR (FD5)	20.9	32.2	45.4	42.1	66.7	140
LSD(0.05)	0.4	0.5	0.6	0.4	0.5	3

†Crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), neutral detergent fiber digestibility (NDFD), total digestible nutrients (TDN), relative forage quality (RFQ). LSD= to compare nutritive value parameter between cultivar means within the same year.

In 2019, the TDN and RFQ values were highest when no K was applied and in the first cut (Table 20, 21). Increase of K application did result in a decrease in nutritive value, due to increased ash content as K fertilizer rates increased (Table 22). The K rate of 336 kg K_2O ha^{-1} applied in full application had the highest ADF and NDF in 2020. Lissbrant et al. (2009) also found an increase in ADF and NDF with increased K rate, which caused an increase in shoot diameter and mass of shoots. The K rates in full had lower TDN and RFQ values from the split-application treatments at the same seasonal rate. This suggests that nutritive value is negatively affected when K is applied in full rate, when compared with split-application.

Table 22. Forage nutritive value analysis for five K treatments averaged across harvests, three cultivars, and two locations in 2019 and 2020.

K_2O rate ($kg\ ha^{-1}$)	CP†	ADF	NDF	NDFD	TDN	RFQ
	------------------------------------			-(%)-		------------------------------------
				2019		
0†	23.8	33.9	45.6	41.2	67.1	141
168	23.0	35.3	47.1	40.8	65.6	132
168S	23.6	34.3	46.2	41.3	66.4	139
336	22.7	35.7	47.6	40.9	65.2	131
336S	23.3	35.1	47.0	40.9	65.5	134
LSD(0.05)	0.3	0.6	0.7	0.4	0.5	3
				2020		
0	21.9	30.7	43.2	42.1	68.5	152
168	20.9	32.0	45.3	42.4	67.0	142
168S	21.0	31.6	44.8	42.3	67.1	143
336	20.6	32.3	45.6	42.2	66.6	140
336S	20.8	32.0	45.3	42.5	66.9	141
LSD(0.05)	0.3	0.5	0.6	0.4	0.4	3

†Crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), neutral detergent fiber digestibility (NDFD), total digestible nutrients (TDN), relative forage quality (RFQ). †Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD= to compare nutritive value parameter between K treatment means within the same year.

The highest nutritive value in 2019 was for the first cut at both locations (Table 23). In the spring, alfalfa grows at a slower rate due to cooler temperatures. Katchunov and Naydenov (1994) found that faster growth of alfalfa is accompanied by a decline in nutritive value. Also, the first cut in 2019 was conducted when alfalfa was at 20% bloom stage, where the second cut occurred at 40% and late-bloom stage for stressed and non-stressed treatments, respectively.

Similarly, Lamb et al. (2012) found that at a late-bloom stage, nutritive value decreases, with an increase in NDF representing a total cell wall concentration increase. In 2020, the highest RFQ value at both locations was at second harvest in July. Alfalfa in Lisbon also had higher TDN and RFQ values than Milnor at all harvest times in 2019, and in 2020 values between locations were similar, though Lisbon still had higher TDN and RFQ at all harvests. This is likely because of the stunted growth that occurred at Lisbon in 2019, decreasing the stem-to-leaf ratio and therefore increasing the digestibility (Albrecht et al., 1987; Sheaffer et al., 2000; Lamb et al., 2003; Grev et al., 2020). The highest CP in 2019 at both locations was at the September harvest (Table 23). This could be explained by the age of the alfalfa harvested, for the July and September harvest occurred between 20-40% bloom stage. The October harvest occurred at late-flowering stage, and therefore had loss of leaves and increased fiber accumulation, decreasing CP value.

Table 23. Forage nutritive value analysis for two locations and three harvest dates in 2019 and five harvest dates in 2020 averaged across five K treatments and three cultivars.

Location	Harvest date	CP†	ADF	NDF	NDFD	TDN	RFQ
		-----------------------------------	-----	-----	(%)	-----	-----------------------------
				2019			
Milnor	29 Jul.	22.1	31.6	44.0	45.7	68.8	154
	19 Sept.	23.0	42.0	52.0	37.4	61.1	105
	16 Oct.	20.5	40.6	52.7	35.2	59.0	94
Lisbon	30 Jul.	25.2	27.0	39.0	48.5	73.3	190
	19 Sept.	26.1	35.7	45.6	39.2	66.7	131
	16 Oct.	23.0	32.3	46.8	40.1	66.7	136
	LSD_1(0.05)	0.4	0.6	0.6	0.4	0.6	3
	LSD_2(0.05)	0.6	1.2	1.3	0.7	1.0	6
				2020			
Milnor	1 Jun.	21.2	32.0	46.4	43.5	68.1	142
	29 Jul.	18.6	31.9	44.5	44.3	65.8	144
	11 Aug.	21.5	35.3	48.0	41.6	66.1	130
	15 Sept.	21.5	35.3	48.0	41.6	66.1	130
	12 Oct.	18.8	32.1	43.9	37.2	63.1	130
Lisbon	2 Jun.	21.8	31.2	46.4	43.6	68.5	143
	30 Jul.	23.3	27.8	41.1	46.8	71.0	172
	11 Aug.	21.8	30.9	43.9	44.7	69.3	154
	15 Sept.	22.6	29.1	42.9	42.6	70.0	156
	12 Oct.	19.4	31.5	43.2	37.2	64.2	134
	LSD_3(0.05)	0.4	0.9	0.9	0.6	0.7	5
	LSD_4(0.05)	0.4	1.0	1.1	0.8	0.8	6

†Crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), neutral detergent fiber digestibility (NDFD), total digestible nutrients (TDN), relative forage quality (RFQ). LSD_1= to compare nutritive value parameter between harvest date means within the same location in 2019; LSD_2= to compare nutritive value parameter between harvest date means across locations in 2019. LSD_3= to compare nutritive value parameter between harvest date means within the same location in 2020; LSD_4= to compare nutritive value parameter between harvest date means across locations in 2020.

Focusing on the mineral aspect of the nutritive analysis, there was a significant effect of location for ash, K, and P in 2019, and ash in 2020 (Table 24 and 25). The cultivar was significant for P in 2019. The K treatment significantly affected ash and K in 2019 and ash, K, and P in 2020 (Table 24). Harvests affected all parameters, in 2020 (Table 25).

Table 24. Analysis of variance and mean squares for alfalfa forage nutritive analysis for two locations (Loc), three cultivars (Var), five K treatments (Trt) and three harvest times (Cut) in 2019.

SOV	df	Ash	K	P
Rep	3	0.82	1.06	0.001
Loc	1	29.58*	10.28*	0.0334*
Rep(loc)	6	2.09	0.73*	0.0012
Var	2	0.17	0.001	0.0048*
Loc x var	2	0.18	0.08*	0.0001
Loc x var x rep	12	0.31*	0.02	0.0006*
Trt	4	2.39*	0.67*	0.0005
Loc x trt	4	0.05	0.01	0.00003
Trt x var	8	0.05	0.003	0.0001
Loc x trt x var	8	0.08	0.01	0.0002
Cut	2	19.83*	0.88*	0.0513*
Loc x cut	2	49.01*	0.64*	0.00001
Var x cut	4	0.16	0.02	0.0004
Trt x cut	8	0.44*	0.13*	0.0009*
Loc x var x cut	4	0.05	0.02	0.0005
Loc x trt x cut	8	0.04	0.02	0.0002
Trt x var x cut	16	0.05	0.005	0.0001
Loc x var x trt x cut	16	0.08	0.004	0.0001
Residual	252	0.10	0.01	0.0002

*Significant at $P \leq 0.05$, level of probability.

Table 25. Analysis of variance and mean squares for alfalfa quality analysis for two locations (Loc), three cultivars (Var), five K treatments (Trt) and five harvest times (cut) in 2020 at Lisbon and Milnor, ND.

SOV	df	Ash	K	P
Rep	3	0.92	0.44	0.0003
Loc	1	18.98*	1.35	0.001
Rep(loc)	6	2.56*	0.36*	0.001
Var	2	0.66	0.03	0.001
Loc x var	2	1.65	0.04	0.0002
Loc x var x rep	12	0.84	0.04*	0.001*
Trt	4	9.13*	2.88*	0.002*
Loc x trt	4	0.18	0.04*	0.0002
Trt x var	8	0.52	0.006	0.0003
Loc x trt x var	8	0.41	0.01	0.0003
Cut	4	36.39*	5.66*	0.070*
Loc x cut	4	5.49*	0.29*	0.036*
Var x cut	8	1.07	0.006	0.0005
Trt x cut	16	0.86	0.05*	0.0004
Loc x cut x var	8	1.03	0.005	0.0004
Loc x trt x cut	16	0.38	0.02*	0.0001
Trt x cut x var	32	0.57	0.006	0.0002
Loc x var x trt x cut	32	0.50	0.005	0.0004
Residual	432	0.66	0.01	0.0004

* Significant at $P \leq 0.05$, level of probability

Alfalfa cultivars affected P in 2019 (Table 26). The cultivar RR Presteez (FD3) had the highest P value, possibly caused by its multifoliolate trait, increasing leaf percentage, which increases the P concentration found in leaves (Juan et al., 1993).

Table 26. Ash, K, and P in three cultivars of different fall dormancy (FD) averaged across harvest times, K treatments, and locations in 2019.

Cultivar (FD)	Ash	K	P
	------------------------(%)------------------------		
RR Presteez (FD3)	8.2	2.25	0.33
RR Stratica (FD4)	8.1	2.24	0.32
L-450 RR (FD5)	8.1	2.24	0.31
LSD(0.05)	0.2	0.04	0.01

LSD= to compare nutritive value parameter between cultivar means in 2019.

The ash and biomass K were highest when K was applied in full at beginning of 2019 and 2020 growing season, the highest ash and biomass K values occurred when applied the K treatments of 336 kg K_2O ha^{-1}, though, the timing of application did not influence the values (Table 27). This was expected with the 336 kg K_2O ha^{-1} at seeding treatment, however, what was surprising was that the 168 kg K_2O ha^{-1} at seeding treatment was significantly higher than the split-application of 336 kg K_2O ha^{-1}. Though the rates applied were the same, this suggests that applying at seeding encourages higher K consumption by the plant than when applied at first cut. In 2020, however, the 336 kg K_2O ha^{-1} rate in split- and in full-application had the highest ash and biomass K. For biomass K values across treatments, the averages were lower in 2020 than in 2019, except for 336 kg K_2O ha^{-1} in full, where the value was the same. This could be explained by higher biomass production in 2020, increasing nutrient demand for the growing season, giving less opportunity for luxury consumption at a given time. A concern with the highest rate of K applied was with luxury consumption, creating toxic levels of K for ruminants. When averaged across treatments, biomass K levels did not surpass toxic levels of 2.5% K (Berger and Drewnoski, 2020).

Table 27. Ash, K, and P with five K treatments averaged across harvest dates, three cultivars, and two locations in 2019 and 2020.

K rate (kg ha^{-1})	Ash	K	P
	---------------------------(%)---------------------------		
	2019		
0†	7.9	2.12	0.317
168	8.2	2.38	0.319
168S	8.1	2.21	0.323
336	8.4	2.38	0.320
336S	8.1	2.25	0.322
LSD(0.05)	0.5	0.48	0.004
	2020		
0†	7.55	1.88	0.277
168	7.9	2.11	0.281
168S	7.8	2.07	0.285
336	8.2	2.26	0.284
336S	8.2	2.25	0.286
LSD(0.05)	0.5	0.02	0.004

†Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD= to compare nutritive value parameter between K treatment means within the same year.

The highest ash content was in the June harvest of 2020 (Table 28). In relation, the K content was also the highest in June at both locations. This is not surprising, because of the expected K^+ availability from weathering over the winter and spring, therefore encouraging more consumption of nutrients. The P content did not correlate with K, but that is also expected considering that soil microbial activity influences P availability, which would likely be more active in the middle of the growing season.

Table 28. Ash, K, and P of harvests and locations averaged across five K treatments and three cultivars in 2019 and 2020.

Location	Harvest date	Ash	K	P
		-----------------------(%)-----------------------		
		2019		
Milnor	29 Jul.	8.2	2.13	0.31
	19 Sept.	7.6	2.16	0.33
	16 Oct.	7.8	1.95	0.29
Lisbon	30 Jul.	7.3	2.30	0.33
	19 Sept.	8.7	2.53	0.35
	16 Oct.	9.3	2.42	0.31
	LSD_1(0.05)	0.1	0.04	0.01
	LSD_2(0.05)	0.4	0.22	0.01
		2020		
Milnor	1 Jun.	8.4	2.26	0.30
	29 Jul.	7.6	1.94	0.25
	11 Aug.	7.5	2.18	0.31
	15 Sept.	7.5	2.18	0.31
	12 Oct.	7.7	1.77	0.25
Lisbon	1 Jun.	9.4	2.40	0.28
	29 Jul.	8.0	2.16	0.31
	11 Aug.	7.6	2.30	0.29
	15 Sept.	7.7	2.17	0.29
	12 Oct.	7.8	1.77	0.24
	LSD_3(0.05)	0.2	0.03	0.01
	LSD_4(0.05)	0.3	0.09	0.01

LSD_1= to compare nutritive value parameter between harvest date means within the same location in 2019; LSD_2= to compare nutritive value parameter between harvest date means across locations in 2019. LSD_3= to compare nutritive value parameter between harvest date means within the same location in 2020; LSD_4= to compare nutritive value parameter between harvest date means across locations in 2020.

The highest RFQ and TDN values were at the first harvest, with treatments 168 kg K_2O ha^{-1} split-application (Table 29). The K, however, was not yet applied at the time of harvest, which would therefore explain why the split-application values were similar values to the control. The lowest RFQ and TDN values were from the samples harvested in October, specifically from the highest rates of applied K.

Table 29. Relative forage quality and total digestible nutrient content between five K treatments and three harvest times averaged across three cultivars and two locations in 2019.

K_2O	RFQ†			TDN		
kg ha^{-1}	1‡	2S	2NS	1	2S	2NS
	---(%)---					
0§	176.7	123.0	123.5	71.8	65.1	64.3
168	166.2	117.7	114.4	70.2	63.9	62.6
168S	179.3	117.9	118.5	72.0	63.9	63.2
336	164.3	115.7	112.4	69.8	63.5	62.2
336S	173.7	114.9	112.8	71.4	63.2	61.9
LSD(0.05)		4.5			0.8	

†Relative forage quality (RFQ); total digestible nutrients (TDN). ‡For harvests 1=29 July; 2S=19 September; 2NS=16 October. §Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD= to compare nutritive value parameter between K treatment means within harvest dates.

Ash content was highest at the lowest values of RFQ and TDN. Ash content increased throughout the growing season across all treatments (Table 30). For K content, the percentage increased from July samples to September, but then decreased from September to October. This would be expected as the plants acclimated for winter, translocating the K in the biomass into their taproots for energy reserves. The P content correlated with the K content, increasing from July samples to September samples, but decreasing from September to October.

Table 30. Ash, K, and P content of five K treatments and three harvest times averaged across three cultivars and two locations in 2019.

K_2O rate	Ash			K			P		
kg ha^{-1}	1†	2S	2NS	1	2S	2NS	1	2S	2NS
0‡	7.5	7.8	8.4	2.11	2.17	2.08	0.31	0.34	0.30
168	7.9	8.1	8.6	2.33	2.34	2.16	0.32	0.34	0.29
168S	7.6	8.1	8.5	2.14	2.31	2.18	0.32	0.34	0.31
336	8.1	8.3	8.7	2.39	2.49	2.26	0.32	0.34	0.30
336S	7.5	8.2	8.6	2.09	2.41	2.25	0.31	0.35	0.31
LSD (0.05)		0.2			0.06			0.01	

†Harvests, 1=29 July; 2S=19 September; 2NS=16 October. ‡Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD= to compare nutritive value parameter between K treatment means within harvest dates.

The lowest K content in the biomass was in October, which was across all K treatments (Table 31). This could suggest that the biomass K was being translocated to the taproots in preparation for dormancy. At harvest in June, the 336 kg K_2O ha^{-1} split-application had significantly higher K concentration than all other treatments and harvest times. This is likely due to the 168 kg K_2O ha^{-1} that were applied in September 2019, that had time to mineralize in spring 2020 and be available for the plant.

Table 31. Alfalfa biomass K concentration of five K treatments and five harvest times averaged across three cultivars and two locations in 2020.

K_2O kg ha^{-1}	1 Jun.	29 Jul.	11 Aug.	15 Sept.	12 Oct.
	------------	------------	(%)	------------	------------
0†	2.11	1.82	1.97	1.94	1.58
168	2.24	2.09	2.26	2.20	1.75
168S	2.30	2.04	2.19	2.09	1.71
336	2.45	2.16	2.42	2.37	1.91
336S	2.55	2.16	2.36	2.28	1.90
LSD (0.05)	0.06				

†Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at first harvest; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD= to compare between K treatment means within harvest dates.

4.6. Alfalfa plant density and persistence

Application time, in full- or split-application did not significantly affect plant density as an indirect measure of persistence (Table 32). This contradicted the findings in Berg et al. (2018), where fertilization with K had less decline of plant density over time. The time of sampling and fall harvest stressing were the only factors studied affecting stand persistence. All other significant interactions involved either or both time of sampling and the stressed treatment.

Table 32. Analysis of variance of plant density for two locations (Loc), three cultivars (Var), five fertility treatments (Trt), and fall harvest stress (Stress) over four samplings (Time) in 2019 and 2020.

SOV	df	MS
Rep	3	2034
Loc	1	16289
Loc(rep)	6	3389
Var	2	3349
Loc x var	2	2350
Loc x var x rep	12	1227
Trt	4	884
Loc x trt	4	524
Var x trt	8	608
Loc x var x trt	8	2231*
Stress	1	20487*
Loc x stress	1	15592*
Var x stress	2	238
Trt x stress	4	1260
Loc x var x stress	2	24
Loc x trt x stress	4	1351
Var x trt x stress	8	2465*
Loc x var x trt x stress	8	2457*
Time	3	323336*
Loc x time	3	21856*
Var x time	6	1570
Trt x time	12	446
Time x stress	3	33287*
Loc x var x time	6	212
Loc x trt x time	12	335
Loc x time x stress	3	14459*
Var x trt x time	24	356
Var x time x stress	6	691
Trt x time x stress	12	513
Loc x var x trt x time	24	658
Loc x var x time x stress	6	176
Loc x trt x time x stress	12	361
Var x trt x time x stress	24	676
Loc x var x trt x time x stress	24	317
Residual	698	849

* Significant at $P \leq 0.05$, level of probability

The L-450 RR (FD5) cultivar had a higher plant density in Lisbon at all K rates than the other cultivars at Lisbon and Milnor, except for 336 kg K_2O ha^{-1} in split-application (Table 33). Plant stands at 336 kg K_2O ha^{-1} in split-application were higher than the full rate for cultivars RR

Presteez (FD3) and RR Stratica (FD4) in Lisbon and L-450 RR (FD5) in Milnor. The plant densities that decreased at 336 kg K_2O ha^{-1} in split-application could be explained by less applied K at the beginning of the growing season, since increasing K rate can increase plant densities (Berg et al., 2007). This would be possible, especially considering that Milnor, expecting to have less soil available K^+, had lower plant density for split-application than in full at the same rate for two of the three cultivars.

Table 33. Alfalfa plant density for five K treatments, three cultivars of different fall dormancy (FD), at two locations averaged across two harvest stress treatments four sampling times in 2019 and 2020.

	Lisbon			Milnor		
K_2O rate kg ha^{-1}	RR Presteez (FD3)	RR Stratica (FD4)	L-450 RR (FD5)	RR Presteez (FD3)	RR Stratica (FD4)	L-450 RR (FD5)
	---plants m^{-2}---------------------------------------					
0†	120	118	118	104	108	110
168	114	101	118	106	111	100
168S	109	103	121	105	97	110
336	113	104	121	106	112	104
336S	123	114	112	93	100	119
LSD_1(0.05)	15					
LSD_2(0.05)	16					

†Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding, 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD_1=to compare between K treatment by cultivar means within same location; LSD_2=to compare between K treatment by cultivar means across locations.

Non-stressed treatments had lower plants density from most treatments, but K treatments did not have a clear interaction with cultivar or stress treatments (Table 34).

Table 34. Alfalfa plant density for five K treatments, three cultivars of different fall dormancy (FD), and harvest stress treatments averaged across two locations and four sampling times in 2019 and 2020.

	Stressed			Non-stressed		
K_2O rate ($kg\ ha^{-1}$)	RR Presteez (FD3)	RR Stratica (FD4)	L-450 RR (FD5)	RR Presteez (FD3)	RR Stratica (FD4)	L-450 RR (FD5)
	--------------------	--------------------	--------------------	plants m^{-2}	--------------------	--------------------
0†	109	117	124	115	109	104
168	119	116	114	101	96	103
168S	112	109	122	102	92	110
336	118	103	119	101	113	107
336S	107	118	111	109	97	119
LSD(0.05)	12					

†Treatments 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding in 2019, at first cut in 2020; 168S=split-application, 84 kg K_2O ha^{-1} applied at first harvest, 84 kg K_2O ha^{-1} applied in mid-September; 336=336 kg K_2O ha^{-1} applied at seeding in 2019, at first cut in 2020; 336S= split-application, 168 kg K_2O ha^{-1} applied at first harvest, 168 kg K_2O ha^{-1} applied in mid-September. LSD= to compare between K treatment by cultivar means across harvest stress treatments.

Plant density significantly decreased from spring 2019 to fall 2020 (Fig. 23). The stressed treatments stand reduction was about 50%, but the non-stressed treatment had a lower decline of 38%. Both the stressed and non-stressed treatments significantly decreased from fall 2019 to spring 2020, similar to results reported by Berti and Samarappuli (2018). The average population across the stressed treatment declined by 78 plants m^{-2}, where the non-stressed treatment declined by 30 plants m^{-2}. This decline on plant density was expected as loss of plants occur in the first winter regardless of initial plant density or weather factors (Berti and Samarappuli, 2018). If plant density is high, alfalfa plants self-thin. Collins et al. (1986) reported a 50% decline in plant density in K fertilized plots from the first to second growing season. Hall et al. (2010) had 120-200 plants m^{-2} in the establishing year, which decreased to less than 100 plants m^{-2} in the first production year.

In spring 2020, the plant density was 70 and 77 plants m^{-2} for stressed and non-stressed treatments, respectively (Fig. 23). Berti and Samarappuli (2018) found that in the first production year, total forage yield was maximized when plant density was 52 plants m^{-2}. Therefore, spring 2020 plant density of 70 and 77 plants m^{-2} for stressed and non-stressed treatments, respectively, are both adequate for a productive stand.

What was surprising to find was that from spring 2019 to fall 2020 the plant density increased, which was not expected. This could be explained by the error in counting plants, when unable to see the belowground roots and estimating number of stems per plant. This error could have also come from a delay in regrowth in spring 2020, with some plants not growing yet when counted. From fall 2020 to spring 2021, the stressed and non-stressed treatments both had plant density decline by 18 plants m^{-2}.

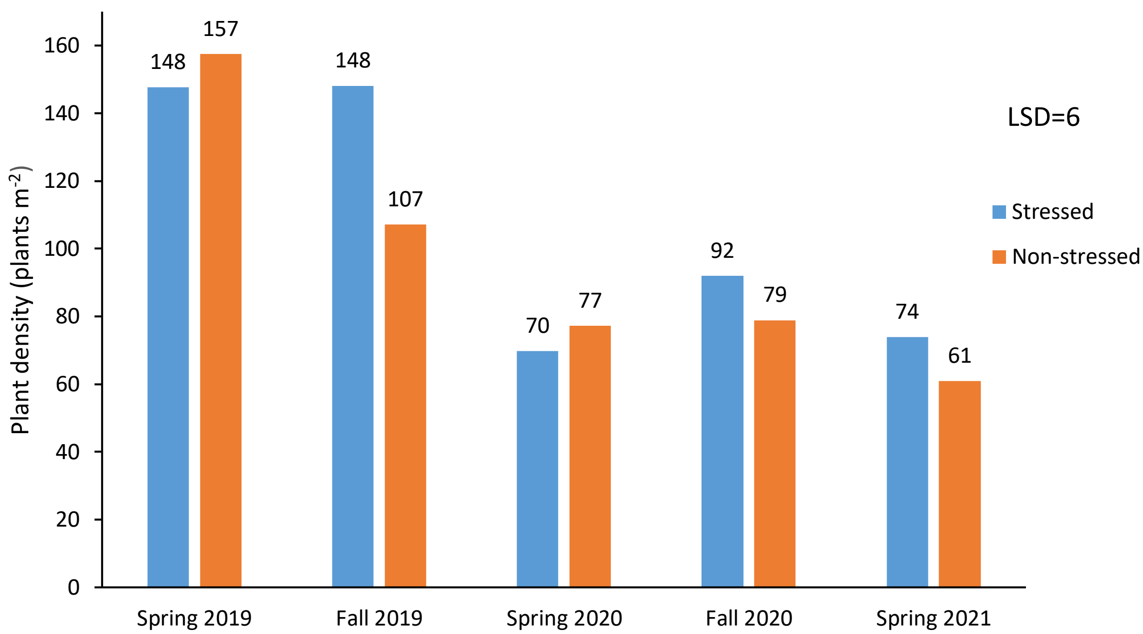

Fig. 23. Average alfalfa plant density of stressed and non-stressed harvests in two growing seasons averaged across two locations, five K treatments, and three cultivars. †Stressed fall harvest was harvested in September, while non-stressed fall harvest was harvested in October prior to first frost. LSD= to compare between means of sampling time by stress vs. non-stress harvest treatment. $P \leq 0.05$.

From fall 2019 to spring 2020, plant density in Lisbon decreased by 60% in the stress treatment compared with Milnor stress treatment decreasing by 42% (Table 35). This could be a product that the soil in Lisbon has a pH of 5.85, below the recommended 6.1 for alfalfa (Undersander et al., 2011), which could have caused more stress in plants.

With the prediction that Milnor location would have less available K with its higher smectite-to-illite clay ratio, it would be expected that there would be a higher decline in stressed plants than in Lisbon. However, Milnor plant density in the stressed treatment had the lowest decline of plants from spring 2019 to fall 2020 (Table 35).

Table 35. Alfalfa plant density between stressed and non-stressed harvest treatments at two locations in 2019 and 2020 averaged across five K treatments and three cultivars.

	Lisbon		Milnor	
Sampling time	Stressed†	Non-stressed‡	Stressed	Non-stressed
	------------------------------plants m^{-2}-------------------------------			
Spring 2019	168	150	128	165
Fall 2019	171	117	125	97
Spring 2020	68	77	72	78
Fall 2020	83	77	101	80
Spring 2021	73	55	75	67
LSD_1(0.05)	9			
LSD_2(0.05)	12			

†Stressed=fall harvest occurred in mid-September; ‡Non-stressed=fall harvest occurred in October prior to first frost. LSD_1= to compare between sampling time means by stress vs. non-stress harvest treatment within the same location; LSD_2= to compare between means of sampling time by stress vs. non-stress harvest treatment across locations.

Applying K did not significantly affect plant density. Berg et al. (2007) concluded that plots fertilized with K had higher plant densities than alfalfa not fertilized with K, though this study was conducted when alfalfa stand was four, five, and six-years-old. Poor persistence due to K deficiency was not found until year five of an eight-yearlong study (Berg et al., 2018). This supports that K fertilization may be more beneficial over long-term production.

4.7. Alfalfa taproot starch and protein reserves

For both 2019 and 2020 root samples, there was a significant interaction between location and fall harvest on starch content (Table 36). There were no interactions among K treatments, cultivar, or harvest stress on taproot protein content for 2019 samples. In 2020, taproot protein content was significant between fall harvests, and for the interaction between locations and fall harvest.

Table 36. Analysis of variance of protein and starch content in alfalfa taproot for two locations (Loc), three cultivars (Var), five fertility treatments (Trt) and two fall harvests (Cut) in 2019 and 2020.

		2019		2020	
SOV	df	Protein	Starch	Protein	Starch
Rep	3	6.5	546.6	25.2	2680.3
Loc	1	1.8	1383.2	215.0	165.3
Loc(rep)	6	24.0	423.7	63.1	6793.7
Var	2	5.3	755.1	10.9	3318.7
Loc x var	2	14.9	145.6	6.0	76.5
Loc x var x rep	12	6.5	625.4	9.1	2408.3
Trt	4	11.2	447.9	0.8	1616.0
Loc x trt	4	2.1	618.5	7.4	2999.7
Var x trt	8	3.5	761.2	2.6	3987.6
Loc x var x trt	8	2.5	409.9	5.2	2993.7
Stress	1	44.5*	198.1	1002.0*	2239.2
Loc x stress	1	17.9	6012.4*	236.2*	44589.0*
Var x stress	2	9.2	77.1	2.0	3715.1
Trt x stress	4	3.2	455.2	0.6	1804.6
Loc x var x stress	2	5.2	458.0	6.8	500.5
Loc x trt x stress	4	3.7	53.6	2.6	991.6
Var x trt x stress	8	3.9	372.5	1.1	638.0
Loc x trt x stress x var	8	3.5	939.6	6.1	1728.2
Residual	162	5.9	621.4	5.8	2238.1

* Significant at $P \leq 0.05$, level of probability.

Alfalfa taproots from the stressed fall harvest in Milnor had significantly lower starch content than the non-stressed fall harvest (Fig. 25). In Lisbon, however, root starch was not different ($P \leq 0.05$). In Milnor, GDD from the stress harvest to the first hard frost were 146 GDD in 2019 and 234 GDD in 2020, likely using starch stored in the root for regrowth. According to

Berg et al. (2018), taproot starch declines until three-weeks post-harvest, then increasing its root starch until around 30-days post-harvest (Fig. 24). The first hard frost was 29-days (146 GDD) after September harvest in 2019, which explains that Lisbon may not have had significant difference between stressed and non-stressed treatments due to the plants having had almost 30-days to replenish its taproot starch.

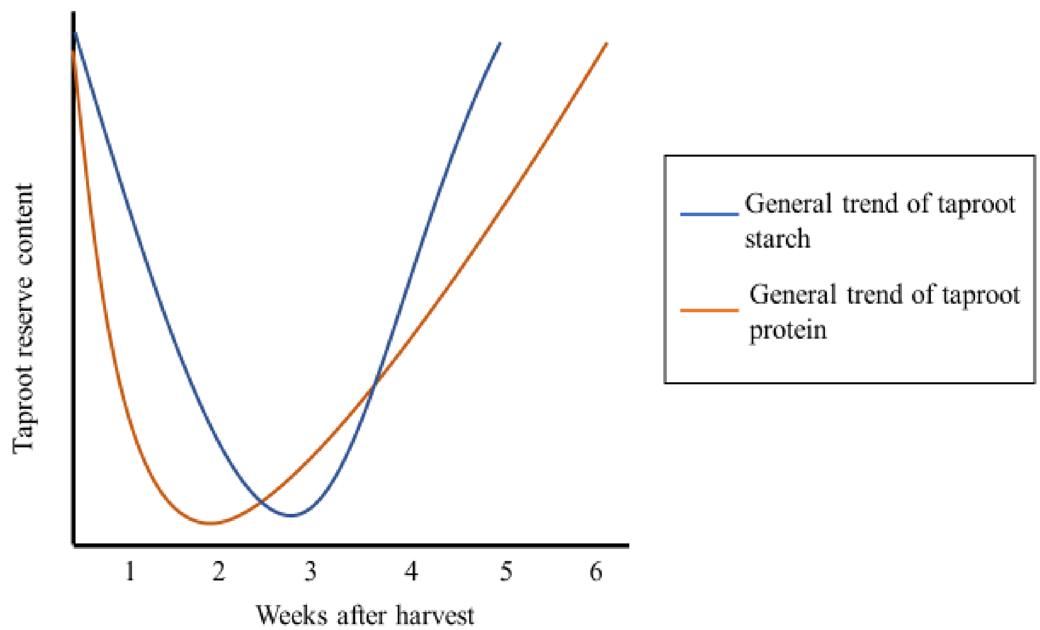

Fig. 24. General trends of alfalfa taproot starch and protein content post-harvest (Avice et al., 1996a; Justes et al., 2002; Dhont et al., 2003; Berg et al., 2018).

In 2020, the non-stressed samples at Lisbon had significantly higher starch content than the stressed samples (Fig. 21). Though locations did not show the same trend between stressed and non-stressed treatments in 2020, the starch contents in the stressed treatments across locations were not significantly different. For the Milnor location, the stressed samples had significantly higher root starch content than the non-stressed samples, which was unexpected. There were 31-days (241 GDD) from September harvest 2020 until first hard frost, which could explain the stressed treatment at Milnor having a higher starch content. It is likely that acclimation was delayed in fall 2020 due to 100 more GDD until first hard frost, along with drier than average conditions. Rainfall in September and October totaled 3.9-cm, which was 13.8-cm less than 2019, not allowing for ideal acclimation for winter. Castonguay et al. (1995) found that during hardening stage, starch concentration decreased as sugar levels increased. The study also found that alfalfa exposed to 2˚C had a decline of starch, but after two-weeks had an increase of starch over time. On 2 October, temperatures were below 2˚C for three nights and on 14 October, temperatures remained below 0˚C at night for the remainder of fall. With roots sampled on 16 October, it is possible that the below 2˚C exposures signaled plants into acclimation, converting starch into sugars (Bertrand et al., 2017). The stressed plant roots may have still been replenishing its starch reserves, before being able to begin its process of starch to sugars conversion.

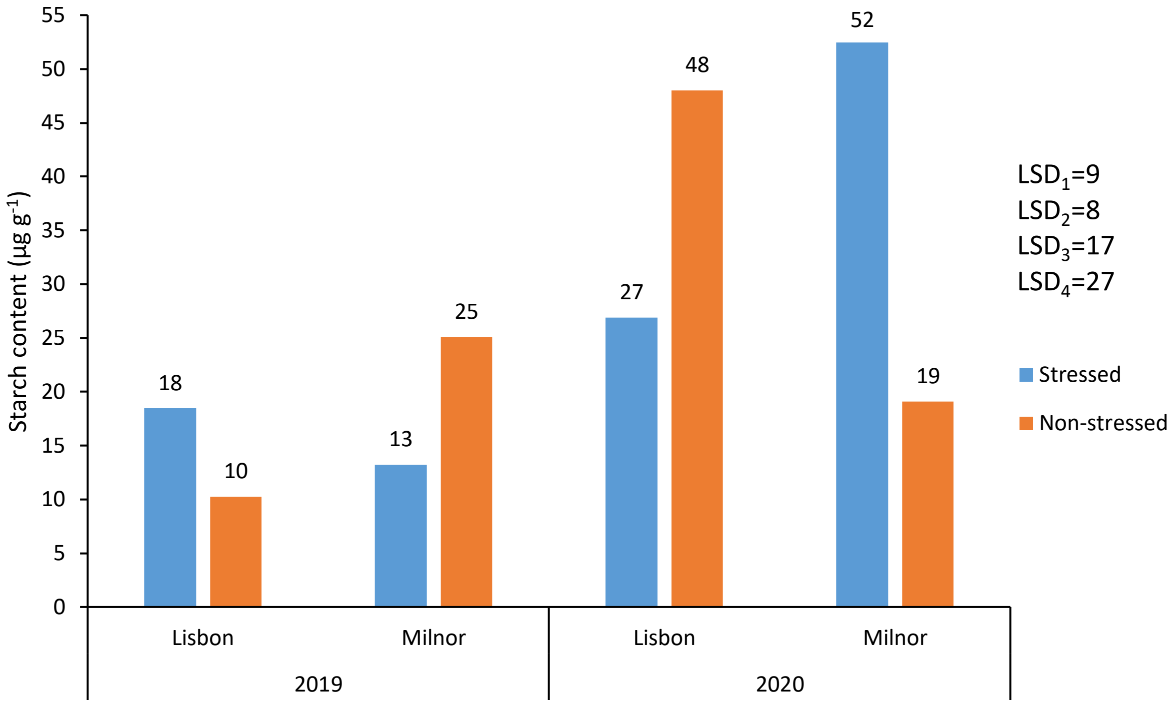

Fig. 25. Root starch content for alfalfa roots sampled at two locations in 2019 and 2020 with stressed and non-stressed fall harvest treatments averaged across five K treatments and three cultivars. LSD_1= to compare starch content between means of stress vs. non-stress within the same location in 2019; LSD_2= to compare between means of stress vs. non-stress treatments across locations in 2019; LSD_3= to compare between means of stress vs. non-stress within the same location in 2020; LSD_4= to compare between means of stress vs. non-stress treatments across locations in 2020. $P \leq 0.05$.

Cultivars did not have an effect on starch content in either year, which was different from the findings in Castonguay et al. (1995). It has been found that taproot starch concentrations are negatively correlated with fall dormancy rating (Bula et al., 1956; Volenec, 1985; Castonguay et al., 1995; Haagenson et al., 2003). These studies, however, found this correlation when comparing dormant, semi-dormant, and non-dormant cultivars. However, the L-450 RR (FD5) cultivar used in this study is sub-categorized as semi-dormant and RR Presteez (FD3) and RR Stratica (FD4) as dormant, the ratings used did not vary as much as past findings suggesting that less dormant cultivars have higher starch contents. Previous studies also sampled taproots throughout the fall, finding that November sampling times had greater variation among cultivars.

The alfalfa taproots from the stress treatment had significantly lower protein content than the non-stressed treatment in both years and locations (Fig. 26). This result was expected, for when plants were harvested in September, the GDD for shoot regrowth robbed the taproot N reserves for supplying shoot regrowth. There were 146 and 241 GDD from September harvest until the first hard frost in 2019 and 2020, respectively. Dhont et al. (2003) found that alfalfa harvested less than 600 GDD from previous harvest had taproot N significantly reduced. The GDD accumulated in the fall in this study would have likely encouraged N use for some shoot regrowth, however, full replenishing of taproot N would have been unlikely. Studies support that approximately six weeks are necessary for full accumulation of root N following defoliation (Lemaire et al., 1992; Dhont et al., 2003).

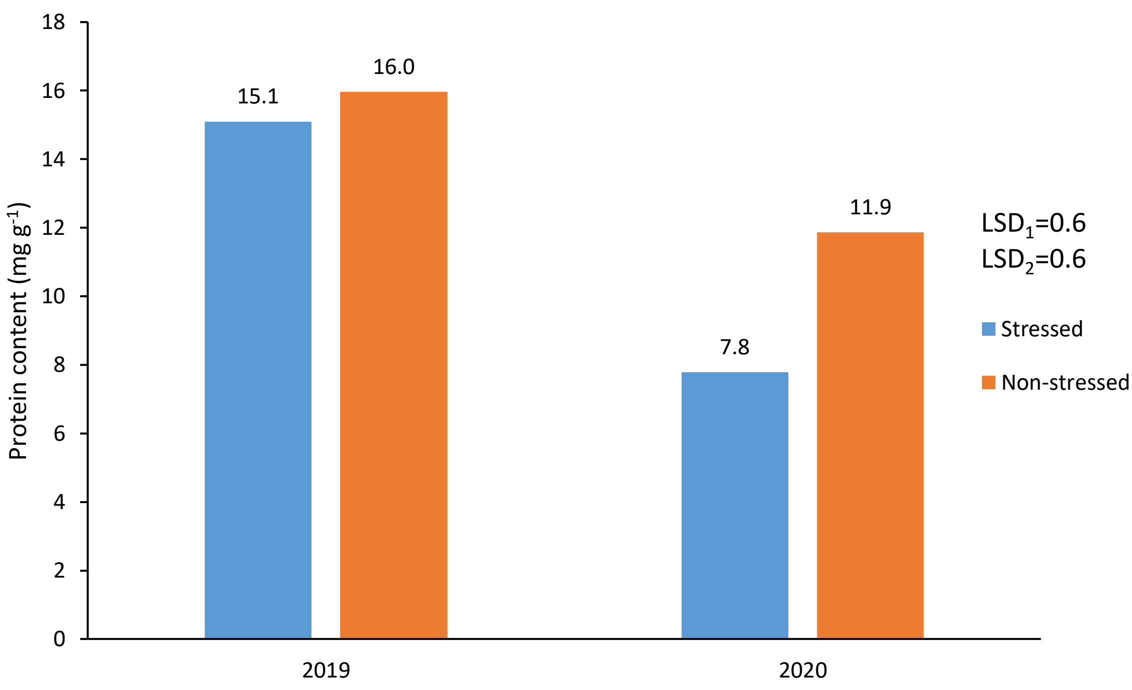

Fig. 26. Root protein content for stressed and non-stressed fall harvest treatments in 2019 and 2020 averaged across five K treatments and three cultivars. LSD_1= to compare between means of stress vs. non-stress treatments in 2019; LSD_2= to compare between means of stress vs. non-stress treatments in 2020. $P \leq 0.05$.

Root protein was significant for the interaction between stress treatment and locations in 2020. The stressed taproots from Lisbon had significantly lower protein content than the stressed taproots in Milnor (Fig. 27). The protein content correlated with the starch content in root samples from Lisbon in 2020. What was interesting was that in 2020, protein content at Milnor was higher in the non-stressed treatment, but the starch content was higher in stressed treatment.

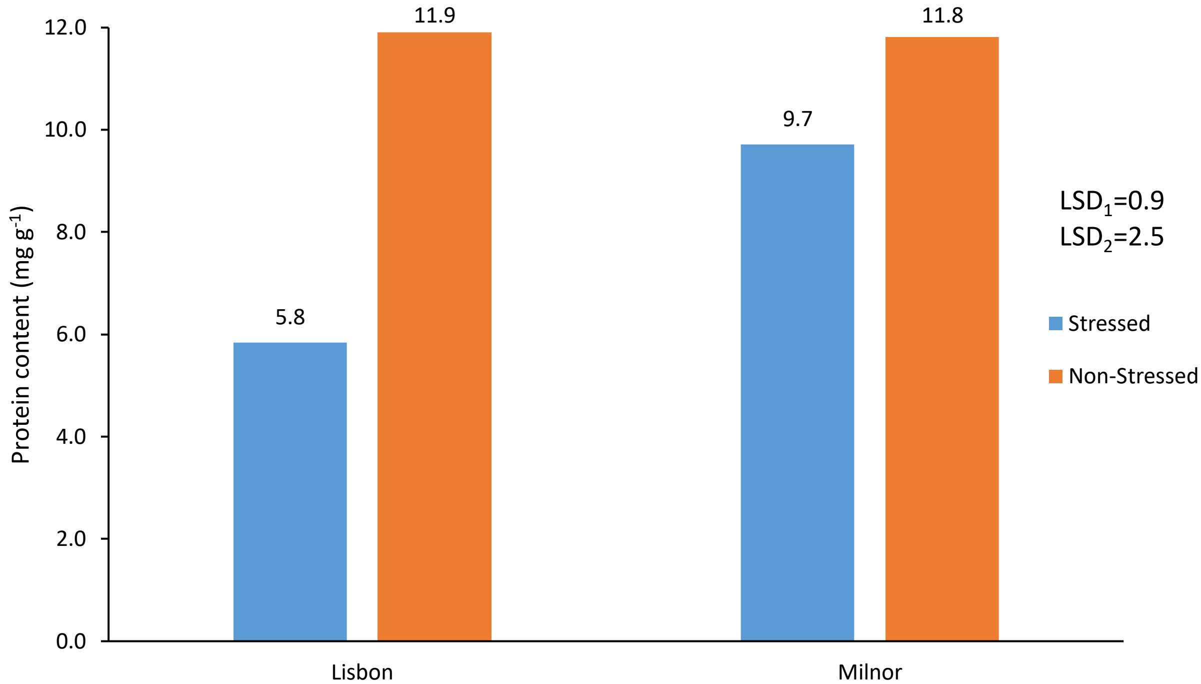

Fig. 27. Root protein content for stressed and non-stressed fall harvest treatments at each location in 2020, averaged across five K treatments and three cultivars. LSD_1= to compare between means of stress vs. non-stress within same location; LSD_2= to compare between means of stress vs. no stress across locations. $P \leq 0.05$.

The K treatments were no different for taproot protein. Oppositely, past findings report that application of K increased total taproot nitrogen and protein (Blevins, 1985; Berg et al., 2018). However, it is important to acknowledge that these past studies were conducted on older alfalfa stands. This study found that fall harvest time was the only factor that significantly influenced starch and protein taproot reserves.

5. CONCLUSION

Lisbon (<3.5 smectite-to-illite) and Milnor (>3.5 smectite-to-illite) did not differ in soil K levels before establishing the experiment, and K treatments did not result in significant differences in soil K between locations. Milnor, a high smectite-to-illite soil did not fix more K^+ from K treatments than in Lisbon as hypothesized. This was likely because both seasons were reasonably well watered with rainfall, decreasing the possibility of greater K fixation at the Milnor site. At both locations, however, the split-application treatments increased soil available K^+ more than the one-time application.

All K fertilization, regardless of rate and time, resulted in significantly greater total seasonal forage yield than the control. The RR Stratica (FD4) cultivar produced the highest forage yield, which was likely the result of it being a recently cultivar with high forage yield potential. An increase in K rate decreased overall nutritive value (TDN and RFQ). Lisbon had higher nutritive value than Milnor, but this may have been due to its lower soil pH, which resulted in more stunted plants and decreased stem-to-leaf ratio. The cultivar with the multifoliolate trait (RR Presteez) had the highest nutritive value compared with the other two cultivars.

Plant density did not increase or decrease with increased K rate and there were no significant differences between differing fall dormancy cultivars' plant densities. Non-stressed plants had a higher decline of plant density than the stressed plants, however, both treatments had plant populations sufficient for a productive stand.

There was no effect between K rates and application timing in alfalfa root protein and starch. There was also no effect of cultivars in protein and starch concentration in the taproot. Harvesting alfalfa in mid-September significantly declined taproot protein reserves in both years.

In 2019, stressed taproots at Milnor had lower starch content than non-stressed, but Lisbon had no difference between harvest stress treatments. In 2020, Lisbon had lower starch content within its stressed treatments, but Milnor had opposite, having lower starch content within its non-stressed treatments. This was unexpected and was potentially caused by an earlier hard frost. Neither K fertilization nor fall dormancy impacted protein and starch amounts in the roots when measured in mid-October.

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