EFFECT OF BORON ON ALFALFA YIELD AND QUALITY AT VARIOUS WATER REGIMES

by

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DEDICATION

To my grandfather, father, mother, brother, and the late grandmother.

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ABSTRACT

Boron (B) application on B-deficient soil may improve alfalfa (*Medicago sativa* L.) yield and quality. The objective of the first study was to identify the effects of foliar-applied B on yield and forage quality of irrigated alfalfa. A two-year study was conducted in 2015/16 at Creston and Dillon, MT. The initial soil B at the Creston and Dillon sites was 0.2 and 0.8 mg kg^{-1}, respectively. The study was designed as a randomized complete block design with four replications of five B rates: 1) 0, split-applications of 2) 0.56, 3) 1.12, 4) 2.24, and a one-time application of 5) 2.24 kg ha^{-1}. Boron fertilization increased ($P < 0.05$) plant B content at both locations. Application of B increased ($P < 0.05$) alfalfa yield only in the second cutting in 2015 at Dillon, but the influence of B was not observed in any other cuttings, nor in the total yields for either years or location. Forage quality was not affected ($P > 0.05$) by B application. The results of this study suggested no effect of foliar-applied B on alfalfa yield or quality. The objective of the second study was to determine the effect of foliar-applied B at various water regimes on alfalfa yield and quality. The study was conducted in 2016-17 at Creston, MT. Soil at this site contained 0.2 mg B kg^{-1}. The experiment was conducted using a split-plot design with three rates of water regimes as main-plot and five B rates as sub-plot factors. The three water regimes were rainfed, 100 percent evapotranspiration (ET), and 50ET. Boron rates and timing of application was the same as in the first study. Irrigation increased total alfalfa yield by 45% and 12% in 2016 and 2017, respectively, with no yield difference between 100ET and 50ET. In 2016, irrigation decreased ($P < 0.01$) forage nutrient quality in the second cutting but had no effect in 2017. There was no effect of B on yield ($P > 0.08$) for either year. Overall, this study suggested that the foliar-applied B on a B-deficient soil did not increase alfalfa yield or quality, regardless of water regime, or year.

CHAPTER ONE

GENERAL INTRODUCTION

Alfalfa, the queen of forages, is a major perennial forage crop that produces high yield and quality forage for livestock (Marita et al., 2003; Bouton, 2012). In the United States, alfalfa is harvested on > 6.7 million ha, producing > 49 million Mg of hay annually (NASS-USDA, 2018). The importance of alfalfa production is paramount due to the increased utilization of alfalfa hay in the dairy and equine industries (Putnam et al., 2000). Recently, the total harvested acreage and production of alfalfa are decreasing in the United States. Between 2015 and 2017, the harvested area decreased by 7% [from 7.2 (2015) to 6.7 (2017) million ha; NASS-USDA, 2018]. Alfalfa hay production declined following the severe drought in 1988 – a counter trajectory from the increasing yield trend for the previous 37 years from 1950 through 1987 (Brummer and Casler, 2014). The increasing demand of alfalfa hay, coupled with the decrease in harvested area and production, expands the need to optimize yield and quality of alfalfa. Fine-tuning high cost production inputs such as irrigation and fertilization can lead to optimal yield and quality in alfalfa (Gauch and Dugger, 1954; Muller and Orloff, 1994; Fereres and Soriano, 2006).

Irrigating alfalfa increases yield, as its response to properly-times available soil moisture demonstrates a linear response to its seasonal crop requirement (Bauder et al., 1978; Donovan and Meek, 1983; Lindenmater et al., 2011; Shewmaker at al., 2013; Holman et al., 2016; Undersander et al., 2016). An untimely irrigation schedule can cause either water-stress or over-application of water, which also has negative impacts on alfalfa yield and quality (Mueller and Orloff, 1994). Nutrient uptake by plants increases with irrigation (Baker and Pilbeam, 2015) because microbial activity increases as moisture availability increases (Zhang et al., 2013), making drought stress problematic for nutrient deficiencies. Most of the reported boron (B) deficiency in crops is reported under drought conditions (Berger, 1962). In alfalfa, B studies are exclusively done under rainfed (Chandler et al., 1946; Razmjoo and Henderlong, 1997) or irrigated conditions (Radtke, 1986; Jackson and Miller, 1998; Dordas, 2006, Kheirkhah et al., 2016), and the interaction between applied B on B-deficient soil and with various water regimes has not been well-studied.

Boron is an essential micronutrient required for the normal growth and development of plants and its deficiency in plants reduces yield and quality of crops (Gupta, 1993; Sakamoto, 2012). Specifically, boron also plays a major role in sugar translocation, carbohydrate metabolism, nucleic acid synthesis, and plant cell growth regulation (Gauch and Dugger, 1954; Gupta, 1980; Howe, 1998; Xiaodong and Yiqin, 1999; Ozturk et al., 2010).The symptoms of B deficiency in alfalfa are yellowing in the leaf tip, retarded plant growth, abnormal tissue structure, dense top canopies, and wilting of leaves (Wright, 1986), which can play a significant role in the reduction of alfalfa yield and quality. Gupta (1993) claimed that crops planted on B-deficient soil do not attain their potential yield and quality. Alfalfa, in particular, removes relatively high 0.35 kg ha^{-1} B compared to other crops like *Brassica napus*, *Gossypium hirsutum*, *Triticum* spp., and *Zea mays* (Shorrocks, 1997). Therefore, B is needed to achieve potential yield of crops (Gupta, 1993; Dordas, 2006).

As irrigation increases yield and influences B availability, evaluating the association between those two factors on alfalfa yield and quality is important. The research in this thesis was conducted at two different locations in Montana to evaluate yield and quality of alfalfa of various water regimes and B fertilization rates. The objectives of this study were to determine the effects of water regimes, rates of B fertilization, and their interaction, on alfalfa yield and forage nutrient quality in Montana. We hypothesized that B uptake in alfalfa following B fertilization on B-deficient soil is dependent upon soil moisture availability.

CHAPTER TWO

LITERATURE REVIEW

Alfalfa Background

History and Origin

Alfalfa is the world's most important forage crop. It is also referred to as "lucerne" in many countries outside of North America (Goplen et al., 1982). Alfalfa, the queen of forage crops, was the first forage crop to be domesticated (Bolton et al., 1972). It is reported that alfalfa was cultivated for over 3,300 years (Bolton et al., 1972; Goplen et al., 1982), and its name is derived from an Iranian word "aspasti" meaning "horse fodder", indicating its high forage quality (Hendry, 1923).

Alfalfa is supposed to originate in Vavilov's "Near Eastern Center" – Asia Minor, Transcaucasia, Iran, and the highlands of Turkmenistan (Hill, 1963; Bolton et al., 1972; Michaud et al., 1988; Attram, 2015). The geographic center of alfalfa is most often mentioned as Iran (Bolton et al., 1972; Goplen et al., 1982), but an investigation by Sinskaya (1950) arguably reported that alfalfa has two distinct centers of origin. The first origin was the mountainous region of Transcaucasia and Asia Minor and adjoining areas of northwestern Iran. Alfalfa from this region is considered to have high winter hardiness ratings (Sinskaya, 1950). The second reported origin was Central Asia where alfalfa germplasms originating from this region were described as susceptible to fungal diseases (Sinskaya, 1950).

Distribution of Alfalfa

It is hard to determine when and how alfalfa dispersed globally. Hendry (1923) states that “The Sumerian merchants from the river villages of Mesopotamia had ships in the Mediterranean as early as 7000 B.C and as late as 4000 B.C., a fully developed sea life was flourishing in the eastern Mediterranean, all of which was favorable to the general dissemination of a plant possessing the conspicuous advantages of alfalfa.” Additionally, alfalfa is supposed to have spread from the plains of Mesopotamia (recently known as Iraq) - a traditional meeting place among Asian, African, and European traders. Hence, the history of the alfalfa distribution and domestication had begun prior to 4-6 B.C and followed the trend of historic civilizations from east to west (Bolton et al., 1972). Klinkowski (1933) indicated that alfalfa spread from Spain to France (1550), Belgium and The Netherlands (1565), England (1650), Germany and Austria (1750), Sweden (1770), and Russia (1800s). The discovery of the Americas and their colonization by Portugal and Spain in the 16th century led to the introduction of alfalfa in America.

Evidence that alfalfa was grown in the southwestern states of the USA was reported as early as 1736 (Stewart, 1926). It is believed that early missionaries from Mexico brought alfalfa to Texas, Arizona, New Mexico, and California (Michaud et al., 1988). Since alfalfa adapted well to the sunny, dry climate and irrigated soils of the southwestern USA, it was immediately cultivated north to Utah. By 1894, domestication of alfalfa began extensively in Kansas and quickly spread towards Montana, Iowa, Missouri, and Ohio in the late 1800’s (Bolton et al., 1972). Alfalfa has since been extensively grown all over the United States. Between 1900 and 1950 increase in alfalfa cultivated area was reported from 2 million acres to 20 million acres (Bagavathiannan and Van Acker, 2009).

Taxonomic Classification of Alfalfa

Taxonomically, alfalfa is classified as:

Kingdom – Plantae
Subkingdom – Tracheobionta (vascular plants)
Superdivision – Spermatophyta (seed plants)
Division – Magnoliopsida (flowering plants)
Class – Magnoliopsida (di-cotyledons)
Subclass – Rosidae
Order – Fabales
Family – Fabaceae (pea family)
Genus – *Medicago*
Species – *sativa*

Growth and General Anatomy

Alfalfa (Fig.2.1) is a bushy, deep tap-rooted perennial crop which grows up to 1-m height (Goplen et al., 1982) with small (1-2 mm long, 1-2 mm wide, and 1 mm thick) kidney-shaped seeds (Teuber and Brick, 1988). Half a kilogram of alfalfa generally consists of up to 225,000 seeds (Undersander et al., 2011b). Each seed of alfalfa has two cotyledons, a radicle, a hypocotyl, and an epicotyl. Carbohydrates, fats, and proteins are stored in the cotyledons. These energy-containing compounds help alfalfa seed during germination until the true leaves begin photosynthesis (Undersander et al., 2011b). Alfalfa seeds start germinating once they absorb about 125% of their body weight in moisture. This process of water absorption causes seed to swell and eventually burst the seed coat (Undersander et al., 2011). The radicle becomes the primary root which provides an initial anchor and also serves as a structure for the first root hairs, structures which are important for water and nutrient absorption (Undersander et al., 2011b). The development of this unbranched taproot (i.e., radicle) originates near the seed hilum during germination (Grove and Carlson, 1972). The epicotyl is the growing point of the future stem and is protected between the two cotyledons until the cotyledons are above ground and separate (Undersander et al., 2011b). As the hypocotyledonary area straightens and elongates, the cotyledonary leaves emerge aboveground (Grove and Carlson, 1972).

Its first leaf is unifoliate just above the cotyledons and proceeds with an alternately-arranged growths of trifoliate leaves (Grove and Carlson, 1972). Trifoliate leaflets are 1.3 – 3.8 cm long with smooth edges and are slightly dented at the tip (Bolton, 1962; Goplen et al., 1982). However, literature suggests that the leaflets vary in shape and size from nearly round to ovate (mostly of *M. sativa*), through to obovate and lanceolate (mostly on *M. falcata*; Goplen et al., 1982).

Alfalfa shoots are indeterminate, producing both vegetative and reproductive organs (Bagavathiannan and Van Acker, 2009). Stems are slender, either solid or hollow, and arise through meristematic activities of the shoot apex (Teuber and Brick, 1988). Contractile growth, which pulls the lowermost buds below ground to form the crown begins one week after emergence and is completed within 16 weeks. This is the source of new buds when alfalfa is harvested or for regrowth during spring (Undersander et al., 2011b). Flowers of alfalfa are usually blue or purple (*M. sativa*), but may be white or yellow (*M. falcata*), and rarely bronze and green (Goplen et al., 1982).

Boron

Boron (B; atomic number five) has an atomic weight of 11 (Brodie, 1859) on the periodic table and is an essential element for animals and plants (Ozturk et al., 2010). Boron is found in the oxidized form in the earth's crust, especially in the oceans, sedimentary rocks, coals, shales, and some soils as borax and colemanite (Howe, 1998; Ozturk et al., 2010). Sedimentary rocks bear more B than igneous rocks (Whetstone et al., 1942). However, B is not found in nature in the elemental form because of its complex chemistry switching between metals and non-metals (Ozturk et al., 2010). Boron in rocks and in the earth's crust are not readily available to plants (Gupta et al., 1985), with the majority of plant-available B in the environment coming from the weathering of rocks (Ozturk et al., 2010) or from the decomposition of soil organic matter (Gupta et al., 1985). Less than 5% of the total soil B is available for crop use (Gupta, 1968), creating a widespread possibility of B deficiency in soils.

Based on the convenience of application, sources of B are divided into two types: a) refined products and b) crushed ores (Table 2.1; Shorrocks, 1997). Refined products are soluble and readily used as solutions or granules, whereas crushed ores contain insoluble gangue (unwanted mineral) and have variable chemical and physical properties (Shorrocks, 1997). Refined products are the primary source of B fertilizer.

Boron and Plants

Boron is an essential micronutrient for plants (Ozturk et al., 2010) and plays an important role in plant growth and development (Gupta and Solanki, 2013). The dicotyledonous plants require more B than monocotyledonous plants; however, the rate of B requirements varies depending upon the plant species and genotypes (Radtke, 1986; Howe, 1998). The range of B deficiency and toxicity is narrow (Gupta et al., 1985; Radtke, 1986; Howe, 1998), with 0.1 mg kg^{-1} being deficient, while 0.4 mg kg^{-1} can be potentially toxic depending upon several factors (Meyer et al., 2007). Boron deficiency has been detected in more than 132 crops in 80 countries (Shorrocks, 1997).

Boron is important not only for high yield but also for high-quality crop production (Blevins and Lukaszewski, 1998) as it plays a major role in various plant physiological and metabolic activities. Gupta (1980) highlighted the importance of a continuous supply of B for proper growth and development of the meristematic regions. Similarly, B plays an important role in the translocations of sugars, carbohydrate metabolism, hormone action, nucleic acid synthesis, reproduction of plants and germination of pollens (Gauch and Dugger, 1954; Howe, 1998; Devirian and Volpe, 2003), and the regulation of plant cell growth (Xiaodong and Yiqin, 1999). Boron also helps in cell wall structure, with B deficiency potentially disturbing the organization of cell wall and the middle lamella (Blevins and Lukaszewski, 1998). Similarly, B is responsible for pectic network in cell walls, and controls the growth of plant cells (Xiaodong and Yiqin, 1999).

In plants, B is also helpful for nodule formation, development, and functionality (Ahmad et al., 2009). Nodules in B-deficient field peas (*Pisum sativum)* were found to be smaller in size and in weight compared to the peas with applied B (0.1 mg L^{-1}). The same study found that B-deficient nodules are mostly nonfunctional after 3 to 4 weeks of B starvation because of inhibition of acetylene reduction activity (Bolanos et al., 1994). A similar response was found in soybean (*Glycine max* L.), where the plant growth, nodule development, and nitrogen fixation was negatively affected in the absence of B. Seed production was decreased along with nodule damage because of low acetylene reduction activities when soybean plants were grown in B-free medium in the greenhouse (Yamagishi and Yamamoto, 1994). In dry bean (*Phaseolus vulgaris*) and alfalfa, deficiency of B decreased nodule numbers and inhibited cell and tissue invasion by rhizobium (Redondo-Nieto et al., 2003).

Boron is reported to be important for carbohydrate metabolism and translocation. Deficiency of B resulted in an accumulation of carbohydrates in the leaves of different plants including tomato (*Solanum lycopersicum*), turnips (*Brassica rapa*), tobacco (*Nicotiana tabacum*), carrots (*Daucus carota*), cotton (*Gossypium* sps.), and alfalfa (Gauch and Dugger, 1954). In cotton, photosynthate reallocation from leaf to the ball was decreased which lowered yield. The decreased reallocation is indicative of the physical barrier to carbohydrate transport as a result of poor transport mechanisms (i.e., disruption of cell organization; Bogiani et al., 2013). In contrast, carbohydrate concentration in the floral bud of cotton was found to be higher in B-applied compared with B-deficient cotton (Zhao and Oosterhuis, 2002). In alfalfa, abnormal accumulation of nitrogen and sugars were found in B-deficient plants (Scripture and McHargue, 1943). This disruptive carbohydrate reallocation from leaves to the other parts of the plant as a result of B-deficiency may affect alfalfa aboveground growth and development. Moreover, carbohydrate reallocation to the roots of alfalfa, essential for winter survival, may be reduced (Gauch and Dugger, 1954; Dhont et al., 2002).

Factors Affecting B Availability in Soil

The relative abundance of micronutrients in soil and their availability to the plants are functions of the parent material, soil type, and climate (Sillanpää and Vlek, 1985). Shorrocks (1997) found that the appearance of B deficiency in the plants are dependent upon factors such as amount of B in the soil, moisture during the growing season, crop requirements, harvest time and the portion of crop harvested, weather, and management practices.

Boron content varies between soil parent materials (Table 2.2; Sillanpää and Vlek, 1985; Aubert and Pinta, 1977; Shorrocks, 1997; Sakamoto, 2001), as sedimentary rocks contain more B than the igneous rocks (Whetstone et al., 1942; Turekian and Wedepohl, 1961; Aubert and Pinta, 1977; Sillanpää and Vlek, 1985). In Table 2.2, the B content in igneous rocks (Basalt) is found to be 5-15 mg kg^{-1} but the B content of sedimentary rocks (Shale) ranges from 20-100 mg kg^{-1} (Turekian and Wedepohl, 1961; Sillanpää and Vlek, 1985).

Soil texture, which is a relative proportion of sand, silt, and clay, is dependent upon parent materials (Brady and Weil, 2017). Boron content in coarse-textured soil is lower than in fine-textured soil, making B deficiency more common in plants growing in coarse-textured soils (Gupta et al., 1985; Goldberg, 1997; Hu and Brown, 1997; Shorrocks, 1997; Niaz et al., 2007). However, availability of B is higher in coarse-textured soil due to soil B being strongly held on clay surfaces or $CaCO_3$ in the fine-textured soils (Niaz et al., 2007). In alfalfa, the absorption of B was found to be greater in coarse-textured soil than on fine-textured soil in optimal climates (Wear and Patterson, 1962). On the other hand, B losses due to leaching is greater in freely-drained coarse-textured soil than on fine-textured soils (Shorrocks, 1997). During drought, coarse-textured soils dry up quickly which causes plants to absorb less B from the topsoil and during extreme rainfall and temperatures, the loss of soil B is increased (Shorrocks, 1997).

Soil pH is another important factor in determining B availability in soil (Wear and Patterson, 1962; Gupta et al., 1985; Sakamoto, 2001). Boron becomes less available to plants when $pH > 7.5$ (Goldberg, 1997; Niaz et al., 2007; Ahmad et al., 2012) and the B adsorption in soil increases with increasing soil pH (Steiner and Lana, 2013). Below pH 7, boric acid ($B(OH)_3$) predominates which has a weak affinity for clay, decreasing soil adherence. As tetrahydroxyborate $(B(OH)_4^-)$ and hydroxide (OH^-) concentrations are low at $pH < 7$, their contribution to total B adsorption is small despite their strong affinity for the clay. However, when the pH is increased to 9, the $B(OH)_4^-$ concentration increases rapidly, increasing the amount of adsorbed B by clay because OH^- concentration is still low compared to B concentration (Keren and Bingham, 1985). Increases in pH above 9 enhanced the OH^- concentration relative to $B(OH)_4^-$, and caused a rapid decrease in B adsorption by clay due to competition by OH^- at the adsorption sites (Keren and Bingham, 1985).

A major factor of soil pH is organic matter content, which heavily influences plant nutrient uptake (McCauley et al., 2017). Soil organic matter (SOM) is an important soil component affecting B availability (Gupta et al., 1985; Goldberg, 1997). The SOM releases B through mineralization, an important source of B in the soil, increasing the risk of B deficiency when SOM content decreases (Shorrocks, 1997; Ahmed et al., 2012). The SOM complexes act both as a B-reserve during mineralization as well as a toxicity buffer when B levels increase through B fertilization (Niaz et al., 2007).

Boron deficiency is mostly observed during drought when microbial activity releasing B from the soil organic matter is reduced (Berger, 1962), and available soil moisture could help the release of B from the SOM. The availability of B to the plants is also influenced by rainfall amounts, temperature, and light intensity (Gupta et al., 1985). Boron is easily leached through the soil layers, with excessive moisture causing gravitational water movement, decreasing its availability in areas of high rainfall or frequent thunderstorms. In contrast, during extreme soil drying, the availability of B decreases and leads to B deficient plants because nutrient uptake by the plant is reduced in dry conditions (Goldberg, 1997).

Water stress affects the incidence and severity of B deficiency more than that of any other micronutrient deficiency (Moraghan and Mascagni, 1991). Absorption or uptake of plant nutrients by roots decreases in water-stressed conditions because of a decrease in transpiration rates, impaired active transport mechanisms, and membrane permeability (Levitt, 1980; Alam, 1999). When B deficiency becomes pronounced, root growth is restricted which further intensifies the drought impacts on the plant (Moraghan and Mascagni, 1991). On the other hand, heavy rainfall also is reported to exacerbate B deficiency mainly on coarse-textured soils, due to increased leaching (Shorrocks, 1997).

The more extreme the condition of rainfall and temperature, the greater the loss of soil B. Commonly, B deficiencies in various crops are associated with hot and dry weather (Shorrocks, 1997) where water demand peaks and drying of the soil is quicker than the ability to irrigate or the occurrence of rainfall events. Crop in this condition undergoes drought avoidance by stomatal closure (Ullah et al., 2017). A dry soil reduces availability and uptake of B from plant roots to the aboveground plant parts (Berger, 1962).

Boron deficient plants are more brittle (due to the poor cell wall integrity) which increases risk of leaf shatter due to wind or management practices like threshing, raking, haying, and baling than B-sufficient plants (Gauch and Dugger, 1954; Shorrocks, 1997). Cutting and transporting of alfalfa hay from production fields to market means that a large proportion of B is removed from the field and thus from the soil (Shorrocks, 1997). Alfalfa harvesting versus graze is the reason why more B is applied to fields in North America compared with South America. In Argentina for instance, alfalfa is mostly grazed so B deficiency is relatively reduced due to nutrient cycling in animal feces (Shorrocks, 1997).

Boron Deficiency and Toxicity in Alfalfa

Boron deficiency in alfalfa can be determined through visual analysis, soil testing, and plant tissue analysis which are described below with probable symptoms and respective values for deficiency, sufficiency, and toxicity.

Visual Analysis: The major symptom of B deficiency in alfalfa is yellowing of petioles or leaf tips (Fig.2.2 and Fig.2.3; Wright, 1986; Meyer et al., 2007; Ottman, 2010;

Undersander et al., 2011a) because B is immobile and deficiencies first appear in younger tissues (Berger, 1962; Brown and Shelp, 1997). Visual cues include retarded growth of plants or shortened internodes, abnormal leaf structure, dense leaf canopy on the top (Meyer et al., 2007; Herrera-Rodríguez et al., 2010; Undersander et al., 2011a), necrosis of terminal buds, and wilting of leaves (Willis and Piland, 1937). Though B deficiency in alfalfa can be visually observed, these symptoms are easily confused with injuries caused by other factors like diseases, salt or water stress, insects, other nutrient deficiencies, and leafhopper damage (Meyer et al., 2007; Undersander et al., 2011a). This might cause significant losses in yield and quality of alfalfa before the B deficiency symptoms are visible (Radtke, 1986; Meyer et al., 2007). Distinctively, B toxicity symptoms in alfalfa are visible at the edges of older leaves as chlorotic or necrotic patches, and in some cases leaves turn yellow leading to defoliation or death in the wost case scenario (Radtke, 1986; Nable et al., 1997).

Soil Test: Soil testing determines nutrient availability and is useful in evaluating the fertility status of the field prior to planting (Meyer et al., 2007). It helps to reveal initial fertility status of the field without waiting for plant damage to occur (Radtke, 1986). In the case of B, the total soil B status is less useful on a short term basis because it is mostly unavailable (Radtke, 1986). As mentioned in the previous section (i.e., Factors Affecting B Availability in Soil), there are a number of factors interacting with each other on soil B concentration.

It remains challenging to point out the optimum rates of B for alfalfa production because B adequacy levels in the soil are interpreted differently in literature (Table 2.3).

However, it is argued that any B level over 0.5 mg kg^{-1} and less than 2.0 mg kg^{-1} is in the optimum range (Table 2.3), with the exception of Meyer et al. (2007) whose optimum range of soil B for alfalfa is 0.2-0.4 mg kg^{-1}. Different soil types and climatic conditions can be a reason for this difference in the optimum level.

Plant Tissue Analysis: Another method to determine the nutrient deficiency of crops, which is considered the most precise method of determining plant nutrient needs, is a plant tissue test (Meyer et al., 2007). However, it can only be measured once the crop is actively growing, so the production loss due to nutrient deficiency is more likely to occur during the first harvest (Radtke, 1986). The soil test method is described as the best approach to prevent nutrient deficiency (Undersander et al., 2011a) whereas the plant tissue test is a precise method to fine-tune the in-season nutrient needs (Meyer et al., 2007). Combining the tissue test with a soil test creates a comprehensive nutrient management of crops (Undersander et al., 2011a). Based on various literature, sufficiency levels of B on top 15 cm of alfalfa when harvested at 10% bloom are listed in Table 2.4.

Addressing B Deficiency and Toxicity in Alfalfa

Boron deficiencies in alfalfa can be corrected by an application of commonly-available B fertilizers (Table 2.1). For B-tolerant crops like alfalfa, previous literature recommends 3.9-7.8 kg B ha^{-1}, however, lower rates of B should be used on sandy soils and higher rates on the fine-textured soils (Meyer et al., 2007). A major problem with B fertilization is the narrow range of concentration from deficient to toxic levels (Gupta et al., 1985; Howe, 1998). Boron application can overcome the deficiency symptoms. In B toxicity scenarios, fields can be heavily-irrigated so that the excess B leaches from the field; however, the irrigated water should be free from B to decrease further introduction (Radtke, 1986). Also, the addition of lime to an acidic soils reduces B toxicity because when soil pH increases, B fixation in the soil also increases (Radtke, 1986; Gupta, 1993).

Alfalfa Yield Response to B Application

Boron is an essential micronutrient for plants and has a major function in crop production and growth (Gupta, 1980). In Oregon (Dregne and Powers, 1942), Connecticut (Brown and King, 1940), Idaho (Mahler and McDole, 1981), and New York (Crowder and Baird, 1958), alfalfa yield increased by 47.7%, 16%, 13%, and 79%, respectively, with applied B fertilizer. Conversely, application of B did not increase alfalfa yield in studies conducted in Ohio, and New York (Chandler et al., 1946; Razmjoo and Henderlong, 1997). Difference in soil types and environmental conditions between the research publications seemed to elicit different responses to B application. Inconsistent responses of alfalfa production to B fertilization in these projects may have been due to various factors including: environmental, plant genotypes, stage of plant growth, soil texture, soil adsorption, and soil texture (Gupta, 1983).

Alfalfa Irrigation

Alfalfa has a high water requirement, using up to 1600 mm of water during the growing season depending upon geographic location, weather, and soil type (Kuslu et al., 2010). The increased water use of alfalfa compared to other crops is mainly due to its long growing season, dense canopy, and deep tap root system that enhances its ability to use moisture deeper in the soil (Irmak et al., 2007; Shewmaker et al., 2013). The amount of water used by alfalfa depends on the type of cultivar, stage of growth, canopy density, and harvest date (Efetha, 2011). Annual water use varies seasonally and geographically; the average daily evapotranspiration for 11 Western States is reported as 5.8 mm, with average peak daily uses of 5.1, 6.4, and 7.6 mm in cool, moderate, and hot climates, respectively (Stanberry, 1955). Alfalfa daily water use in Nebraska has been reported to range between 8 to 9 mm for the months of July and August, respectively, and can be as high as 12 mm on hot, windy, and dry days (Irmak et al., 2007). Shewmaker et al. (2013) indicated that alfalfa grown at Kimberly, Idaho uses about 900 mm of water per year and under extreme conditions 10 mm per day in mid-summer.

The roots of a well-irrigated alfalfa plant have an effective root zone of about 120 cm with root distribution mainly concentrated near the soil surface. Alfalfa typically receives 40% of its seasonal water from the upper 30 cm, 70% from the upper 60 cm, and 90% from the upper 90 cm of the 120 cm root zone (AIMM, 2016). It is important that particular attention is given to the moisture status of the soil profile during irrigation scheduling so that irrigation is initiated when about 50-60% of the plant available water in this soil depth is depleted. This will avoid water stress which can lead to yield potential loss.

Irrigation Scheduling for Alfalfa

Developing an irrigation scheduling (IS) strategy can help to plan for and properly irrigate alfalfa based on crop water demands (Irmak et al., 2007). Establishing an irrigation strategy will help to determine when and how much water to apply and when it is needed (Peterson, 1972). An IS also assures consistent availability of water to plants based on the daily crop water requirement.

There are various factors influencing irrigation decisions: weather, crop variety, soil fertility, rainfall events, and interactions of these factors that all contribute to the decision of IS (Peterson, 1972). Attram (2015) indicated that soil texture, soil water holding capacity, effective root zone and allowable water depletion (sometimes known as management allowable depletion) by the crop are also responsible in determining IS. Through proper irrigation, crop yield and quality can be improved and profitability and water productivity increased. Proper IS also prevents excessive water application where water is lost through deep percolation and runoff, optimizing pumping costs (Attram, 2015).

The IS methods for alfalfa and other crops are classified into three categories: plant-based, soil-based, and ET-based methods (AIMM, 2016).

Plant-Based Methods: The plant-based approach to IS can be the simplest possible approach. Irrigation in this method is based on plant wilting or visual conditions of the crop. Various water stress indicators for IS are stomatal conductance, leaf conductance, and net CO_2 assimilation or net photosynthesis (Fernández, 2017). These methods of measurement may be quick, popular, and convenient, but it does not indicate how much water to apply, is labor intensive, less precise, and causes economic yield loss before the water stress symptoms are visible or indicated (Jones, 2004; AIMM, 2016). The principle of using plant-based methods of IS is that the plant’s response using the aforementioned processes is directly related to plant water status and is indirectly related to soil moisture and atmospheric conditions (AIMM, 2016).

Soil-Based Methods: Measuring soil moisture can help to determine the amount of water needed to bring the soil to field capacity (AIMM, 2016). With this method, a "soil moisture" or "soil water potential" sensor specifies when and how much to irrigate based on soil moisture profile (Osroosh, 2014). There is a wide range of soil moisture measuring instruments available including: dielectric sensors, tensiometers, gypsum blocks, gravimetric methods, psychrometers, and granular matrix sensors (Jones, 2004; Osroosh, 2014). It is convenient, precise, and indicates how much water to apply; however, in this method it is hard to position the soil probe at a depth that is representative of the root-zone. Additionally, sensors may not measure water status at the root surface as it can be difficult to locate roots, and the point of selection might not be a good representative of the entire field (Jones, 2004; Osroosh, 2014). Moreover, recent reviews by Evett et al. (2007) asserted that most of the commercially-available soil moisture sensors signaling moisture availability are imprecise.

Evapotranspiration (ET) - Based Methods: The ET-based methods are also referred to as weather-based methods of IS (AIMM, 2016). Allen et al. (1998) defined ET as the sum of water loss from both the soil surface evaporation and the water loss from the crop through transpiration. The ET is influenced by various factors like weather parameters, crop characteristics, soil fertility, soil moisture, management aspects, and interaction of these major parameters (Peterson, 1972; Allen et al., 1998). The ET rate from a well-watered grass reference surface is referred to as reference evapotranspiration (ETc) – which is affected by actual weather variables (Allen et al., 1998). Crop evapotranspiration (ETc) is the evapotranspiration from an ideal plant which is disease-free, well-fertilized, grown in a large field with optimum soil water conditions, and is producing the highest yield at any given weather conditions (Allen et al., 1998). Daily ETo (grass), and other reference ET, can be obtained from local weather stations. Similarly, daily ETc can be estimated by multiplying ETo by the alfalfa crop coefficient (K_c; Eq. I). The Kc values are affected by various factors like crop type, stage of growth, soil moisture, plant health, and cultural practices and are determined under highly controlled conditions of adequate soil moisture, good plant health, and cultural practices (Hanson, 2016).

$$\mathrm{ET_c} = \mathrm{ET_o} \times \mathrm{K_c} \qquad \text{(Eq. I; Allen et al., 1998)}$$

Daily soil moisture depletion can be calculated by subtracting daily ETc, as well as any drainage and runoff, and adding irrigation and rainfall received, starting with 100% field capacity at spring growth or planting for each day of plant growth (Torrion and Stougaard, 2017).

$$\mathrm{PAW_i} = \mathrm{PAW_{i-1}} - \mathrm{ET_c} + \mathrm{I} + \mathrm{R} - \mathrm{D} - \mathrm{RO} \text{ ----------- (II; Broner, 2004)}$$

Where,

i = Current day plant available water today (inches)
$\mathrm{PAW_{i-1}}$ = Prior day plant available water (inches)
$\mathrm{ET_c}$ = Crop evapotranspiration (inches)
I = Irrigation (inches); R = rainfall (inches); D = drainage (inches); RO = surface run-off

It is difficult to estimate runoff and deep percolation in field conditions so whenever the water addition (I+ R) to the root zone is greater than $\mathrm{PAW_{t-1}} + \mathrm{ET_c}$, soil water depletion can be adjusted to zero (Andales et al., 2015). Equation II can be simplified to

$$\mathrm{PAW_i} = \mathrm{PAW_{i-1}} - \mathrm{ET_c} + \mathrm{I} + \mathrm{R} \text{ ------------- (III)}$$

In Heermann et al. (1990) the water balance equation has been illustrated differently than that is presented above.

$$\mathrm{D_{p_i}} = \mathrm{D_{p_{i-1}}} + \mathrm{K_{c_i}} * \mathrm{E_{tp_i}} + \mathrm{E_{tr_i}} - (\mathrm{R_i} - \mathrm{RO_i}) + \mathrm{W_{d_i}} ------(\mathrm{IV})$$

Where,

$\mathrm{D_{p_i}}$ = the depletion on day i,
$\mathrm{K_{c_i}}$ = crop coefficient (a function of crop development),
$\mathrm{E_{tp_i}}$ = the reference evapotranspiration,
$\mathrm{E_{tr_i}}$ = additional soil evaporation following an irrigation or rain,
$\mathrm{R_i}$ = the sum of effective rainfall and net irrigation on day i,
$\mathrm{RO_i}$ = surface runoff,
$\mathrm{W_{d_i}}$ = the drainage below root zone or upward flow (–) from the groundwater.

An important aspect of IS using an ET based soil-water-balance approach (equations, II-IV) is determining the soil water holding capacity (WHC). The WHC is defined as the difference between field capacity and permanent wilting point (PWP; Brady and Weil, 2010). The WHC determines the total PAW at FC of a soil depth where roots are actively growing (Brady and Weil, 2010). Though the method is straightforward and indicates when and how much to irrigate, it requires an estimate of evaporation and rainfall, frequent calibration with change in plant growth and root development, and accurate estimates of local precipitation or runoff. Errors may be cumulative which alter the supposedly precise irrigation in the long run, if in-season adjustment or calibration is not carried out (Jones, 2004).

Alfalfa Yield Response to Irrigation

Alfalfa stands can fully recover from drought stress once irrigation is resumed, making alfalfa an ideal forage species in regions where water is limiting (Rogers et al., 2016). It is drought tolerant but is also well-adapted to irrigated or high rainfall environments, producing yields in proportion to water supply (Peterson, 1972; Shewmaker et al., 2013). It produces an annual yield up to 17-26 tons ha^{-1} when irrigated (Rogers, 2001; Greenwood et al., 2006; Lawson et al., 2009) compared to 3-11 tons ha^{-1} under dryland conditions (Hirth et al., 2001).

A number of studies have been conducted evaluating the importance of irrigation in alfalfa. Alfalfa yield was highest when the moisture levels were managed to meet its daily ETc in Las Cruces, New Mexico (Abdul-Jabbar et al., 1982), increasing linearly with increasing water supply (Donovan and Meek, 1983). Effects of water-stressed conditions were found to severely affect alfalfa forage yield primarily due to reduced transpiration which affects growth performance and consequently reduces yield (Ball et al., 2001; Hanson et al., 2007; Al-Naeem, 2008; Slama et al., 2011).

Peterson (1972) mentioned that alfalfa used to be irrigated only in arid climates of the world because its daily ET demand is high enough to reduce yield. Cultivation of irrigated alfalfa is expanding to semi-arid environments due to market-demand (Fereres and Soriano, 2006). Irrigation sustains production of alfalfa even in humid regions during years where rainfall events and amounts are erratic. In semi-arid regions, production of alfalfa increases from 19.8 to 26.5 tons ha^{-1} through adequate supply of water, compared to production of just 2.4-7.5 tons ha^{-1} in similar rain-fed locations (Peterson, 1972).

Research in Foggia, Italy on a black loam soil recorded a significant increase in alfalfa stem number with irrigation. Stem number varied between 320.5-472.1 m^{-2} for irrigated fields and was only 44.4-230.1 m^{-2} under rainfed conditions. This increased stem density had a concurrent increase in alfalfa yield, attributed mainly to the four-fold increase in the density of stems. In this project, irrigation was applied from April to the beginning of seed filling. The total amount of water applied was 378, 373, and 328 mm ha^{-1} for the years 1995, 1996, and 1997, respectively (Iannucci et al., 2002).

Alfalfa yield increased linearly with irrigation in a study conducted in northern Victoria, Australia. In the 5-years of study, annual production ranged from 1.4-17.7 tons ha^{-1} with the highest dry matter recorded under fully-irrigated plots (Rogers et al., 2016). Linear increases in yield with increasing water application was also found in a study done in North Dakota, USA on a Maddock sandy loam soil. Four-year average yield was 5,800 kg ha^{-1} under rainfed conditions, and increased to 10,350 kg ha^{-1} when optimal irrigation was applied (Bauder et al., 1978). Shewmaker et al. (2013) also found that yield response to water application is linear for alfalfa (Fig.2.4). However, the linearity may vary regionally (Hill et al., 1983), seasonally, and even down to in-season cutting events (Myer et al., 1991).

Water stress, just like in any other crop, limits alfalfa production more than any other management practice (Undersander et al., 2011a). In a well-managed IS, yields may have a linear response to ETc as long as water is provided at the required ETc (Undersander et al., 2016). Studies in various parts of the world reported linear yield responses to increasing but properly managed IS (Saeed and El-Nadi, 1997; Greenwood et al., 2006; Jafarian et al., 2016; Maona et al., 2016); however, failure to provide sufficient supplemental irrigation during reproductive development or during peak demand recorded significant yield penalties (Guitjens, 1993; Orloff et al., 2014).

Factors Affecting Forage Quality

Forage quality is defined as the extent to which a forage has the potential to produce a desired animal response (Ball et al., 2001). It is also a function of nutrient concentration, amount of forage consumed or intake, digestibility, and partitioning of metabolized products within animals (Buxton, 1996). Additionally, forage quality is also defined as the broader term that includes nutritive value, anti-quality factors, and forage intake (Adesogan et al., 2002, Newman et al., 2006). Forage quality is determined based on leaf-stem ratios, degree of lignification, fiber, and crude protein (CP) content of the species (Elliott et al., 1972). There are various factors affecting forage quality including: maturity, species, temperature, and management (Elliott et al., 1972; Buxton, 1996; Ball et al., 2001; Adesogan et al., 2002).

Plant maturity impacts forage quality (Ball et al., 2001) due to decrease leaf-to-stem ratio with increase maturity, which also decrease intake potential, and rate of fiber digestion (Fick et al., 1994; Buxton, 1996; Ball et al., 2001). Reduced leaf-to-stem ratio causes a decline in forage quality because leaves are more nutrient-dense than stems (Ball et al., 2001, Fick et al., 1994), containing on average approximately 70% of plant nutrients. With increases in maturity, or as reproductive development of plants begin, leaf-to-stem ratio decreases, and the overall forage nutritive quality also decreases (Ball et al., 2001). Leaves are high in nutritional value and have a strong positive correlation to CP and other forage qualities (Fick et al., 1994).

Crude protein is determined by measuring the amount of Nitrogen (N) in the forage and multiplying the obtained value by 6.25, with the assumption that N constitutes about 16% of tissue protein in the forage (Newman, et al., 2006). The CP content varies between forage types, with legumes having the highest CP concentration, averaging 120-250 g kg^{-1}, followed by cool-season grasses (80-230 g kg^{-1}), and then warm-season grasses (50-180 g kg^{-1}; Newman, et al., 2006). When leaf-stem ratio decreases, fiber content increases and CP and overall quality decreases (Fick et al., 1994; Ball et al., 2001).

Fiber refers to the cell wall constituents of hemicellulose, cellulose, lignin, pectin and other CHO components (Newman et al., 2006), which is partitioned into acid detergent fiber (ADF), and neutral detergent fiber (NDF). The NDF contains cellulose, hemicellulose, and lignin; whereas ADF is made up of only cellulose and lignin (Newman et al., 2006). With increases in NDF value, animal dry matter intake decreases. Similarly, with increases in ADF value forage digestibility decreases. Low ADF and NDF are desirable for good quality forage and increased animal performance (Newman et al., 2006).

Legumes generally are considered higher quality forage than grasses when compared at similar maturities due to legumes having less fiber, higher CP, and higher animal intake rate due to a greater percentage of rapidly digestible leaves (Ball et al., 2001; Adesogan et al., 2002). As plants reach their reproductive stage, leaves senesce due to the carbon reallocation from the leaves to the seeds (Chardon et al. 2014), causing the reduction of leaves and quality at harvest.

Plant environment is also an important determinant of forage quality. The most important environmental factors affecting forage quality are: temperature, water stress, and solar radiation (Buxton, 1996). Increases in temperature hastens plant development, enhances leaf drop (i.e., lowers stem-leaf ratio), and lowers digestibility (Buxton, 1996); a 1°C increase in temperature decreases digestibility of cool-season forages by 3-7 g kg^{-1} (Wilson and Minson, 1983; Buxton, 1996). Additionally, the NDF of forages grown under high temperatures is less digestible than forages grown in cool temperatures because of increased lignification due to the increased temperatures (Buxton, 1996). Similarly, changes in photoperiod affect forage quality; an increase of 1 hour in day length increases digestibility by 2 g kg^{-1} (Buxton, 1996).

Water is equally important for alfalfa yield and forage quality. Under water-stressed conditions, leaf mass is reduced because of accelerated senescence of older leaves. As leaves senesced and die, protein, Nitrogen, and carbohydrates are transported out of the leaves, reducing yield and forage quality significantly (Buxton, 1996). Water-stressed conditions are generally accompanied with high atmospheric temperatures, which hastens crop maturity and decreases the forage quality under natural conditions (Vough and Marten, 1971).

Managing both water and nutrients can increase yield and quality of crops, minimizing impacts of water and nutrient stresses (Shiferaw, 2017). Additionally, management factors such as water application impact nutrient uptake to the plants because water drives root proliferation in the soil profile. The concentration of root hairs per unit volume of soil impacts the nutrient absorption which is in tandem with availability of water (Wimmer et al., 2015).

Irrigation is one of the major yield-limiting factors for alfalfa (Putnam, et al., 2000) and B is required for alfalfa growth and development. Boron deficiency can affect water uptake due to the reported xylem damage which then reduces transpiration rates even in well-watered conditions (Wimmer and Eichert, 2013).Though irrigation and B application plays an important role in alfalfa production, research to evaluate the interaction of irrigation and B has not been quantified yet for alfalfa. The research described in this thesis was conducted to bridge the gap and evaluate the effects of full- and deficit-irrigation, differing rates of B, and the interaction effect of irrigation and B on alfalfa yield and quality.

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Table 2.1: Most commonly used agricultural borates. Reproduced from Shorrocks (1997).

Types	Formula	Name	B (%)
Refined products	$Na_2B_4O_7 \cdot 5H_2O$	Sodium tetraborate pentahydrate	14.9
	$Na_2B_8O_{13} \cdot 4H_2O$	Solubor	20.8
	$Na_2B_4O_7 \cdot 10H_2O$	Sodium tetraborate decahydrate	11.3
	$Na_2B_4O_7$	Sodium tetraborate	21.4
	$B(OH)_3$	Boric acid	17.5
Crushed ores	$2CaO \cdot 3B_2O_3 \cdot 5H_2O$	Colemanite	Variable
	$Na_2O \cdot 2CaO.5B_2O_3\ 16H_2O$	Ulexite	
	$2CaO \cdot B_2O_3 \cdot 2SiO_2 \cdot H_2O$	Datolite	
	$CaO \cdot MgO \cdot 3B_2O_3 \cdot 6H_2O$	Hydroboracite	
	$2MgO \cdot B_2O_3 \cdot H_2O$	Ascharite	

Table 2.2: Boron content in soil differs between parent materials. (Source: Sillanpaa and Vlek, 1985)

Element	Igneous rocks		Sedimentary rocks		
	Granite	Basalt	Limestone	Sandstone	Shale
Boron (mg kg^{-1})	15	5	20	35	100

Table 2.3: Interpretation of Boron concentration in the soil.

	Boron (mg kg^{-1})				
Source	Very Low	Low	Optimum	High	Excessively High
(Rehm et al., 1993)	-	<0.9	1.0-5.0	>5.0	-
(Kelling, 1999)	<(0.2-0.5)	0.3-1.0	0.5-2.0	1.1-4.0	>(2.5-4.0)
(Koenig et al., 1999)	-	<0.25	>0.5	-	-
(Meyer et al., 2007)	0.1	0.1-0.2	0.2-0.4	>0.4	-
(Kaiser et al., 2011)	-	<1.0	1.0-5.0	>5.0	-

Table 2.4: Interpretation of Boron concentration of top 15 cm of plant when harvested at 10% bloom.

	Boron (mg kg^{-1})			
Source	Deficient	Low	Sufficient	High
(Rehm et al., 1993)	<20	20-30	31-80	>80
(Kelling, 1999)	<20	20-30	30.1-80	>80
(Undersander et al., 2000)	-	<25	25-60	>60
(Meyer et al., 2007)	<15	15-20 (marginal)	20-40	>200
(Schwab et al., 2007)	-	-	20-80	-

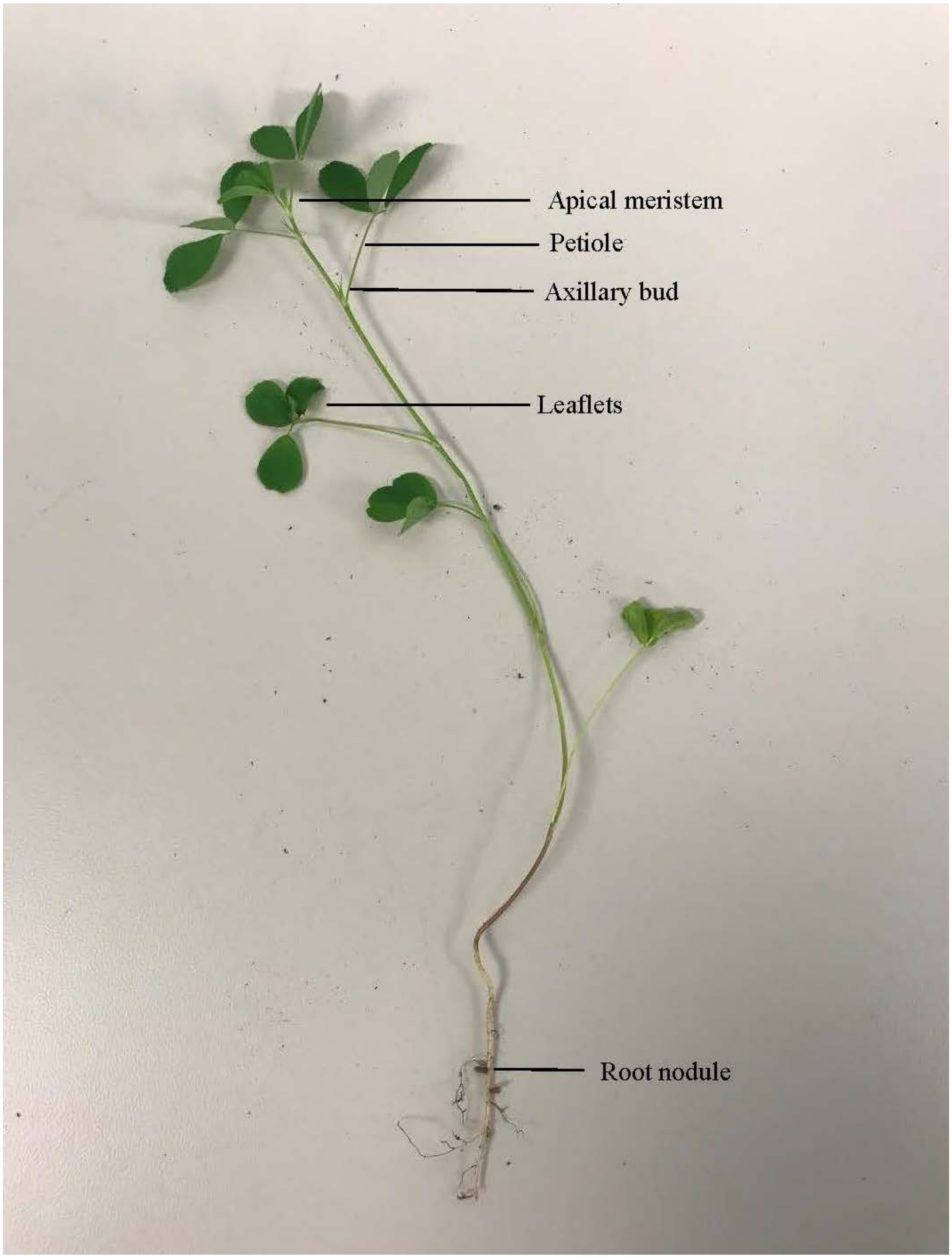

Figure 2.1. General anatomy of alfalfa showing various parts

Figure 2.2: Alfalfa plants with symptoms of possible boron deficiency.

Figure 2.3. Alfalfa trifoliate with possible boron deficiency symptoms. Photo by Angie Peltier, University of Illinois Extension (available at http://bulletin.ipm.illinois.edu/print.php?id=1634, accessed on 25 Dec. 2017)

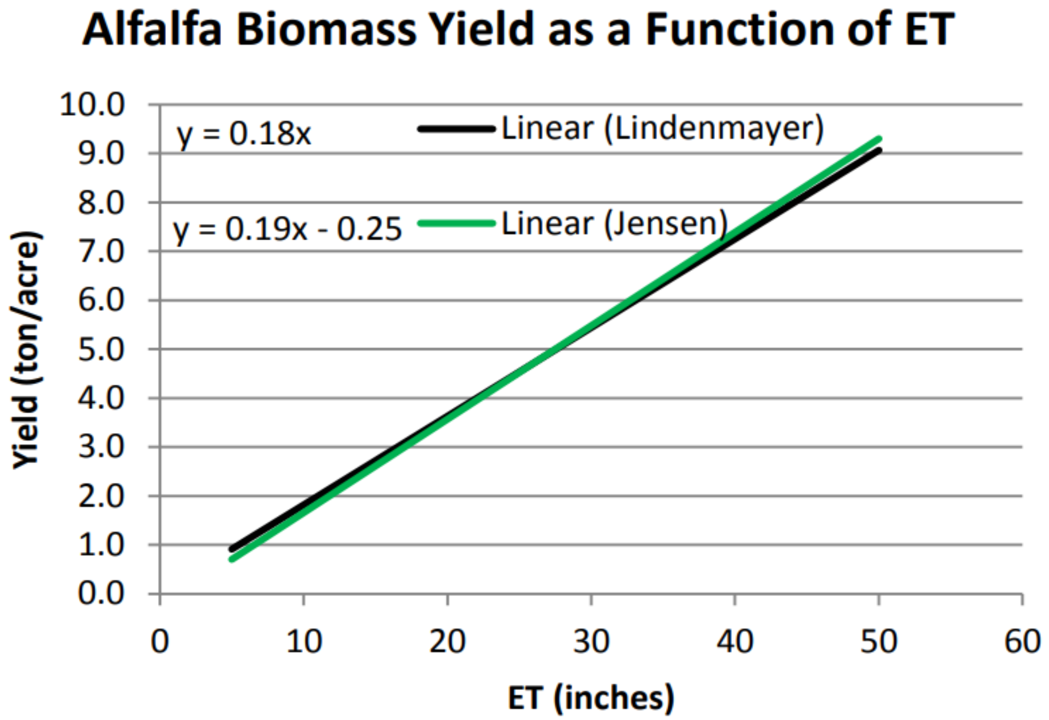

Figure 2.4. Linear increase in alfalfa yield with application of irrigation water. Graph retrieved from Shewmaker et al. (2013)

CHAPTER THREE

BORON FERTILIZATION OF IRRIGATED ALFALFA IN MONTANA

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

Author: Anish Sapkota

Contributions: Main author responsible for data collection, statistical analysis, interpretation, and drafting of this thesis.

Co-Author: Emily C. Meccage

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Abstract

A boron (B) deficient soil may negatively impact irrigated alfalfa (*Medicago sativa* L.) plant tissue sufficiency; thereby compromising yield and quality in a short-season environment such as Montana. The objective was to identify the impacts of B fertilization on irrigated alfalfa yield and quality conducted in 2015/2016 at Creston and Dillon, MT on fine sandy loam and silt loam soils, respectively. The initial soil B at the Creston and Dillon sites were 0.2 and 0.8 ppm, respectively. The study was conducted as a randomized complete block design with five B levels: 1) 0, split- applications of 2) 0.50, 3) 1.00, 4) 2.00, and a one-time application of 5) 2.00 lb acre^{-1} on four replications. Full doses of treatment five and half the dose of treatments 2-4 were applied in early spring at 3-inch regrowth height. The other half dosage of treatments 2-4 was re-applied at 3-inch plant height after the first cutting. A liquid 10% B Agrisolution^{TM} was foliar-applied as B fertilizer. All the cuttings were performed at 10% bloom. Application of B increased ($P < 0.05$) plant B content in both locations. Boron increased ($P < 0.05$) crop yield for the second cutting in 2015 at Dillon, but application of B did not influence all the other cuttings nor total yields for either year or location. No significant effect of B on forage quality was observed. This research suggests that foliar B fertilization based on a low B soil test is not beneficial for irrigated alfalfa producers in Montana.

Keywords: *Medicago sativa, yield, forage, quality, boron, irrigation, fertilizer*

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Introduction

Alfalfa (*Medicago sativa* L.) is a widely-grown perennial forage crop because of its adaptability to varied environmental conditions (Iannucci et al., 2002). It is effective at fixing atmospheric nitrogen (Peterson and Russelle, 1991) and is known for its high dry matter yield and forage quality (Brown, 2004). It is a preferred forage for livestock as it has low fiber content (Balliette and Torell, 1993), is highly digestible (Rodiek, 2001), and has high protein (Higginbothan et al., 2008). In the U.S, alfalfa is grown on almost 17 million acres of cropland (USDA-NASS, 2017a). It is an important forage source for the livestock and dairy industries as many producers depend heavily upon alfalfa as a mainstay crop. Additionally, the dairy and equine industries are continuing to expand their utilization of alfalfa hay (Putnam et al., 2000). To meet this increasing demand for alfalfa hay, growers can strategically apply the required fertilizer, along with other agronomic practices, to increase hay yield and quality (Mueller and Orloff, 1994).

Boron is an essential micronutrient for plant growth and development (Dear and Weir, 2004). Boron deficiency impairs plant growth and reduces quantity and quality of crops (Sakamoto, 2012). Boron helps in the translocation of sugars, carbohydrate metabolism, hormone action, nucleic acid synthesis, reproduction of plants, root growth, and germination of pollen (Dell and Huang, 1997; Howe, 1998; Devirian and Volpe, 2003). Nutrient removal is high in hay production, particularly in productive areas, and can eventually suppress alfalfa yield and quality if not replenished (Dordas, 2006). Shorrocks (1997) reported that the removal of B is markedly higher in alfalfa (0.31 lb acre^{-1}) compared to other crops such as *Beta vulgaris* (0.27 lb acre^{-1}), *Gossypium* *hirsutum* (0.13 lb acre^{-1}), *Triticum* spp. (0.02 lb acre^{-1}), and *Helianthus annuus* (0.09 lb acre^{-1}). Boron deficiency in alfalfa causes chlorotic leaves (Ottman, 2010, McCauley et al., 2011, Undersander et al., 2011), stunted growth (Herrera-Rodríguez et al., 2010), abnormal leaf structure, necrosis of terminal buds, and excessive wilting (Willis and Piland, 1937), all of which negatively impact hay production. Moreover, B-deficient alfalfa produces relatively low root biomass and root nodule number and size, which reduces nitrogen-fixing performance (Bonilla et al., 2009). Wright (1986) reported that leaves of B-deficient alfalfa dry much faster than leaves of B-sufficient alfalfa, so leaf loss (while raking and baling) of the former is greater compared with the later. Increased leaf loss decreases leaf to stem ratio, lowering the protein content and digestibility of the harvested hay (Wright, 1986).

While addressing the negative impact of B deficiency in alfalfa, an excessive amount of applied B also reduces crop yield and quality (Nable et al., 1997; Hong et al., 2009). An abundance of B that exceeds 6 ppm in the soil can cause toxicity (Orloff, 1995). Boron toxicity symptoms on alfalfa are generally visible in lower trifoliates of the stem as marginal or leaf-tip chlorosis (Gupta, 1991) to yellowing of an entire leaf that leads to defoliation (Bradford, 1966).

Alfalfa response to B fertilization is conflicting. In Oregon, B fertilization led to an increase in alfalfa production. The application of boric acid (17.5% B) at the rate of 30 lb acre^{-1} (i.e., 5.3 lb B acre^{-1}) on William silt loam soil in a dryland setting increased alfalfa yield by 47.7% in comparison with the control treatment (with 0.6 to 0.9 ppm soil B) which yielded 3.4 ton acre^{-1} (Dregne and Powers, 1942). Research on B-deficient soil conducted in Connecticut,found that application of B decreased the prevalence of "yellowing" in alfalfa from 25% to 3% and increased yield by 16% when 2.3 lb B acre^{-1} was applied, compared with a no-fertilizer control (Brown and King, 1940). Similarly, in Idaho, application of 1.0 lb B acre^{-1} in B-deficient soil increased alfalfa yield by 13% (Mahler and McDole, 1981). A study performed in New York in a dryland setting also found a significant yield increase when borax (11.3% B) was applied. Total production averaged 1.24 ton acre^{-1} with a 3.4 lb acre^{-1} B application versus 0.67 ton acre^{-1} in the 0 lb acre^{-1} B-applied check (Crowder and Baird, 1958). Conversely, application of B did not increase alfalfa yield in studies conducted in Ohio and New York. Razmjoo and Henderlong (1997) found application of up to 1.8 lb B acre^{-1} did not affect alfalfa dry matter yield in Columbus, OH on Crosby silty clay loam soil. Similarly in New York, NY, no yield response was observed with B application rates of up to 4.4 lb acre^{-1} (Chandler et al., 1946).

Fertilization based on soil tests at the beginning of the season is generally used to correct nutrient deficiency to avoid a negative impact on yield and quality of alfalfa. According to the available B amendment guidelines applicable for this region (Mahler and Mcdole, 1981; Jacobsen et al., 2005) which are based on soil test results, verification of the soil test-based B fertilization recommendation for irrigated alfalfa production is lacking in MT. This project aimed to evaluate the response of foliar application of B on the yield and forage quality of irrigated alfalfa in Montana. We hypothesized B fertilization would improve yield and forage nutritive value on soils testing low or very low in B.

Site Description

A two-year study was conducted in 2015 and 2016 at two different irrigated locations in western Montana with low initial soil B (described in Table 1). These sites represent two of the relatively higher alfalfa-producing regions in the state, producing an average of 3.0 and 4.0 ton acre^{-1}, for northwest and southwest region of Montana, respectively (USDA-NASS, 2017b). Based on the alfalfa fertilization guide for Montana (Jacobsen et al., 2005), other nutrient requirements for the research sites in this study were met via soil amendments following early spring soil sampling. The second year of the study continued from the first year’s experimental sites.

Boron Treatments and Experimental Design

The experiment was laid out in a randomized complete block design with five B treatments replicated four times. The plots measured 10 x 15 ft with a 5 ft alley between replications and around the experimental field to allow easy access for plot maintenance and harvesting. Boron treatments and their timing of application are described in Table 2. Treatment randomization and the B application protocol were determined using Agriculture Research Manager (Gylling Data Management, 2016). Both research sites were located within production fields. In the production field in Dillon, no mowing was done on the perimeter of the experimental plots. At the Creston site, the perimeter of the plot was maintained via mowing for easier access of the forage harvester; however, border plots were retained around the experimental site.

A liquid B formulation (10% B AgriSolutions^{TM}) was used as the B fertilizer source, as it is widely-available to producers in Montana. A liquid formulation was chosen for the ease in correct application to a small plot area. The volume of solution for each treatment was 43 oz which was then divided into four equal parts (i.e., 10.8 oz) for each experimental unit. Each 43 oz of solution contained 0.6, 1.2, 2.6, and 5.1 oz of 10% liquid B fertilizer for the respective treatments B_1, B_2, B_3, and B_4 (Table 2). The first application was made to treatments B_1-B_4 in spring when plant height was 3-inch and the second application was made to treatments B_1-B_3 when regrowth height after the first cutting was 3-inch. The amount of B fertilizer was the same during first and second application for the respective treatments (Table 2). A CO_2-powered backpack sprayer equipped with a 10-ft flat nozzle was used to apply the prepared B solution.

Yield and Dry Matter Analysis

Alfalfa was harvested at 10% bloom at both locations for all harvests. A forage harvester (ALMACO^{TM}, Nevada, Iowa) was used to harvest alfalfa plots at Creston. Harvesting was confined to the middle of the plot (5 x 15 ft strip) to avoid an edge effect. Three cuttings were taken in Creston in both years. A battery-powered, hand-held electric shear (Black and Decker^{TM}, New Britain, Connecticut) was used in Dillon with a 3.3 x 3.3-ft quadrat which was randomly thrown into the middle of each plot. Only two cuttings were made in Dillon in both years.

Petioles from the top 6-inches (Kelling 1999; Undersander et al., 2011) were randomly collected from 20 plants in each plot for petiole B analysis, and 20–whole plants were bagged from each plot for whole tissue forage nutrient quality. The fresh biomass samples collected for yield determination were immediately weighed and then dried in a forced-air oven at 140º F for 48 h or until they reached a stable dry weight. The whole plant for forage quality and top 6-inch for petiole B samples were dried, ground to pass through a 2-mm screen, and sent to Midwest Labs. (Omaha, NE) and Agvise Labs. (Northwood, ND), respectively. Forage quality was evaluated utilizing wet chemistry methods to determine crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrient (TDN), and relative feed value (RFV). In the first year of the study (2015), hay quality and B tissue tests were composited by treatment due to funding limitations, thus statistical analysis for 2015 tissue tests was not possible.

Statistical Analysis

Data were analyzed using the PROC GLIMMIX procedure of SAS (9.4, SAS Institute Inc., Cary, NC) as the majority of the variables were not normally-distributed based on the Shapiro-Wilk test of normality. For yield data, the year and B treatments were considered fixed effects whereas replications within year were random effects. For petiole B and hay quality, only the 2016 data were analyzed because in 2015, petiole B and quality were pooled data from all the replications within a B treatment and variance cannot be analyzed. For 2016 petiole and hay quality, B treatments and replications were considered fixed and random effects, respectively. The LINES option was used to establish significance between means using Fisher's protected least significance difference test at $\alpha = 0.05$.

Irrigation, Rainfall and Temperature

These fields, which are within a commercial production field, were irrigated using a wheel-line sprinkler from mid-June to August of each year with 1-2 inches of water per week. The irrigation events were adjusted accordingly with the rainfall events. Seasonal rainfall patterns differed between years and locations (Table 3). For Dillon, rainfall averaged 9.7 and 9.1 inches for 2015 and 2016 respectively, which is similar to the 30-year average (9.3 inches; NOAA, 2016). For Creston, rainfall was 63% less in 2015 (average 4.9 inches) and was slightly higher (15%) in 2016 (average 15.1 inches) than the 28-year average (USBR, 2016) due to 3.7 inches more rainfall than normal in October. The 2015 seasonal temperature was slightly higher than 2016 for both sites. Temperatures in 2016 for both sites were near-identical to the 1989-2016 average (NOAA, 2016; USBR, 2016).

Effect of Boron on Petiole Boron Content

In 2015, tissue tests for both sites were pooled for each of the treatments due to budget constraints and the data mentioned are from the pooled treatment petiole B, not from least square means. In 2015, the average petiole B for each treatment was numerically higher with B application relative to the control in Creston (data not shown), although the treatments could not be statistically analyzed. In Dillon, no observable trend of petiole B with B application in relation to the control was observed (data not shown, B tissue test > 43 ppm). An increased B application rate significantly increased ($P<0.05$) the B content of alfalfa petioles at both locations in 2016, except the first cutting in

Dillon (Table 4). At Dillon, the maximum amount of petiole B was 60.3 ppm at the second cutting when 2.0 lb acre^{-1} (B_4) of B was applied at the beginning of spring growth. However, it was not significantly different (P>0.05) from the lower split-applied B (B_1 and B_2) rates. This pattern is similar to the rest of the cuttings in 2016 at either site. The data obtained show that petiole B concentration at both sites was within or close to sufficiency level reported in the literature (Kelling, 1999 [>30 ppm]; Meyer et al., 2002 [20-40 ppm]; Undersander et al., 2011 [>25 ppm]; Table 4). The third cutting B treatments in Creston and all cuttings and treatments in Dillon had higher petiole B than the recommended sufficiency range according to Meyer et al. (2002). However, no visual toxicity symptoms were observed. The range between sufficiency and toxicity is narrow (Wimmer et al., 2015) and continues to be debated.. Both sites in 2016 showed a numerical increase of the later cuttings over the earlier cutting, regardless of the B treatments. In the second cuttings at both sites, the majority of the irrigation events were scheduled. For the third cutting in Creston, on top of the planned irrigation events, ~5 inches of rain was received in October prior to the last cutting (Table 3). Increased availability of moisture should increase nutrient uptake from dry soil (Kramer and Boyer, 1995).

Miller and Smith (1977) found a similar increase in alfalfa petiole B content with increased B application in Illinois. Petiole B content was found to be 40, 46, and 55 ppm when alfalfa was treated with 0, 1.4, and 2.8 lb B acre^{-1} respectively (Miller and Smith, 1977). A study from Connecticut also showed alfalfa petiole B increased from 21 to 62 ppm with an application of 2.3 lb acre^{-1} B in granular form (Brown and King, 1940). A field study in northern Greece also suggests that alfalfa tissue B increased in the top 6 inches of the plant in both 2003 and 2004 when 80, 160, or 240 ppm B ($Na_2B_8O_{13}4H_2O$; 20% B) were applied foliarly (Dordas, 2006). Similar increased B levels in plant tissue with B application was also observed in other crops such as melons (*Cucumis sativus* L.; Goldberg et al., 2003) and soybean [*Glycine max* L. Merr.; Sutradhar et al., 2017); however, none of these studies evaluated foliar application. Only a few of the above studies reported an associated yield increase with the corresponding increase in petiole B (Brown and King, 1940; Dordas, 2006) as discussed in the preceding section; only one evaluated foliar application.

Effect of Boron on Yield

With increased B rates, dry matter yield at Dillon significantly increased up to the B_2 (split 1.0 lb B acre^{-1}) rate (P <.05; Table 5) during the second harvest in 2015. No further increases were observed at rates >B_2 treatment. The total dry matter yield during that season showed a numerical increase in yield, but was insignificant (P>0.05). Rannnah et al. (1984), Haby and Leonard (2005), and Kheirkhah et al. (2016) all found positive impacts of B on alfalfa production. However, our data showed that B fertilization did not influence the total yield of alfalfa in either year. Razmjoo and Henderlong (1997) also found no effects of B on alfalfa yield when 0.9 and 1.8 lb B acre^{-1} was foliar-applied on a silty clay loam soil. Additionally, a study in Iowa on a Readlyn loam soil showed no effect on alfalfa yield when 2.0 lb acre^{-1} B was applied (Pecinovsky and Lang, 2012).

No yield response to B fertilization was observed at Creston regardless of cutting or year (Table 6). Further, no yield response to B was observed at Dillon in 2016 across all cuttings (Table 5). Petiole B concentration was found to be generally at sufficient levels (30.1 – 80 ppm), except for the control treatment (B_0) in 2015 at Creston. Alfalfa yield did not respond to B application despite the very low soil B (0.2 ppm) in 2015. This implies that low soil B content was not indicative of a need to apply B under an irrigated environment in Montana. Based on this two-year study, the risk of economic loss with an application of B fertilizer via soil test results is high considering the market price of the product used (i.e., ~USD 10.00 at an application rate of 0.5 lb acre^{-1}), although, cheaper alternatives are available. Regardless of fertilizer source, any fertility amendment increases farm input cost and thus, B fertilization decision requires careful assessment. If soil test results show low B in early spring in MT or similar environment, B application may not be necessary under irrigated alfalfa production, consistent with other reports (Meyer et al., 2002; Meyer et al., 2007; Orloff et al., 2010), but rather should be further evaluated using in-season plant tissue testing. In-season visual symptoms of B deficiency should be verified with petiole testing, which is more diagnostic and is of better value for alfalfa producers than soil testing. Meyer et al. (2002) argued that plant tissue testing should be utilized to determine the B nutrient deficiency in alfalfa.

The difference in seasonal weather pattern (Table 3) did not influence total yield in Creston site (i.e., no year or year x B interaction). In Dillon, on the other hand, year was significant ($P<0.05$) for total yield. For this site, the 2015 yield, on average, was

0.7±0.2 ton $acre^{-1}$ higher than 2016. Year impacts on the petiole B and hay quality are not presented here due to our inability to estimate the variation in 2015.

Effect of Boron on Forage Quality

Boron application had no significant effect on alfalfa forage quality ($P > 0.05$) at either site in 2016 (Table 7), possibly because the tissue tests did not indicate a low petiole tissue B (Table 4) based on the Meyer et al. (2002) B sufficiency range. While the third cutting B_1-B_4 treatments in Creston, and all of the treatments across cuttings in Dillon had petiole B above the sufficiency range (Table 4), no visual toxicity symptoms were observed as mentioned in the previous section. The literature suggests inconsistent rate responses of B on forage quality. The application of B in a semi-arid region of eastern Turkey increased alfalfa crude protein when $Na_2B_4O_7.10H_2O$ fertilizer at the rate of 0, 0.1, 0.3, and 0.9 lb B $acre^{-1}$ was applied on aridisols deficient in B (Turan et al., 2010). Similarly, Wright (1986) claimed that B application improved the forage quality of harvested alfalfa. When 2.0, and 4.0 lb B $acre^{-1}$ was applied on a Darco loamy fine sand in Texas, there was no consistent increase in forage quality (i.e., crude protein); and an increase in forage quality was only evident at one out of the four cuttings (Rouquette et al., 2001).

Conclusion

A two-year analysis showed that foliar B application did not influence total yields for either of the irrigated sites with low initial soil B, except for the second harvest in 2015 at one location. Alfalfa quality also was not affected by B at either site during the study. There was a significant increase in plant tissue B content with increasing B application, which implies that foliar application of liquid B is effective but did not positively impact alfalfa yield or quality. This research shows that low soil B does not limit the yield of irrigated alfalfa in Montana, and B enhancement may be unwarranted. Additionally, petiole B concentrations met the sufficiency range despite the initial low soil B levels from soil test results. Thus, using soil-tests are not necessarily a reliable measure of the need to amend B using foliar-application at irrigated sites in Montana. Diagnosing symptoms of B deficiency coupled with in-season petiole B analysis may be of better value to producers.

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Table 3.1. Study site descriptions: Northwestern Agricultural Research Center (NWARC) at Creston, MT and Producer field at Dillon, MT

	Creston, MT	Dillon, MT
Field Coordinates	48° 11’ N, 114° 8’ W (Northwest region)	45°17’ N, 112° 38’ W (Southwest region)
Soil Type	Fine sandy loam	Silt loam
Initial B level in 2015	0.2 - 0.3 ppm (very low)	0.8 ppm (low)
Recommended B (Jacobsen et al., 2005)	2 lb acre^{-1}	1 lb acre^{-1}
Initial Soil pH in 2015	6.5 – 6.8	8.0 – 8.2
Initial Organic Matter (%) in 2015	1.9 – 2.1	1.0 – 1.6
Year from alfalfa establishment	Second year	Third year
Cultivar	Pioneer58V09 (Pioneer)	Standout (Green Genes, Inc.)
Seeding rate	12 lb acre^{-1}	

Table 3.2: Boron (B) treatments, amounts, and timing of application.

Treatments	Total B (lb acre^{-1})	Application time
B_0	0	Untreated check
B_1	0.5	Split: 0.25 lb acre^{-1} applied at 3 inches spring growth + 0.25 lb acre^{-1} at 3 inches regrowth after first cutting
B_2	1.0	Split: 0.50 lb acre^{-1} applied at 3 inches spring growth + 0.50 lb acre^{-1} at 3 inches regrowth after first cutting
B_3	2.0	Split: 1.0 lb acre^{-1} applied at 3 inches spring growth + 1.0 lb acre^{-1} at 3 inches regrowth after first cutting
B_4	2.0	2.0 lb acre^{-1} applied at 3 inches spring growth

Table 3.3: Monthly, seasonal, and 28-year total growing season rainfall and mean air temperature at Creston (USBR, 2016) and Dillon (NOAA, 2016), MT.

	Creston, MT					
	Rainfall (inch)			Temperature (°F)		
	2015	2016	1989-2016	2015	2016	1989-2016
April	0.6	1.7	1.8	44.8	49.3	43.0
May	0.6	2.8	2.3	54.7	53.2	51.1
June	1.0	1.9	3.6	65.1	59.9	57.2
July	0.4	1.5	1.4	66.3	63.7	64.8
August	0.2	1.0	1.1	65.2	63.8	63.5
September	0.9	0.8	1.4	53.7	53.3	54.3
October	1.4	5.3	1.6	47.5	43.5	42.4
Season	4.9	15.1	13.2	56.8	55.3	53.8

	Dillon, MT					
	Rainfall (inch)			Temperature (°F)		
	2015	2016	1989-2016	2015	2016	1989-2016
April	1.8	1.3	1.5	41.5	46.5	42.6
May	2.5	1.9	2.1	51.5	50.0	51.1
June	0.7	1.3	1.9	64.5	62.5	58.6
July	2.0	0.7	1.0	65.5	65.0	66.4
August	0.4	0.1	1.0	65.0	63.0	64.2
September	1.6	1.6	0.8	57.5	54.0	56.3
October	0.8	2.3	1.0	49.5	45.5	44.8
Season	9.7	9.1	9.3	56.4	55.2	54.9

Table 3.4: Effect of boron (B) on plants tissue B concentration in the year 2016 at Creston and Dillon, MT. Treatments include: B_0 (no applied B, control check); split-applied B_1 (0.25 + 0.25 lb acre^{-1}), B_2 (0.5+ 0.5 lb acre^{-1}), and B_3 (1.0 + 1.0 lb acre^{-1}); and B_4 (single applied 2.0 lb acre^{-1}).

	Creston			Dillon	
	B (ppm)				
Treatments	First cutting	Second cutting	Third cutting	First cutting	Second cutting
B_0	21.25^{c}	25.75^{c}	34.25^{b}	47.25	48.25^{c}
B_1	25.75^{b}	31.50^{bc}	44.75^{ab}	50.75	54.50^{abc}
B_2	30.00^{a}	35.50^{ab}	53.50^{a}	55.75	55.75^{ab}
B_3	29.50^{a}	39.00^{a}	54.50^{a}	49.00	50.00^{bc}
B_4	30.00^{a}	38.25^{ab}	55.25^{a}	53.25	60.25^{a}
P-value	***	**	**	n.s.	*

Values in columns with different superscripts are significantly different (*, **, and *** indicate significance at α = 0.05, 0.01, and 0.001, respectively); n.s., not significant.

Table 3.5: Effect of boron (B) on dry matter yield of alfalfa during 2015 and 2016 in Dillon, MT. Treatments include: B_0 (no applied B, control check); split-applied B_1 (0.25 + 0.25 lb acre^{-1}), B_2 (0.5 + 0.5 lb acre^{-1}), and B_3 (1.0 + 1.0 lb acre^{-1}); and B_4 (single applied 2.0 lb acre^{-1}).

Year	Treatments	First cutting	Second cutting	Total
		------------------ ton acre^{-1} -----------------		
2015	B_0	2.2	1.7^b	3.9
	B_1	2.3	1.9^{ab}	4.2
	B_2	2.5	2.0^a	4.5
	B_3	2.6	2.0^a	4.6
	B_4	2.4	2.1^a	4.5
	P-value	n.s.	<0.05	n.s.
2016	B_0	2.0	1.6	3.6
	B_1	2.1	1.5	3.6
	B_2	1.9	1.5	3.4
	B_3	2.3	1.6	3.9
	B_4	2.2	1.6	3.8
	P-value	n.s.	n.s.	n.s.

Values in columns with different superscripts are significantly different at $\alpha = 0.05$; n.s., not significant.

Table 3.6: Effect of boron (B) on dry matter yield of alfalfa during 2015 and 2016 in Creston, MT. Treatments include: B_0 (no applied B, control check); split-applied B_1 (0.25 + 0.25 lb acre^{-1}), B_2 (0.5 + 0.5 lb acre^{-1}), and B_3 (1.0 + 1.0 lb acre^{-1}); and B_4 (single applied 2.0 lb acre^{-1}).

Year	Treatments	First cutting	Second cutting	Third cutting	Total
		---------------------------- ton acre^{-1}	----------------------------	----------------------------	----------------------------
2015	B_0	2.9	1.8	1.5	6.2
	B_1	3.1	1.6	1.5	6.2
	B_2	2.9	1.7	1.4	6.0
	B_3	3.0	1.6	1.3	5.9
	B_4	3.0	1.6	1.3	5.9
	P-value	n.s.	n.s.	n.s.	n.s.
2016	B_0	2.0	1.6	1.4	5.0
	B_1	2.6	1.6	1.4	5.6
	B_2	2.6	1.5	1.4	5.4
	B_3	2.5	1.6	1.5	5.6
	B_4	2.4	1.5	1.5	5.4
	P-value	n.s.	n.s.	n.s.	n.s.

n.s., not significant at $\alpha = 0.05$.

Table 3.7: Effects of boron (B) on crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrient (TDN), and relative feed value (RFV) in 2016 at Creston and Dillon, MT. Treatments include: B_0 (no applied B, control check); split-applied B_1 (0.25 + 0.25 lb acre^{-1}), B_2 (0.5 + 0.5 lb acre^{-1}), and B_3 (1.0 + 1.0 lb acre^{-1}); and B_4 (single applied 2.0 lb acre^{-1}).

	2016 Creston					2016 Dillon				
	Second cutting					First cutting				
	CP	ADF	NDF	TDN	RFV	CP	ADF	NDF	TDN	RFV
Treatment	---------------------%-------------------					------------------%-----------------				
B_0	22.8	33.5	42.4	62.6	138.0	23.6	33.9	41.3	62.1	140.8
B_1	27.0	33.0	39.0	63.0	151.5	22.5	37.3	44.6	58.5	125.0
B_2	26.7	32.0	41.2	64.1	144.8	23.5	34.2	42.7	61.8	137.8
B_3	25.8	34.0	41.6	62.0	140.2	24.2	33.6	41.6	62.4	141.0
B_4	24.1	35.0	42.0	61.0	137.0	22.6	36.3	43.9	59.6	128.8
P-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s., not significant at α = 0.05.

CHAPTER FOUR

ALFALFA RESPONSE TO BORON APPLICATION AT VARIOUS WATER REGIMES

Contribution of Authors and Co-Authors

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Contributions: Main author responsible for data collection, statistical analysis, interpretation, and drafting of this thesis.

Co-Author: Emily C. Meccage

Contributions: Critical in achieving funds for this research, experimental design, data collection, analysis and revisions for this thesis.

Co-Author: Robert N. Stougaard

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Manuscript Information

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____ Accepted by a peer-reviewed journal
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Abstract

Boron (B) fertilization on B-deficient soil may avoid negative impact to alfalfa (*Medicago sativa* L.) yield and quality. The objective of this study was to determine the effect of foliar-applied B, various water regimes, and their interaction on alfalfa yield and quality. Alfalfa was planted in 2016 at Creston, MT in sandy loam soil that tested 0.2 mg kg^{-1} B. The experiment was designed as a split-plot, with water regime as the main-plot and B rates as sub-plot factors, with four replications. Irrigation was applied when plant available water was depleted by 35%. Irrigation increased total alfalfa yield by 45% and 12% in 2016 (seeding year) and 2017, respectively, with no yield difference between the 100 percent evapotranspiration (ET) and 50ET treatments. In 2016, irrigation decreased ($P < 0.01$) forage nutrient quality in the second cutting but had no effect in 2017. There was no effect of B on yield ($P > 0.05$) in either year. Both irrigation and B fertilization increased petiole B but did not correlate ($0 \geq R^2 \leq 0.06$) with yield or quality. In 2017, water and B levels influenced yield ($P = 0.02$) in the second cutting, as well as relative feed value, neutral detergent fiber, and petiole B content in the third cutting ($P < 0.05$). The foliar B application on a B-deficient soil did not increase alfalfa yield or quality regardless of water regimes.

Keywords: *Medicago sativa*, deficit irrigation, boron, yield, forage quality

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Introduction

Alfalfa is a major perennial forage crop, producing high yield and increased nutrient concentrations (Marita et al., 2003; Bouton, 2012). It is adapted to harsh environmental conditions (Iannucci et al., 2002) while remaining a highly digestible forage with low fiber content when harvested between bud and early flower (Balliette and Torell, 1993; Rodiek, 2001; Marita et al., 2003). North America (the United States and Canada) is the major alfalfa producer, accounting for 41% of the world's total alfalfa production, of which the United States is the leading country in terms of area of alfalfa production (Yuegao and Cash, 2009). In the United States, alfalfa was harvested on over 6.7 million ha in 2017, producing > 49 million Mg of hay (NASS-USDA, 2018). However, the total harvested area and production of alfalfa is reportedly decreasing in the United States. The yield per hectare of alfalfa hay production has been decreasing following a severe drought in 1988; a counter trajectory from the increasing yield trend for the previous 37 years (from 1950 through 1987; Brummer and Casler, 2014) with harvested area decreasing by 7% (from 7.2 to 6.7 million ha; NASS-USDA, 2018). This decrease in alfalfa hay cultivation and production increased the need to optimize yield by fine-tuning high-cost production inputs such as fertilization and irrigation (Gauch and Dugger, 1954; Mueller and Orloff, 1994; Fereres and Soriano, 2006).

Irrigating alfalfa increases yield (Peterson, 1972; Rogers, 2001; Lawson et al., 2009; Li and Su, 2017). Its yield response is linear to an appropriately-timed available soil moisture (Bauder et al., 1978; Donovan and Meek, 1983; Lindenmayer et al., 2011; Shewmaker et al., 2013; Holman et al., 2016; Undersander et al., 2016). The degree of the response (i.e., slope) of this linear relationship between ‘transpirational’ water and yield can vary according to climatic regions (Hill et al., 1983), seasons, and number of harvests within the growing season (Myer et al., 1991). The availability of moisture for transpiration drives the amount of nutrients taken up by plants (Levitt, 1980; Adam, 1999; Wimmer et al., 2015). This in part is due to the increased volume of nutrient uptake (Kramer, 1981), and increased microbial activity associated with increasing moisture availability (Berger, 1962; Zhang et al., 2013b). Untimely irrigation causes either water stress or excessive water when it is not needed reduces alfalfa yield and quality (Mueller and Orloff, 1994). Specifically, water stress is the highest yield-reducing factor of production as transpiration and nutrient uptake are significantly reduced (Kramer, 1981; Boyer, 1982).

Most of the reported B-deficiency are under drought conditions (Berger, 1962). Strategic B amendments depending on moisture availability perhaps is a valuable to producers to increase forage yield. Fine tuning fertilizer amendments, such as B, may be dependent upon the availability of moisture. Since B studies in literature were conducted either under rainfed (Chandler et al., 1946; Razmjoo and Henderlong, 1997) or irrigated condition, exclusively (Radtke, 1986; Grant and Miller, 1998; Dordas, 2006; Kheirkhah et al., 2016; Sapkota et al., 2017), it is important to investigate the interaction between the applied B on a B-deficient soil and water regimes, as it has not been well-studied.

Boron is an essential micronutrient for plants growth and development as it plays role in sugar translocation, carbohydrate metabolism, nucleic acid synthesis, and pollen germination (Gauch and Dugger, 1954; Gupta, 1980; Howe, 1998; Devirian and Volpe, 2003; Ozturk et al., 2010), and plant cell growth regulation (Xiaodong and Yiqin, 1999). Shorrocks (1997) reported that alfalfa B removal is high (0.35 kg ha^{-1}) compared with other crops like *Brassica napus* (0.08 kg ha^{-1}), *Gossypium hirsutum* (0.15 kg ha^{-1}), *Triticum* spp. (0.03 kg ha^{-1}), and *Zea mays* (0.03 kg ha^{-1}). This large removal of B in alfalfa can lead to yield-impacting B deficiency (Dordas, 2006). The major symptoms of B deficiency in alfalfa is yellowing of petioles or leaf tips (Wright, 1986; Undersander et al., 2000; Ottman, 2010) because of its immobility in the plant tissues. (Berger, 1962; Brown and Shelp, 1997). Additionally, retarded growth of plants, abnormal leaf structure, dense top canopy (Undersander et al., 2000; Herrera-Rodríguez et al., 2010), necrosis of terminal buds, and wilting of leaves (Willis and Piland, 1937) are also symptoms of B deficiency in alfalfa.

We hypothesized that the uptake of B from the fertilization of B-deficient soil is impacted by soil moisture availability. The objectives of this study were to: a) determine the effect of water regimes on alfalfa yield and forage quality, b) evaluate the effect of different rates of B on alfalfa production and quality, and c) determine the interaction effect of B and water regimes on alfalfa yield and quality.

Materials and Methods

Site Description

This study was conducted in 2016 and 2017 at the Northwestern Agricultural Research Center in Creston, MT (48°11’10” N lat., 114°8’39” W long., 894 m elevation). The soil type was a sandy loam (USDA-SCS and MAES, 1959) with 1.5% organic matter and a pH of 6.7. The research field was fall-plowed (John Deere^{TM} 975, Moline, IL) after the previous crop of spring wheat (*Triticum aestivum* L.) was harvested, and then disked the following spring (John Deere^{TM} 4000, Moline, IL). Soil samples were collected from three depths, 0-15, 15-60, and 60-90 cm, using a soil probe (Giddings^{TM}, Windsor, CO) and submitted to a commercial laboratory for soil nutrient analysis. The NPK fertilizers were applied following the fertilizer guideline for Montana crops (Jacobsen et al., 2005) to meet the soil nutrient requirements. Pre-seeding spring soil analysis for B (DTPA-sorbitol method; Miller et al., 2001) indicated that B content of the experimental site was very low (0.2 mg kg^{-1}; Kelling, 1999).

Monthly and seasonal mean temperature and rainfall during the growing season and the average of 30-yr (1988-2017) are shown in Table 4.1. The year 2016 had near-average rainfall and temperature. Mean seasonal precipitation was 17% greater whereas the mean air temperature was similar (i.e., < 1 °C) to the 30-yr average. The year 2017 had low precipitation and high air temperature. Precipitation for this year diminished from May to Aug. which was 36% lower seasonally compared with the 30-yr average. The mean air temperature from May to Aug. was consistently higher than the 30-yr average. This low rainfall and higher air temperature in 2017 was classified as a severe drought occurrence by the U.S Drought Monitor (2017) as reflected with the minimal rainfall events after the first cutting (Fig. 4.1B) than the 2016 near-normal year (Fig. 4.1A).

Table 4.1. Monthly and seasonal precipitation and air temperature and the 30-year average obtained at the Creston weather station (USBR, 2017a) located ~100 m from the research site.

	Rainfall (cm)			**Temperature (°C)**		
Months	**2016**	**2017**	**1988-2017**	**2016**	**2017**	**1988-2017**
Apr	3.8	5.9	4.6	8.8	4.7	6.0
May	7.1	1.8	5.9	10.8	11.4	10.6
June	5.3	6.7	8.9	14.7	15.3	14.1
July	3.9	0.2	3.5	17.0	20.0	18.3
Aug	2.8	0.5	2.7	17.1	17.9	17.5
Sept	2.0	2.5	3.4	11.9	12.2	12.4
Oct	13.5	3.3	3.9	6.4	5.2	5.8
Season	5.5	3.0	4.7	12.4	12.4	12.1

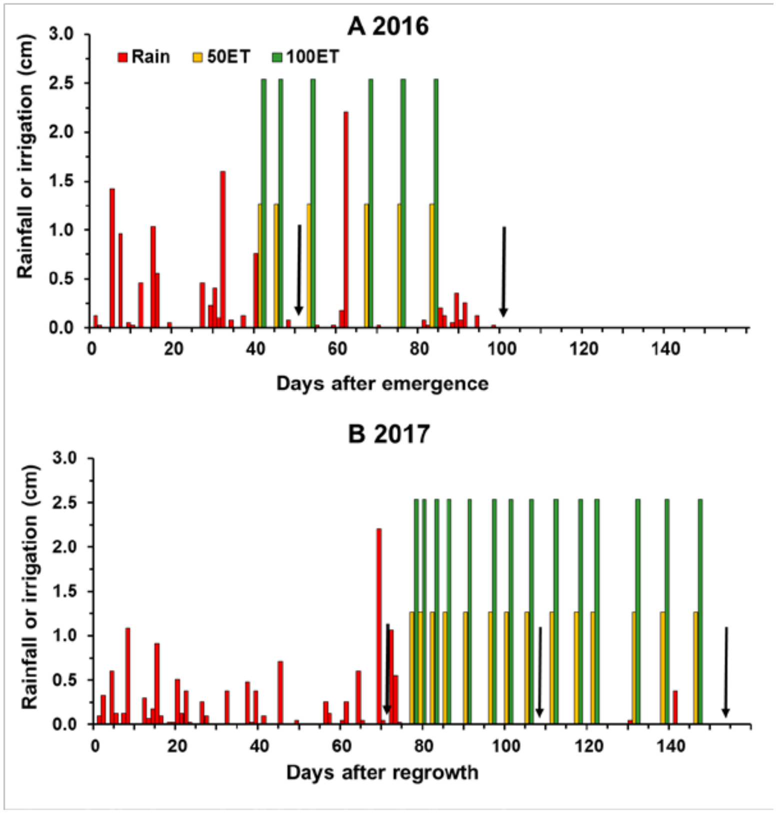

Figure 4.1. Irrigation and rainfall events during the growing seasons in 2016 (A) and 2017 (B). Downward arrows indicate the harvest events. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET.

Treatments and Experimental Set-up

Alfalfa variety ‘Hybriforce-3400’ (fall dormancy 4, Dairlyland Seed) was broadcast seeded at the rate of 22.4 kg ha^{-1} on 24 May 2016. Moxy herbicide (active ingredient, a.i.: 33.4% octanoic acid ester of bromoxynil) at the rate of 1.2 L ha^{-1} and Pursuit (a.i.: 22.87% ammonium salt of imazethapyr) at the rate of 0.4 L ha^{-1} were applied post-emergence in 2016 for weed control.

The experiment was laid out in a split-plot design with three irrigation treatments as the main-plot and five B treatments as the subplot factors (Table 4.2), and was replicated four times. The B treatments (amount and time of application; Table 4.2) were adapted from Sapkota et al. (2017). The plots measured 3.05 x 4.57 m, surrounded by border plots, with a 1.5 m alley between replications and around the experimental plots to allow easy access for plot maintenance and harvest. Treatment randomization and the prescribed volume of B for each experimental unit were determined using Agriculture Research Manager (Gylling Data Management, 2016).

Table 4.2: Irrigation and boron (B) treatments, amounts, and timing of application. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET.

Treatments	**Irrigation (cm)**		**Description**
Water regimes	**2016**	**2017**	
Rainfed	0	0	No irrigation (Check)
100ET	+15.2	+35.6	Irrigate 2.54 cm of water whenever 35% plant available water was depleted
50ET	+7.6	+17.78	Irrigate 1.27 cm (half to 100ET) of water applied the same day as the 100ET
Boron	**Total B (kg ha^{-1})**		
B_0	0		Untreated (Check)
B_1	0.56		Split: 0.28 kg ha^{-1} applied at 8 cm spring growth + 0.28 kg ha^{-1} at 8 cm regrowth after first cutting
B_2	1.12		Split: 0.56 kg ha^{-1} applied at 8 cm spring growth + 0.56 kg ha^{-1} at 8 cm regrowth after first cutting
B_3	2.24		Split: 1.12 kg ha^{-1} applied at 8 cm spring growth + 1.12 kg ha^{-1} at 8 cm regrowth after first cutting
B_4	2.24		2.24 kg ha^{-1} applied at 8 cm spring growth

A locally-available liquid B fertilizer (10% B Agrisolutions^{TM}) was foliar-applied as the B source using a 3.05 m flat nozzle boom-mounted CO_2-pressurized backpack sprayer. Liquid B was chosen for the ease in the precise application on a small experimental area. When spraying the solution, the boom was maintained at 0.6 m above the ground level. The spray volume was 187 L ha^{-1}. The volume of solution (water and B) for each experimental unit was 318 ml with liquid B amounts of: 4.8, 9.5, 19, and 38.1 ml B corresponding to treatments B_1, B_2, B_3, and B_4, (Table 4.2), respectively. For both years, the initial application of B fertilizer was made in spring to all the B treatments (B_1 to B_4) at 8 cm plant height. The same amount of B was re-applied only to treatments B_1 to B_3, after the regrowth reached a height of 8 cm.

The irrigation treatments are described in Table 4.2. A 100 percent evapotranspiration (100ET), and 50 percent evapotranspiration (50ET) were included to evaluate alfalfa response to B under non-water-stressed (100ET) and simulated water stress by deficit water application (50ET). The daily crop ET (ETc) and soil water balance approach were used to schedule irrigation. The grass-based reference ET (ETo) was retrieved from the Creston Weather Station (USBR, 2017a) located ~100 m from the experimental site. The crop coefficient (Kc) values used for the specific growth stages of alfalfa were: 0.2 (beginning of the spring growth), 0.4 to 0.7 as alfalfa growth increases, and 0.9 after the full canopy cover. These Kc values are based on the curve developed on lysimeter plots, Kimberly, ID 1969-75 (USBR, 2017b). Daily ETc was calculated by multiplying ETo and the alfalfa Kc (Allen et al., 1998). Daily soil water depletion was determined by subtracting daily ETc of the soil water balance with an assumed full profile in spring due to spring snowmelt and rainfall events (Torrion and Stougaard, 2017). The rooting depth to calculate depletion during the 2016 early establishment was 0 to 60 cm which was adjusted to 0 to 90 cm as soon as alfalfa plants were near full canopy in the second year (2017). During thunderstorms, the water in excess of field capacity was considered lost by deep drainage as there was no evidence of surface run-off in the field. Hence soil water depletion can be adjusted to zero or a full soil profile (Andales et al., 2015). No temporary water was assumed withheld above field capacity due to the coarse soil texture of the study site. The boron content of the irrigated water was below the detection limit of 0.1 mg kg^{-1}.

To irrigate, seven drip tapes were laid on each irrigated plot at equal distances (30 cm apart) leaving 60 cm on each side of the plot to avoid lateral movement of water to adjacent plots. The alfalfa field was irrigated whenever 35% plant available water was depleted from the field capacity of 100ET treatment. The 50ET treatments were irrigated on the same day the 100ET plots received irrigation but at only half the applied amount of the 100ET treatment. For each irrigation event, 2.54 and 1.27 cm of water was applied in 100ET and 50ET treatments, respectively (Table 4.2). Due to the full soil profile in spring and the amount of rainfall received, alfalfa was not irrigated for the first cutting of 2017, but irrigation began soon after first cutting. Irrigation and rainfall events in 2016 and 2017 are shown in Fig. 4.1. Irrigation water productivity (IWP), which is defined as the amount of yield produced per unit of irrigation water use (Molden, 1997) was calculated using Eq. 1.

$$\mathrm{IWP}\ (\mathrm{kg\ ha^{-1}cm^{-1}}) = \frac{(\mathrm{Yield_{ET}} - \mathrm{Yield_{rainfed}})}{\mathrm{IW}} \quad \mathrm{Eq.\ 1\ (Molden, 1997)}$$

Where,
$\mathrm{Yield_{ET}}$ is the alfalfa production (in kg ha^{-1}) when irrigated, ET is either 50 or 100
$\mathrm{Yield_{rainfed}}$ is the alfalfa production (in kg ha^{-1}) from the rainfed plots, and
IW is the irrigation water applied (in cm)

Data Collection

Alfalfa was harvested at 10% bloom for all cuttings in both years. Plant height and number of trifoliates on the main stem were recorded before each cutting. A forage harvester (ALMACO$^{\mathrm{TM}}$, Nevada, IA) was used to harvest the alfalfa plots. A 1.5 x 4.6 m strip was harvested in the middle of the 3 x 4.6 m plot leaving a 5-cm stubble height. Collected strips were weighed for fresh weight production.

Fresh biomass subsamples were collected from the harvested strip, immediately weighed, and then oven dried (forced-air, 60 °C) for at least 72 h or until a stable weight was reached to determine the dry matter percentage for plot yield determination. The top 15 cm petioles of 20 plants were randomly collected from each plot for petiole B analysis. Another 20 whole plant subsamples were also collected from each plot for forage quality analysis. The petiole subsamples were dried and sent to a commercial laboratory to determine petiole B concentration. Whole plant samples were also sent to a commercial lab to determine: crude protein (CP; method 990.03; AOAC International, 2016), acid detergent fiber (ADF; modified AOAC, 2016), neutral detergent fiber (NDF; Van Soest et al., 1991), total digestible nutrients (TDN), and relative feed value (RFV). Values of ADF and NDF were used to estimate TDN and RFV values.

Statistical Analysis

Data were analyzed using PROC GLIMMIX in SAS version 9.4 (SAS Institute, 2014, Cary, NC) because the majority of the data were not normally distributed as per the Shapiro-Wilk hypothesis. Since 2016 was the year of establishment, it is likely that many variations between 2016 (year of establishment) and 2017 (established year) would occur. Therefore, year effect was removed, and data were analyzed within each year and with B and water regimes as the fixed effects. Block and its interaction with irrigation were set as random effects. An additional analysis testing the effect of cutting events was also analyzed as a fixed effect. Year, irrigation, B rates, and cutting were defined as categorical variables in the CLASS statement. The denominator degree of freedom was estimated by using the option DDFM=KR in the model statement. Mean separation for both years was determined using the LINES option in the LSMEANS statement. Treatment effects were considered significant at the $P \leq 0.05$ probability level. Correlations among response variables were assessed using PROC CORR.

Results

Alfalfa Response to Irrigation

Total dry matter yield (sum of in-season yields from all cuttings) of alfalfa was significantly influenced by water regimes in both years (Table 4.3). Plant height ($P < 0.01$), dry matter yield ($P < 0.05$), petiole B concentration ($P < 0.001$), and trifoliate counts on the main stem ($P < 0.05$) were positively affected for three out of the four total harvests when irrigation was applied (Table 4.3). Effects of irrigation on forage nutrient values (CP, ADF, NDF, TDN, and RFV) were significant only in 2016 (Table 4.3).

Irrigation increased alfalfa total dry matter yield by 45% ($P < 0.001$) and 12% ($P = 0.012$) compared to rainfed production in 2016 and 2017, respectively (Fig. 4.2). Of all cuttings, only the second cutting of 2017 showed no difference ($P = 0.372$) in irrigation vs. rainfed on yield. There was no significant difference between the 50ET and 100ET irrigation treatments (Fig. 4.2) on total yield in any harvest, and as a result, IWP of 50ET was greater than 100ET (Fig. 4.2) translating to the higher efficiency of 50ET vs. 100ET.

Table 4.3. Analysis of Variance (ANOVA) table showing *P*-values of the effect of water regimes (denoted as evapotranspiration [ET]), boron (B) rates, and their interaction on plant height (height), dry matter yield, petiole boron content (PB), number of trifoliates on the main stem (Tri), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrients (TDN), and relative feed value (RFV). The table also shows the numerator and denominator degree of freedom (ndf and ddf) for each source of variations.

2016	**ndf**	**ddf**	**Height^1**	**Height^2**	**Height^3**	**Yield^1**	**Yield^2**	**Yield^3**	**Total yield**	**PB^1**	**PB^2**	**PB^3**	**Tri^1**	**Tri^2**	**Tri^3**
ET	2	6	0.001	<0.001	-	0.034	<0.001	-	<0.001	0.133	<0.001	-	0.030	<0.001	-
Boron	4	36	0.399	0.766	-	0.083	0.840	-	0.457	<0.001	<0.001	-	0.407	0.661	-
ET*Boron	8	36	0.610	0.905	-	0.329	0.986	-	0.787	0.587	0.074	-	0.185	0.517	-
2017															
ET	2	6	-	0.149	0.002	-	0.372	0.002	0.012	-	<0.001	0.002	-	0.200	0.001
Boron	4	36	0.493	0.365	0.671	0.198	0.440	0.753	0.058	0.024	0.001	<0.001	0.387	0.598	0.296
ET*Boron	8	36	-	0.703	0.949	-	0.020	0.573	0.164	-	0.626	0.049	-	0.945	0.791

2016	**ndf**	**ddf**	**CP^1**	**CP^2**	**CP^3**	**ADF^1**	**ADF^2**	**ADF^3**	**NDF^1**	**NDF^2**	**NDF^3**	**TDN^1**	**TDN^2**	**TDN^3**	**RFV^1**	**RFV^2**	**RFV^3**
ET	2	6	0.355	<0.001	-	0.505	<0.001	-	0.439	0.001	-	0.508	<0.001	-	0.396	<0.001	-
Boron	4	36	0.530	0.200	-	0.042	0.764	-	0.048	0.361	-	0.043	0.760	-	0.067	0.495	-
ET*Boron	8	36	0.427	0.637	-	0.212	0.873	-	0.103	0.714	-	0.212	0.871	-	0.074	0.755	-
2017																	
ET	2	6	-	0.690	0.318	-	0.738	0.551	-	0.862	0.383	-	0.740	0.555	-	0.820	0.322
Boron	4	36	0.343	0.367	0.694	0.578	0.326	0.091	0.132	0.504	0.076	0.587	0.323	0.094	0.195	0.451	0.033
ET*Boron	8	36	-	0.533	0.217	-	0.067	0.168	-	0.306	0.040	-	0.066	0.168	-	0.172	0.017

$^{1,\ 2,\ \text{and}\ 3}$ are first, second, and third cuttings, respectively. Alfalfa was harvested on 27th July and Sep. 17th in 2016, and it was harvested on 15th June, 21st July, and 9th Sep. in 2017.

Total yield is the sum of in-season yields from all the cuttings

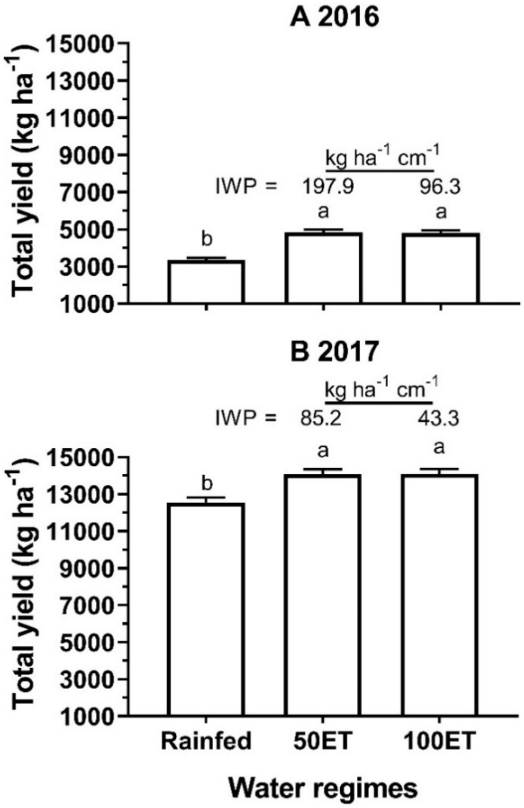

Figure 4.2. Effects of irrigation on alfalfa total dry matter production in 2016 (A) and 2017 (B). Error bars denote standard error of the means and different letter assignments indicate significance at $P \leq 0.05$. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET. Irrigation water productivity (IWP; kg ha^{-1} cm^{-1}) for respective irrigation treatments are also shown above the bars in the graph.

Irrigation significantly increased ($P < 0.01$) plant heights for all harvests except the second cutting of 2017 (Fig. 4.3). Plant height increased 47% and 23% in irrigated treatments in 2016 and 2017, respectively. Plant height for irrigated plots was numerically higher in the second cutting of 2017, but was insignificant ($P = 0.149$). The number of trifoliate on the main stems was significantly increased ($P < 0.05$) in all irrigated plots in both years, except the second cutting of 2017; however 50ET and 100ET had a similar number of trifoliates on the stem.

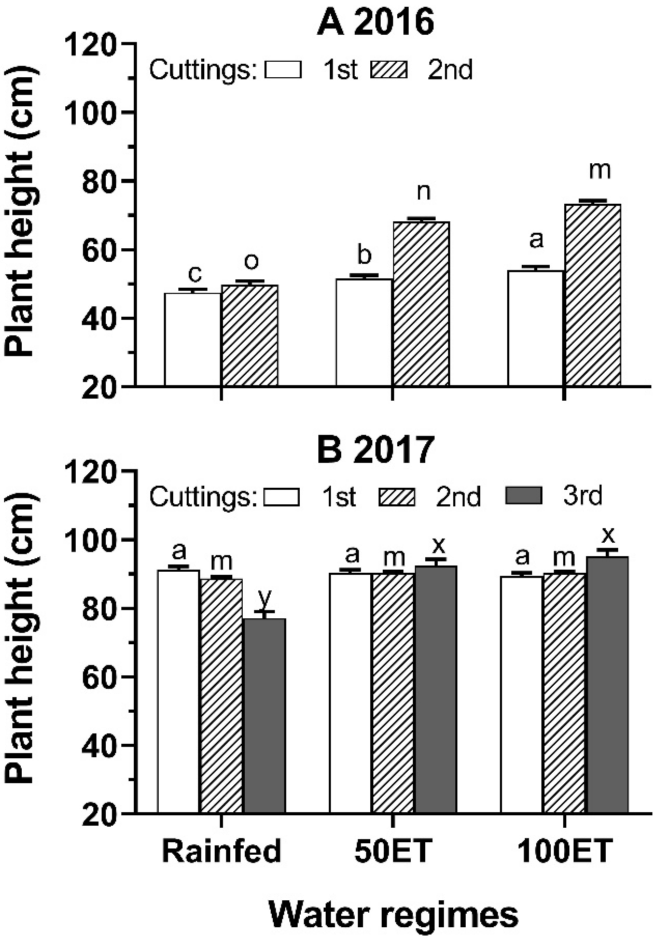

Figure 4.3. Alfalfa plant height in response to water regime in 2016 (A) and 2017 (B). Error bars indicate standard error of the means within each cutting. Different letter assignment indicates significance at $P \leq 0.05$ within harvest. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET.

In an exception to the first cutting of both years, petiole B concentration increased significantly ($P < 0.01$) with irrigation (Fig. 4.4). In 2016, petiole B concentration was close to or above the sufficiency level (> 30.1 mg kg^{-1}) as defined by Kelling (1999), despite the very low soil B level of this study site and was not different amongst water regimes. As the season progressed in 2016 (i.e., 1st to 2nd cutting), the petiole B content increased significantly ($P < 0.001$) in the irrigated plots (Fig. 4.4A). However, it decreased slightly in the rainfed plots. Petiole B remained below the reported sufficiency levels for all irrigated plots in 2017 except for 100ET in the second cutting. The petiole B increased ($P < 0.002$) with irrigation in 2017 as the season progressed (i.e., from 1st to 2nd and 3rd cuttings), although the increases barely or did not meet the sufficiency line (Fig.4.4B).

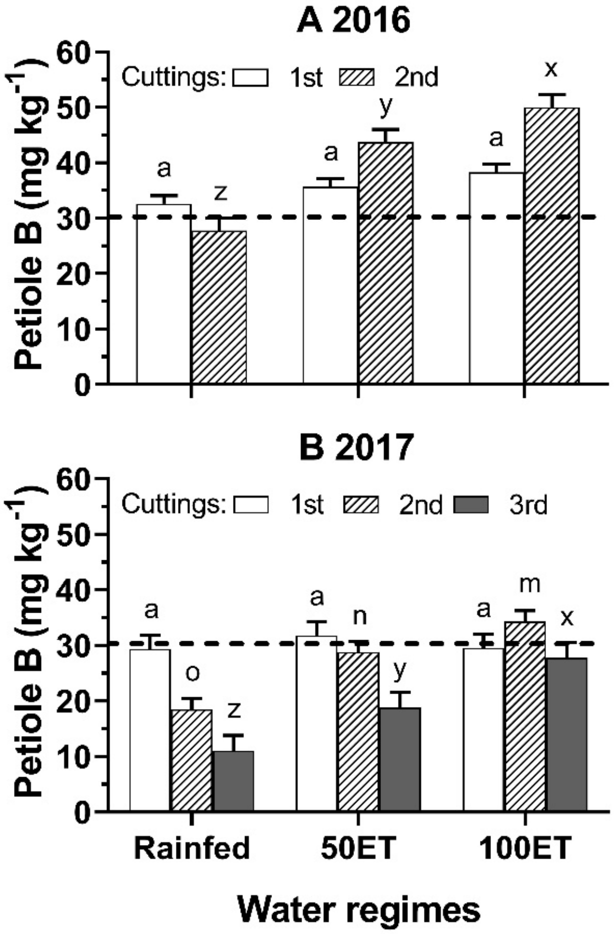

Figure 4.4. Effects of water regimes on petiole boron (B) concentration (top 15 cm of alfalfa plant at 10% bloom) in 2016 (A) and 2017 (B). Horizontal dashed-lines indicate the reported B sufficiency level in the alfalfa petiole (Kelling, 1999). Error bars are the standard error of the means within each cutting. Different letter assignment indicates significance at $P \leq 0.05$ within each cutting. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET.

Forage quality was significantly influenced (*P* < 0.05) by water regimes in the second cutting of 2016 (Fig. 4.5) but not in the first cutting. No difference in forage quality between 50ET and 100ET was observed in any of the harvest (Fig. 4.5). None of the forage quality parameters (i.e., CP, ADF, NDF, and TDN) were significantly different (*P* > 0.9) in 2017 (Supplemental Table 1).

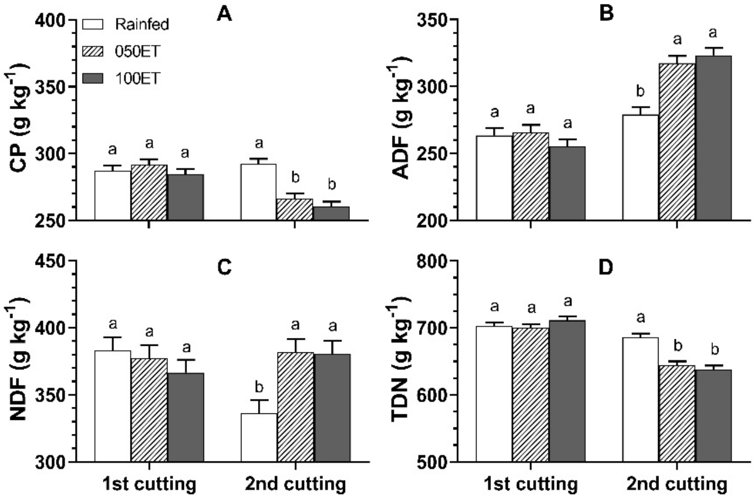

Figure 4.5. Effects of irrigation and cutting event on A) crude protein (CP), B) acid detergent fiber (ADF), C) neutral detergent fiber (NDF), and D) total digestible nutrients (TDN) in 2016. Error bars are the standard error of the means across water regimes within each cutting. Different letter assignment indicates significance at $P \leq 0.05$ within each harvest. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET.

<u>Alfalfa Response to Boron Application</u>

Effect of B fertilization on plant height, yield, CP, and trifoliate number on main stems was insignificant (*P* > 0.05; Table 4.3) for all cuttings in both years.

Petiole B increased (*P* < 0.001) with B rates in the first cutting of 2016. Similar increases were observed in the second cutting as well but only up to the twice-applied

1.12 kg B ha^{-1} (Fig. 4.6A) for both cuttings. Overall, the petiole B content decreased as the season progressed from 2016-2017 (Fig. 4.6). Within each cutting of 2017, petiole B concentration was increased significantly with increasing B rates, although petiole B remained at or below the sufficiency range (Fig. 4.6B). The highest petiole B was in the B_3 and B_4 treatments whether it was split-applied or applied in a single dose (Fig. 4.6) for all cuttings.

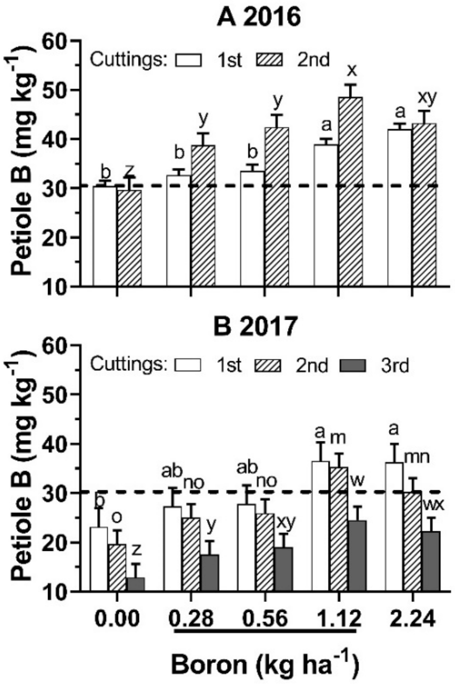

Figure 4.6. Effects of boron (B) fertilization on alfalfa petiole B concentration (top 15 cm of alfalfa plant at 10% bloom) in 2016 (panel A) and 2017 (panel B). Underlined B rates were re-applied after the first cutting reached 8 cm height regrowth in each year. The horizontal dashed-line indicates the reported B sufficiency level in the alfalfa petiole (Kelling, 1999). Error bars are the standard error of the means within each cutting. Different letter assignment indicates significance at $P \leq 0.05$ within cutting.

Only the ADF, NDF, TDN, and RFV were significant ($P < 0.01$) with applied B in 2016 (Fig. 4.7) when the data were averaged within each year. In 2016, the improvement of NDF and ADF and the decrease in RFV and TDN (Fig. 4.7) occurred at the two highest B rates. In 2017, only the split-applied highest B rate had a significantly lower NDF (P = 0.037; Fig. 4.7B) and the highest RFV (P = 0.028; Fig. 4.7D). The difference between years is the plant establishment and the weather condition (Table 4.1), these are two fairly significant events.

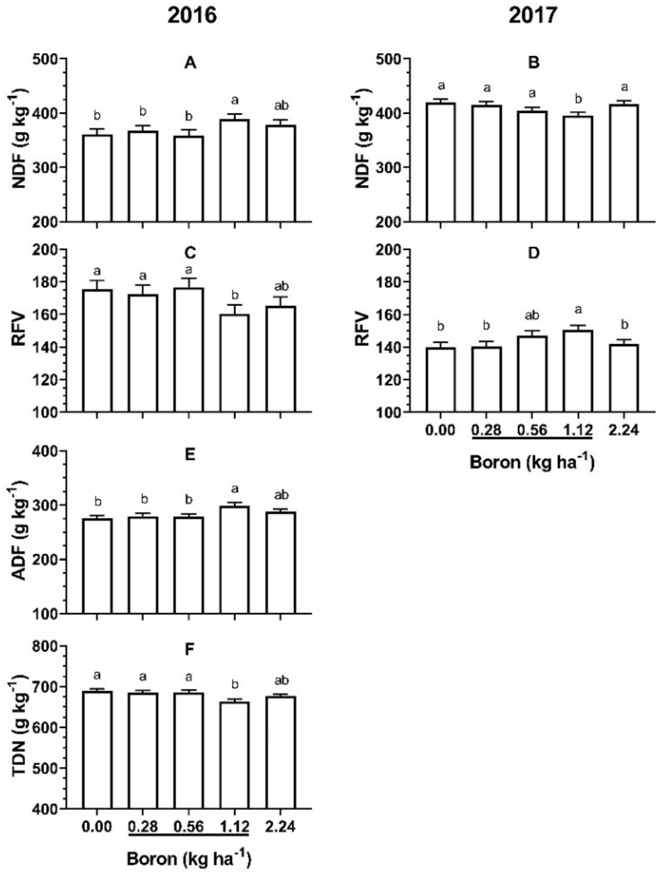

Figure 4.7. Effects of boron (B) application in 2016 (left) and 2017 (right) on alfalfa neutral detergent fiber (NDF; Fig. A and B), relative feed value (RFV; Fig. C and D), acid detergent fiber (ADF; Fig. E), and total digestible nutrients (TDN; Fig. F). Different letters denote significance at $P \leq 0.05$. Underlined B rates were re-applied after first cutting reached 8-cm height regrowth in each year. Data were averaged within years. Error bars are the standard error of the means.

Interaction Effect of Boron and Water Regimes

The interaction of B and irrigation was insignificant for any response variables in the year of establishment (i.e., 2016; Table 4.3), but was significant for four response variables in 2017: the second cutting yield (Fig. 4.8A; $P = 0.020$), along with NDF (Fig. 4.8B; $P = 0.040$), RFV (Fig. 4.8C; $P = 0.017$), and petiole B (Fig. 4.8D; $P = 0.049$) from third cutting of 2017 were significantly influenced by B and irrigation treatments. However, the treatment rate response was inconsistent. Yield in the second cutting of 2017 increased with increasing B rates up to first three B treatments in rainfed plots. At B rates of 1.12 kg ha^{-1} (which was re-applied after first cutting), all irrigation rates resulted in statistically similar yields (4,741±137 kg ha^{-1}; $P < 0.020$). With increase in application of B rates, RFV increased significantly ($P < 0.017$; Fig. 4.8C) and NDF decreased ($P = 0.040$; Fig. 4.8B) in 2017 third cutting, with an exception at 0.56 kg B ha^{-1}. At any given B rates, the availability of moisture increased ($P = 0.049$; Fig.4.8D) petiole B, although the increase in petiole B with B rates in the deficit irrigation relative to the rainfed check is hard to decipher. When moisture is non-limiting (i.e., 100ET), increase in petiole B as B rate increase is straightforward (Fig. 4.8D).

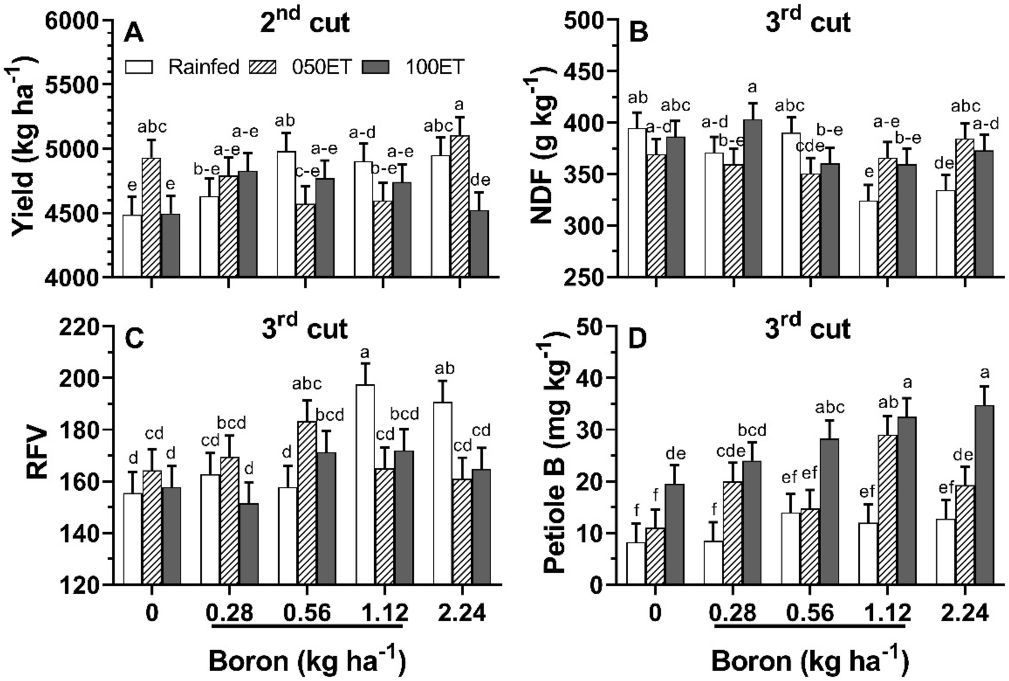

Figure 4.8. Interaction effects of water regimes and boron in 2017 on A) alfalfa yield, B) neutral detergent fiber (NDF), C) relative feed value (RFV), and D) petiole B concentration. Underlined B rates were re-applied after first cutting reached an 8 cm height in each year. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET. Error bars are the standard error of the means within each cuttings across ET and B interaction means. Different letter assignment indicates significance at $P \leq 0.05$ within the interaction events.

Correlations between Response Variables

Petiole B concentration was significantly influenced by both water regime and B factors, therefore correlation analysis was performed to determine the relationship between petiole B and all other response variables. No correlation (figure not shown; $0 \geq R^2 \leq 0.06$) was found for yield, CP, ADF, NDF, TDN, RFV, plant height, and trifoliate numbers which suggest that petiole B has a limited impact in alfalfa production and quality, as discussed in the previous section.

Discussion

Yield

Alfalfa responds to moisture (Carter et al., 2013; Jafarian et al., 2016; Cavero et al., 2017; Li and Su, 2017) as shown by the results of this study. Irrigation increased alfalfa yield with no yield impact in the 50ET plots compared to 100ET. Other researchers evaluated deficit irrigation from 90ET through 60ET with varied outcomes. Deficit irrigation strategy down to 70ET did not impact alfalfa yield in Kansas (Harmoney et al., 2013), but lowering further to 60ET reduced alfalfa yield in China (Li et al., 2016). Employing a riskier approach than those reported above, irrigating alfalfa down to 50ET did not reduce yield in northwestern Montana, correspond to Harmoney et al. (2013). This approach increases the opportunity to capture rainfall events and increases the chance for efficient soil moisture storage as evident in 2016 (Fig. 4.1). In the second year (2017) of the establishment, rainfall events from 80 days after regrowth through the final cutting were scarce (Fig. 4.1B), yet the alfalfa yield at 50ET remained similar to 100ET that year. It is known that alfalfa roots extends deep in the soil profile (Zang et al., 2013a), and possible water stress due to 50ET approach was likely minimized by the compensating effect of root growth beyond the 90-cm soil depth. The supposed water-stressed plots (50ET and rainfed) had high yields in 2017 relative to 2016 (Fig. 4.2), though the former (50ET) and the latter (rainfed) were insignificantly and significantly different, respectively, with 100ET. The yield impact of 50ET resulted in a three- and two-fold irrigation water productivity in 2016 and 2017, respectively, as compared to the well-watered 100ET treatment (Fig. 4.2).

Alfalfa yield had no significant effect with B fertilization in either year. The cutting effect in 2017 showed that the yield of the second cutting had a BxET interaction (Fig. 4.8A) due to the significant yield increase on the twice-applied 0.56 kg B ha^{-1} under the rainfed condition, whereas, the BxET in other treatment condition is hard to decipher. Overall, the impact of B on total yield is insignificant, similar to the reported findings in literature (Razmjoo and Henderlong, 1997; Grant and Miller, 1998; Pecinovsky and Lang, 2012; Sapkota et al., 2017), but is contradictory with Sherrell and Toxopeus (1978) and Kheirkhah et al. (2016).

These contrasting findings may be due to environmental factors, and efficacy of B application may be location- and weather-specific. The yield increase on the rainfed plots during the 2017 second cutting with B up to the twice-applied 0.56 kg ha^{-1} rate (Fig. 4.8A) may be due to the 2017 drought condition. Just after the 2017 first cut, no rainfall events were received (Fig. 4.1B), which was also hotter period relative to 2016 and the first cutting (Table 4.1). As soil dries, the availability of B in the soil decreases (Gupta et al., 1985; Goldberg, 1997), along with decreases in the absorption and uptake of plant nutrients during water-stressed conditions (Alam, 1999) yet data in Fig. 4.8A showed a response in yield on the rainfed treatments. Due to drought, available soil B may be limited and plants may have been able to absorb the second foliar-applied B this year. Still, the assumed efficacy of B of the rainfed treatment at the second cutting of 2017 remained inferior in terms of petiole B concentration than with the irrigated treatments

(Fig. 4.4B) and as well as at the second cutting during the near-average rainfall and mean air temperature year (2016; Fig. 4.4A). This implies that there is a decreased risk of lower petiole B in irrigated conditions or in areas with high rainfall than under rainfed or dryland production systems in the semiarid environment. However, in this study, application of B did not increase yield regardless of water regime or year.

Forage Quality

Forage quality in this study was not affected by irrigation in 2017 but the quality decreased in 2016 when irrigation was applied similarly to Li and Su (2017) and Holman et al. (2016). Decreased CP and increased NDF and ADF in 2016 (Fig. 4.5) were similar to the findings of Undersander et al. (1987) where CP content of alfalfa was greater under water-stressed conditions, and that ADF and NDF content increased as water-stress decreased. With irrigation, plant height significantly increased (Fig. 4.3A), which resulted in a decreased leaf-to-stem ratio and increased fiber content. Since the leaf to stem ratio has a significant impact on forage quality (Elliott et al., 1972), decreases in leaf-to-stem ratio cause a concurrent decrease in forage quality. Leaves contain high nutritional value and have a strong positive correlation between protein content and forage nutrient quality (Fick et al., 1994).

Boron fertilization increased the fiber content of alfalfa (ADF and NDF) in 2016. This was supported by the Rouquette et al. (2001), who found that with an application of 2.24 and 4.48 kg B ha^{-1}, fiber content increased. Crude protein was not affected by B in this study or the research performed by Rouquette et al. (2001). Another study in

Pennsylvania showed no effect of foliar-applied B products on alfalfa yield and quality (Hall et al., 2002). In 2017 of this current study, NDF decreased, and the RFV increased at 1.12 kg B ha^{-1} (the same amount of B was re-applied after 1st cutting regrowth); however, ADF and TDN were not significantly affected which are strongly correlated to NDF and RFV (data not shown). Since this effect was only observed when the data at both years were combined, this was not the case on all the cuttings in 2017.

Summary

Petiole B concentration increased with irrigation and B application, but it did not provide evidence for increased alfalfa yield and quality. The total yield of alfalfa significantly increased with irrigation; however, the 50ET and 100ET treatments were not significantly different from one another. By applying only 50% of required irrigation amounts, we may be able to produce a similar yield to full irrigation under a semi-arid condition in northwestern MT. Foliar application of B only resulted to an increased petiole B concentration which was not translated to an improvement of either yield or quality. Irrigation significantly decreased forage quality in the establishment year (2016) but not in 2017. Irrigation also led to increased plant height, which decreased leaf-to-stem ratio, in the establishment year. The effect of B on forage quality parameters such as ADF, NDF, TDN, and RFV was inconsistent with years. Crude protein, in particular, was not affected by B fertilization in either year. Application of foliar-applied B on a B-deficient soil is impractical regardless of water regimes.

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Table 4.1. Monthly and seasonal precipitation and air temperature and the 30-year average obtained at Creston weather station (USBR, 2017a) located ~100 m from the research site.

	Rainfall (cm)			**Temperature (°C)**		
Months	**2016**	**2017**	**1988-2017**	**2016**	**2017**	**1988-2017**
Apr.	3.8	5.9	4.6	8.8	4.7	6.0
May	7.1	1.8	5.9	10.8	11.4	10.6
June	5.3	6.7	8.9	14.7	15.3	14.1
July	3.9	0.2	3.5	17.0	20.0	18.3
Aug.	2.8	0.5	2.7	17.1	17.9	17.5
Sept.	2.0	2.5	3.4	11.9	12.2	12.4
Oct.	13.5	3.3	3.9	6.4	5.2	5.8
Season	5.5	3.0	4.7	12.4	12.4	12.1

Table 4.2: Irrigation and boron (B) treatments, amounts, and timing of application. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET.

Treatments	**Irrigation (cm)**		**Description**
Water regimes	**2016**	**2017**	
Rainfed	0	0	No irrigation (Check)
100ET	+15.2	+35.6	Irrigate 2.54 cm of water whenever 35% plant available water was depleted
50ET	+7.6	+17.78	Irrigate 1.27 cm (half to 100ET) of water applied the same day as the 100ET
Boron	**Total B (kg ha^{-1})**		
B_0	0		Untreated (Check)
B_1	0.56		Split: 0.28 kg ha^{-1} applied at 8 cm spring growth + 0.28 kg ha^{-1} at 8 cm regrowth after first cutting
B_2	1.12		Split: 0.56 kg ha^{-1} applied at 8 cm spring growth + 0.56 kg ha^{-1} at 8 cm regrowth after first cutting
B_3	2.24		Split: 1.12 kg ha^{-1} applied at 8 cm spring growth + 1.12 kg ha^{-1} at 8 cm regrowth after first cutting
B_4	2.24		2.24 kg ha^{-1} applied at 8 cm spring growth

Table 4.3. Analysis of Variance (ANOVA) table showing P-values of the effect of water regimes (denoted as evapotranspiration [ET]), boron (B) rates, and their interaction to the plant height (height), dry matter yield, petiole boron content (PB), number of trifoliate on the main stem (Tri), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrients (TDN), and relative feed value (RFV). The table also shows the numerator and denominator degree of freedom (ndf and ddf) for each source of variations.

2016	**ndf**	**ddf**	**Height^{1}**	**Height^{2}**	**Height^{3}**	**Yield^{1}**	**Yield^{2}**	**Yield^{3}**	**Total yield**	**PB^{1}**	**PB^{2}**	**PB^{3}**	**Tri^{1}**	**Tri^{2}**	**Tri^{3}**
ET	2	6	0.001	<0.001	-	0.034	<0.001	-	<0.001	0.133	<0.001	-	0.030	<0.001	-
Boron	4	36	0.399	0.766	-	0.083	0.840	-	0.457	<0.001	<0.001	-	0.407	0.661	-
ET*Boron	8	36	0.610	0.905	-	0.329	0.986	-	0.787	0.587	0.074	-	0.185	0.517	-
2017															
ET	2	6	-	0.149	0.002	-	0.372	0.002	0.012	-	<0.001	0.002	-	0.200	0.001
Boron	4	36	0.493	0.365	0.671	0.198	0.440	0.753	0.058	0.024	0.001	<0.001	0.387	0.598	0.296
ET*Boron	8	36	-	0.703	0.949	-	0.020	0.573	0.164	-	0.626	0.049	-	0.945	0.791

2016	**ndf**	**ddf**	**CP^{1}**	**CP^{2}**	**CP^{3}**	**ADF^{1}**	**ADF^{2}**	**ADF^{3}**	**NDF^{1}**	**NDF^{2}**	**NDF^{3}**	**TDN^{1}**	**TDN^{2}**	**TDN^{3}**	**RFV^{1}**	**RFV^{2}**	**RFV^{3}**
ET	2	6	0.355	<0.001	-	0.505	<0.001	-	0.439	0.001	-	0.508	<0.001	-	0.396	<0.001	-
Boron	4	36	0.530	0.200	-	0.042	0.764	-	0.048	0.361	-	0.043	0.760	-	0.067	0.495	-
ET*Boron	8	36	0.427	0.637	-	0.212	0.873	-	0.103	0.714	-	0.212	0.871	-	0.074	0.755	-
2017																	
ET	2	6	-	0.690	0.318	-	0.738	0.551	-	0.862	0.383	-	0.740	0.555	-	0.820	0.322
Boron	4	36	0.343	0.367	0.694	0.578	0.326	0.091	0.132	0.504	0.076	0.587	0.323	0.094	0.195	0.451	0.033
ET*Boron	8	36	-	0.533	0.217	-	0.067	0.168	-	0.306	0.040	-	0.066	0.168	-	0.172	0.017

$^{1,\ 2,\ \text{and}\ 3}$ are first, second, and third cuttings, respectively. Alfalfa was harvested on 27th July and Sep. 17th in 2016, and it was harvested on 15th June, 21st July, and 9th Sep. in 2017.

Total yield is the sum of in-season yields from all the cutting

Supplemental table 1. Analysis of variance (ANOVA) table showing *P*-values of the effect of cutting events within year to water regimes (denoted as evapotranspiration [ET]), boron (B) rates, and its interaction with B and ET to the plant height, dry matter yield, crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrients (TDN), relative feed value (RFV), petiole boron (B) concentration, and trifoliate number on the main stem. The table also shows the numerator and denominator degree of freedom (ndf and ddf) for each source of variations.

2016

Source of variation	**ndf**	**ddf**	**Plant height**	**Yield**	**CP**	**ADF**	**NDF**	**TDN**	**RFV**	**Petiole B**	**Trifoliate**
Cut	1	81	<0.001	<0.001	<0.001	<0.001	0.163	<0.001	0.179	<0.001	0.945
ET x Cut	2	81	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.065
Boron x Cut	4	81	0.588	0.393	0.495	0.496	0.190	0.500	0.332	0.006	0.750
ET x Boron x Cut	8	81	0.714	0.894	0.265	0.390	0.282	0.385	0.249	0.213	0.108

2017

Source of variation			**Plant height**	**Yield**	**CP**	**ADF**	**NDF**	**TDN**	**RFV**	**Petiole B**	**Trifoliate**
Cut	2	126	0.072	<0.001	0.599	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ET x Cut	4	126	<0.001	<0.001	0.202	0.617	0.677	0.615	0.432	<0.001	<0.001
Boron x Cut	8	126	0.637	0.988	0.484	0.475	0.713	0.482	0.430	0.964	0.103
ET x Boron x Cut	16	126	0.786	0.370	0.402	0.338	0.654	0.343	0.215	0.999	0.725

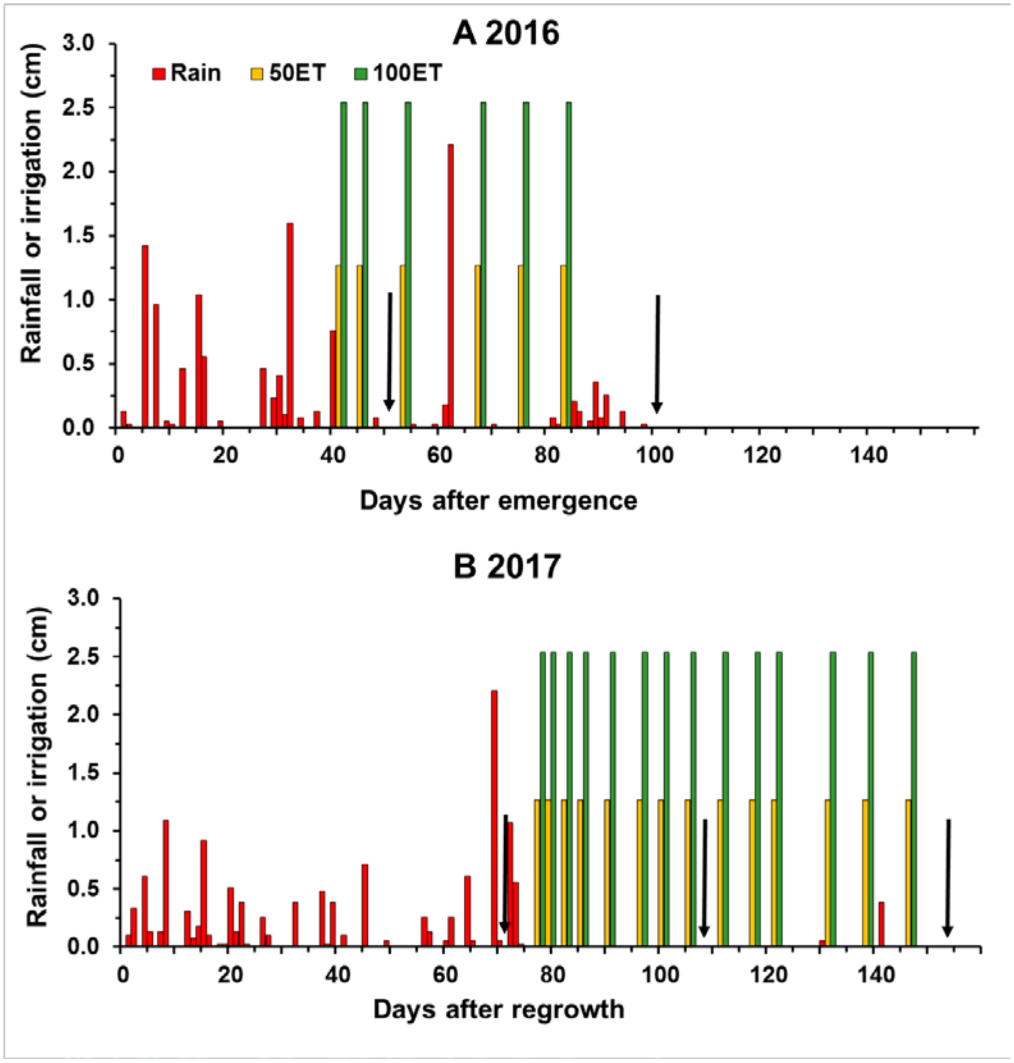

Figure 4.1. Irrigation and rainfall events during the growing seasons in 2016 (A) and 2017 (B). Downward arrows indicate the harvest events. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET.

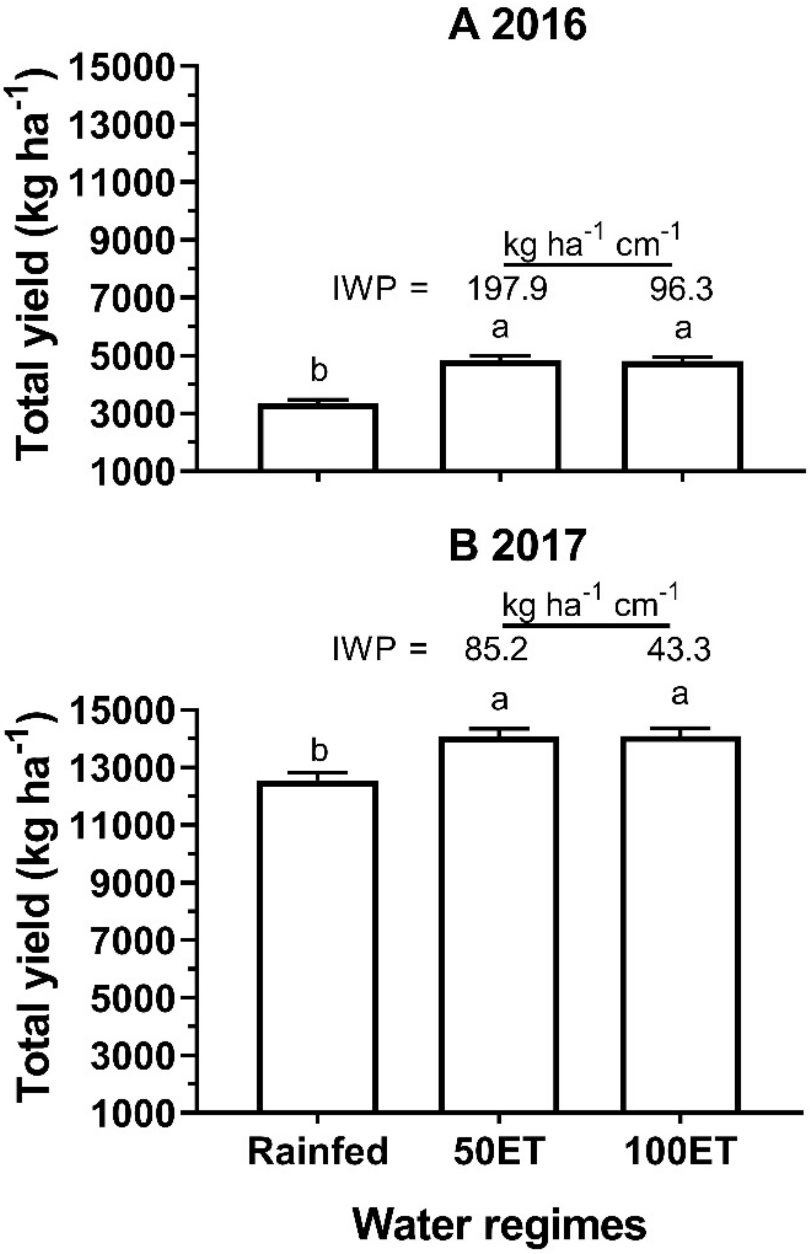

Figure 4.2. Effects of irrigation on alfalfa total dry matter production in 2016 (A) and 2017 (B). Error bars denote the standard error of the means and different letter assignment indicates significance at $P \leq 0.05$. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET. Irrigation water productivity (IWP; kg ha^{-1} cm^{-1}) for respective irrigation treatments are also shown above the bars in the graph.

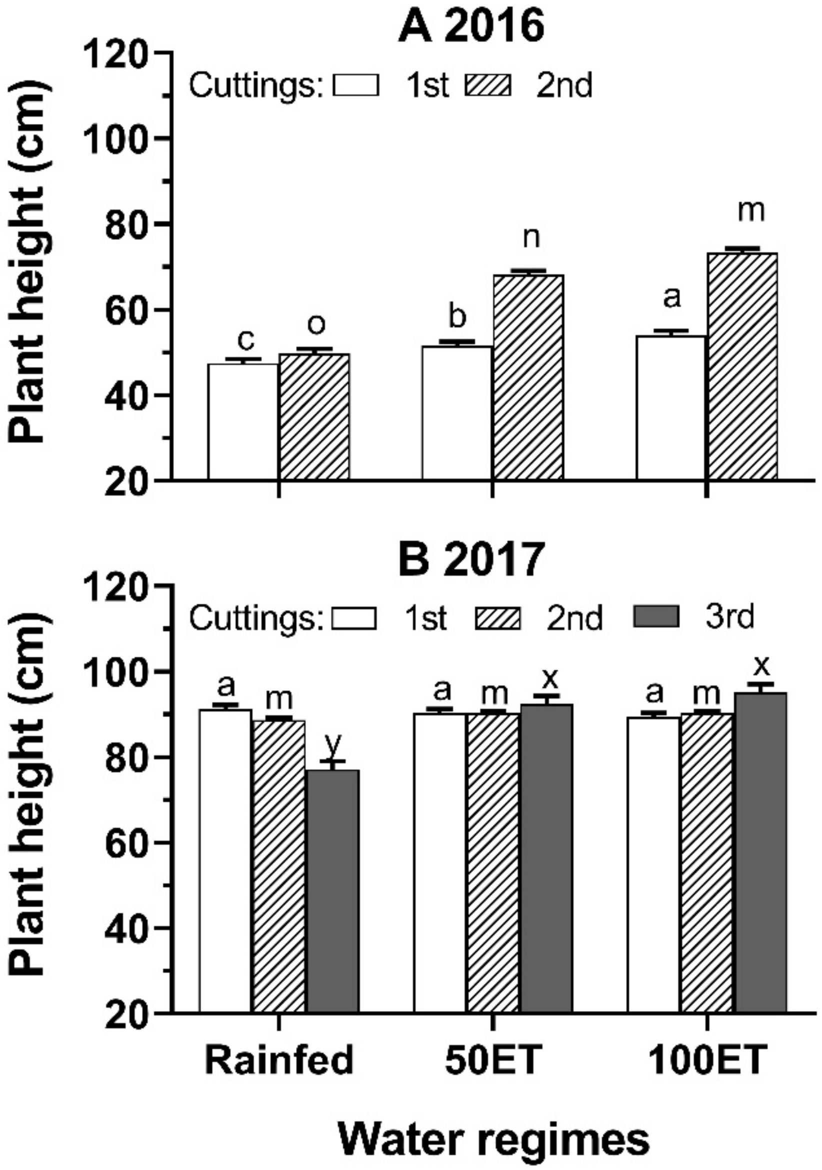

Figure 4.3. Alfalfa plant height in response to water regime in 2016 (A) and 2017 (B). Error bars indicate standard error of the means within each cutting. Different letter assignment indicates significance at $P \leq 0.05$ within harvest. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET.

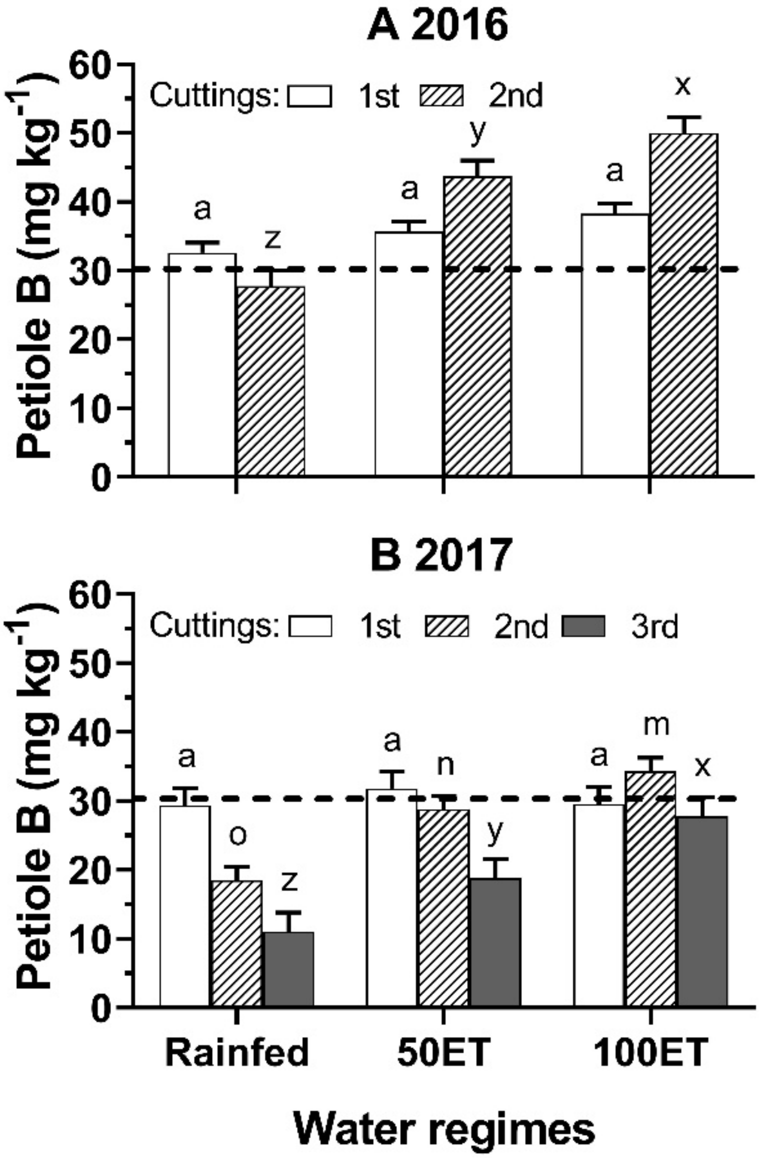

Figure 4.4. Effects of water regimes on petiole boron (B) concentration (top 15 cm of alfalfa plant at 10% bloom) in 2016 (A) and 2017 (B). Horizontal dashed-lines indicate the reported B sufficiency in the alfalfa petiole (Kelling, 1999). Error bars are standard error of the means within each cutting. Different letter assignment indicates significance at $P \leq 0.05$ within each cutting. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET.

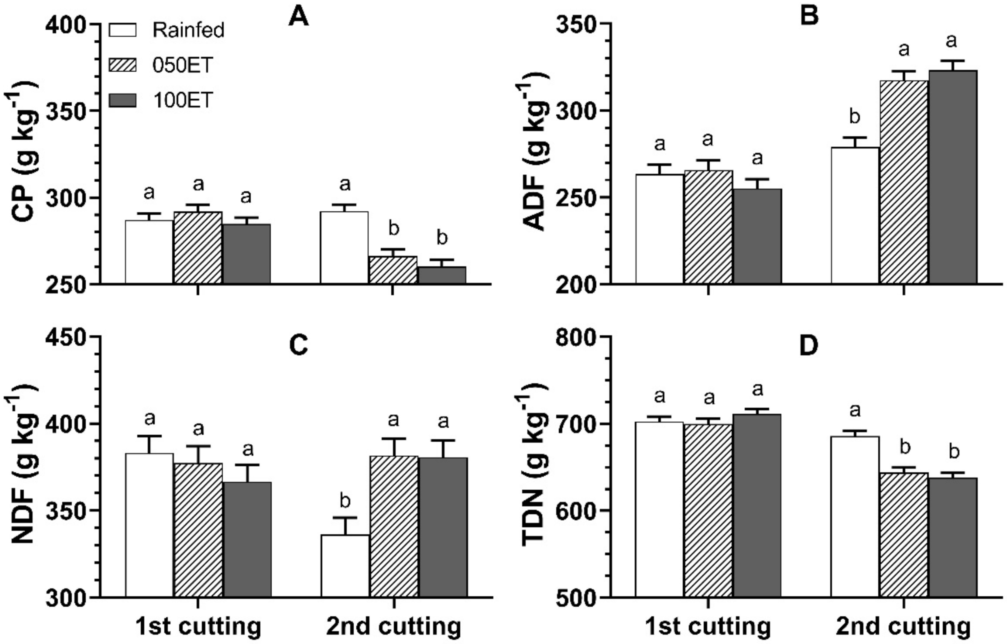

Figure 4.5. Effects of irrigation and cutting event on A) crude protein (CP), B) acid detergent fiber (ADF), C) neutral detergent fiber (NDF), and D) total digestible nutrients (TDN) in 2016. Error bars are the standard error of the means across water regimes within each cutting. Different letter assignment indicates significance at $P \leq 0.05$. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET.

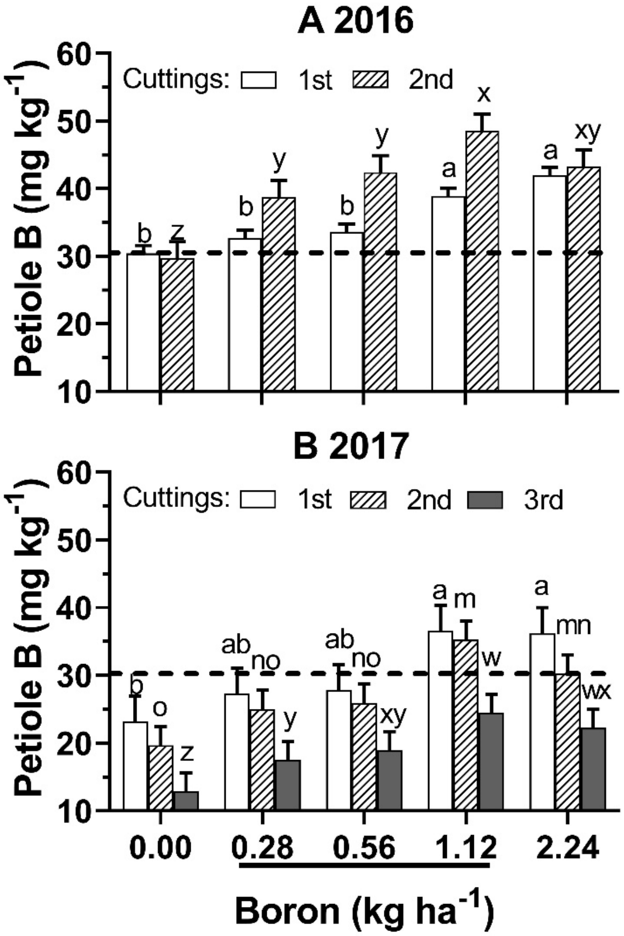

Figure 4.6. Effects of boron (B) fertilization on alfalfa petiole B concentration (top 15 cm of alfalfa plant at 10% bloom) in 2016 (panel A) and 2017 (panel B). Underlined B rates were re-applied after first cutting reached 8 cm height regrowth in each year. The horizontal dashed-line indicates the B sufficiency level in alfalfa petiole (Kelling, 1999). Error bars are the standard error of the means within each cutting. Different letter assignment indicates significance at $P \leq 0.05$ within cutting.

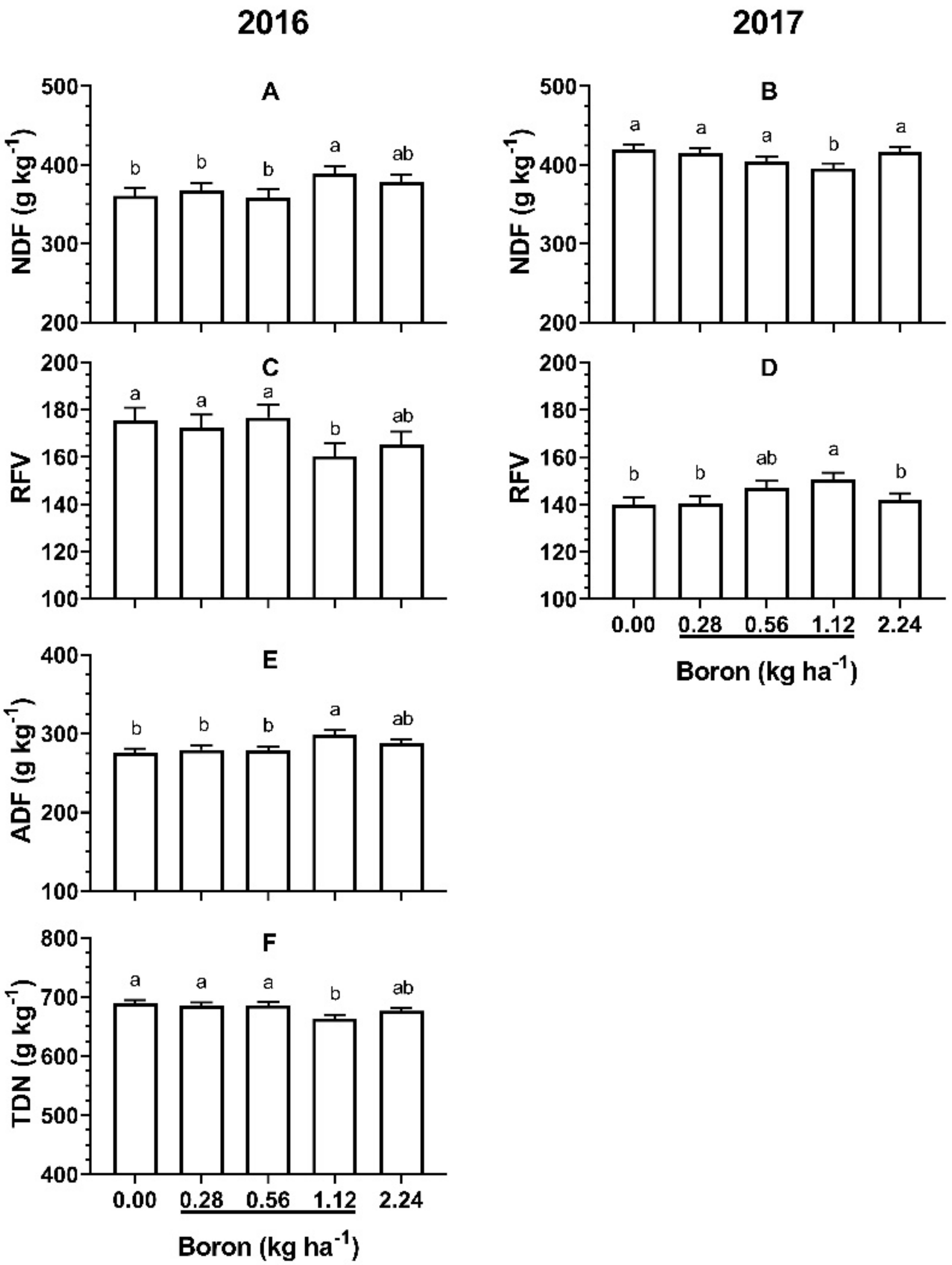

Figure 4.7. Effects of boron (B) application in 2016 (left) and 2017 (right) on alfalfa neutral detergent fiber (NDF; Fig. A and B), relative feed value (RFV; Fig. C and D), acid detergent fiber (ADF; Fig. E), and total digestible nutrients (TDN; Fig. F). Different letters denote significance at $P \leq 0.05$. Underlined B rates were re-applied after first cutting reached 8 cm height regrowth in each year. Data were averaged each years. Error bars are standard error of the means.

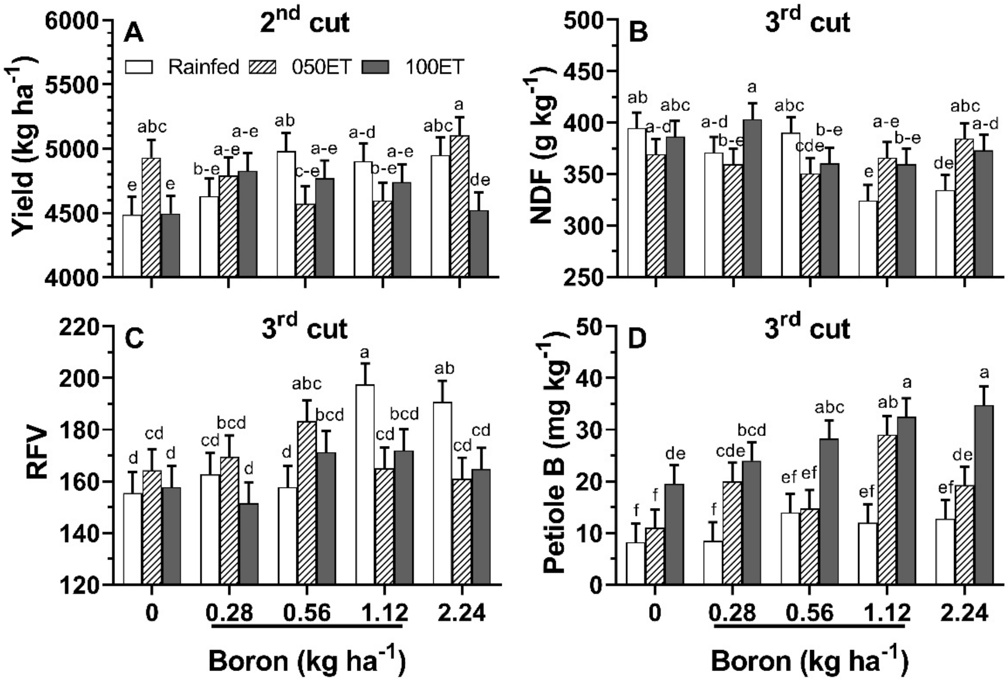

Figure 4.8. Interaction effects of water regimes and boron in 2017 on A) alfalfa yield, B) neutral detergent fiber (NDF), C) relative feed value (RFV), and D) petiole B concentration. Underlined B rates were re-applied after first cutting reached 8 cm regrowth height in each year. The full irrigation treatment is designated as 100ET, while the deficit irrigated treatment (50% water application) is designated as 50ET. Error bars are the standard error of the means within each cuttings across ET and B interaction means. Different letter assignment indicates significance at $P \leq 0.05$ within the interaction events.

CHAPTER FIVE

CONCLUSIONS

The effects of Boron (B) application at various water regimes on a B-deficient soil was studied in two different locations in Montana. In the first study, the objective was to evaluate the response of foliar application of B on the yield and forage quality of irrigated alfalfa. The two-year (2015/16) analysis showed that foliar application of B did not influence total yields for either year, except for the second harvest in 2015 at Dillon, MT. Alfalfa quality also was not affected by B at either site during the study. There was a significant increase in plant tissue B content with increasing B application, which implies that foliar application of liquid B is effective but did not positively impact yield or quality. This research shows that low soil B does not limit the yield of irrigated alfalfa in Montana.

In the second study, the objectives were to study the effect of water regimes (rainfed, 100% evapotranspiration [100ET], and 50ET), different rates of B, and their interaction on alfalfa yield and quality. The two-year (2016/17) study showed that total yield of alfalfa significantly increased with irrigation, however, production from 50ET and 100ET treatments were not significantly different from one another. Applying only 50% of required water amounts had similar yield to full irrigation under semi-arid conditions in northwestern Montana. Foliar application of B indicated only an increased petiole B concentration but was not translated to yield. Irrigation and B application also increased petiole B concentration, but they did not provide evidence to contribute to alfalfa yield and quality in this study. Overall, the foliar application of B on a B-deficient soil did not increase alfalfa yield or quality regardless of water regimes. A decision to apply B on a B-deficient soil should be assessed in-season and verified using a tissue test.

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