SCREENING OF A DIVERSITY OF LEGUMES AND NON-LEGUMES FORB SPECIES AT FIVE STRATEGIC LOCATIONS ACROSS UTAH FOR THE DEVELOPMENT OF SMART FOODSCAPES

by

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ABSTRACT

Screening of a Diversity of Legumes and Non-Legumes Forb Species at Five Strategic Locations Across Utah for the Development of Smart Foodscapes

by

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Grass-dominated rangelands in the Western United States provide sufficient forage quality for cowherd in spring and early summer, but protein falls below beef cow requirements (7.5%) as plants mature. Supplemental hay or protein cubes add significant feed costs. This study aimed to identify legumes and non-legume forbs with elevated protein and reduced fiber for cultivation in rangeland resource islands to more economically supplement late-season grazing. Plant species were chosen for their nutritional content, their appeal to grazing ruminants, and their ability to improve animal health and environmental sustainability. Species evaluations included a seed stratification study for hard-to-germinate seeds, a two-years evaluation of the establishment of 27 perennial herbaceous forages at five Utah locations, and assessments of yield and concentrations of primary and secondary nutrients of select species from June through October. Stratification enhanced germination in seeds of the most dormant species. Across field sites, 'Ladak' alfalfa, sainfoin, and small burnet established most rapidly and consistently; these introduced species are commonly for extensive rangeland plantings, but they persisted in dense monoculture stands. Other promising species were falcata alfalfa, cicer milkvetch, crownvetch, Maximillian sunflower, showy goldeneye and Utah sweetvetch. Protein concentrations decreased by late summer in all assayed legumes and forbs, with only Ladak alfalfa and Utah sweetvetch maintaining protein concentrations above 7.5% through October (9% and 8.5%, respectively). Acid and neutral detergent fiber values increased with maturity, but concentrations remained below 50 and 60%, respectively, for the species with the greatest yield (Ladak alfalfa and sainfoin), and lower-yielding species had even lower fiber, which would result in greater intake and digestibility. The introduced species sainfoin and birdsfoot trefoil had condensed tannin concentrations of about 20 $mg \cdot g^{-1}$ dry matter. The native species showy goldeneye and Utah sweetvetch had slower establishment and moderately dense stands. They contained both condensed tannin concentrations of 20 and 80 $mg \cdot g^{-1}$ DM, respectively, and hydrolysable tannin concentrations of 25 and 35 mg·g-1 DM, respectively. The introduced species small burnet maintained a hydrolysable tannin concentration of about 25%. About one-third of the evaluated plant species are good prospects for inclusion in rangeland resource islands.

(197 pages)

PUBLIC ABSTRACT

Screening of a Diversity of Legumes and Non-Legumes Forb Species at Five Strategic Locations Across Utah for the Development of Smart Foodscapes

Surbhi Verma

Grass-dominated rangelands in the Western United States often lack sufficient protein for pregnant cows after grasses mature in mid- summer, leading ranchers to rely on expensive feed supplements such as protein cubes or alfalfa hay. The goal of this study was to screen 27 perennial legume and forb species with the potential to remain green into late summer and provide sufficient protein to supplement cows on grass-dominated rangeland. Three experiments were carried out to understand germination of species, plant establishment and persistence at five Utah locations, and agronomic characteristics. The seed of five hard-to-germinate species were studied for the ability of extended cold treatment to improve germination. The germination of Lewis flax and Utah sweetvetch was improved by stratification. Arrowleaf balsamroot and fernleaf biscuitroot required stratification for germination, but germination percentage remained lower than the seed tag-based expectation. At five field sites across Utah, sainfoin, small burnet, and 'Ladak' alfalfa consistently became established in dense plant stands. Other species established moderate stands while many failed to establish at all. Ladak alfalfa, birdsfoot trefoil, small burnet, and sainfoin were studied for nutritive value characteristics from June to October of 2023 and 2024. In 2024, crested wheatgrass, falcata alfalfa, showy goldeneye, and Utah sweetvetch were also evaluated for nutritive value and dry matter yield. Additionally, Ladak alfalfa and sainfoin were evaluated for leaf-to-stem ratio over the season. Only Ladak alfalfa (2023) and Utah sweetvetch (2024) maintained protein levels above the minimum cattle requirement (7.5%) through October. Birdsfoot trefoil and sainfoin, contained condensed tannins and small burnet contained hydrolysable tannins. Showy goldeneye, and Utah sweetvetch contained both tannins. Further, all four species tested in 2023 accumulated a unique range of secondary metabolites. Ladak alfalfa contained a higher leaf-to-stem ratio than sainfoin from June to August. Crested wheatgrass, Ladak alfalfa, falcata alfalfa, and sainfoin maintained higher dry matter yield through October, demonstrating their ability to extend forage availability on rangeland. Secondary metabolites such as tannins can shift nitrogen excretion from urine to feces, reduce methane emissions, and lower parasitic burdens in cattle while other flavonoids richness can provide antioxidant and anti-inflammatory properties to ruminants.

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Surbhi Verma

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CHAPTER I

LITERATURE REVIEW

Introduction

Rangelands in the Western United States have a high prevalence of grass species that provide excellent forage quality during the spring season. However, forage quality on rangeland declines rapidly as grass species become reproductive in early summer and continue as the season progresses toward late summer and fall (Ganskopp & Bohnert, 2001; Holechek et al., 1989). These changes are associated with forage maturity and an increased stem-to-leaf ratio. Additionally, the nutritional requirement of the cowherd also changes throughout the reproductive calendar. The high nutrient requirement of gestating and lactating cows is not met solely by grazing on grass-dominated rangeland with the seasonal drop in forage quality, especially the decline in digestible energy and protein, hampering ruminants' ability to perform at their potential (George et al., 2001). Concomitantly, inadequate maternal nutrition can lead to intrauterine growth retardation of the fetus (Bazer et al., 2004). If sufficient forage is available, supplementing the basal diet with protein concentrates can enhance energy status as insufficient protein negatively impacts the digestion of low-quality forage in the rumen, which in turn slows intake. When forage crude protein content is above 10%, the forage intake of cows is sufficient to meet the requirements of all production stages, but dormant grasses fail to meet this requirement (Adams et al., 1996). To maintain forage intake and the utilization of metabolizable energy that is fundamental to animal health and production, protein supplementation is commonly provided to cattle (Mccollum & Horn, 1990). The issue lies in the economically draining cost of the feed including supplements that can account for ~35-40% of the total variable cost of production (Adams et al., 1996)

The creation of smart foodscapes (SFS) involves incorporating legumes and forbs capable of becoming established in dense stands, persisting in a rangeland environment, and supplying adequate forage quality and medicinal or bioactive compounds for an extended period of time, while reducing the environmental impacts of beef production systems. These islands of perennial legumes and forbs at strategic locations within extensive, grass-dominated landscapes can serve as sources of protein supplementation. The core idea is to reduce the impact of seasonal declines in forage quality on ruminant nutrition and health while mitigating production costs associated with protein supplementation during the late grazing season and maintaining or increasing environmental sustainability.

Distel et al. (2020) reviewed the nutritional and environmental benefits of grazing on phytochemically diverse pasturelands, which is attributed to positive associative relationships among biochemically and taxonomically different forages. Because they often have deep taproot systems, legume, and forb species can access moisture more effectively than grasses. Moreover, the nitrogen-fixing ability of legumes makes them rich sources of proteins, enhancing dietary nutritional profiles for cattle. However, there are challenges associated with increasing the availability of rangeland legumes and forbs. In a survey conducted by Matches (1989), difficulty in establishment and concerns about bloat risk were the major barriers to the use of legumes on rangeland in the western United States. High temperature, multi-year drought, and a lack of information on successful establishment and management of legumes and forbs on semiarid rangelands due to variation in seed germination and seedling vigor hinder the use of nutrient-rich plant species. These characteristics underscore the value of screening these species for their potential to successfully alleviate challenges of nutritional deficiency and greenhouse gas emissions while supporting ruminant production on rangelands.

There is wide acceptance that forage diversity provides more opportunities and beneficial feed choices for grazing livestock to fulfill their physiological requirements as well as to express their inherent explorative and selective grazing behavior (Zanon et al., 2022). When ruminants have access to a mixture of forage species with diverse nutrient profiles, they can benefit from selecting nutrients they prefer compared to being offered only one type of forage (Provenza et al., 2003). The adoption of introduced forage species is sometimes avoided in preference to native species or even specific ecotypes of native species, but legumes and forbs native to the northern Mountain West are usually not found growing in dense stands.

Rangelands typically receive minimal management as compared to cultivated, irrigated land, and common rangeland management practices may be deleterious to desirable plant species (Wilton et al., 1978). The species under study for inclusion in smart foodscapes (SFS) were screened for germination, establishment and persistence under semi-arid rangeland conditions in Utah. A subset was assessed for yield, forage nutritive value, and content of beneficial secondary metabolites.

The plant species selected for evaluation for the SFS project were tested at a range of altitudes and associated climates. Replicated experiments were used to compare the establishment and persistence of approximately 24 legumes and non-legume forbs

(Tables 1.1 & 1.2), with the most promising plant species tested further for forage nutritive value and secondary metabolite concentration during summer and into fall

Potential benefits of smart foodscapes

Provision of protein supplementation during late summer and fall

Owning to high temperatures during mid- to late-summer, plant development is accelerated and leaf to stem ratio is reduced (Buxton, 1996). Semi-arid rangeland forages are grazed once every year or two, and do not have access to soil water to support regrowth (Briske et al., 2008). The quality of un-harvested forages decreases from spring through fall on rangelands, and rangeland grasses in late reproductive growth do not meet the minimum crude protein requirements of ruminants to maintain bodily functions (Mccollum & Horn, 1990). These values are 7 or 7.5% for a non-lactating cow and 9.6% for cows producing 4.5 kg milk per day (Hersom, 2020; Lalman & Holder 2023; Sprinkle, 1996). The rumen microbial colonization of dry, dormant, fibrous grasses is inhibited during the late summer and fall when protein concentration falls below 5%, limiting forage intake. Therefore, nutritionally suboptimal forage necessitates supplementation.

Energy supplements do not require rumen fermentation, so they can directly improve animal performance but are not cost-effective. Thus, protein supplements are commonly provided (Holechek & Herbel, 1986). They complement the carbohydrates available as fiber in mature grasses, and facilitate microbial colonization and digestion in the rumen, boosting forage consumption, providing essential nutrients for growth and lactation, and improving body condition and reproductive performance (Sprinkle, 1996).

Adequate protein supplementation not only benefits mother cows but improves the potential carcass composition of beef calves and lambs (Minson, 1990).

Incorporating legumes and forbs into low-quality grasses would bridge the gap in seasonal inadequacy of protein supply with minimal inputs; see Ch. IV for protein concentrations of unstressed greenhouse-grown validation plants of legumes and forbs as well as selected field-grown plant species. Although crested wheatgrass and legumes were both expected to decline in crude protein as the season progressed, the crude protein concentration of legumes was expected to remain above 7%, as was reported for native legumes on rangeland in Texas (Muir et al., 2011).

Beneficial plant secondary metabolites

The excessive excretion of protein nitrogen as urea in urine is one of the constraints on meeting the crude protein requirements of ruminants. This occurs when the supply of protein to the rumen exceeds the readily available carbohydrates needed for microbial colonization, and results in the lysis of the amino group from amino acids to recover carbon skeletons that can be used for energy (Satter & Roffler 1975). For dairy cattle, optimal circumstances involve a substantial proportion of forage plant protein bypassing rumen degradation, which is digested to amino acids in the abomasum that are absorbed in the intestines (Buxton, 1996). On average, 75% of forage protein is degraded in the rumen and only about 25% escapes ruminal fermentation and passes to the intestine as bypass protein (Minson, 1990). On rangeland, excessive protein excretion is generally not problematic, but efficient use of available nitrogen and minimal losses to the environment are relevant considerations.

Temperate forage legumes are beneficial ruminant feeds because of their relatively high protein concentrations and digestibility. However, bloat can be caused by the same characteristics, and it is inadvisable to graze pastures with proportions of alfalfa or clovers greater than 25%. Condensed tannins (CT) accumulated by some temperate legumes such as sainfoin can prevent bloat while increasing bypass protein by protecting excess plant protein from proteolysis in the rumen (Sottie et al., 2014). These naturally occurring plant polyphenols bind to proteins to form stable complexes. The CT-protein interaction depends on the structures and concentrations of both the CT and the protein, and is pH-dependent (Lorenz et al., 2014). Condensed tannins bind to proteins in the rumen at pH 5.5 to 7, leaving the rumen following precipitation, thereby reducing microbial degradation of protein and the release of ammonia to the rumen environment. The CT-protein complex dissociates in the abomasum, where the pH is typically 2.0 to 2.5. This is followed by gastric and pancreatic digestion and absorption of amino acids in the small intestine, shifting nitrogen excretion from urine to faeces (Jones and Mangan, 1977). An *in vivo* study conducted by Hymes-Fecht et al. (2013) demonstrated a 24% reduction in rumen NH_3 when *Lotus corniculatus* silage with a CT concentration between 73 and 95 $mg \cdot g^{-1}$ was substituted for *Medicago sativa* silage containing 0% dietary total CT in a dairy cattle ration.

Some CT, especially at higher concentrations, can function as anthelmintics to reduce internal parasite loads and improve ruminant health (Naumann et al., 2017). Condensed tannin-containing legumes exhibited anthelmintic properties in both *in vivo* (Molan et al., 1999) and *in vitro* studies. Condensed tannin and hydrolysable tannin (HT) both reduced egg hatching and the motility of larvae associated with gastrointestinal nematode parasites, specifically in small ruminants (Naumann et al., 2017). Including optimal concentrations of beneficial plant secondary metabolites, such as tannins, in diets can confer a range of health benefits to ruminants.

There are CT and HT that are not beneficial or that may interfere with the productive use of protein and are therefore considered “antinutritional”. They function by decreasing diet palatability, impeding intake, and inhibiting digestibility of nutrients such as protein, carbohydrates and fats, thereby depressing feed efficiency and production of animal products (Naumann et al., 2017). None of the tannin-containing perennial legumes and forbs used in this study are reported to have antinutritional effects on ruminants.

Potential to increase rangeland biodiversity and benefits for the environment

It is well documented that a diversity of productive plants from different functional groups and with a range of beneficial secondary metabolites supports healthy above- and below-ground ecosystems. Evidence supports the benefits of plant diversity for soil health, plant health, ruminant health, the health of the environment, and, consequently, the economic health of ranchers. In contrast, rangelands are dominated by grass species and depleted in legumes and forbs (Schaub et al., 2020). The establishment of phytochemically diverse resource islands planted with desirable legumes and non-legume forbs in strategic locations can be used to supplement protein and supply secondary metabolites while facilitating restoration in dryland ecosystems through natural distribution of seed (Hulvey et al., 2017). The development of SFS is anticipated to yield direct benefits for sustainable beef production while concurrently promoting biodiversity within rangeland ecosystems.

Potential reduction in greenhouse gas emissions

The inclusion of legumes with beneficial plant secondary metabolites (PSM) such as CT on rangeland can positively impact the environment by mitigating the carbon and nitrogen footprints of cattle through reducing methane and ammonium emissions (Rochfort et al., 2008). Methane (CH_4) is the primary source of greenhouse gas (GHG) emissions from beef cattle, accounting for 56% of all GHG from the beef industry and 39% of all GHG emissions from the livestock sector (Rotz et al., 2019). In a life cycle assessment of beef production in western Canada, 63% of CO_2-equivalent GHG was from CH_4, and the cowherd was found to be the source of 61% of CO_2-equivalent GHG (Beauchemin et al., 2010).

Methane emission is a key factor in climate change, as it exerts a global warming potential 28 times greater than that of CO_2 (Kozłowska et al., 2020). Increasing the quality or the tannin concentration of forage diets can reduce methane; examples include Roca-Fernández et al. (2020), who used in vitro ruminal fermentation to demonstrate a reduction in enteric CH_4 production from diets consisting of 50:50 grass-legume with an optimal level of CT as compared to alfalfa and MacAdam et al. (2022) who demonstrated methane reductions as great as 60% for cattle grazing legume pastures compared with grass pastures.. While high forage quality on semiarid rangelands is not sustained beyond the spring, legumes and forbs can provide significant levels of protein, CT and other secondary metabolites, that contribute to both nutritional and environmental quality.

Forage quality

Forage nutritive value is used to predict the performance of a diet for a given class of ruminants, such as the cowherd. Alternatively, forage quality includes nutritive value as well as the appeal of a feed to ruminants resulting from the growth environment, such as soil fertility, and plant stage of growth, impacting animal health and productivity(Nelson & Moser, 1994). Forage quality also includes animal responses to secondary metabolites, including secondary metabolites. Plant adaptation and stage of growth interact with the chemical composition and digestibility of forage. Rangeland and pasture forage quality can be improved by increasing the availability of adapted, nutrient-dense forages with beneficial secondary metabolites (Nelson & Moser, 1994).

Environmental and physiological factors play a role in determining how the maturity of herbage influences forage quality (Buxton, 1996). In forage species, where the entire aboveground biomass is the crop, the highly palatable vegetative stage with high protein and low fiber concentration transitions to a highly fibrous and low protein concentration mature reproductive stage (Oelberg, 1956; Vera-Velez & Lamb, 2022). At lower temperatures in spring, cell wall materials at a given stage of growth are less lignified and easier to digest, while at higher temperatures, lignin synthesis is augmented, resulting in less digestible forage (Nelson & Moser, 1994). Forage plant maturation occurs more rapidly under higher temperatures and longer photoperiods during summer, which increases the rate of plant development (Field et al., 1976). Existing plant nutrients are translocated to seeds or storage, and as stem growth continues and lower leaves cease to function and senesce, the leaf-to-stem ratio decreases (Oelberg, 1956). This decline in nutritional quality is routinely seen in rangeland forages that are grazed annually rather than being harvested at a given stage of growth. Our study of the seasonal change in nutritive value of seeded legumes and forbs assessed unharvested plants from summer through autumn.

Seasonal change in forage quality and availability drive corresponding changes in cattle diet selection as shown by McCollum et al. (1985), where a dietary shift was observed in cattle. As the forage quality of grasses decline by October, cattle showed greater dependence on forb species (83% compared to 17% in August). This reflects the change in diet as a compensatory strategy for nutrition driven by maturity of grass species. This dietary shift was likely driven by higher nutritional quality and less maturity of forb species during the late season (Scasta et al., 2016).

In selecting plant materials for densely planted resource islands, we were interested in identifying species that maintained optimal forage quality or had the most gradual decline throughout the grazing period, especially during the late season. Protein concentration is a key determinant of forage quality as it provides essential amino acids for the animal as well as rumen microbes to function properly and can be used for energy. If more protein could be supplied by forage, protein supplementation could be reduced.

Protein is generally expressed as crude protein, which is calculated from the nitrogen concentration of the forage (N × 6.25). A minimum crude protein concentration of 7% is required to meet the daily requirement of an adult cow during lactation and gestation (Hersom, 2020). In a study by Holechek et al. (1989) the average crude protein concentration of five wheatgrass species fell below the minimum requirements of ruminants during late summer and fall. In the Canadian Great Plains, where summer rainfall fluctuated between 5 and 15 inches (130-390 mm), crested wheatgrass had the lowest protein concentration (5%) among the cool-season grasses tested in late summer (Biligetu et al., 2014). In the same study, mixtures of crested wheatgrass with sainfoin or cicer milkvetch were also below 6% and only mixtures with alfalfa reached the critical protein concentration of 7.5%.

Similar to other forage nutritive components, CT also changes over the season and with maturation. Dry and warm weather is thought to promote accumulation of tannins in plants (Lees et al., 1994) and in a study by Berard et al. (2011), CT concentration was found to be greater in all species in the mature samples than in the vegetative samples. In another study, the contents of extractable condensed tannin and total condensed tannin increased in whole plants of purple prairie clover and white prairie clover with plant maturity (vegetative to seed maturity stage). However, sainfoin exhibited the reverse trend by containing more CT in vegetative than in mature samples (Li et al., 2014). In mature rangeland-grown plants, leaf loss may occur as unharvested plants continue to mature in the field. Loss of leaves may cause a decrease in CT concentration of whole plant samples.

The objective of screening both native and introduced legume and forb species in seeded, replicated studies was to identify plants with sufficiently high crude protein in late summer to successfully supplement a widely established rangeland grass, such as crested wheatgrass, along with acceptable levels of establishment and persistence under dryland conditions. Previous studies had identified alfalfa, sainfoin and small burnet as having the greatest likelihood of succeeding in relatively dense stand (Kneebone, 1959; Monsen et al., 2004), but a number of other legumes and forbs, including many native plant species, were recommended as palatable and nutritious for cattle, including Utah sweetvetch (Johnson et al., 1989), Maximillian sunflower (Wennerberg, 2004), prairie coneflower (Carr, 2009), and showy goldeneye (Monsen, 2004). To address the goal of reducing environmental impacts and benefitting ruminant health, plants selected for this study also contained some level of beneficial secondary metabolites (Table 1.2). The screening commenced with the evaluation of species establishment and persistence in the field to determine agronomic suitability, followed by an appraisal of forage quality and secondary metabolite type and concentration to determine the degree to which the species would benefit the health and nutritional of ruminants while reducing the environmental footprint of rangeland beef production.

Plant species

Extending the grazing season on rangeland into late summer and autumn could be achieved by identifying forbs that maintain their nutritive value as they mature and that can become established and persist in relatively dense stands. Given their ability to fix nitrogen, legumes could compensate for the decline in forage quality of rangeland grasses by supplying greater crude protein and more digestible fiber (Sleugh et al., 2000). Including non-legume forbs with the desired nutrient profile and high yield could also accomplish this aim in the semi-arid rangelands of the western United States.

Legumes

The success of rangeland grass seedings have been studied far more extensively than seedings of perennial legumes and forbs. Recently work on the germination of 20 native forb species by Jensen et al. (2022) is, to quote the authors, "unique in that it provides data on seedling emergence rates for forb species of restoration interest in the Intermountain West". The two species included in the Jensen et al. (2022) study and also studied for this thesis research were *Achillea millefolium* (western yarrow) and *Heliomeris multiflora* Nutt. (showy goldeneye), which had germination of 0.25 and

0.20%, respectively. This thesis reports plant numbers established from a constant number of pure live seed planted and followed over a two-year period and is also unique in studying establishment of a number of legumes and forbs in relatively dense plantings in the Mountain West, based on our literature review. The most relevant similar study was carried out by the USDA in Oklahoma (Kneebone, 1959). The following 27 species have been evaluated to harness the benefits of legumes and forbs on rangelands and strategically utilize complementary relationships among different species.

Alfalfa

Often referred to as the "queen of forages", its wide adaptation, high protein content and ability to fix N make it a commendable choice for rangelands. The downside of grazing solely on this species is the rapid degradation of protein content in the rumen, inducing poor dietary efficiency and nitrogen loss detrimental to the environment. There are now many documented cases of suitability of alfalfa in dryland conditions, e.g., at Nephi, UT, alfalfa showed strong persistence, by surviving the extreme arid conditions and significantly increased the total forage yield and protein when planted with crested wheatgrass as compared to only grass plots (Rumbaugh et al., 1981). Stewart et al. (2019) found that cows and heifers feeding on alfalfa hay had greater excretion of N in urine as compared to cows and heifers feeding on small burnet, sainfoin, cicer milkvetch, meadow bromegrass, or birdsfoot trefoil.

Alfalfa, purple flower (*Medicago sativa* L.), cv. Ladak

***Establishment and persistence**:* In an establishment study of legume species at five locations in northern Colorado, *Medicago sativa* showed excellent seedling emergence at all locations (Townsend & McGinnies, 1972). Excellent establishment and rapid first-year growth of alfalfa has also been reported by Kneebone (1959) in western Oklahoma rangelands.

***Forage quality**:*Rumbaugh et al. (1982) reported a mean protein concentration of 21.2% in May for alfalfa grown under dryland conditions in Utah, which declined to 5.2% in August.

PSM: While the study was not conducted on rangeland, Nowacka & Oleszek, (1994) noted saponins in both aerial and root parts in this species, with concentrations of 9.5 $mg \cdot g^{-1}$ and 24 $mg \cdot g^{-1}$, respectively. Notably, the presence of saponins in this species has been demonstrated to reduce methane production in ruminants (Kozłowska et al., 2020).

Alfalfa, yellow flower (*Medicago sativa* L. ssp. *falcata* (L.) Arcang)

Forage quality: Peel et al. (2013) reported 8.9% crude protein during mid-summer. The study further demonstrated the superior nutritional value of alfalfa during mid-summer when compared to dormant grasses.

Birdsfoot trefoil (*Lotus corniculatus* L)

Birdsfoot trefoil is a desirable species for rangeland owing to its nutritional profile and ability of condensed tannins in its tissues to complex with dietary protein in the rumen, thus increasing the proportion of bypass protein in the small intestine (Waghorn et al., 1987).

Establishment and persistence: In a study conducted under dryland conditions in New Zealand, birdsfoot trefoil matched the annual and seasonal dry matter production of perennial ryegrass and white clover. Furthermore, established stands thrived for three years, outyielding traditional pasture by 10% and showing superior summer productivity (Barry et al., 2003). Similarly, Bush (2002), indicated, that as a dryland pasture legume, its growth is routinely 20% more than most dryland grass-legume mixtures after July 1, indicating its potential to be a promising species on rangelands.

Forage quality: Crude protein content of 36% is reported by Sleugh et al. (2000) in a study conducted in Iowa. Although a significant decline in crude protein content and an increase in cell wall content was observed with the progression of the season in research conducted on assessing the impact of maturity on the nutritive profile of birdsfoot trefoil in Turkey (Karabulut et al., 2006), the crude protein level did not fall below the minimum required level for mature cow (7%) reported by Hersom (2020). That being reported, our study evaluated the forage quality of this potential candidate within western rangeland conditions.

PSMs: Birdsfoot trefoil contains a concentration of CT that effectively decreases rumen degradable protein (RDP) proportion as compared with *Medicago sativa* (0 $mg \cdot g^{-1}$ CT) and hence enhances nutrient utilization in ruminants (Hymes-Fecht et al., 2013). Furthermore, in a study conducted by Coblentz & Grabber (2013), CT-containing birdsfoot trefoil showed a linear decrease in the immediately soluble crude protein fraction and rate of degradation *in situ*. These characteristics ultimately enhanced ruminant performance (Barry et al., 2003). In an *in vivo* study by Stewart et al. (2019), cows and heifers consuming birdsfoot trefoil hay, rich in procyanidin CT, showed higher N retention than animals consuming sainfoin, small burnet, alfalfa, cicer milkvetch, and meadow bromegrass, by reducing N excretion in urine.

Other benefits: While increasing levels of birdsfoot trefoil in mixtures with perennial ryegrass increased the partitioning of N from urine to feces in a study by Woodward et al. (2009), birdsfoot trefoil had no impact on the partitioning of nitrogen excretion from urine to faeces in a study by Kapp-Bitter et al. (2023). However, in a study testing nutrient utilization in dairy cattle fed alfalfa silage versus one of three birdsfoot trefoils with increasing concentrations of CT (6 $mg \cdot g^{-1}$, 12 $mg \cdot g^{-1}$, and 17 $mg \cdot g^{-1}$), cattle showed increased production when fed on the 3 birdsfoot trefoil diets (Hymes-Fecht et al., 2013).

Canadian milkvetch (*Astragalus canadensis* L.)

Establishment: No information was found on the establishment of this species.

Forage quality: An irrigated study reported 24.2% and 15.2% crude protein and crude fiber, respectively in this species (Davis, 1982). This species was observed to have a toxic effect by ranchers in Oregon due to the high nitrite content (Coburn et al., 1974).

PSMs: Davis (1982) reported the presence of 5.9 $mg \cdot g^{-1}$ CT in an irrigated study of this species.

Cicer milkvetch (*Astragalus cicer* L.)

Establishment and persistence: Although it has beneficial characteristics like drought tolerance, compatibility with other species and high forage quality, its utilization is limited. This is attributed to its slow establishment (Acharya et al., 2006). In a multi-location study in northern Colorado, stands showed fair to good establishment at seed depths of 0.013 to 0.025 m by the end of the growing season due to its rhizomatous characteristic (Townsend & McGinnies, 1972). The slow establishment is due to the hard/impermeable seed coat, slow growth at the seedling stage, and poor vigor (A. Johnston, 1975; Ogle et al., 2003). In contrast, when evaluated for adaptability in dryland conditions in Colorado, it outperformed other legumes and was second best after alfalfa (Wilton et al., 1978). Seed should be scarified before planting.

Forage quality: Cicer milkvetch forage has optimal nutrients for ruminants, especially during the late grazing season due to its slow growth (Acharya et al., 2006; Tilley et al., 2008). Rumbaugh et al. (1982) reported a decline in average protein concentration from 21.8% to 5.8% from May through August. Notably, this species has much lower neutral detergent fiber (NDF) concentration than alfalfa and birdsfoot trefoil (Kephart et al., 1990). It grows slower than alfalfa but remains green for a longer period in the fall (Johnston et al., 1971; Tilley et al., 2008) indicating its usefulness in pasture during the late season. In a five-year study conducted in a semi-arid prairie region of Canada, Loeppky et al. (1996) demonstrated this species exhibited 48% and 60% higher crude protein content than alfalfa during September and October, respectively.

Cicer milkvetch was observed to have in vitro dry matter digestibility (IVDMD) equivalent to alfalfa during spring, with cicer milkvetch exhibiting a 20% higher IVDMD than alfalfa during October. These findings indicated the potential of cicer milkvetch to provide a nutrient-rich diet during the late season (Loeppky et al., 1996).

PSMs: Cicer milkvetch is reported to contain isoflavonoids, saponins and CT (Butkutė et al., 2018). In a study, CT concentration declined from 12 to 7 $mg \cdot g^{-1}$ with the progression of the season from August to October (Villalba et al., 2013). In a study by Stewart et al. (2019), cows and heifers consuming protein rich but non-CT containing cicer milkvetch exhibited high N excretion in urine. However, it showed better N retention than other non-CT containing species such as alfalfa and meadow bromegrass in both cows and heifers, likely by balancing the energy and protein levels, supporting better utilization of N by microbes in rumen. Cicer milkvetch was found to contain a substance that inhibited cellulosic digestion (Weimer et al., 1993) and was later shown to accumulate an arabinogalactan protein (Weimer, 1998).

Grazing preference: Another study from the semi-arid prairie region of Canada revealed the preference of grazing animals for cicer milkvetch during late summer as compared to alfalfa harvested at a similar time, due to its superior palatability (Lardner et al., 2018). In a study by Pitcher et al. (2019) steers grazing irrigated cicer milkvetch pastures did not gain as well as steers grazing irrigated birdsfoot trefoil, but gains were equal to steers grazing irrigated meadow bromegrass (*Bromus biebersteinii*) pastures.

Crownvetch (*Securigera varia* L. Lassen)

Previously, crownvetch was regarded as an undesirable forage species for ruminants due to the presence of astringent substances (Burns et al., 1967). However, subsequent studies showed it to be an acceptable legume species for grazing as it was readily grazed by heifers (Burns et al., 1972).

Establishment and persistence: In a study conducted under northeastern conditions by Burns et al. (1977), stands were unproductive and could not survive continuous grazing after establishment due to poor vigor.

Forage quality: Forage quality assessment of first growth crownvetch in the northeastern USA revealed ~30% crude protein during May that declined to ~18.2% by the end of June (Shenk & Risius, 1974). Burns et al. (1977) illustrated significant gain in cows and calves grazing on crownvetch as compared to tall fescue irrespective of the season.

Grazing Preference: The grazing preference of cattle for this species was suspected to be season-dependent due to the fluctuations in components such as total phenols (Burns et al., 1977).

PSMs: The presence of CT has been confirmed in this species, and it showed an inverse relationship with IVDMD (Burns and Cope, 1974). Another greenhouse study confirmed the presence of epicatechin and catechin (CT) in this species (Sarkar et al., 1976).

Kura clover (*Trifolium ambiguum* M. Bieb)

Kura clover shows great promise as a persistent, competitive legume under a wide range of environmental conditions. It can tolerate low winter temperatures and recovers quickly after drought (Dear & Zorin, 1985).

Forage value: In a study conducted by Sleugh et al. (2000), among three species: alfalfa, birdsfoot trefoil and kura clover, kura clover exhibited the highest average crude protein (46%) in monoculture. Neutral detergent fiber concentration was lowest in the kura clover monoculture.

PSMs: This species is reported to contain an average of 0.1 and 4.1 $mg \cdot g^{-1}$ CT during the vegetative stage and maturity, respectively (Berard et al., 2011).

Leadplant (*Amorpha canescens* Pursh)

Leadplant is a slow-growing species that is well-grazed by livestock (Kneebone, 1959).

Establishment and persistence: Leadplant showed poor establishment and slow growth in the first year of western Oklahoma rangelands (Kneebone, 1959).

PSMs: This species is reported to accumulate flavonoids, isoflavonoids, and various flavonoid glycosides (Burton et al., 2021).

Purple prairie clover (*Dalea purpurea Vent*)

Purple prairie clover is readily grazed by sheep (Peprah et al., 2022).

Establishment and persistence: Prairie clovers are known for their protracted germination resulting in sparse plant stand density (Mischkolz et al., 2013). Kneebone

(1959), reported poor establishment and slow growth of purple prairie clover in the first year on western Oklahoma rangelands. Another study demonstrated a comparatively lower yearly yield of purple prairie clover (1423 ± 479, 2014 ± 348, and 2297 ± 942 $kg\cdot ha^{-1}$ at vegetative, full-flower, and late-flowering stages, respectively) than the conventional legume forages under dryland conditions (Wang et al., 2019). The yield poses a significant limitation to the use of this nutritionally rich species on rangeland as a bloat-free alternative to alfalfa for late summer and fall (Peprah et al., 2022). Despite these drawbacks, due to its ability to extend the grazing season and enhance forage quality, purple prairie clover is considered a desirable species in pasturelands (Mischkolz et al., 2013).

Forage quality: As a maturity characteristic, the samples collected from native pasture from June to August in Saskatchewan showed a decline in crude protein (13.7 to 8.43%) and increase in acid detergent fiber (ADF, 35.0 to 53.4%) and NDF (43.8 to 54.7%) (Peng et al., 2020).

PSMs: In a dryland study conducted under semi-arid conditions, leaves had a higher concentration of extractable condensed tannin than stems, with whole plant levels increasing from the vegetative stage to the seed pod formation stage (~51 to ~70 $mg\cdot g^{-1}$) (Li et al., 2014). The Terrill et al. (1992) methodology can be used to measure three fractions of condensed tannins: extractable, protein-bound, and fiber-bound, which can then be summed. Since these fractions do not predict in vivo rumen CT function, an alternative is the total CT assay (Grabber et al., 2013). Incorporation of 50% purple prairie clover in diets at full flower stage holds promise for providing exceptional forage quality (depending on animal selectivity) and positively impacting rumen fermentation and digestibility when grazed before the seed pod stage (Peng et al., 2020).

Other: In *in vitro* rumen digestion studies, as the proportion of purple prairie clover in mixtures with cool-season grass increased, the accumulation of ammonia-N production increased, due to its high protein concentration, and was not inhibited by purple prairie clover CT. Higher ammonia-N production was observed when purple prairie clover was at the vegetative stage than at flowering and seed pod stages, indicating a direct association of high protein concentration with ammonia-N production (Peng et al., 2020).

Sainfoin (*Onobrychis viciifolia* Scop.)

Establishment and persistence: Kneebone (1959) demonstrated the excellent establishment and rapid first-year growth of sainfoin on western Oklahoma rangelands. Similar findings were reported in a multi-location study evaluating establishment in northern Colorado, where sainfoin showed good to excellent seedling emergence at all locations (Townsend & McGinnies, 1972).

Forage quality: Sainfoin has superior forage value compared with forages such as alfalfa at a similar growth stage in terms of greater feed intake, and dry matter digestibility, but lower ruminal protein degradability (Wang et al., 2015). However, in a dryland study conducted by Carlton et al., (1968), the protein concentration in sainfoin was 11.9% at 10% bloom stage and 10.3% at 100% bloom stage. Crude protein content in alfalfa was higher than sainfoin at similar stages of maturity. Due to the high percent of non-structural carbohydrates supplied by sainfoin, it has superior forage quality.

PSMs: In western Canada, an average of six varieties resulted in 46 $mg \cdot g^{-1}$ CT (values ranging from 16.3 to 94.4 $mg \cdot g^{-1}$, Berard et al., 2011). The concentration of CT in sainfoin (50 mg·g^{-1}) does not affect palatability and feed intake. Heifers feeding on sainfoin hay showed higher N retention than heifers fed on alfalfa, small burnet and meadow bromegrass and shifted N excretion from urine to feces (Stewart, et al. (2019).

Grazing preference: Lambs previously grazing on sainfoin preferred sainfoin over cicer milkvetch when parasitized, and lambs previously grazing on cicer milkvetch later increased preference for sainfoin when parasitized, likely due to its anthelmintic properties (Villalba et al., 2013).

Utah sweetvetch (*Hedysarum boreale* Nutt.)

Once established, this forage species emerges early in the season, initiating growth at the onset of spring, and offers palatable pasture without any harmful effects (Bassendowski et al., 1989; Johnson et al., 1989).

Establishment and Persistence: There was a decrease in the forage abundance of this species from early spring to late fall and winter, attributed to its prostrate growth during autumn and restricted forage productivity during this season (Johnson et al., 1989).

Forage quality: Utah sweetvetch produces leaves early in spring and the lower leaves remain green throughout winter (Ogle et al., 2003). In the northern Canadian prairies, Bassendowski et al. (1989) reported that leaves contain twice the concentration of crude protein (24.99%) compared to stems (12.87%). Conversely, stems have twice as much ADF (33.81%) and NDF (47.1%) as leaves.

PSMs: Utah sweetvetch had higher tannin concentration than birdsfoot trefoil and alfalfa with comparable protein and fibre fraction. The CT concentration, reported as catechin equivalents, was 126.4 mg·g^{-1} in leaves, which is twice the concentration of tannins in stems (63.2 mg·g^{-1}, Bassendowski et al., 1989).

White prairie clover (*Dalea candida* Michx. ex Willd.)

Establishment and persistence: In a study conducted on western Oklahoma rangeland, this species showed poor establishment and slow growth in the first year (Kneebone, 1959). In an irrigated study this species exhibited 64 to 88% plant survival in different populations after 4 years of establishment (Khanal et al., 2018).

Forage quality: The forage nutritive value of white prairie clover is superior in the bloom stage as compared to post-seed harvest maturity. In an irrigated study, crude protein content ranged from 18.3 ± 0.52% to 14.9 ± 0.59% at the bloom stage, declining to 7.1 ± 0.38% to 6.2 ± 0.35% as the plant matured further (Khanal et al., 2018). Yet, mature white prairie clover stands have the potential to extend the grazing season. Incorporating this species along with grass increased crude protein content in the feed during the fall season and reduced the fiber content during all seasons (Khanal et al., 2018).

PSMs: Extractable condensed tannin concentration of white prairie clover increased from vegetative to maturity stage ($7.64\ \mathrm{mg \cdot g^{-1}}$ to $42.06\ \mathrm{mg \cdot g^{-1}}$). Mainly, CT is present in the inflorescence and seed pod parts (Li et al., 2014).

Non leguminous forbs

Nutrient-rich non-leguminous forb species are also prominent on rangelands in the Western United States. The forage value of non-leguminous forbs is less frequently considered than their value to habitat diversity and wildlife feed, even though these species have the potential to contribute to the diets of cattle on native rangelands. Some forb species (small burnet, Lewis flax) have the potential to extend the grazing period by remaining green during the fall and winter. However, except for small burnet, their use is limited on rangeland due to low dry matter yield and the high cost of seed (Shaw & Monsen, 1983). The forb species considered for inclusion in SFS are described below.

Arrowleaf balsamroot (*Balsamorhiza sagittata* (Pursh) Nutt.)

Establishment and persistence: Arrowleaf balsamroot is the most widespread species of balsamroot. Although the seed germinates and establishes quickly under the right climatic conditions, plants are slow to develop. The slow growth of this species would make it an unsuitable component of resource islands; three to four years are required for arrowleaf balsamroot to reach full production, and establishment may take up to eight years (Ogle et al., 2003).

Forage quality: In a study conducted for four years, crude protein declined from ~30 to 3% from May to November, with a coupled increase in non-structural carbohydrate and crude fiber (Blaisdell et al., 1952).

PSMs: Sesquiterpene lactones are reported in this species (Bohlmann et al., 1985).

Blanketflower (*Gaillardia aristata* Pursh)

Establishment and persistence: Blanketflower establishes readily when seeded in the spring (Winslow, 2011).

PSMs: The presence of sesquiterpene lactones has been reported in this species (Bohlmann et al., 1984).

Fernleaf Biscuitroot (*Lomatium dissectum*)

Renowned for its significant biomass production and towering stature, it is a highly valuable early-season forage option, commencing growth in the early spring (Tilley et al., 2012).

Establishment and persistence: There is slow above-ground seedling growth because the priority is the development of a substantial taproot, and thus an entire year is required for initial establishment of this species. Plants grow from early spring to summer, entering dormancy in mid-summer, which may give the appearance of mortality. In their first year, most plants only produce a few leaves and will not produce flowers or fruit during the first three to four years of growth. It should be planted in late fall as a dormant seeding to allow for the natural stratification of the seed over winter (Tilley, 2012).

Forage quality: No study was found in the literature on this specific species, however, in other *Lomatium* sp., a decline in crude protein content from 9 to 3.5% and change in ADF and NDF from 31.1 and 52.2 to 32.2 and 54.4%, respectively has been reported with transition of season from spring to fall (Wagoner, 2011).

PSMs: Chaurasia (2022) documented the presence of flavonoids, saponins, terpenoids, and steroids in this species.

Lewis's flax (*Linum lewisii* Pursh), 'Maple Grove'

Lewis flax is considered a semi-evergreen forb capable of providing year-round forage (Monsen et al., 2004).

Establishment and persistence: Seedling vigor is good, but not comparable to most grasses. Germination typically occurs in the first growing season but could be delayed until the subsequent growing season, and full flowering cannot be anticipated sooner than the second growing season (Ogle et al., 2002a). Lewis flax establishes well and exhibits a moderate growth rate even under arid conditions. Increasing the number of seeds of this species can potentially enhance the percentage of plants that survive and establish (Monsen et al., 2004).

Forage quality: Lewis flax is used as an early season forage since it initiates growth early in the season and provides fair forage value for livestock and wildlife during spring and winter (Ogle et al., 2002a).

PSMs: This species is reported to contain lignans (1.6 to 3 $mg \cdot g^{-1}$) (Konuklugil et al., 2007).

Maximillian sunflower (*Helianthus maximiliani* Schrad.)

Maximillian sunflower provides palatable forage and remains green until late fall. However, it loses flavour later in the season (Wennerberg, 2004).

Establishment and persistence: Maximilian sunflower exhibited a sparse density of ≤1.0 plant per square meter when planted with an established stand of big bluestem/Indiangrass as part of a blend of 11 native species (Richwine et al., 2024).

Forage quality: Myer (2022) reported transition from 22 to 16%, 21 to 24%, and 21 to 25% in crude protein, ADF, and NDF concentration, respectively, from June to September.

PSMs: The presence of sesquiterpene lactones in this species has been confirmed by Gershenzon and Mabry (1984).

Penstemon species (*Penstemon palmeri* A. Gray and *Penstemon strictus* Benth.)

'Cedar' Palmer’s penstemon (*Penstemon palmeri*) and 'Bandera' Rocky Mountain penstemon (*Penstemon strictus*) are the only released penstemons noted to have any forage value (Ogle et al., 2013).

Establishment and Persistence: *Penstemon palmeri* requires two to three years to establish, mature and flower and is also considered semi-evergreen, providing year-round forage, while *Penstemon strictus* demonstrates early spring growth (Monsen et al., 2004; Shaw & Monsen, 1983).

Forage quality: *Penstemon sp.* have been reported to contain 41.01 ± 2.56% and 24.74 ± 1.45% NDF and ADF, respectively in Montana (Bhattacharyya & Ray, 2015).

PSM: Penstemons are short to long-lived perennial species reported to contain CT in a concentration of 60.63 ± 6.09 mg quebracho CT eq·g^{-1} (Bhattacharyya & Ray, 2015).

Prairie coneflower (*Ratibida columnifera* (Nutt.) Wooton & Standl.)

Prairie coneflower is a perennial native forb and provides palatable forage during the early grazing season (Carr, 2009).

Forage quality: In a dryland study, this species showed a transition in ADF, NDF, and crude protein from 28 to 35%, 29 to 40%, and 13 to 17%, respectively, from June to September (Myer, 2022).

Prairie aster (*Machaeranthera tanacetifolia* (Kunth) Nees)

This species exhibits rapid growth rate and medium seedling vigor (USDA NRCS Plant database).

Showy Goldeneye (*Heliomeris multiflora* Nutt.)

Establishment and persistence: Showy goldeneye demonstrates vigorous seedling development and requires two to three years for establishment. It develops later in the season and can compete well with other species (Monsen et al., 2004). Despite its ability to occur at elevations ranging from 914 to 3,597 meters (Stevens & Monsen, 2004), a study conducted by Hull (1974) demonstrated the inability of this species to become established at two locations in Utah, namely Monte Cristo and LaBarge, attributable to other climatic and edaphic constraints.

Forage quality: Showy goldeneye is a highly palatable species, promptly consumed by ruminants, and can provide succulent forage from April to October (Monsen, 2004).

PSMs: Buschmann & Spring (1997) have demonstrated the presence of sesquiterpene lactones in this species.

Small burnet (*Sanguisorba minor* Scop.)

Small burnet has good palatability and is a crucial species for early spring, late summer, and late winter. However, selective grazing can reduce the plant stand and plant vigour during these periods (Shaw & Monsen, 1983).

Establishment and persistence: Rapid germination and seedling development generally facilitate successful establishment through spring seedings, resulting in good ground cover within one to two years, attributed to fast growth after germination. It establishes well at dry sites but requires adequate spring moisture (10 inches/254 mm). The plants demonstrate high persistence, maintaining high density for up to 25 years (Monsen et al., 2004).

Forage quality: Small burnet is a pivotal forage in late winter, early spring, and late summer when other species supply limited green forage (Monsen et al., 2004). In an irrigated study, Elgersma et al. (2014) reported 29.5, 24.0 and 13.2% NDF, ADF, and crude protein, respectively, in this species. The maturity of plants has a significant effect on the chemical composition of the plant. An increase in crude protein content from seedling to pre-flowering followed by a decline in these components from pre-flowering to flowering (6.7%, 20.7%, and 13.7%) has been observed. Both ADF (36.2%, 17.4%, 29.4%) and NDF (54.5%, 36.2%, 49.2%) follow the reverse trend during the same stages showing the general trend of an increase in less digestible cell contents (NDF and ADF) coupled with a decrease in crude protein at maturity. Further, in an *in vitro study,* a decrease in the fermentable fraction of plants with maturity due to increased cell wall content caused reduction in gas and methane production (Kaplan et al., 2014).

PSMs: There is evidence of the presence of CT in this species, which increases from the seedling to the pre-flowering stage, followed by a decline from pre-flowering to flowering (4 mg·g^{-1}, 16 mg·g^{-1}, and 9 mg·g^{-1}, Kaplan et al., 2014). Kapp-Bitter et al. (2023) demonstrated the potential of this species to reduce urinary N excretion by 30% when included in the diet at a proportion of 80 mg·g^{-1}. Another study by Stewart et al. (2019) found less methane emission when small burnet hay containing 45 mg·g^{-1} HT) was the diet of heifers compared with other hay treatments: sainfoin, cicer milkvetch, birdsfoot trefoil, alfalfa, and meadow bromegrass. Furthermore, *in vivo* results showed a significant impact of this species on shifting the nitrogen excretion from urine to feces in cows and heifers (Stewart et al., 2019).

Smooth blue aster (*Symphyotrichum laeve* (L.) Á. Löve & D. Löve)

Smooth blue aster provides palatable and nutritious forage to white-tailed deer (Ogle et al., 2003; Wennerberg, 2004a).

Establishment and Persistence: It is one of the first species to green up with the onset of spring (Ogle et al., 2003).

Forage quality: Samples collected from Saskatchewan exhibited a decline in crude protein content from May to October (20 to 6%), an increase in NDF (19 to 43%) and ADF (~13 to 30%). With the progression of the season, this species was found to exceed the minimum requirement of 7% crude protein until September (~9 %) declining to 7% in

October (Vera-Velez & Lamb, 2022). Additionally, the low fiber content in this species makes it a promising candidate for inclusion in resource islands.

Western yarrow (*Achillea millefolium* L. var. occidentalis DC)

Establishment and persistence: Western yarrow establishes and proliferates effectively in sites occupied by annual weeds. If not grazed for a minimum of two years, it persists under heavy grazing and usually recovers well by natural seeding (Monsen et al., 2004).

Forage quality: As with other the species, there was a decline in crude protein concentration with maturity of the plant from May to October from ~13 to 4 % and an increase in NDF and ADF from 31 to 38% and 22 to 39%, respectively (Vera-Velez & Lamb, 2022) indicating limited ability to extend the grazing period as a nutritious feed for ruminants.

PSMs: There is evidence for the presence of a diversity of PSM in this species including sesquiterpenes, sesquiterpene lactones, and flavonoids including flavonoids, flavonols and flavones (Lim, 2014).

Grazing preference: According to Winslow (2011), domestic sheep and goats obtain significant forage value from western yarrow, while cattle and horses predominantly graze only on the flower heads. It exhibits relatively high digestibility (45.0% IVDMD) during late summer (Darambazar et al., 2013). Even so, in a study by Dwyer et al. (1964), it was left completely ungrazed, when the preference of steers for various species was evaluated.

White (Louisiana) sagebrush (*Artemisia ludoviciana* Nutt.)

White sagebrush is generally considered a poor forage (Shaw and Monsen, 1983), probably due to its pungent taste in more northern areas, and it is typically not grazed until after the first frost. Its maturation period spans three years (Ogle et al., 2003).

PSMs: In an irrigated study, the leaves of this species had total phenolic compounds in a concentration of 0.36 g gallic acid equivalent (GAE)·100 g^{-1} of dry matter out of which 0.10 g GAE·100 g^{-1} of dry matter are flavanols such as quercetin, myricetin and kaempherol (Carvalho et al., 2011).

Grass

Crested Wheatgrass (*Agropyron cristatum* (L.) Gaertn)

This long-lived, cool season introduced grass species is extensively used on pasturelands throughout the U.S. due to its relative ease of establishment, persistence, and palatability to a myriad of wildlife and livestock (Ogle, 2002).

Forage quality

Protein supplements are required along with this species to meet the protein requirements of ruminants (Ogle, 2002). In a study conducted over six years, the average NDF, ADF, and crude protein values were 60.2, 34.2, and 5.3%, respectively (Biligetu et al., 2014). It showed rapid growth and attained maturity earlier than the other cool-season, adapted grasses included in the study, and was high in quality in early spring, but the quality plummeted much more rapidly in comparison to other cool-season perennial grass species, necessitating the incorporation of nutrient-rich species on rangeland (Hart et al., 1983). Rumbaugh et al. (1982) reported a decline in crude protein content of crested wheatgrass from 10.9 to 3.3% from May to August. Inclusion of legumes such as cicer milkvetch and alfalfa augmented the forage yield and protein content in crested wheatgrass monocultures (Rumbaugh et al., 1982).

Conclusions

Extensive rangeland restoration plantings routinely include forbs, but there are advantages of augmenting traditional grass-dominated forage stands with concentrated stands of legume and non-legume broadleaf species that can be managed to enable ruminants to choose a more varied and higher-protein diet while reducing the environmental impact of livestock production (Distel et al., 2020). The development of resource islands would shift grass-dominated rangelands toward greater diversity and harness the benefits of bioactive secondary metabolites for ruminant production and environmental health. A comprehensive understanding of diverse forage species and their nutritional profiles from small-plot studies will augment the evidence collected from rangeland restoration studies and help to provide a nutritionally balanced diet to cowherds grazing rangeland. For this, an extensive assessment of the establishment, persistence, changes in forage quality and metabolic profiles of plant species are required. This evaluation of multifunctional forage species will help in strategically selecting the species capable of supporting environmental and economically sustainable beef production by matching forage production and quality with cattle requirements and mitigating methane emissions.

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Table 1.1

Agronomic traits and planting locations of prospective candidates for Smart foodscapes

Common name	**Flowering time**	**Planting time**	**Seeds per pound***	**Palatability for grazing animal***	**Planting locations**
		Introduced Legumes			
Alfalfa, purple flower^{L}	Spring	Spring	225,000	High	CGCCP^{+}
Alfalfa, yellow flower^{M}	Spring	Spring to early summer	225,000	_	
Birdsfoot trefoil^{M}	Early spring	Early spring or late summer	370,000	High	CGCCP
Cicer milkvetch^{L}	June- August	_	145,000	Medium	CGCCP
Kura Clover^{M}	Spring	_	152,212	High	P
Sainfoin^{M}	Early spring	Spring or fall	19,000	High	CGCCP
		Introduced Forbs			
Small burnet^{L}	May to October	Late fall to very early spring	55,000	High	CGCCP
		Introduced Grass			
Crested wheatgrass^{L}	Early spring	Early spring to late fall	200,000	Medium	CGCCP
		Native Legumes			
Utah sweetvetch^{ML}	Late spring	Early spring or late fall	33,600	High	CGCCP
Canadian milkvetch^{S}	June – July	_	3,00,000	Low	CGCC
Crownvetch^{L}	Late spring	Spring	110,000	Medium	CGCCP
Leadplant^{L}	Early summer	Spring or fall	123,000	Low	GCC
		Native Forbs			
Arrowleaf balsamroot^{L}	Late spring	Late fall	58,000	Medium	CCP
Blanketflower^{S}	Mid-summer	Early spring or fall	220,700	Low	CGCCP
Fernleaf biscuitroot^{S}	Early summer	Late fall	45,000	High	CGCCP
Lewis flax^{S}	Mid-May to early July	Late fall to very early spring	170,000	High	CGCCP
Maximillian sunflower^{M}	Late summer	Spring	1,96,360	Medium	CGCC
Prairie aster	_	_	_	_	CGCCP
Palmer's penstemon^{M}	Late spring to early summer	Late fall or early winter	600,000	Low	CGCCP
Prairie coneflower^{L}	Mid-summer	Early spring or fall	600,000	Medium	CGCCP

Purple prairie clover^{M}	Summer	Fall	290,000	Medium	CGCCP
Rocky Mountain penstemon^{L}	Late spring	Very early spring	285,000	Low	CGCCP
Showy goldeneye^{L}	Mid-summer	October-November	10,00,000	_	CGCCP
Smooth blue aster^{L}	Mid-spring to mid-summer	Sep. to Nov. or April to May	880,000	Low	CGCCP
Western yarrow^{M}	May- July	Early spring	2,800,811	Low	CGCCP
White prairie clover^{M}	_	Early spring	374,000	High	CGCCP
White (Lousiana) sagebrush^{S}	Early spring	Spring to early summer	2,495,000	Medium	CGCC

— Information Unavailable, ^{s}Short longevity, ^{M}Medium longevity, ^{L}Long longevity, ^{ML}Medium to long longevity,

*Source: USDA NRCS Plant Database

CEGCCP^{+} : Clarkston, Ephraim, Greenville, Cedar City, Panguitch

Table 1.2

Secondary metabolites in prospective candidates for SFS

Species	Secondary metabolites	Reference
Alfalfa, purple flower	Saponins	(Nowacka and Oleszek, 1994)
Alfalfa, yellow flower	Saponins	(Nowacka and Oleszek, 1994)
Arrowleaf balsamroot	Sesquiterpene lactones	(Bohlmann et al., 1985)
Birdsfoot trefoil	Condensed tannins	(Hymes-Fecht et al., 2013)
Blanketflower	Sesquiterpene lactones	(Bohlmann et al., 1984)
Canadian milkvetch	Condensed tannins	(Davis, 1982)
Cicer milkvetch	Isoflavonoids, Condensed tannins, Saponins	(Butkutė et al., 2018)
Crested wheatgrass	__*	__
Crownvetch	Condensed tannins	(Burns and Cope, 1974)
Fernleaf biscuitroot	Flavonoids, Saponins, Terpenoids, and Steroids	(Chaurasia, 2022)
Leadplant	Flavonoids, Isoflavonoids, and Flavonoid glycosides	(Burton et al., 2021)
Lewis flax	Lignans	(Konuklugil et al., 2007)
Maximillian sunflower	Sesquiterpene lactones	(Gershenzon and Mabry, 1984)
Palmer's penstemon	Condensed tannins	(Bhattacharyya and Ray, 2015)
Prairie aster	__	__
Prairie coneflower	__	__
Purple prairie clover	Condensed tannins	(Li et al., 2014)
Rocky Mtn. penstemon	Condensed tannins	(Bhattacharyya and Ray, 2015)
Sainfoin	Condensed tannins	(Berard et al., 2011)
Showy goldeneye	Sesquiterpene lactones	(Buschmann and Spring, 1997)
Small burnet	Hydrolysable tannins	(Finimundy et al., 2020)
Smooth blue aster	__	__
Utah sweetvetch	Condensed tannins	(Bassendowski et al., 1989)
Western yarrow	Flavonoids: Flavonol and Flavone	(Lim, 2014)
White prairie clover	Condensed tannins	(Li et al., 2014)
White sagebrush	Flavonol	(Carvalho et al., 2011)

–*Information unavailable

CHAPTER II

EFFECT OF COLD STRATIFICATION ON SEED GERMINATION IN FIVE NATIVE FORAGE SPECIES

Introduction

Seed germination is an important stage in the plant's lifecycle through its influence on plant establishment. For dormant species, a comprehensive understanding of the requirements for breaking seed dormancy and inducing germination is required for the successful establishment of a plant stand. Seed dormancy is the physiological state of temporary absence of germination under favorable environmental conditions which can be released by natural or artificial mechanisms, stimulating germination (Simpson, 1990). A seed germinates when it perceives an appropriate set of environmental conditions within its range of requirements (Vleeshouwers et al., 1995). There are gaps in the understanding of dormancy and germination requirements of species capable of enhancing the nutritional richness of rangelands; this knowledge deficit may impede their use on rangeland. Failure to germinate could be due to a mismatch between the planting date and favorable environmental conditions for breaking dormancy.

Cold stratification plays a critical role in breaking seed dormancy for many species native to challenging temperate environments, such as semi-arid rangelands. This process emulates the natural encounter of seed with winter conditions, exposing imbibed seed to a period of cold temperatures, which is crucial for breaking dormancy and triggering germination in many native species. In addition, there exist variabilities in the cold stratification requirement of species highlighting their adaptation to various environmental conditions (Kitchen, 2001). Therefore, prior to planting dormant seed, it is crucial to determine the specific conditions required for breaking dormancy and inducing germination. The insights gained from seed germination studies can be used to ensure that an adequate number of seeds have been planted, based on germination percentage. It also informs optimum planting time where cold stratification is required for seed germination (Jones & Kaye, 2014). Additional pre-treatments can also be performed if required, e.g., scarification in the case of physical dormancy.

This study was conducted to evaluate the effect of cold stratification on seed germination of five native forage species. Other species used in the Smart Foodscapes small plot herbaceous plant species study germinated readily at room temperature, or with scarification. We hypothesized that cold stratification of the five dormant plant species would significantly improve their germination, except for Utah sweetvetch, which exhibits only physical dormancy (hard seededness, Redente et al., 1982).

Materials and Methods

A germination test was conducted on five species (Table 2.1) under *in vitro* conditions for 60 days; two species were studied for 92 days. One replication consisted of 24 seeds in a 10-mm petri dish. Four replications of 24 seeds were subjected to chilling treatment, in which sterilized and imbibed seeds were stored at 3-4 °C in the dark in a forced-air refrigerator. Additionally, seed germination was tested for one replication of 24 seeds under control conditions, where seeds were kept in a dark cupboard at room temperature (20 °C). Seeds were obtained from Bruce Seed Farm Inc. (Townsend, MT). Germination test results recorded on seed labels are included in Table 2.1.

Before starting the study, hard seeds were scarified for 10 sec. using an electric seed scarifier (Forsbergs, Inc., Thief River Falls, MN). All seeds were sterilized before initiating the germination study using 1% sodium hypochlorite (Perry et al., 2005) by manually agitating the seed by hand in the solution for 10 minutes followed by rinsing three-four times with deionized water. Twenty-four sterilized seeds were placed on top of one layer of 90-mm-diameter blotter paper in 100-mm petri dishes. Blotting papers were pre-treated with 4-5 drops of Apron fungicide using a 0.67% w/v aqueous solution. Seeds were placed apart from each other to reduce the spread of mold in petri dishes. Blotting papers were moistened to saturation with distilled deionized water and the petri dish was closed to minimize evaporation. Stacks of petri dishes of the same species were kept in plastic zip-top bags comprising each of four replications to minimize moisture loss. A blank dish (blotting paper but no seed) was placed on top of each stack to ensure uniform side illumination for all seeds (Meyer and Kitchen, 1992). Germination was recorded for the date when a radicle emerged ≥ 1 mm in length (Bujak, 2015). Cumulative germination was counted every day for the first week, every other day for the second week, and once a week for the rest of the study period. Germinated or moldy seeds were removed and discarded. Since, for most species, the seeds started molding after two months, germination percentages were calculated at 60 days as the proportion of the total number of seeds that had germinated for each experimental unit (petri dish). However, the non-moldy seeds of fernleaf biscuitroot and arrowleaf balsamroot were kept under cold treatment for an additional 32 days to observe trends in the germination of these species.

Statistical analysis

Statistical analysis was performed on cumulative weekly seed germination under cold treatment using the PROC GLIMMIX procedure of SAS studio (version 3.8; 2024).

LSMeans were compared pairwise using the Tukey-Kramer method, adjusting for multiplicity with a significance level specified at 0.05. PROC PLM was used to perform the post-hoc analysis. It sliced the LSMeans of cumulative seed germination from 0 to 60 days for each species. Control germination is included in graphs for comparison with mean cumulative weekly germination under cold treatment.

Results

Germination under cold conditions differed from the room-temperature control for each species. No germination was observed under control conditions for arrowleaf balsamroot or fernleaf biscuitroot. For the other species, stratification delays germination relative to control.

Utah sweetvetch (scarified): Control seed germinated between days 4 and 8 while most cold-treated seed germinated between days 8 and 16. Under cold treatment, seeds started germinating from day 8 with 5.21% germination and continued at the same rate through day 16. From day 16 (69.79%) to day 40 (76.04%), germination of stratified seed continued but at a lower rate, while germination of control seeds had reached a maximum of about 70.82% in 12 days remaining constant afterward. In cold treatment, an average germination of 79.17% was achieved in 44 days after the onset of the trial, while maximum germination (82.29%) was achieved in 60 days. The control group reached maximum germination of 70.83% on day 12, (Fig. 2.1).

Lewis flax (scarified): Cold treatment improved germination relative to the control in this species. The cold-treated seed started germinating on day 20 with 31% germination and reached an average of 80.2% in 56 days, showing no further germination. In the control group, seeds started germinating from day 8, achieving a maximum of 66.66%, with no further germination thereafter (Fig. 2.1).

Showy goldeneye: Germination of showy goldeneye began on day 8 for both the cold treatment and the control, but the germination of cold-treated seeds was slowed. Ultimately, the same total germination was reached by both treatments. The cold-treated seed exhibited 6.25% germination on day 8 and reached the maximum of 44.79% in 56 days. Control-treated seeds exhibited 33.33% germination on day 8 and reached 45.83% on day 12, with no further germination throughout 60 days (Fig. 2.1).

Arrowleaf Balsamroot: Seeds started germinating on day 44, exhibiting 4.17% initial germination, and reaching a maximum of 9.38% in 60 days. The non-moldy seeds of this species were allowed to continue germination and an average of 67.71% was reached in 92 days. No germination was observed under control conditions (Fig 2.1).

Fernleaf Biscuitroot: No germination was observed until 36 days under cold treatment and only 26.04% of seed germinated by 60 days. The non-moldy seeds were allowed to continue germination, and an average germination of 61.4% was reached by 92 days. No seed germination was observed under the control treatment (Fig 2.1).

Discussion

Germination plays an important role in determining species establishment on rangeland. Seed dormancy inhibits germination even under favorable conditions of soil moisture and temperature if a germinated seedling is unlikely to survive and reproduce (Vleeshouwers et al., 1995). For arrowleaf balsamroot and fernleaf biscuitroot, fall dormancy that results in germination following months of cold, moist conditions result in seeds germinating in soil containing moisture from snowmelt, which has become an environment for establishment. In the field, fall planting synchronizes planting with environmental conditions that help break dormancy, a period of natural stratification before warm temperatures in spring. We compared the germination of five native forage species under cold conditions to determine the impact of cold temperature on germination compared with room-temperature control. Species other than showy goldeneye exhibited higher germination under cold conditions, while showy goldeneye exhibited slower but ultimately comparable germination under stratification.

Under the control (room temperature) treatment, the seed of Utah sweetvetch, showy goldeneye, and Lewis flax germinated within the first 8 days and reached maximum germination by day 12. In a previous study by Redente (1982), the seed of Utah sweetvetch germinated across a wide range of temperatures from 5º to 30º C, with rapid germination at 20 ºC. Similarly, we observed rapid germination under control conditions of 20 ºC for this species, with no significant increase in germination under cold stratification. The rapid germination of the control treatment indicates that most seeds had no physiological dormancy, although the gradual additional germination in cold temperatures indicates that some seeds of Utah sweetvetch and Lewis flax had physiological dormancy that was overcome by stratification (Meyer & Kitchen, 1992).

According to Gucker et al. (2018), showy goldeneye exhibits intra-specific variability in dormancy and germination behavior, with some populations responding positively to stratification, while others may not benefit from cold treatment. This is due to environmental factors including seed source, which may strongly influence the germination requirements for showy goldeneye.

The delayed yet higher germination of Lewis flax under cold treatment aligns with the observations of Meyer & Kitchen (1994), where 5 weeks of cold treatment induced primary dormancy in seed that was reversed in the prolonged cold treatment of 24 weeks and resulted in enhanced germination percentage. Hence, Lewis flax interacts with the environment, waiting out cold periods and ultimately achieving higher germination (Babb, 1959).

Under natural winter conditions, arrowleaf balsamroot seeds would experience freezing as well as periods of warming, so a steady temperature of 4 °C in the dark did not replicate natural conditions. A study by Young & Evans (1979) demonstrated the important role of cold temperature in breaking seed dormancy and inducing germination in the seed of arrowleaf balsamroot. In this study, maximum germination reached 50-55% and 45-50% at 5 °C and 2 °C, respectively, after an extended cold period of 12 weeks. The germination increased up to 87% after incubating the seeds at 10 °C following stratification. The germination response of fernleaf biscuitroot is consistent with the findings of Scholten et al. (2009) who reported optimal germination occurred at a temperature of 3-4 °C. This is attributed to the large seed, dispersed with immature embryos that require cold treatment to complete development. The natural physiological immaturity of harvested seed can be addressed by fall planting, resulting in germination when environmental conditions are favorable.

Conclusion

Cold stratification had varying influence on seed germination highlighting varying requirements among species. Compared to control conditions, germination increased by around 14.5% in Utah sweetvetch and 18.4% in Lewis flax under cold treatment. In contrast, showy goldeneye germinated more rapidly under control conditions but ultimately achieved comparable germination percentages under cold treatment, indicating cold exposure delayed but did not prevent germination. Fernleaf biscuitroot and arrowleaf balsamroot exhibited strong physiological dormancy, requiring longer than 60 days of cold stratification period to achieve maximum germination. For these two species, no germination occurred under control conditions, indicating their dependence on prolonged cold exposure, consistent with fall planting on rangeland were seeds experience months of cold temperature before germination.

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Table 2.1

Description of species evaluated for effect of cold stratification

Species	Scientific names	Legume/Forb	Germination tests[*]		Pure seed (%)
			Germination (%)	Tetrazolium (%)	
Arrowleaf balsamroot	*Balsamorhiza sagittate*	Forb	_[**]	94	99.96
Fernleaf biscuitroot	*Lomatium dissectum*	Forb	_	68	97.07
Lewis flax	*Linum lewisii*	Forb	71	21	94.87
Showy goldeneye	*Heliomeris multiflora*	Forb	_	83	79.67
Utah sweetvetch	*Hedysarum boreale*	Legume	_	95	99.9

[*] The germination % for each species was provided by Bruce Seed Farm Inc. based on germination or tetrazolium tests for each species.

[**] Seed germination was determined only by tetrazolium test.

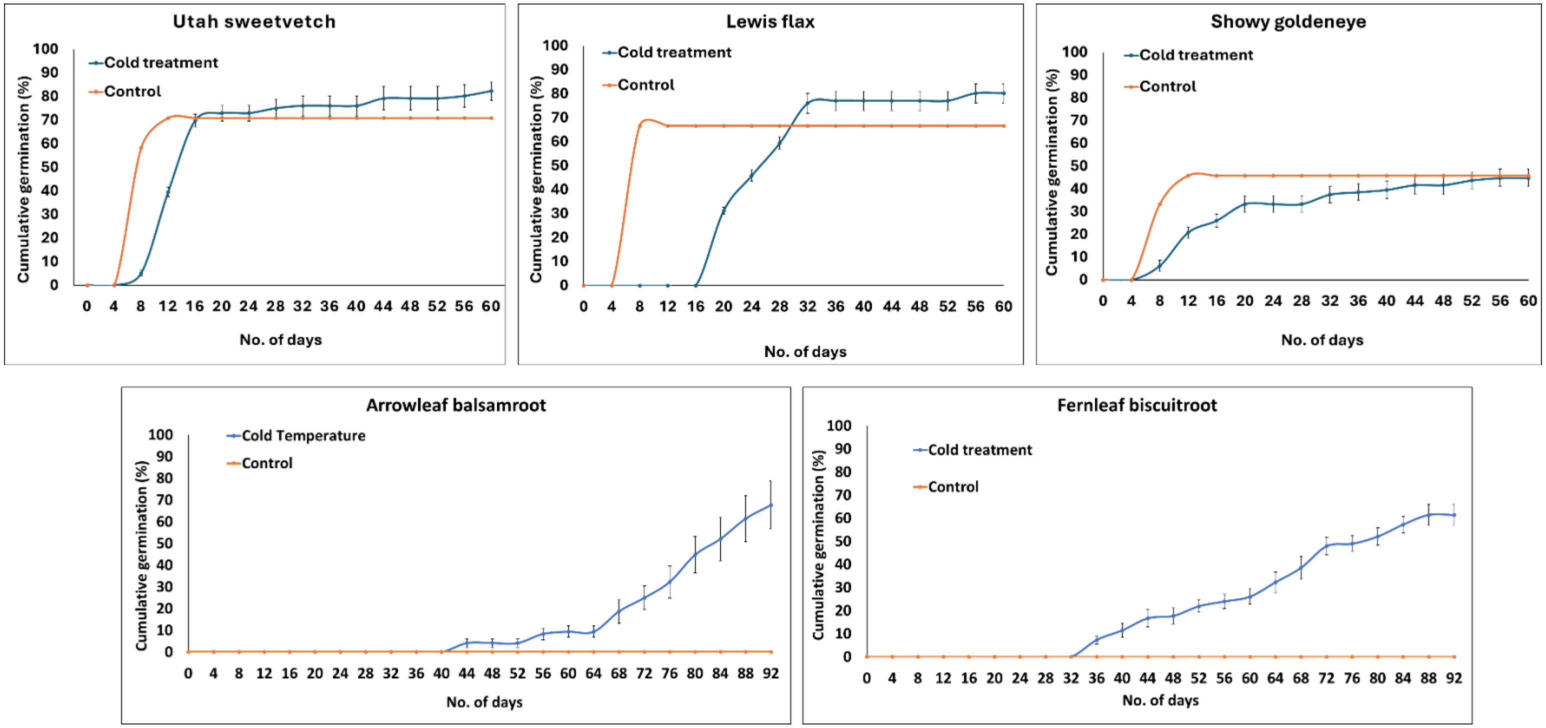

Figure 2.1. Comparison of LSMeans for cumulative seed germination in species under cold treatment vs. control (room temperature). Error bars indicate standard error.

CHAPTER III

EVALUATION OF 27 FORAGE SPECIES FOR ESTABLISHMENT AT FIVE LOCATIONS ACROSS UTAH

Introduction

Grazing lands in the United States encompass approximately 588 million acres, with rangelands consisting of around 404 million acres (Natural Resources Conservation Service, National Resources Inventory, 2017). Within this total, the Rocky Mountain West comprises 69% of the nation's rangeland (Mitchell, 2000). These rangelands are historically dominated by grass monocultures, which are highly resilient under the semi-arid conditions of these rangelands and exhibit relatively high dry matter yield under these conditions (Robins et al., 2020). It has been recognized that the incorporation of legumes and forbs into rangelands can enhance forage production and improve animal performance. Adaptability and ease of establishment are of utmost importance when selecting a species for rangeland grazing. However, relatively few forb species can persist under rangeland conditions, much less thrive with the reliability of adapted grasses. Research on rangeland forbs has documented the use of native forb species by grazing and browsing ruminants, and the value of these plants to wildlife and the ecosystem (Shaw & Monson, 1983).

A goal of the Smart Foodscapes project is to identify both native and introduced plant species that can be successfully established at relatively high densities at strategic sites to serve as supplemental nutrition stations termed "resource islands" for use by cowherds that commonly consume a diet of mature grass during late summer and early autumn. This approach to the use of legumes and other forbs is in contrast to the extensive seeding of these forage plant species at rates reflective of the occurrence of native forbs across grass-dominated rangelands.

Given the benefits of biological nitrogen fixation and the nutritive value of legumes, which routinely exceed that of grasses at the same stages of growth, perennial legumes that can become established and persist on semi-arid rangeland are of particular interest (Kneebone, 1959; Peel et al., 2011). There are also non-legume forbs that we will refer to simply as "forbs" in this chapter, that are persistent and nutrient-dense and would also enrich the diets of cowherds. A further benefit of many legumes and forbs is the production of secondary metabolites that are beneficial for ruminants by improving nitrogen retention, reducing methane emissions, or reducing parasite loads. Secondary metabolites and other substances accumulated by some legumes or forbs can be harmful, but only plants that accumulate secondary metabolites with the potential to benefit ruminants, the environment or both are of interest to this project.

The overall goal of this study was to identify beneficial plant species with successful establishment that can be further evaluated for forage quality and beneficial secondary metabolites with the aim of incorporating them into Smart Foodscapes resource islands to improve the performance of cowherds grazing rangeland ecosystems. Since altitude, aspect, dry summers, and highly variable winter precipitation are typical of Western rangeland environments, our approach was to screen a large number of legumes and forbs at multiple locations to identify the most reliable and valuable candidates for resource islands.

Previous studies, such as Wilton et al. (1978), have documented the poor establishment of native legumes and the high adaptability of introduced species including alfalfa, cicer milkvetch, and sainfoin that outperform native species under dryland conditions. However, the current literature lacks replicated studies that document the establishment, dry matter production, and nutritive value of a wide range of native and introduced legumes and forbs on semi-arid rangelands. To address this perceived gap in the literature, we screened 27 species including one grass (crested wheatgrass), nine introduced, and 17 native legume and forb species for establishment and persistence at five different locations across Utah (Table 3.1). Due to constraints on land area at university farms, it was only possible to include the grass and 20 of the legumes and forbs at all locations. This study provides novel insights into the establishment, persistence, productivity, nutritive value, and secondary metabolites of candidate plant species for use in resource islands in support of sustainable ruminant production on rangeland.

The legumes and forbs were selected for their lack of toxicity, late summer foliage quality, beneficial secondary metabolites as well as their nutritive value but with little information on their ability to establish, be productive, and persist in relatively dense stands (Table 3.1). We hypothesized that alfalfa, sainfoin, crested wheatgrass, small burnet, and birdsfoot trefoil would demonstrate high plant density across all locations based on reports in the literature (Rumbaugh, 1983; Wilton, 1978). Our objective was to assess the establishment, productivity, and persistence as well as nutritive value of species adapted to semi-arid rangelands.

Materials and Methods

This research was conducted at five locations across Utah including Greenville Farm (North Logan), Godfrey Dryland Experimental Farm (Clarkston), Snow College land at Ephraim, Southern Utah University land in Cedar City, and the USU Research Farm at Panguitch. See Table 3.2 for information on location, elevation, and soil type.

Plant species selection

Plant species were selected for inclusion based primarily on their value to grazing ruminants. The productivity of a few of these species have been studied on rangeland, but there is very limited information in the literature on the ability of most to become established in dense stands, as for seed production, on the quality of their foliage in late summer, or on the quantification of their secondary metabolites. There is scarce information in the literature on other characteristics such as the rate of germination and establishment, productivity, and forage quality, although we have studied some of the species including birdsfoot trefoil, sainfoin and small burnet. We chose perennial species because it would be challenging for ranchers to replant multiple annual species in resource islands every year, and because of the potential for perennial resource islands to serve as habitat for pollinators, birds, and other wildlife. Further, resource islands could serve as a source of legume and forb seed distribution and dispersal across the wider landscape dominated by grass species.

Soil amendment

Before planting, soil samples from each location were tested. The samples from the Ephraim and Greenville research sites were tested at the USU Analytical Lab (Logan, UT), and samples from the Cedar City site were analyzed by Ward Laboratories (Kearney, NE). Soil testing at Clarkston and Panguitch was conducted by USU extension. Pre-plant fertilization was provided at each site to address nutrient deficiencies based on soil-test recommendations. At the USU Greenville Research Farm, 23 $kg \cdot ha^{-1}$ Fe, 5.6

kg·ha^{-1} Zn, and 22.4 kg·ha^{-1} S was applied. The Clarkston research farm, which had previously been used for small grain production, received 42 kg·ha^{-1} P_2O_5, 20 kg·ha^{-1} N, 25 kg ha^{-1} S, and 13 kg·ha^{-1} Fe. The Ephraim site received 11 kg·ha^{-1} Zn, 11 kg·ha^{-1} Fe, and kg·ha^{-1} S. No nutrient deficiencies were identified by soil tests at the Cedar City site which had recently been used for irrigated alfalfa production. The soil at the Panguitch research farm was brought to optimum nutrient levels before planting by adding 34 kg·ha^{-1} Fe, 76 kg·ha^{-1} S, and 4.5 kg·ha^{-1} Zn. In addition, 67.25 kg·ha^{-1} of mycorrhizal inoculant (Basin and High Plains Suite, Reforestation Technologies International, Gilroy, CA, USA) providing approximately 1,500,000 living propagules ha^{-1} was applied at all five locations. Legume seeds were inoculated with required rhizobium strains (Exceed Inoculant; Visjon Biologics, Henrietta, TX) before sowing to ensure adequate nitrogen supply.

Experimental design

At Cedar City, Greenville, and Panguitch, plots received irrigation immediately following planting to support germination, on the advice of our project's advisory committee. No irrigation was available at the Clarkston location, and none was applied at Ephraim. In Cedar City, approximately half of the plot area was also irrigated during the two-year-long study; the amount of irrigation is noted in Table 3.6. This occurred because the adjacent studies required irrigation in both 2023 and 2024. No. of replications per species receiving irrigation are mentioned in Table 3.7. At all other locations, no irrigation was applied during the rest of the study.

The study at Cedar City was planted as a randomized complete block design with three replications for all species except for the three species planted in replication of four

(Table 3.7), and plots were 3 × 40 ft. (0.91 × 12.2 m). The other four locations employed a randomized complete block design replicated four times at Greenville, Ephraim, and Panguitch, and five times at Clarkston. Plot sizes were selected to accommodate the maximum number of species on the land area available, and 20 of the 27 species were evaluated at all sites. Plot sizes were as follows: Greenville, 6 × 31 ft. (1.83 × 9.45 m), Ephraim, 6 × 100 ft. (1.83 × 30.48 m), Panguitch, 8 × 34 ft., (2.44 × 10.36 m) and Clarkston 8 × 95 ft. (2.44 × 28.96 m). Planting at Clarkston was carried out in November of 2021, and this site was replanted in early May 2022 following unsuccessful emergence. Ephraim was planted in mid-August of 2022, and Greenville and Cedar City were planted in mid-September of 2022. Panguitch, at 2000 m, was planted in mid-August of 2023 to complement sites at 1400 m (Clarkston and Greenville) and 1700 m (Ephraim and Cedar City) (Table 3.3 & 3.4).

A 120 × 200 ft. stand of crested wheatgrass was planted on the northern end of the Godfrey Farm legume and forb plots at Clarkston. Crested wheatgrass was included at all locations; it was planted adjacent to middle alleys at Greenville and Ephraim and as a separate series of plots at Cedar City and Panguitch to facilitate anticipated herbicide applications. Due to bindweed encroachment and other weed issues at Clarkston, glyphosate (using a solution of 0.8% active ingredient) was applied to large patches of bindweed and to plots with no establishment of seeded species using backpack sprayers. Prowl H_2O (pendimethalin at 0.8l ha^{-1} active ingredient) and clethodim (0.3 l ha^{-1} active ingredient) were applied in the fall of 2023 at Greenville to reduce cheatgrass infestation. At Ephraim, Weedmaster (active ingredients 0.28 kg dicamba and 0.80 kg 2,4-D ha^{-1}) were applied in the spring of the planting year to control perennial weeds, including alfalfa encroachment from an adjacent pivot. Before planting, 25 mm of irrigation water was applied to leach herbicide below the seedling root zone.

Seed and seeding equipment

Certified seed of plant species was obtained from Bruce Seed Farm Inc. (Townsend, MT), except kura clover which was obtained from Welter Seed and Honey Co. (Onslow, IA). Germination tests were obtained for all species from Bruce Seed Farm. Field sites with the exception of Panguitch were cultivated to create a firm seed bed, and seed at all sites was drilled at a rate of 1 million pure live seed (PLS) per acre (Table 3.1) (Hardegree et al., 2016) using a Great Plains 6 ft. drill (Model 3P600) at Greenville and Ephraim or a Great Plains 8 ft. no-till drill (Model 3P806NT) at Clarkston and Panguitch. Both drills were equipped with double disk openers and press wheels (Great Plains Ag., Salina, KS), and the no-till planter had coulters to slice the soil ahead of the double-disk openers. The row spacing was 15.24 cm (6 inches) and 7.5 cm for the 6 ft and no-till 8 ft. planters, respectively. A cone seeder was used to plant at Cedar City.

Data collection

Plants per unit area were counted beginning the year after planting at Greenville, Ephraim, and Cedar City, during the first or second week of each month from May to July in 2023 and 2024, and in 2024 at Panguitch. At Clarkston, a legacy site from a seed grant received the year before the NIFA grant funding the balance of this study, plant counts were taken in May and June of 2023 and 2024. In 2023, all five replications of four plant species at Clarkston (‘Ladak’ alfalfa, birdsfoot trefoil, sainfoin, and small burnet) were sampled monthly for forage nutritive value, tannin concentration, and identity of secondary metabolites. In 2024, the same four plant species were collected along with falcata alfalfa, showy goldeneye, and Utah sweetvetch (Tables 3.3 & 3.4).

The establishment of plants was evaluated using plant counts scaled to plant establishment density, since a single sampling area was not appropriate to the wide range of population densities. As suggested by Sawyer (1989), the spatial distribution of populations can have a profound impact on the relationship between the sample mean and variance described by Taylor's power law.

$$\mathbf{s^2=amb}$$

s^2: Sample variance (spread of plant counts).

m: Sample mean (average plant count per unit).

a: Scaling factor (influenced by sampling unit size).

b: Index of aggregation:

b=1: Random distribution,

b>1: Aggregated/clumped distribution,

b<1: Uniform distribution.

The size of the sampling unit could influence the estimation accuracy of this relationship. Larger sampling instruments can capture populations that are sparse or aggregated in distribution, while smaller sampling instruments are better for denser and more uniformly distributed populations. Thus, using a smaller measuring instrument in denser plots prevented the overestimation of density due to aggregation effects by capturing the finer-scale variations. On the other hand, the use of a larger measuring instrument across sparsely populated areas avoided an underestimation of counts as could result from using small sample units.

Plots were long and relatively narrow, so counting instruments were placed in representative locations after considering the entire plot's plant density. Different sizes of hoops [small (0.2082 m^2), medium (0.342 m^2), and large (0.52 m^2)] were selected according to plant density, and all plants within the area enclosed by a hoop were counted and scaled up to determine the number of plants (100 m^2)$^{-1}$(Robinson & Conely, 2014). In sparsely populated plots, all of the plants in the entire plot were counted, so the plot became the counting instrument. In a few intermediate plots, data were obtained by standing at a representative spot and counting individual plants within a one-meter radius (3.14 m^2). Different plant counting methods selected from those described above were employed for each half plot when plant stand density was not uniform across the plots.

Climate data

Daily weather data were obtained from the Utah Climate Center for the following parameters: maximum temperature (°C), minimum temperature (°C), precipitation, (mm), and ETr (Reference Evapotranspiration) (mm). Precipitation measurements were obtained using one of two instruments depending on location: a weighing precipitation gauge (WPG) or tipping bucket (TB). ETr is calculated by the ASCE method, also known as the Penman-Monteith method (ASCE-EWRI 2005). The temperature data for all five locations was averaged monthly while the ETr and precipitation were summed by month (Fig. 3.1). Precipitation data was obtained using a WPG instrument (precipWPG), for Clarkston, Cedar City, and Panguitch, and from a tipping bucket (TB) instrument (precipTB) for Greenville and Ephraim, where no WPG was installed. The data for precipTB data can be less accurate than precipWPG because the TB is dependent on the natural melting of snow before measurement as precipitation. Precipitation recorded by the TB is generally comparable to that recorded by the WPG but may be less accurate in winter.

Data analysis

Statistical analysis was performed using the PROC GLIMMIX procedure in SAS Studio (version 3.8; SAS Institute, Cary, NC, USA). The data analysis was done individually for each site to ensure results were not confounded by differences in environmental conditions across the different sites. For each site, the analysis compares species performance within each month as well as across months. Year-to-year comparisons were made for each location by considering the average plant establishment across all three months (two months for Clarkston). Further, a comparison of the average performance of each species over two years was performed at each location. All data were subjected to log transformation. The stated LSMeans and standard errors estimated by the model were back transformed from the log scale. Standard error estimates depended on the choice of covariance structure when performing the mixed model analysis. Different structures were used when required to make the model fit appropriately including heterogeneous first-order autoregressive and variance components. LSMeans were compared pairwise for species x month interactions to assess the differences between the species within a given month and across different months for a given species. Also, the species x year interactions were tested to determine the differences in the performance of the species over years using the Tukey-Kramer method, adjusting for multiplicity with a significance level specified at $\alpha \leq 0.05$. PROC PLM was used to perform the post-hoc analysis. It sliced the LSMeans by different combinations of variables, i.e., species and months, to generate visual contrasts.

Results

Climate data

A summary of the average monthly temperature, precipitation and evapotranspiration demand for all five locations, starting from the year the study began at each site up to 2024 is shown in Figure. 3.1 and Table 3.5 provides annual mean data for each location.

Plant establishment

The response of plant counts to season varied across species ($p < 0.0001$) at all time points and locations. To visualize the best-performing species, heat maps were generated using log-transformed plant count values, (log (plant density +1)) plants (100 $m^2)^{-1}$. Since the original plant count values varied on a wide range from 0 to thousands of plants (100 $m^2)^{-1}$, log transformation was applied to normalize the data and help visualization by compressing the higher values and expanding the lower values. Figure. 3.2 shows the trends in plant density of each species by month and year.

Locations by Month and Year

Clarkston

Monthly plant counts during 2023 and 2024 are presented in Figure. 3.3. During 2023, there was a significant increase in plant density of showy goldeneye ($p < 0.0001$) from May to June, while cicer milkvetch, crested wheatgrass, crownvetch, Lewis flax, white sagebrush and Maximillian sunflower exhibited a significant decline over the months ($p = 0.008$, $p < 0.0001$, $p = 0.0003$, $p = 0.01$, $p = 0.01$, and $p = 0.005$, respectively). Notably, a single prairie aster plant was observed in May 2023. Fernleaf biscuitroot emerged for the first time in June 2023. At the same time, zero establishments were observed for blanketflower, Palmer's penstemon, and Rocky Mountain penstemon. Smooth blue aster established just one plant in 2024. There was no significant increase in plant density over months during 2024; except for, purple prairie clover, white prairie clover, and prairie aster as the seedlings became established for the first time in June 2024 ($p < 0.0001$). The fernleaf biscuitroot plants started establishing in May 2024 and aboveground desiccation was observed during June, indicating plants entering dormancy

Ephraim

The monthly plant counts during 2023 and 2024 are presented in Figure. 3.4. During 2023, there was no significant increase in plant density over the months for the majority of species. However, there was a noticeable increase in plant density of blanketflower and Maximillian sunflower as these species started emerging in June, and July, respectively. The apparent plant density of arrowleaf balsamroot declined significantly from May to July ($p = 0.01$) as plants became dormant. During 2024, there was a significant increase in plant density of cicer milkvetch ($p = 0.003$) while a significant decline in plant density was observed in arrowleaf balsamroot ($p = 0.009$) and showy goldeneye ($p < 0.0001$) from May to July. Notably, arrowleaf balsamroot plants started entering dormancy in June and a continuous decline was observed in the apparent plant population during both years.

Greenville

The monthly plant counts for 2023 and 2024 are presented in Figure. 3.5. During 2023, a significant increase in plant density was observed from May to July for species including birdsfoot trefoil, Maximillian sunflower, and Rocky Mountain penstemon ($p < 0.0001$). However, the plant density declined significantly from June to July for Canadian milkvetch ($p < 0.0001$), and Utah sweetvetch ($p = 0.0003$). White sagebrush emerged for the first time in July, while Palmer's penstemon, prairie coneflower, showy goldeneye, and western yarrow started emerging in June and remained stable in density through July. During 2024, the plant density did not vary significantly across months except for birdsfoot trefoil and Canadian milkvetch, where a significant decline ($p = 0.009$, $p = 0.009$, respectively) was observed from May to June. Additionally, a distinct pattern was observed for fernleaf biscuitroot, which produced seedlings in June and went into dormancy in July (Fig. 3.3).

Cedar City

The monthly plant counts during 2023 and 2024 are presented in Figure. 3.6. Since the western side of the field received supplemental irrigation in both years at this location (Table 3.6) and planting was done in a completely randomized design. Thus, a random subset of plots for each species received irrigation (Table 3.7). During 2023, a significant increase in plant density was observed in Maximillian sunflower, Palmer's penstemon, prairie coneflower, Rocky Mountain penstemon, and showy goldeneye ($p < 0.0001$), from May to July. During 2024, a significant increase in plant density was observed from May to July in blanketflower ($p < 0.0001$) and prairie coneflower, while there was a significant decline in plant density of Falcata alfalfa ($p = 0.007$) and fernleaf biscuitroot ($p = 0.03$). Purple prairie clover was not established at this location.

Panguitch

The monthly plant count during 2024 is presented in Figure. 3.7. There was a significant increase in plant density of birdsfoot trefoil ($p = 0.0003$), cicer milkvetch ($p = 0.03$), and Lewis flax ($p = 0.0004$) from May to July, whereas the plant density of kura clover ($p < 0.0001$), and prairie coneflower ($p = 0.006$) declined significantly over the months.

Kura clover experienced a sharp decline in plant population from May to June, demonstrating weak establishment during the first year after planting. No establishment was observed for arrowleaf balsamroot, fernleaf biscuitroot, prairie aster, purple prairie clover, and smooth aster with no plants observed during the first year after planting.

Trends Between Years at All Locations

A statistical comparison of the LSMeans for species performance was conducted between years for each location as shown in Figure. 3.8. The heat map compares the differences in LSMeans of plants (100 m^2)$^{-1}$ between 2023 and 2024 for each species and location.

At Clarkston, significant increase in average plant density (calculated across May and June) was observed from 2023 to 2024 in blanketflower ($p = 0.0007$), cicer milkvetch ($p < 0.0001$), fernleaf biscuitroot ($p < 0.0001$), Maximillian sunflower ($p < 0.0001$), Palmer's penstemon ($p = 0.007$), prairie aster ($p = 0.0043$), prairie coneflower ($p = 0.04$), Rocky Mountain penstemon ($p < 0.0001$), showy goldeneye ($p = 0.0002$), western yarrow ($p < 0.0001$), and white prairie clover ($p = 0.04$). Based on the average plant density across 2023 and 2024, showy goldeneye and sainfoin exhibited the greatest plant density among all species with over 5000 and >3900 plants (100 m^2)$^{-1}$. Overall, a very sparse establishment of white sagebrush, Canadian milkvetch, Palmer's penstemon and smooth blue aster was observed with fewer than 20 plants (100 m^2)$^{-1}$ across two years.

At Ephraim, the average plant density (calculated across May, June, and July) significantly increased from 2023 to 2024 for arrowleaf balsamroot ($p = 0.02$), showy goldeneye ($p = 0.0005$), Maximillian sunflower ($p = 0.004$), and cicer milkvetch ($p = 0.0033$). Whereas, plant density declined in blanketflower ($p < 0.0001$), Lewis flax ($p = 0.01$), prairie coneflower ($p = 0.0007$), and western yarrow ($p = 0.0005$), while remaining nearly stable in others from 2023 to 2024. Based on average plant density across 2023 and 2024, sainfoin exhibited the greatest number of plants (>3000 plants $(100\ m^2)^{-1}$, $p < 0.0001$). Negligible establishment was observed for Palmer's penstemon, fernleaf biscuitroot, Canadian milkvetch, leadplant, white sagebrush, prarie aster, purple prarie clover, Rocky Mtn penstemon, smooth aster, and white prairie clover with ≤3 plants $(100\ m^2)^{-1}$.

At Greenville, a significant increase in average plant density (calculated across May, June, and July) was observed for the majority of species from 2023 to 2024 except for Ladak alfalfa, falcata alfalfa, Canadian milkvetch, sainfoin, Utah sweetvetch, white prairie clover, purple prairie clover, and prairie aster. Based on the average plant density across 2023 and 2024, sainfoin consistently exhibited significantly greater plant density among all species with >3500 plants $(100\ m^2)^{-1}$ ($p < 0.0001$; Fig. 3.8). However, negligible establishment was observed for prairie aster across two years. Fernleaf biscuitroot, smooth aster, white prairie clover, and purple prairie clover only showed establishment during 2024, and the latter three species established fewer than three plants $(100\ m^2)^{-1}$.

At Cedar City, a significant increase in plant establishment (calculated across May, June, and July) was observed from 2023 to 2024 in falcata alfalfa ($p = 0.02$), cicer milkvetch ($p = 0.01$), crownvetch ($p < 0.0001$), Lewis flax ($p = 0.003$), Maximillian sunflower ($p < 0.0001$), prairie coneflower ($p = 0.04$), Utah sweetvetch ($p = 0.002$), and western yarrow ($p < 0.0001$), while there was significant decline in plant density of smooth blue aster ($p = 0.02$). The greatest average plant density among all species across 2023 and 2024 was observed in sainfoin with >3000 plants (100 m^2)$^{-1}$ ($p < 0.0001$). No establishment was observed for Canadian milkvetch, fernleaf biscuitroot, leadplant, white sagebrush, prairie aster, Rocky Mountain penstemon, smooth aster, and white prairie clover at this location, while Palmer's penstemon, Lewis flax, blanketflower, and Maximillian sunflower exhibited negligible average establishment of less than 8 plants (100 m^2)$^{-1}$ across two years.

Average Performance of Plant Species

Figure 3.9 summarizes plant density for each of the five locations across three counts in one (Panguitch) or two years in order from greatest to least plant count. While locations were not compared statistically, it is worth noting that sainfoin produced the thickest stand at all locations. At Clarkston, which was planted in the spring of 2022, the mean plant count of showy goldeneye was no different from sainfoin. At Panguitch, while variation in the first year was too high to distinguish among plant species, sainfoin had the numerically greatest mean plant density. In addition to sainfoin, four other species were found among the top 12 mean plant counts for all five locations: Ladak and falcata alfalfa, small burnet, and western yarrow. The next tier of plants occurring in the top 12 legumes and forb plant counts at four of the five locations include birdsfoot trefoil, cicer milkvetch, crownvetch, Maximillian sunflower, showy goldeneye, and Utah sweetvetch. The penstemons (Palmer's and Rocky Mountain) did well at Greenville and Cedar City, and yellow prairie coneflower did well at Cedar City and Ephraim. Data on yield and quality, and factors such as the cost of seed and ease of planting will need to be considered, but the species listed above will be considered further as candidates for rangeland resource islands.

Discussion

Temporal trends

Plant densities fluctuated rather than simply increasing or decreasing for many species, which underscores their different establishment strategies. At Ephraim, plots with arrowleaf balsamroot had the highest plant density in May but declined with the progression of the season due to an apparent return to dormancy (Fig. 3.2). The stand density early in the year demonstrates that the species is expanding its crown and roots below ground each spring (Ogle et al., 2003). Fernleaf biscuitroot followed a similar growth habit, showing early-season growth during the third year after planting at Clarkston, followed by dormancy by mid-summer (Fig. 3.2). This growth habit is well documented for this species as being repeated for the first three to five years after-planting (Tilley et al., 2012). The slow establishment of these native species makes them poor candidates for supplemental protein resource islands for cowherds, but once established, they produce nutritious forage and provide valuable services for wildlife and the rangeland ecosystem

Adding diversity from legumes and forbs to grass monocultures has the potential to significantly enhance ruminant health and productivity while benefiting the environment. There is growing interest in diversifying plant species on rangelands (Distel et al., 2020; Muir et al., 2011). While legumes and other herbaceous forbs are nutritionally richer than grass species, their incorporation into rangelands is challenging due to poor establishment and persistence (Matches, 1989). While it is common to document efforts to seed degraded rangeland in order to restore historical plant communities (Zerga, 2015), it is rare to find accounts of the level of plant establishment that has resulted, in either plant establishment or persistence. Seed germination cannot succeed without moisture, and it is not uncommon for rangeland to require multiple reseeding before the establishment succeeds (Torres et al., 2024). This study was conducted under dryland conditions to address this gap, with irrigation only used immediately after seeding at three sites. At Cedar City, the western side of the plot area received supplemental irrigation from an adjacent study during the entire study (Tables 3.6 & 3.7; Fig. 3.14). Some plant species in that section of the study responded with greater establishment and growth, but not all species responded to irrigation. While the Cedar City site will not be included in publication on this rangeland study, it serves as an interesting case study for the ability of species to respond to irrigation.

Our findings demonstrated significant differences in the density of established plants even though all species were seeded at the same number of PLS ha^{-1}. Differences were primarily due to plant species rather than latitude, elevation, or precipitation, highlighting the ability of well-adapted species to become established and persist.

Species performance across different locations

Sainfoin maintained the greatest plant density in all locations and across months except at Clarkston, where showy goldeye was the best performer (Fig. 3.9), indicating its wide adaptation across northwestern rangeland soils and climates. According to Tilley et al. (2008), sainfoin requires a minimum of 355.6 mm (14 inches) mean annual precipitation for optimum establishment. In our study, its performance was satisfactory at all locations, even with less-than-optimal precipitation except at Greenville, where it received >355 mm precipitation during both years (Table 3.5). However, plants at Ephraim were desiccated by June of each year due to an extremely dry and warm climate (Fig. 3.20).

Ladak alfalfa also maintained dense plant stands throughout the period of evaluation at all sites, and falcata alfalfa also achieved moderately dense plant stands across all locations, except at Cedar City (Fig. 3.9). Our findings were in accordance with those of Rumbaugh & Pedersen (1979) regarding alfalfa's resilience and robust establishment in semi-arid conditions.

Small burnet also maintained consistently dense plant stands (Fig. 3.9). Sainfoin, the alfalfas and small burnet all started growth early in the season and their shoots entered the maturity phase by late summer (July), after which new basal growth was apparent (Fig 3.10). While the relationship between regrowth and stand persistence was not tested in our study, the commencement of growth in early season, and ability to regrow might have contributed to the persistence of dense plant stands. As highlighted by Blumenthal and McGraw (2015), birdsfoot trefoil plant stands in our study were sparser than the best performing species at all locations aligning with their slow seedling growth habit. However, under extremely dry conditions at Ephraim, birdsfoot trefoil maintained moderate plant density compared to the average density of other species across two years (Fig. 3.9).

Cicer milkvetch also exhibited slow but robust establishment and growth at all locations especially during 2024 (Fig. 3.8). As documented by previous studies, due to its high drought tolerance and rhizomatous characteristic, the species achieved high plant density and demonstrated potential to extend the grazing period by remaining green during late summer and fall (Acharya et al., 2006; Townsend & McGinnies, 1972).

During both years, showy goldeneye significantly increased its population at Clarkston in June (Fig. 3.2) and established the thickest average plant density across two years at this location (Fig. 3.9). Its slow-growing habit has been documented by previous studies (Monsen et al., 2004). In some dense stands, the growth of showy goldeneye became stagnant, and shoots appeared desiccated by July. The species western yarrow showed a significant increase in plant density during the second year after planting, especially at Greenville and Cedar City (Fig. 3.8). Species that develop relatively dense stands but that are slow establishing could be seeded into resource islands to add long-term diversity as the rapidly established species begin to thin. White prairie clover, purple prairie clover, and fernleaf biscuitroot only became established three years after planting at Clarkston, but their densities were low whereas the densities of showy goldeneye and Maximillian sunflower were greater. The slow growth and poor establishment of white prairie clover and purple prairie clover after the first year of planting is in line with findings in Oklahoma by Kneebone (1959).

In contrast, many species such as Canadian milkvetch, prairie aster, and smooth blue aster demonstrated negligible or zero establishment at all locations (Fig. 3.9). This could be attributed to various factors such as suboptimal environmental conditions for germination and establishment, poor seed or seedling vigor, or the inability of resources to meet plant growth requirements on uniformly semi-arid rangeland, even though there are microenvironments where those plant species are found in Utah. For instance, prairie aster (minimum precipitation = 406 mm), Canadian milkvetch, and smooth aster (minimum precipitation = 508 mm) have precipitation requirements that are greater than available moisture on Utah rangelands (USDA, Plant database). Consequently, their suboptimal performance, including the appearance and subsequent disappearance of individual plants, might be attributed to insufficient precipitation relative to their physiological needs. However, all three of these species were in irrigated plots at Cedar City (Table 3.7) but did not establish noticeably more plants under irrigation than at dryland sites, suggesting their poor establishment was due to causes other than suboptimal soil water availability. While the establishment of plant species could not be statistically compared among sites, species that appeared to have a greater density of established plants in irrigated plots at Cedar City than at sites without irrigation were cicer milkvetch, Maximilian sunflower, Palmer’s penstemon, prairie coneflower, Rocky Mountain penstemon, and western yarrow.

The species showy goldeneye failed to establish at Ephraim during 2023, possibly due to the dry conditions. Cicer milkvetch also showed improved establishment in some locations during the later season while failing to reach high density at Ephraim. The herbicide Weedmaster (2,4-D and dicamba) had been used in the spring of 2022 at Ephraim to control kochia and alfalfa that had invaded from an adjacent field under pivot irrigation. The recommended 2.54 cm of irrigation water had been applied before planting, but it is possible that some of the seeded species were sensitive to herbicide residue that had resisted leaching, or that they germinated but were killed when their roots reached the leached herbicide.

Factors responsible for plant count inconsistency

Each species demonstrated a unique growth habit, ranging from erect forms (Table 3.1), such as penstemons and prairie coneflower to semi-erect forms, such as birdsfoot trefoil and Utah sweetvetch (Fig. 3.10). Additionally, decumbent species, i.e., cicer milkvetch (Fig. 3.11), exhibited extensive ground spread. The choice of employing suitable and different plant count methods was necessary to capture the variability in plant stand density. Because of the challenging conditions and high seeding rate, there was considerable variability in plant distribution within each plot as well as between reps. Inconsistent establishment within plots contributed variation to the statistical analysis but was consistent across the sites included in the study. The plant counting method employed was designed to minimize bias and provided the most accurate estimate of plant density for the vastly different species and levels of establishment seen in this study.

Conclusion

Based on the literature, we expected sainfoin, Ladak alfalfa, and small burnet to establish at relatively high densities and be the best prospects among the species we tested for use in protein supplementation resource islands for cowherds. Overall, sainfoin consistently exhibited the highest plant density across all sites except Clarkston, highlighting its strong adaptability to dryland environments. Other top-performing species included small burnet and Ladak alfalfa, each achieving high plant density, indicating their suitability for rangeland restoration and forage production.

Species with moderate establishment included falcata alfalfa, cicer milkvetch, and Utah sweetvetch. Showy goldeneye was a top performer at Clarkston where it was in the third year of establishment, and it was a moderate performer at Greenville.

Species such as prairie aster, purple prairie clover, smooth blue aster, and white sagebrush exhibited poor establishment, with low densities. Crownvetch, Maximillian sunflower, Rocky Mt. penstemon, and Western yarrow showed moderate performance after two years of planting but were limited to specific locations. Arrowleaf balsamroot and fernleaf biscuitroot showed highly variable performance as the season progressed. These species showed slower establishment due to prioritizing root and crown development and would not be well-suited to use in resource islands.

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Table 3.1

Description of species planted at five locations across Utah

Species	Planting locations	Origin	Scientific names	Cultivar	Pure live seed** kg ha^{-1}	Growth habit***
Falcata alfalfa^{S}	CEGCCCP^{+}	Introduced^{L}	*Medicago sativa L. ssp. falcata* (L.) Arcang	Don	5.02	Erect
Ladak alfalfa	CEGCCP	Introduced^{L}	*Medicago sativa* L.	Ladak	5.61	Erect
Arrowleaf balsamroot^{D}	ECCP	Native^{NL}	*Balsamorhiza sagittata* (Pursh) Nutt.	VNS*	27.73	Erect
Birdsfoot trefoil	CEGCCP	Introduced^{L}	*Lotus corniculatus* L.	Bruce	7.26	Semi-erect
Blanketflower^{DS}	CEGCCP	Native^{NL}	*Gaillardia aristata* Pursh	VNS	5.54	Erect
Canadian milkvetch^{S}	CEGCC	Native^{L}	*Astragalus canadensis* L.	MN Native	4.6	Prostrate
Cicer milkvetch^{S}	CEGCCP	Introduced^{L}	*Astragalus cicer* L.	Monarch	55.25	Decumbent
Crested wheatgrass	CEGCCP	Introduced^{G}	*Agropyron cristatum* (L.) Gaertn.	Fairway	6.15	Erect
Crownvetch^{S}	CEGCCP	Introduced^{L}	*Securigera varia* (L.) Lassen	Penngift	11.37	Decumbent
Fernleaf biscuitroot^{D}	CEGCCP	Native^{NL}	*Lomatium dissectum* (Nutt.) Mathias & Constance	VNS	37.73	Erect
Kura clover^{S}	P	Introduced^{L}	*Trifolium ambiguum M. Bieb.*	VNS	6.15	Prostrate

Leadplant[D]	EGCC	Introduced[L]	*Amorpha canescens* Pursh	VNS	13.24	Semi-erect
Lewis flax[D]	CEGCPC	Native[NL]	*Linum lewisii* Pursh cv. Maple Grove	Maple Grove	9.79	Erect
White (Louisiana) sagebrush[D]	CEGCC	Native/ Introduced	*Artemisia ludoviciana* Nutt.	VNS	0.52	Erect
Maximillian Sunflower	CEGCC	Native[NL]	*Helianthus maximiliani* Schrad.	Medicine Creek	12.18	Erect
Palmer's penstemon[D]	CEGCCP	Native[NL]	*penstemon palmeri* A. Gray	Cedar	3.76	Erect
Prairie aster[DS]	CEGCCP	Native[NL]	*Machaeranthera tanacetifolia* (Kunth) Nees	VNS	3.51	Erect
Prairie coneflower, yellow	CEGCCP	Native[NL]	*Ratibida columnifera* (Nutt.) Wooton & Standl.	Stillwater	3.11	Erect
Purple prairie clover	CEGCCP	Introduced[L]	*Dalea purpurea* Vent.	Bismarck	4.55	Erect
Rocky Mtn. penstemon[D]	CEGCCP	Native[NL]	*Penstemon strictus* Benth.	Bandera	8.62	Erect
Sainfoin	CEGCCP	Introduced[L]	*Onobrychis viciifolia* Scop.	Remont	70.38	Erect
Showy goldeneye[D]	CEGCCP	Native[NL]	*Heliomeris multiflora* Nutt.	VNS	1.68	Erect
Small burnet	CEGCCP	Introduced[NL]	*Sanguisorba minor* Scop.	Delar	22.17	Erect
Smooth blue aster[D]	CEGCCP	Native[NL]	*Symphyotrichum laeve* (L.) Á. Löve & D. Löve	VNS	2.08	Erect
Tall fescue (alleys)			*Schedonorus arundinaceus* (Schreb.) Dumort.	Sidewinder	5.21	Erect

Utah sweetvetch[DS]	CEGCCP	Introduced[L]	*Hedysarum boreale* Nutt.	Timp	35.14	Semi-erect
Western yarrow[D]	CEGCCP	Native[NL]	*Achillea millefolium* L. var. *occidentalis* DC	Eagle	0.57	Erect
White prairie clover	CEGCCP	Introduced[LNL]	*Dalea candida* Michx. ex Willd.	VNS	3.83	Decumbent

[S] Seed were scarified before planting

[D] Seed were found to be dormant in a germination study conducted invitro as well as in NRCS plant guides; [*] Variety not stated

[**] Pure live seeding rate ($kg \cdot ha^{-1}$) was calculated by multiplying seed germination and purity percentage obtained from seed tags;

[***] Source: USDA Plant database

CEGCCP[+] – Clarkston, Ephraim, Greenville, Cedar City, Panguitch

[L] – Legume, [NL] – Non legume. [G] – Grass

Table 3.2

Description of research sites and dominant soil type at each location

Field site	Elevation (m)	Soil	Location (latitude - longitude)	Location
Clarkston	1382	Mendon - Silt Loam, Fine-silty, mixed, mesic Calcic Pachic Argixerolls	41.895°N ,112.048°W	Northern Utah
Ephraim	1686	Woodrow - Fine-silty, mixed, superactive, calcareous, mesic Xeric Torrifluvents	39.37037°N ,111.58161°W	Central Utah
Greenville Farm	1413	Millville - Coarse-silty, carbonatic, mesic Typic Haploxerolls	41.76509°N ,111.81413°W	Northern Utah
Cedar City	1685	Medburn- Coarse-loamy, mixed (calcareous), mesic Xeric Torriorthents	37.673°N ,113.137°W	Southern Utah
Panguitch	1996	Notter - Fine-loamy, mixed Aridic Argiborolls	37.86831°N ,112.4355°W	Southern Utah

Table 3.3

Schedule of planting and counting of plants for each location.

	2021	2022				2023				2024			
	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec
Planting													
Clarkston	Planted		Replanted				C1 C2 S1	S2 S3 S4	S5		S1	S2 S3 S4	S5
Ephraim				Planted			C1 C2	C3			C1 C2	C3	
Greenville				Planted			C1 C2	C3			C1 C2	C3	
Cedar City				Planted			C1 C2	C3			C1 C2	C3	
Panguitch								Planted			C1 C2	C3	

C = Counting, S = Sample collection

Table 3.4

Planting and plant count schedule for all five locations

Location Activity	Planting	2023 Plant Count	2024 Plant Count
Clarkston	18-Nov 21	31-May	9-May
	7-May 22	29-Jun	10-Jun
Ephraim	18-19 Aug 22	5-May	4-May
		6-Jun	3-Jun
		6-Jul	12-Jul
Greenville	20-Sep-22	11-May	8-May
		8-Jun	10-Jun
		7-Jul	8-Jul
Cedar City	18-Sep-22	6-May	3-May
		7-Jun	3-Jun
		5-Jul	11-Jul
Panguitch	15-Aug-22		2-May
			3-Jun
			11-Jul

Table 3.5

Description of average annual climatic conditions at each research site

Field site	Precipitation (mm)			Evapotranspiration (mm)			Maximum temperature (°C)			Minimum temperature (°C)		
Year	**2022**	**2023**	**2024**	**2022**	**2023**	**2024**	**2022**	**2023**	**2024**	**2022**	**2023**	**2024**
Clarkston	284	254	184	1395	120	1417	15	13	16	-1	-1	1
Ephraim	252	240	342	1129	628	725	17	16	18	-1	-1	1
Greenville farm	387	559	405	1258	1227	1329	15	14	16	2	2	3
Cedar City	328	214	368	1838	782	2010	18	17	19	1	1	2
Panguitch*	__	248	132	__	1383	1564	__	15	17	__	-5	-4

*Study was commenced in 2023 at this location

Table 3.6

Water applied as irrigation (mm) at Cedar City

Month	2023	2024
May	203	102
June	127	102
July	254	152

Table 3.7

Number of irrigated replications for each species at Cedar City, UT

Species	Irrigated Reps
Alfalfa, Ladak (purple)	1 of 3
Alfalfa, falcata (yellow)	0 of 3
Arrowleaf balsamroot	2 of 3
Birdsfoot trefoil	3 of 3
Blanketflower	3 of 3
Canadian milkvetch[*]	2 of 4
Cicer milkvetch	3 of 3
Crested wheatgrass[*]	0 of 3
Crownvetch	2 of 3
Fernleaf biscuitroot	2 of 3
Leadplant	2 of 3
Lewis flax	2 of 3
White sagebrush	1 of 3
Maximillian sunflower[*]	3 of 4
Palmer's penstemon	2 of 3
Prairie aster	2 of 3
Prairie coneflower	2 of 3
Purple prairie clover	3 of 4
Rocky Mtn. penstemon	2 of 3
Sainfoin	2 of 3
Showy goldeneye	1 of 3
Small burnet	1 of 3
Smooth aster	1 of 3
Utah sweetvetch	3 of 3
Western yarrow	1 of 3
White prairie clover	2 of 3

[*] Species planted in four replications.

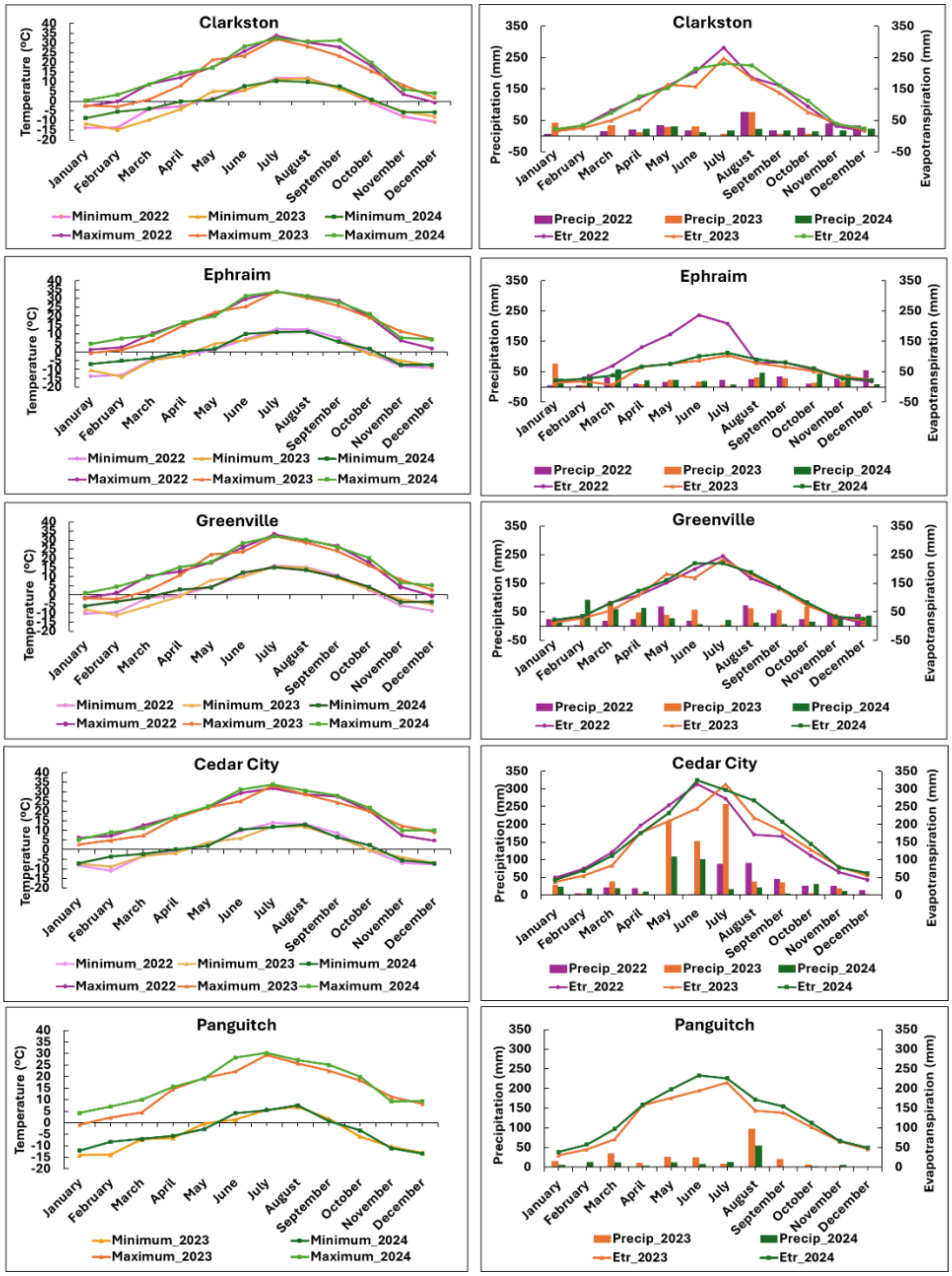

Figure 3.1. Comparison of total daily precipitation and evapotranspiration (summed over the months), and mean daily maximum and minimum temperature averaged over the months at Clarkston, Ephraim, Greenville, Cedar City, and Panguitch, UT obtained from the Utah Climate Center

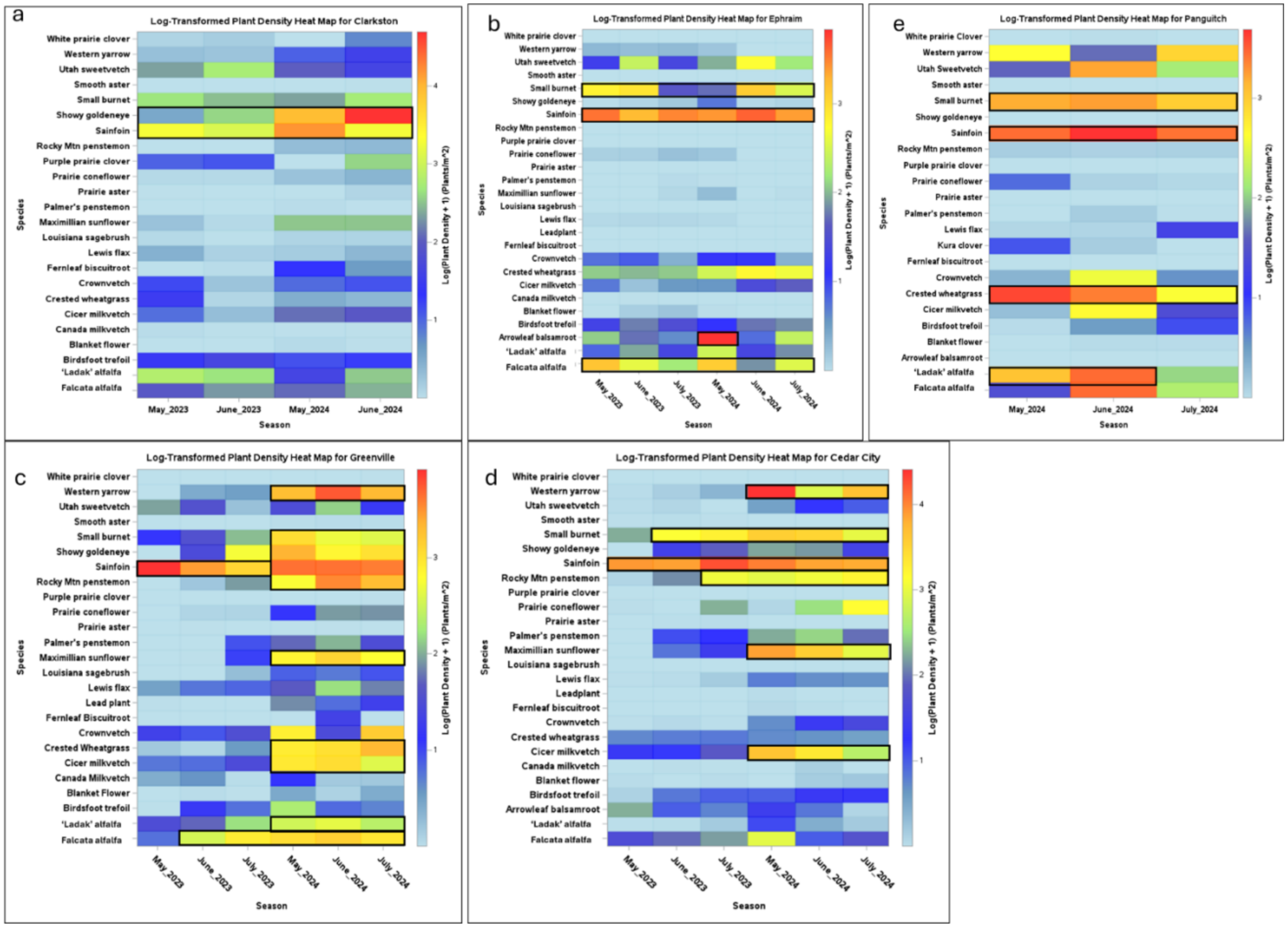

Figure 3.2. Heat maps showing the plant density for each species across months for all five research sites. The black outlined cells highlight particular species and time points with dense plant stands

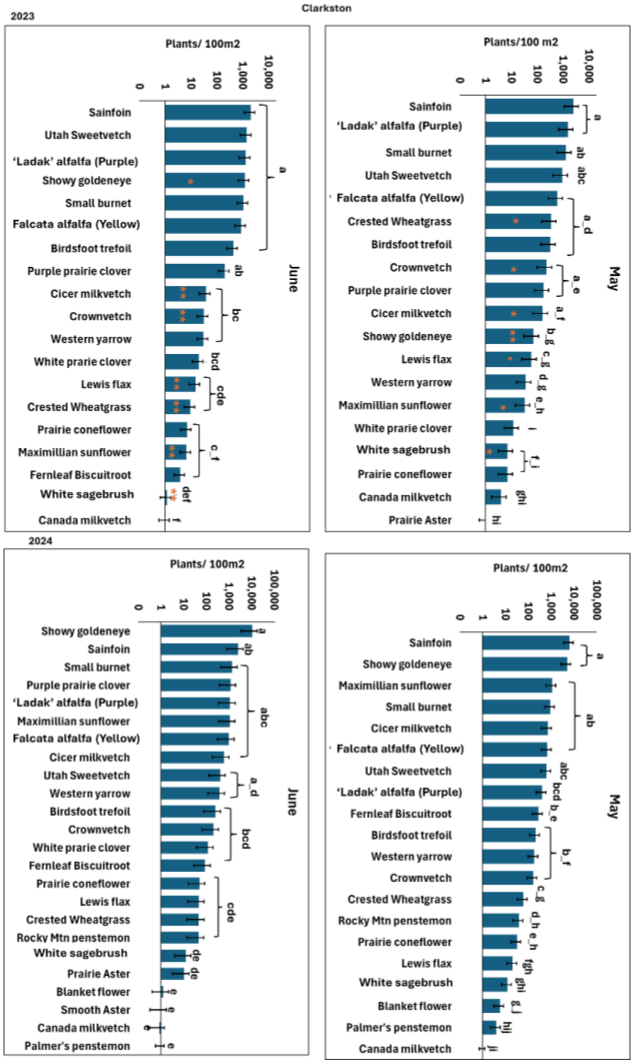

Figure 3.3. LSMeans ± standard error of number of plants $(100 \text{ m}^2)^{-1}$ across five replications at Clarkston, Utah, during May and June 2023 and 2024. Different lowercase letters indicate significant differences in species performance within each month based on Tukey-Kramer's multiple comparison test ($\alpha \leq 0.05$). An asterisk (*) indicates the species whose performance varied significantly after establishment across months. * = A, ** = B. The species with zero establishment have not been included in the graphs. The y-axis represents $\log_{10}$-transformed values to enhance visualization of differences across magnitudes

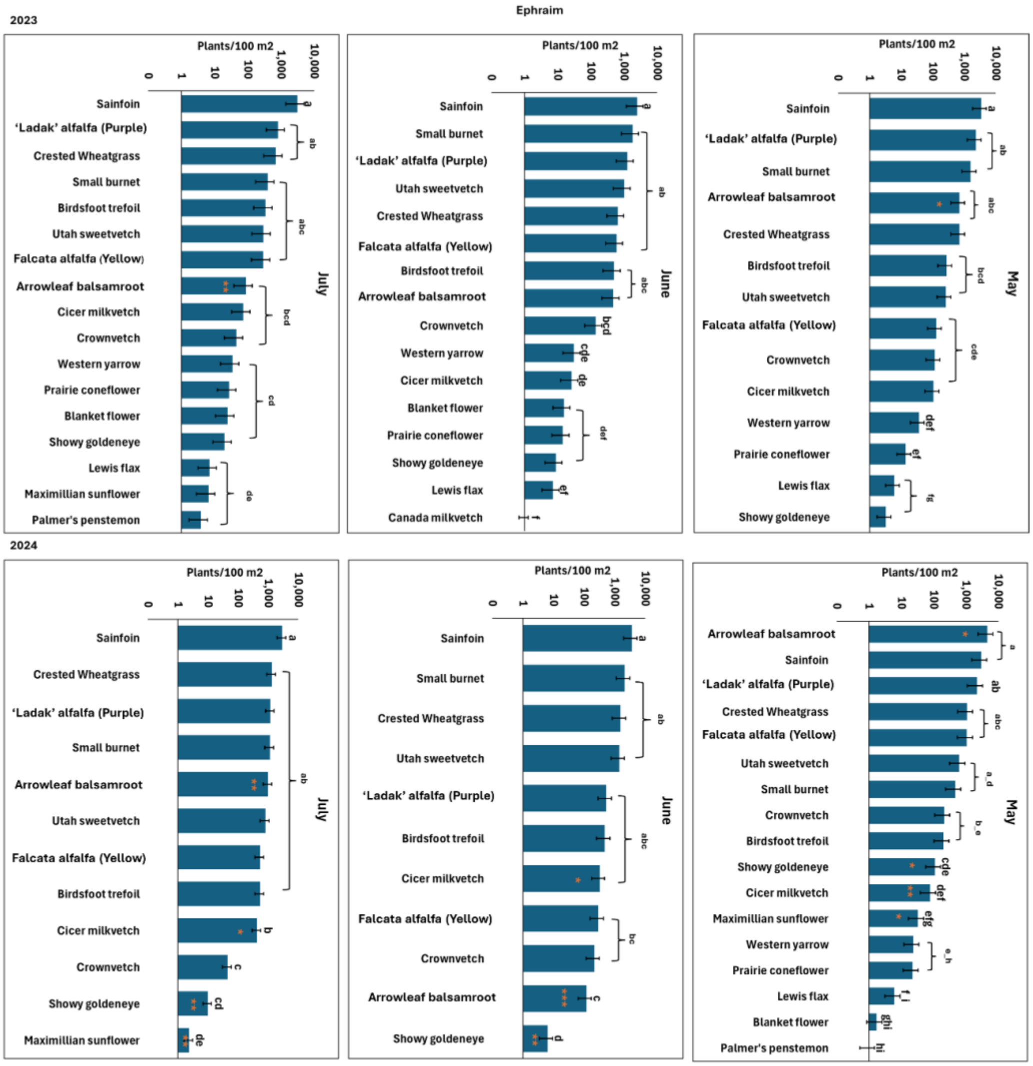

Figure 3.4. LSMeans ± standard error of number of plants $(100\ \mathrm{m}^2)^{-1}$ across four replications at Ephraim, Utah, during May and June 2023 and 2024. Different lowercase letters indicate significant differences in species performance within each month based on Tukey-Kramer's multiple comparison test ($\alpha \leq 0.05$). An asterisk (*) indicates the species whose performance varied significantly after establishment across months. * = A, ** = B, *** = C. The species with zero establishment have not been included in the graphs. The y-axis represents $\log_{10}$-transformed values to enhance visualization of differences across magnitudes

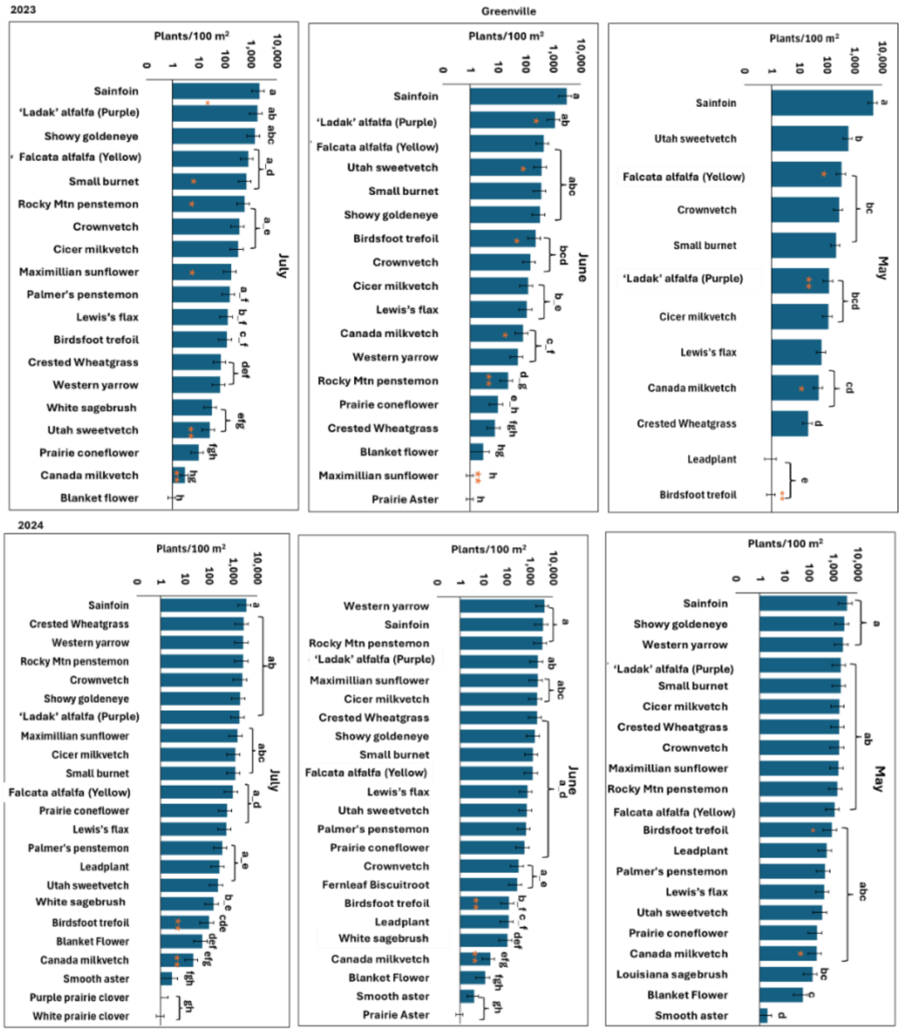

Figure 3.5. LSMeans ± standard error of number of plants $(100\ m^2)^{-1}$ across four replications at Greenville, Utah, during May, June, and July of 2023 and 2024. Different lowercase letters indicate significant differences in performance among species within each month based on Tukey-Kramer's multiple comparison test ($\alpha \leq 0.05$). An asterisk (*) indicates a species whose performance varied significantly across months after establishment. * = A, ** = B. The species with zero establishment have not been included in the graphs. The y-axis represents $\log_{10}$-transformed values to enhance visualization of differences across magnitudes.

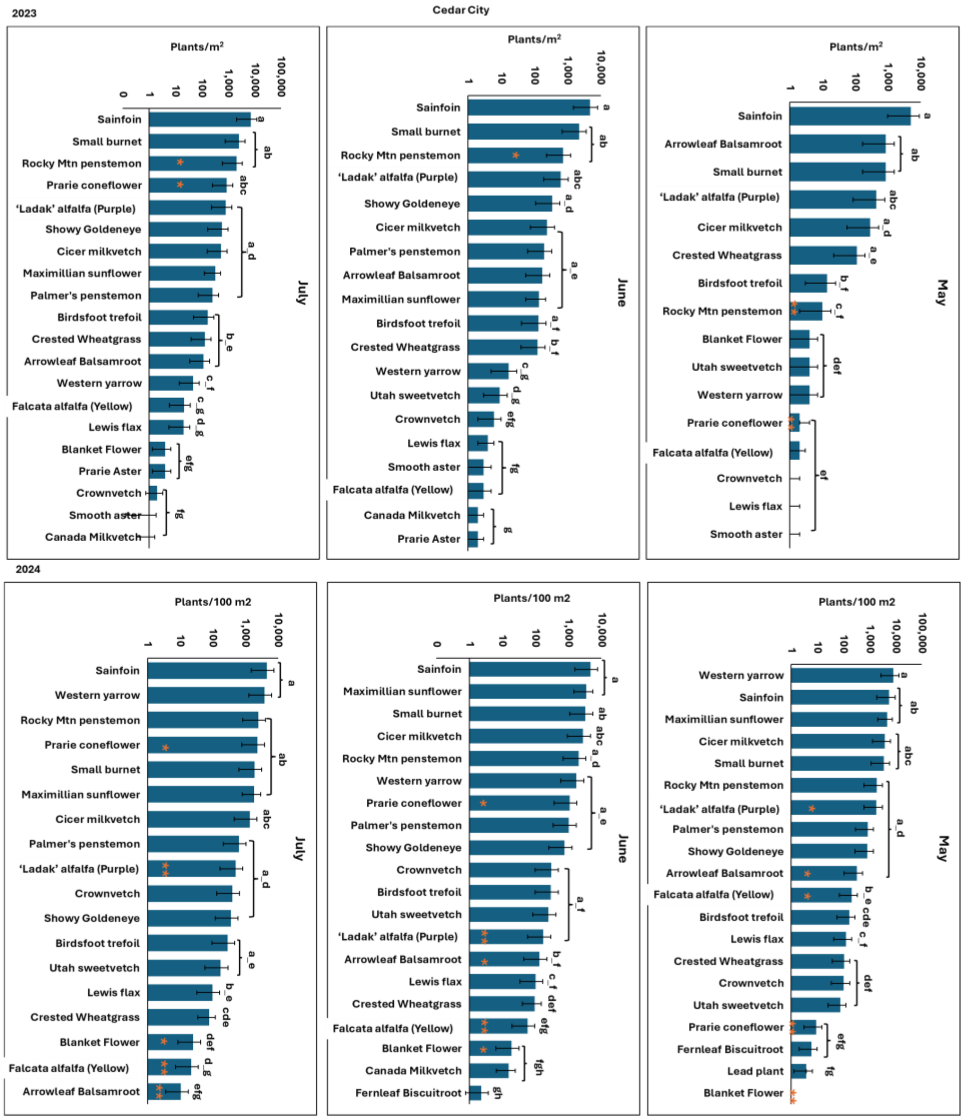

Figure 3.6. LSMeans ± standard error of number of plants $(100\ m^2)^{-1}$ across four replications at Cedar City, Utah, during May, June, July 2023, and 2024. Different lowercase letters indicate significant differences in species performance within each month based on Tukey-Kramer's multiple comparison test ($\alpha \leq 0.05$). An asterisk (*) indicates the species whose performance varied significantly after establishment across months. * = A, ** = B, *** = C. The species with zero establishment have not been included in the graphs. The y-axis represents $\log_{10}$-transformed values to enhance the visualization of differences across magnitudes

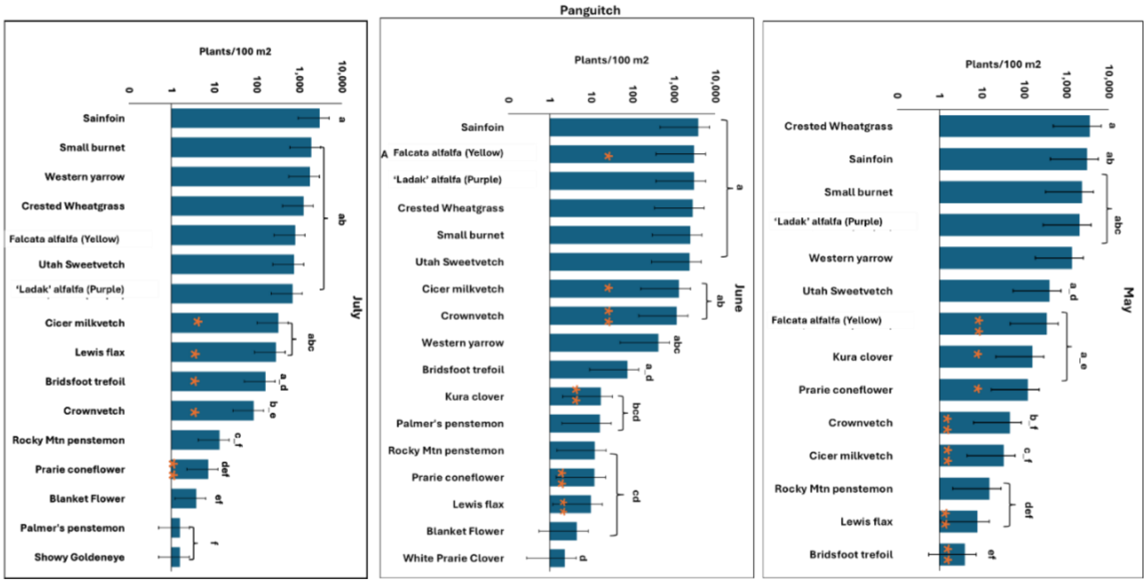

Figure 3.7. LSMeans ± standard error of number of plants emerging $(100\ m^2)^{-1}$ across four replications at Panguitch, Utah, during May, June, and June 2024. Different lowercase letters indicate significant differences in species performance within each month based on Tukey-Kramer's multiple comparison test ($\alpha \leq 0.05$). An asterisk (*) indicates the species whose performance varied significantly after establishment across months. * = A, ** = B, *** = C. The species with zero establishment have not been included in the graphs. The y-axis represents $\log_{10}$-transformed values to enhance the visualization of differences across the magnitude

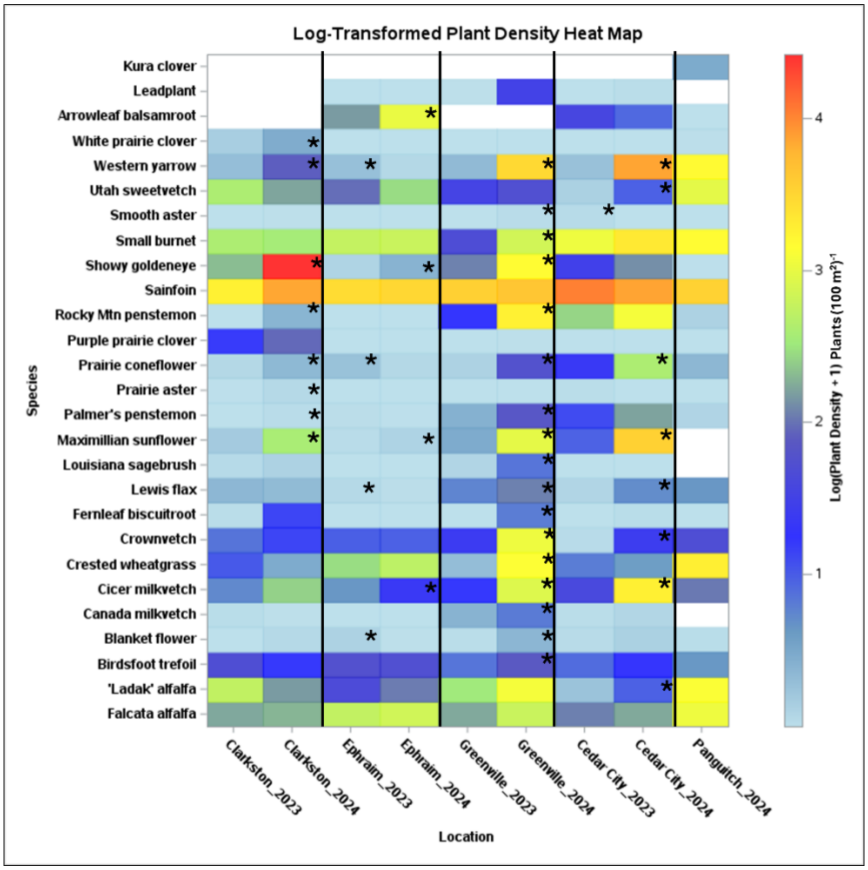

Figure 3.8. Summary heat map representing the LSMeans comparison of averaged plant density calculated as (log (plant density +1)) plants (100 m2)$^{-1}$, over months during 2023 and 2024 for all locations and only during 2024 at Panguitch. *Indicates significantly higher plant density for a species between 2023 and 2024 at each location; White boxes indicate species not planted at the specific locations

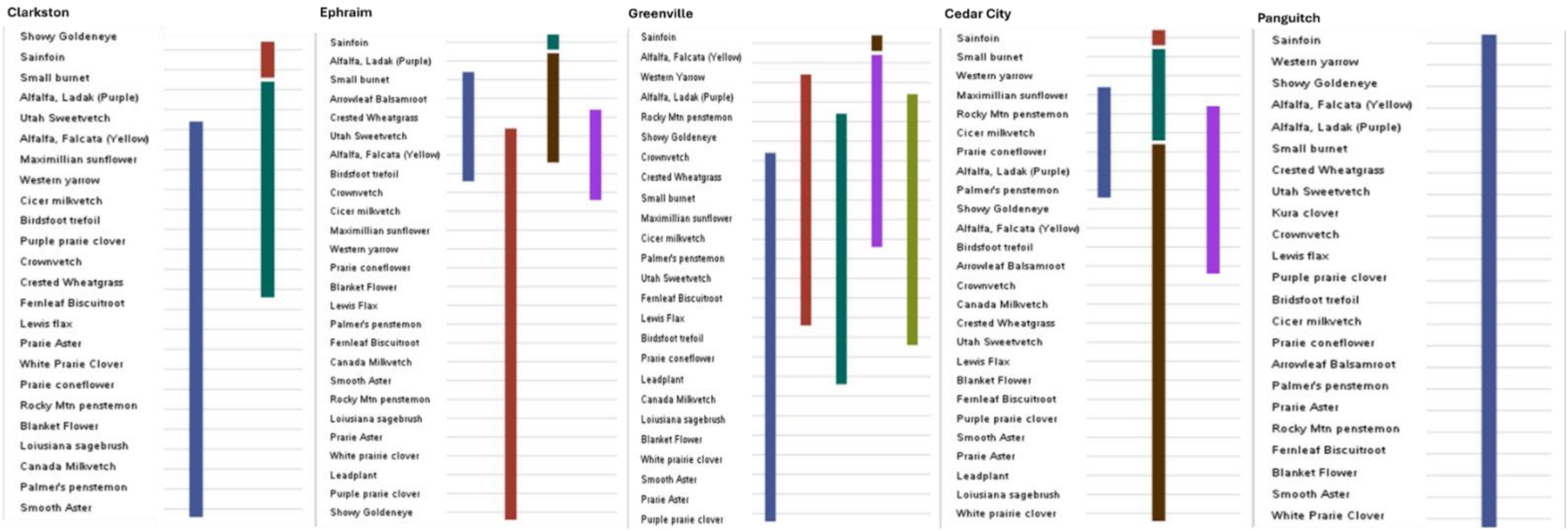

Figure 3.9. LSMeans separation of average plant density across 2023 and 2024 (Clarkston, Ephraim, Greenville, Cedar City) and during 2024 (Panguitch) for all species. Bars represent LSMeans separation suggesting species with similar or significantly different plant density at each location. The numerical value of plant density is not shown, as the figure is intended to illustrate the relative groupings of species. Overlapping bars of different colors suggest no significant difference in plant density between species at each location.

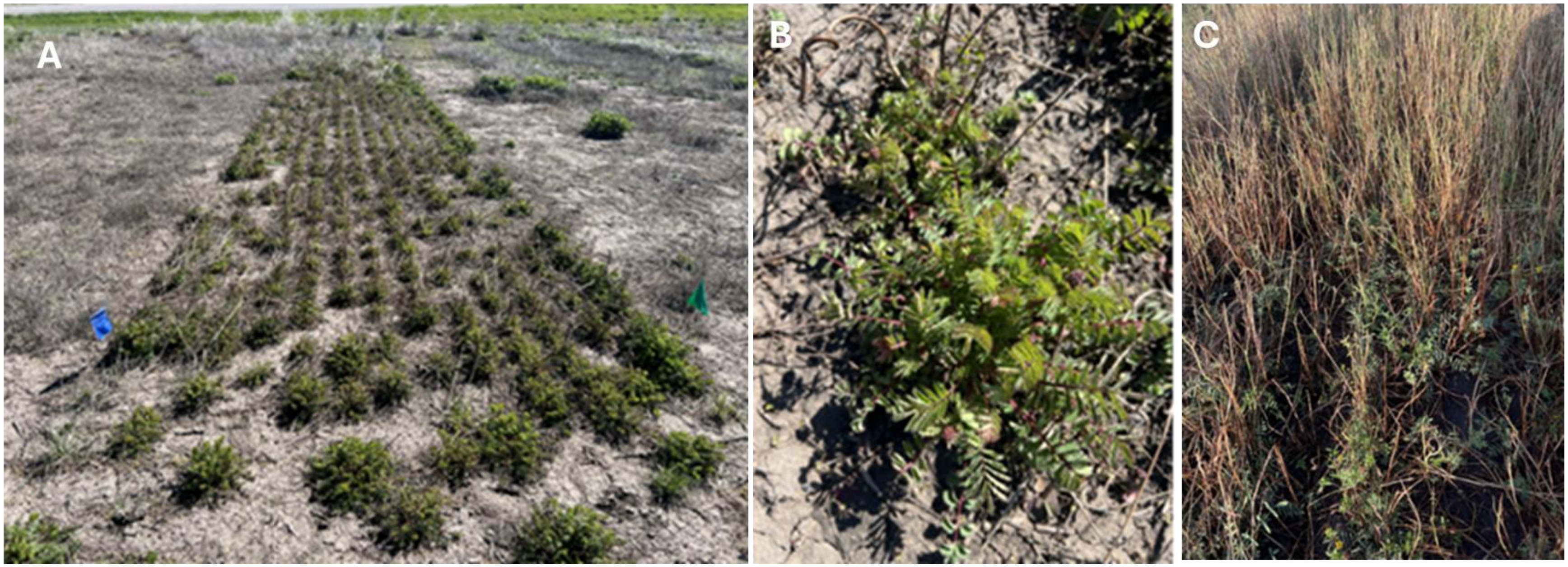

Figure 3.10. Dense plant stand of small burnet (A), vigorous vegetative growth of individual small burnet plants (B) in May 2024, and senescent sainfoin plant stand commencing new growth in July 2024 (C), at Clarkston

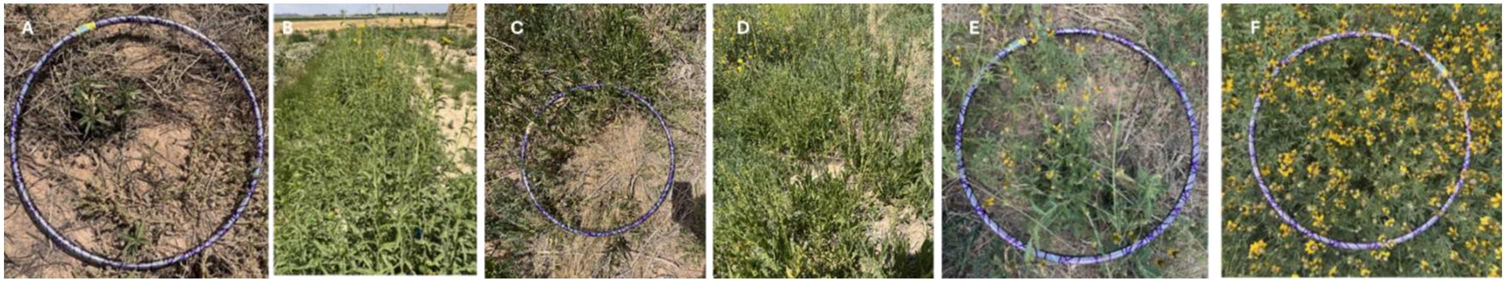

Figure 3.11. Comparison in plant growth and density of unirrigated (left) vs irrigated (right) plots of Maximillian sunflower (A & B), Rocky Mountain penstemon plots (C & D), prairie coneflower (E & F) at Cedar City during July 2024. Here, the area under the hoop represents the whole plant density in an unirrigated plot

Figure 3.12. Prairie coneflower (A) and Palmer’s penstemon (B) exhibited erect growth, Utah sweetvetch (C), and birdsfoot trefoil (D) exhibited semi-erect growth capturing different numbers of plants multiple plants in a small-sized hoop at Cedary City, 2024

Figure 3.13. Single cicer milkvetch plant exhibiting decumbent growth captured in small size hoop at Cedar City

101 Lewis Flax	201 Purple P. Clover	301 Prairie Aster	401 Falcata Alf	501 Showy Goldeneye	601 Lead Plant
102 Showy Goldeneye	202 Small Burnet	302 Canada Milkvetch	402 F. Biscuitroot	502 Rocky Mtn Penst	602 Western yarrow
103 Canada Milkvetch	203 Prairie Coneflower	303 Smooth Aster	403 Max Sunflower	503 Western Yarrow	603 Small Burnet
104 White P Clover	204 Falcata Alf	304 Ladak Alf	404 Sainfoin	504 A. Balsamroot	604 Crownvetch
105 Palmers Penst	205 Ladak Alf	305 Louisiana Sage	405 Falcata Alf	505 Smooth Aster	605 Louisiana Sage
106 Prairie Aster	206 Prairie Aster	306 Lewis Flax	406 Sinfoin	506 Canada Milkvetch	606 Lead Plant
107 Smooth Aster	207 Lewis Flax	307 BFT	407 A. Balsamroot	507 Sainfoin	607 Purple P Clover
108 Rocky Mtn Penst	208 Louisiana Sage	308 Blanket Flower	408 Max Sunflower	508 BFT	608 Ladak Alf
109 A. Balsamroot	209 Utah S-vetch	309 Palmer penstemon	409 Prairie Coneflower	509 F. Biscuitroot	609 Blanket flower
110 Max Sunflower	210 Purple Clover	310 Cicer Milkvetch	410 F. Biscuitroot	510 Cicer Milkvetch	610 Utah S-vetch
111 Cicer MV	211 Prairie Clover	311 Palmer Penstemon	411 Rocky Mtn Pents	511 Lead Plant	611 Western Yarrow
112 Crownvetch	212 BFT	312 Blanket flower	412 Utah S-vetch	512 White P Clover	612 Small Burnet
113 Crownvetch	213 White P Clover	313 Showy Goldeneye	413 Purple P Clover	513 Canada Milkvetch	613 Max Sunflower

Figure 3.14. Plots receiving irrigation (in blue) on the western side of the plot area at Cedar City.

CHAPTER IV

ASSESSMENT OF TEMPORAL TRANSITION IN FORAGE QUALITY AND SECONDARY METABOLITES IN LEGUME, FORB, AND GRASS SPECIES

Introduction

In the Western United States, the ability of grazing ruminants to meet their nutritional requirements depends on the nutritional profile of species growing on rangelands throughout the year. Like other grasslands, these rangelands are dominated by grass species. Grasses, including crested wheatgrass, peak in forage quality during spring and early summer followed by a decline during late summer and early fall (Cook & Harris, 1952; Ganskopp & Bohnert, 2001), eventually falling below the physiological requirements of grazing cattle as the season progresses (Hersom, 2020). This decline in forage quality has a negative impact on cowherd performance. To compensate for a nutritionally deficient grass monoculture during the late season, costly supplements are often provided to cattle to ensure adequate nutrition.

Forage quality encompasses a feed's nutritional value, palatability, and digestibility, all of which determine livestock performance (Buxton, 1996). Crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), and lignin are key indicators of forage quality. Crude protein refers to protein concentration and is estimated by nitrogen content in plants, using the equation, crude protein = nitrogen × 6.25. This factor is derived from the nitrogen content of the predominating protein present in different foods (Merill et al., 1955). Acid detergent fiber and NDF are the fibrous portions of the plant, defined based on the detergent analysis system of van Soest et al. (1991). ADF measures the lignified cellulose content of cells and is negatively related to digestibility. NDF quantifies hemicellulose, lignin, and cellulose and is negatively related to intake potential due to physical fill limitations (Buxton, 1996; Stokes & Prostko, 2024). Lignin is the most indigestible fibrous fraction in plant cell walls. While animals can digest cellulose, high lignin content in forages hinders the digestibility by forming complex bonds with cellulose. Overall, a high fiber content is undesirable for digestibility and feed intake (Stokes & Prostko, 2024).

The environmental conditions, plant maturity, and their interaction play crucial roles in determining forage quality (Nelson & Moser, 2015). Because leaves contain more cell content including protein than stems, a decreasing proportion of leaves with plant maturity and higher temperature is responsible for the decline in forage quality (Buxton, 1996; Nelson & Moser, 2015). Furthermore, lignin synthesis is preferentially increased with rise in temperature making the forage less digestible (Nelson & Moser, 2015).

In addition to the transitions in nutrient composition of different species at different times, the nutritional requirements of cows also change depending on their physiological conditions (Vavra & Raleigh, 1976). All stages of the life cycle of cattle have protein and energy requirements beyond maintenance to support growth, gestation, and lactation (Hersom, 2020). Herbaceous perennial forages and forbs generally begin growth in spring, go to seed in early summer, and mature in late summer and autumn as they fill seed and accumulate carbohydrates and proteins to support spring growth the following year. During periods of high energy demand for cattle, such as gestation and lactation, grass-dominated rangelands are becoming increasingly mature, and are generally insufficient to meet the nutritional requirements of ruminants. At these stages, cattle require increased forage intake to meet higher caloric needs, but if the crude protein concentration of forages is insufficient to support rumen microbial growth, the rate of digestion of low-quality forages is slow and forage intake is constrained. Supplementing mature, low-protein grasses with costly protein supplements addresses this problem, but another way to address the imbalance between the forage nutritional profile and dietary requirements of cattle throughout the year (Adams et al., 1996) would be to provide alternative forages with high nutritive value, such as leafy green legumes or forbs.

According to Hersom (2020), a crude protein concentration of 7.5% or greater is adequate to provide a mature cow with sufficient protein, but grass monocultures do not meet the minimal protein threshold during the late season. This could be addressed by incorporating a diversity of protein-rich forbs and legumes on rangeland that retain green foliage during late summer and fall. It would allow ruminants to select a nutritious diet. This notion was previously supported by Provenza et al. (2003), suggesting that herbivores have evolved to prefer grazing on a diversity of plant species, likely due to the nutritional benefits inherent in diverse diets compared with plant monocultures. Grazing cattle can select plants that, while possibly less nutritious individually, may include beneficial nutrient composition and enhance overall cattle nutrition. This strategy has the potential to support efficient, lower-cost beef production throughout the year.

One salient issue among the key challenges in sustainable beef production systems is the alarming levels of methane emission attributed to ruminants. Methane is released as a byproduct during fermentation in rumens that is facilitated by methanogens. Methane has 28 times higher greenhouse warming potential than CO_2 (USEPA, 2013) and its relatively short lifespan in the atmosphere makes it an effective target for emissions reduction strategies aimed at mitigating environmental impacts (Króliczewska et al., 2023; Muller & Muller, 2017). In 2020, beef and dairy cows were the largest sources of methane (CH_4) emissions in the livestock sector, responsible for 72% of total emissions (FAOSTAT, 2022). Enteric fermentation alone accounted for 88% of global methane emissions from livestock (FAO, 2017), and about 28% of anthropogenic methane emissions (Cleveleland, 2025).

The challenge of inefficient nitrogen utilization in ruminants arises when rumen microbes cannot utilize the excessive protein in their diets and excrete a high proportion of nitrogen intake as urea in urine or milk. Increasing the amount of protein that escapes enteric fermentation in the rumen and reaches the small intestine can reduce ammonia formation and change the fate of nitrogen excretion from urine to feces (Jonker & Yu, 2016), thereby increasing organic matter in the soil.

Synthetic treatments such as glycerol and nitrate supplements are usually administered to ruminants to improve rumen fermentation and reduce methane emissions. However, synthetic supplements raise consumer concerns about chemical residues in animal products (Aerts et al., 1999). Therefore, there is increasing demand from policymakers and consumers for improved food quality and a reduced environmental footprint in beef production, underscoring the need for the adoption of sustainable, non-pharmaceutical approaches to beef and dairy production. Among the various approaches are dietary modifications, feed additives, selective breeding, and genetic approaches (Buddle et al., 2011). The incorporation of biological feed additives such as secondary metabolites has emerged as a promising strategy for mitigating methane emissions (Króliczewska et al., 2023).

Tannins are high molecular weight polyphenols that are defined by their ability to form complexes with proteins in a pH-dependent manner (Hagerman et al., 1992). Tannins also possess antiparasitic and anti-methanogenic properties, providing natural protection to grazing ruminants (Besharati et al., 2022). A linear reduction in ammonia production in rumen and in methane emissions was observed with increasing dietary condensed tannin concentration (Williams et al., 2011). Reduced ammonia production may be attributed to the protein precipitation property of tannins reducing protein degradation in the rumen, and reduced methane emissions may be due to an alteration in the composition of the microbial community, reducing fermentation in the rumen. Tannins can also lead to a shift in fermentation end products favoring propionate and valerate that utilizes H^+ over H^+-producing acetate and butyrate synthesis, or to direct suppression of the growth of methanogenic bacteria (Williams et al., 2011).

Tannins are categorized into (1) hydrolysable tannins (HT) or (2) condensed tannins (CT), and some complex tannins also exist as combinations of these basic structures (McSweeney et al., 2001). Hydrolyzable tannins are polyphenolic compounds where a gallic acid is esterified to a central polyol, typically glucose. The oxidative coupling of galloyl groups converts gallotannins into ellagitannins. Condensed tannins are widely known as proanthocyanidins (PA), produced through condensation of flavan-3-ol subunits (Hagerman, 2011). The most frequently occurring flavan-3-ol subunits include catechin, epicatechin, gallocatechin, and epigallocatechin. Catechin and epicatechin are referred to as procyanidin (PC) subunits because, upon oxidation, both give rise to cyanidin. Similarly, gallocatechin and epigallocatechin are referred to as prodelphinidin (PD) subunits because, upon oxidation, both give rise to delphinidin.

Proanthocyanidins (PC) are typically formed by the polymerization of PC and PD subunits through interflavan linkages (Salminen, 2018). Typically, the PAs that comprise PC tend to be mixtures of oligomers and smaller polymers, and PAs comprising PDs are usually mixtures of larger polymers (Mueller-Harvey et al., 2019). The heterogeneity of PA should be considered, because PA from different plant species may vary in response to nutrient availability and utilization by ruminants, even at the same concentrations.

Contrary to early views of an antiherbivore role for tannins, including for tannin-containing forages grazed by ruminants, current knowledge is built on demonstrated beneficial effects on ruminant nutrition and environmental sustainability (Mueller-Harvey et al., 2019). Tannin-rich forages in ruminant diets help naturally address challenges with beef and dairy production by limiting protein deamination and ammonia release in the rumen, enhancing protein digestion in the small intestine, and preventing parasite infections, without relying on synthetic chemicals (Aerts et al., 1999; Besharati et al., 2022; Naumann et al., 2017). An adequate concentration of CT can exert positive effects such as better nitrogen utilization, reduced methane emission, and anthelmintic activity. The role of CT in eliminating bloat in cattle grazing nutrient-dense legumes, and in partitioning dietary nitrogen to feces from urine, also benefits cattle, consumers, and the environment. This study was conducted to evaluate the transition in forage quality and secondary metabolites in perennial legumes and non-legume forbs from June to October under semi-arid rangeland conditions. We hypothesized that a selection of the legume and non-legume forage species recommended for rangeland ruminants would provide significantly greater concentrations of protein and secondary metabolites to support sustainable beef production as compared to traditional grass species on rangelands, especially during late summers and fall

Materials and Methods

Greenhouse study (unreplicated)

Out of 27 species planted in the field for establishment evaluation, 24 were planted in single one-gallon pots in the greenhouse (GH) as validation plants. This was not designed as a replicated study. The greenhouse environment, inhospitable for two species (arrowleaf balsamroot and fernleaf biscuitroot), and kura clover were only selected for field evaluation at Panguitch, during 2023, leaving 24. Species that became established in the greenhouse were harvested multiple times, at the flowering stage except for crested wheatgrass, white sagebrush, Palmer’s penstemon, Rocky Mountain penstemon, Maximillian sunflower, small burnet, smooth blue aster, and western yarrow, because greenhouse conditions did not meet their vernalization or daylight requirements. Plants that remained vegetative were harvested when foliage created a closed canopy. Harvested foliage was frozen, freeze-dried, composited for all dates, milled and used in preliminary studies of forage nutritive value, tannin type and concentration, and in vitro rumen fluid digestion.

Field study (replicated)

Experimental Design and Plant Establishment

This study used a randomized complete block design with five blocks (replications). It was conducted under dryland conditions at the Godfrey Research Farm, Clarkston, Utah (lat. 41.895°, long. 112.048, elev. 1506 m). The soil was a Mendon fine silty, (mixed, mesic Calcic Pachic *Argixerolls*) and was amended by applying 42 $kg \cdot ha^{-1}$

P_2O_5, 20 kg·ha^{-1} N, 25 kg·ha^{-1} S, and 13 kg·ha^{-1} Fe per soil test recommendations. In addition, 67.25 kg·ha^{-1} of the Basin and High Plains Suite mycorrhizal inoculant providing approximately 1,500,000 living propagules ha^{-1} (Reforestation Technologies International, Gilroy, CA, USA) was applied to improve nutrient uptake.

Within each block, 24 species including legumes, forbs, and the grass species crested wheatgrass were randomly assigned to plots (experimental units) that were 2.44 x 29 m (8 × 95 ft.). A 37 x 61 m (120 × 200 ft.; 0.55 acre) sward of crested wheatgrass was planted on the northern end of the plot area. The seed of species with hard seeds, determined by informal germination tests, were sacrificed, and legume seeds were treated with the specifically required rhizobium strain (Exceed Inoculant; Vision Biologics, Henrietta, TX) before planting. Planting was carried out in November 2021 (plots were replanted in May 2022) after cultivating in fertilizers and mycorrhizae and creating a firm seed bed using a Great Plains 8-ft. no-till drill (Model 3P806NT) equipped with coulters, double disk openers and press wheels (Great Plains Ag., Salina, KS) at a seeding rate of one million pure live seed per acre. The row spacing was 6 inches (15.24 cm). However, due to a lack of germination of seeded plant species the following spring, plots were replanted in early May of 2022. Among the original species selected, no commercial seed was available of three species (basalt milkvetch, Searle’s prairie clover, and western prairie clover), so these species along with leadplant were replaced with Canadian milkvetch, fernleaf biscuitroot, Maximillian sunflower, and showy goldeneye. All other species were replanted at the Godfrey farm in the originally assigned plots.

Plant Species Selection

Selection of species established under dryland growth conditions for forage quality analysis from mid-summer into fall was based on their previously reported nutrient profile and establishment potential. Forage samples were collected for species including ‘Ladak’ alfalfa, birdsfoot trefoil, sainfoin, and small burnet during 2023. In addition to these, in 2024, showy goldeneye, Utah sweetvetch, crested wheatgrass and falcata alfalfa samples were also collected for forage quality and yield estimation owing to their robust establishment that was evident during 2023. Additionally, to address differences in protein concentration with the advancement of the season, Ladak alfalfa and sainfoin samples were also collected for estimation of change in leaf and stem dry matter proportion. Harvesting occurred monthly, during the first or second week of each month, from June through October of both years (Table 4.1). Crested wheatgrass failed to establish as part of the randomized complete block study, and also initially failed to establish in the northern sward area from plantings in 2021 and 2022 but formed a stand in 2023 on the north end of the study area. Monthly crested wheatgrass samples were collected from July to October in 2024 from this stand.

Harvest

All forage samples were harvested, collected in zip-lock bags, and forage quality samples were immediately covered with ice packs and stored in coolers for transport, then stored at -20 °C until freeze-dried. Leaf-stem samples were refrigerated until leaves and stems could be separated. Samples were freeze-dried using a Labconco freeze dryer at 0.120 mBar vacuum and -50 ºC until samples reached room temperature, in two to four days depending on the moisture present in the plant samples. Dry matter yield samples were harvested using a quadrat of 0.1073 m^2. These samples were dried after harvesting in a 60 °C oven until a constant weight was reached in two to three days. After drying, samples were milled to pass the 2-mm screen of a UDY cyclone mill, or the 1-mm screen of a Willey Mill.

Forage quality evaluation

Milled legume and forb samples were scanned with a scanning monochromator (Model DS2500F, FOSS NIR Systems Inc.) to predict sample composition including crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin. The calibration equation available from the NIRS Consortium (NIRS Forage and Feed Testing Consortium, Hillsboro, WI, USA) for legume hay was used for legume species, the mixed hay equation was used for non-legume species, and the grass hay equation, which is calibrated using various C_3 grasses, was used for crested wheatgrass. Selection of the NIRS equations for predicting the forage quality parameters was based on global H (GH) and neighborhood H (NH) values. A smaller value for these parameters indicates closer alignment of sample with the calibration dataset. Alfalfa and sainfoin were included for calibrating the legume hay equation. Crested wheatgrass and other cool-season C_3 grasses were used for calibration of grass hay equation. So, these equations were applied to their respective species. Since small burnet was not represented in any of the calibration datasets, the mixed hay equation was chosen for this species based on its lower values for GH and NH, indicating a better suitability in predicting the parameters of forage quality. The GH values indicate the distance measure to the average spectrum of the calibration set and NH is a distance measure to the most similar spectrum in the calibration set. In a general sense, a global H value <3 is considered acceptable; however, higher GH and NH values do not exclude use of an equation to predict forage quality parameters. The GH and NH values obtained using the above-described equations for sample screened using NIRS, collected from Clarkston, 2023, are presented in Table 4.2. Creating credible new nutritive value equations for a plant species requires many plants from many locations and stages of growth, which was not feasible at this stage of a screening study.

Since the NIRS equations used for predicting a wide range of forage quality parameters were not specifically calibrated for all the species under study, we expected predictions to be accompanied by higher GH and NH values. Therefore, we chemically determined crude protein of field samples using an elementar combustion analyzer (Elementar Americas, Ronkonkoma, NY) to measure N and calculate crude protein, and we determined amylase-assisted NDF (aNDF) concentration for field grown and greenhouse grown species using the standard protocol for ANKOM A200 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA). These are methods used for the development of NIRS calibration equations.

Condensed tannin

All 24 species grown under GH conditions were analyzed for total CT. Forage samples of Ladak alfalfa, birdsfoot trefoil and sainfoin collected in 2023 and 2024 and crested wheatgrass, falcata alfalfa, showy goldeneye, Utah sweetvetch collected in 2024 from the Godfrey Farm were analyzed for total CT using the butanol-HCl-acetone method of Grabber et al. (2013). Tannin assay solution contained 0.15% w/v ammonium iron (III) sulfate dodecahydrate, 3.3% v/v water, 5% v/v concentrated HCl, 41.7% v/v butyl alcohol, and 50% v/v acetone. Briefly, triplicate 0.030 g dry matter of ground plant tissue was suspended in 15 mL of tannin assay solution and heated for 2.5 h in a 70 °C water bath. The samples were mixed periodically during heating. Standard, blank solutions and check samples were included in each run. After tubes cooled, they were centrifuged at 5,000 *x g* for 10 minutes and the absorbance of the supernatant was determined at 554 nm. The CT concentration was determined by using birdsfoot trefoil standard curve for all species except sainfoin where sainfoin standard curve was used.

Tannin standards for the spectrophotometric assay were isolated from samples of the tannin-containing plant material being assayed based on Hagerman (2011). Briefly, a suspension of 10% w/v finely ground plant material in 1% v/v acetic acid, 24% v/v water and 75% v/v acetone were sonicated for 30 minutes with periodic mixing. Mixtures were centrifuged for 10 minutes at 3000 x g and the supernatant filtered through a coarse fritted disk; plant material was extracted a total of three times and supernatants combined. The supernatant was mixed with an equal volume of ethyl ether and the aqueous layer was retained; the supernatant was extracted a total of three times with equal volumes of ethyl ether. The acetone and ethyl ether remaining in the aqueous solution were removed by rotary evaporation. The aqueous solution was mixed with Sephadex LH 20 resin equilibrated in a 4:1 v/v ethyl alcohol:water solution, rinsed with 95% ethyl alcohol, and extracted with a 3:1 v/v acetone:water solution. The acetone was removed by rotary evaporation, and the aqueous solution was frozen and freeze-dried.

Hydrolysable tannin

Twenty-four species grown under GH conditions, the small burnet samples collected in 2023 and 2024, along with it, showy goldeneye and Utah sweetvetch collected in 2024 at Clarkston were evaluated for total HT concentration following the protocol of Hartzfeld et al. (2002). Triplicate 0.1-g samples were weighed into 16 x125 mm (15-mL) Pyrex screw top tubes with Teflon cap liners to which 4 mL methanol and 0.4 mL concentrated sulfuric acid were added. Tubes were heated at 85 °C for 20 h for methylation. After cooling and centrifugation, the pellet was rinsed three times and discarded, and 0.4 mL ethanolamine was added to the supernatant followed by 1 mL 3.7 M ammonium acetate. The pH of each sample was adjusted to 5.5 and the final volume was brought to 10 mL. One set of 0.2 mL aliquots of samples, blanks, and standards was used for tannin assay, and a second set for background measurements. Two mL of methanol and 0.7 mL of 0.3 N HCl were added to the background set while 2 mL of methanol and 0.7 mL of water was added to the tannin assay set. Then 0.08 mL of a 5% potassium iodate solution was added to each tube and absorbance was read at 525 nm after 50 to 60 min incubation at 30 °C. Samples were read against methyl gallate standards of 5, 10, 15 and 20 mg and a 4 mg tannic acid check was included with each run.

Polyphenolic profiling of samples

Chemicals

UHP-LC-MS-grade acetonitrile (ACN), dimethyl sulfoxide (DMSO), methanol (MeOH), tert-butyl methyl ether (MTBE), formic acid (FA) and water (H2O) (all, Supelco LiChrosolv®) were ordered from Sigma-Aldrich (St. Louis, MO, USA). Non-labeled standards of compounds and stable-isotopically labeled (IS) standards including 4-hydroxybenzoic acid-13C6, apigenin-D5, benzoic acid-D5, genistein-D5, phenol-13C6, quercetin-D3, and tryptamine-D4 were purchased from Sigma-Aldrich (St. Louis, MO, USA) and/or Cayman Chemical (Ann Arbor, MI). All individual compounds were dissolved at a stock concentration of 1 to 10 mM in the reagents mentioned (or a combination thereof) depending on their solubility. All compounds were optimized one by one by direct infusion after having been diluted to 1 µM) to determine optimal MS-parameters, such as declustering potential (DP), collision energy (CE), cell exit potential (CXP), and Q3-transitions.

Sample extraction

For extractions, 0.027 to 0.033 g of ground plant samples of 24 greenhouse grown species and Ladak alfalfa, birdsfoot trefoil, sainfoin, and small burnet, collected montly from Clarkston in 2023 were weighed out in of 2 mL tube (02-681-375, Thermo Fisher Scientific Waltham, MA). A 5 mm stainless steel bead and 1200 µl of pre-cooled (-20 °C) internal standard spiked extraction solvent containing MTBE:MeOH (2:1, *v*:*v*) was added to each sample, after which they lysed at 30 oscillations/sec for 5 min (Qiagen Retsch Tissue Lyser II, Germantown, MD). Samples were subsequently sonicated in an ice-cold water bath for 30 minutes and stored at -80 °C for 4 hours to facilitate protein precipitation. The samples were mixed with 750 µl of water, vortexed, and centrifuged at 18,000 rcf for 10 min at 4 ˚C to induce phase separation. The MTBE layer was removed and 1000 µl of the lower phase was collected in a new set of tubes, which represents the free phenolics. The protein pellet was subsequently washed with 1000 µl of MeOH, and 1 mL of internal standard spiked 2M NaOH was added to the protein pellet and shaken for 16 h in a water bath at 30 ° C for alkaline hydrolysis. Thereafter, the hydrolyzed sample that contained the 'bound' phenolics was acidified to pH 3 with concentrated formic acid. Samples were then filtered (pore size, 0.45 µm) and subjected to solid phase extraction. Samples were loaded into a Strata-X-Pro 96-well plate (Phenomenex, Torrance, CA, USA), washed with 1000 µl of water in 0.1 % formic acid, and eluted using 1000 µl of

MeOH in 0.1 % formic acid. Both the free and bound samples were subsequently evaporated using brief nitrogen drying (40 min at 30°C), followed by drying using a Speed Vac concentrator (1 h at 30°C). Samples were resuspended in 200 µl of H_2O:MeOH (1:1, *v*:*v*) in 0.1% formic acid and subjected to UPLC-MS/MS analysis.

LC-MS/MS conditions

Compounds were simultaneously detected as precursor ion/product ion pair using multiple reaction monitoring (MRM) using UPLC-MS/MS. The platform utilized an SCIEX Hybrid Triple Quad 7500 (Framingham, MA) with a front-end Shimadzu Nexera 40 Series (Kyoto, JP) liquid chromatography system. The sample extracts were kept at 4°C in an auto-sampler and compounds were separated at 30°C using a reverse phase Kinetex F5 100A column (2.1 mm x 150 mm, 2.6µM) column from Phenomenex (Torrance, CA) with binary mobile phases of water (A) and acetonitrile (B), both containing 0.1% formic acid (*v*:*v*). Samples were run in both negative and positive electrospray ionization mode mixture of purified standards of target compounds was injected using an unscheduled method to determine their retention times for the scheduled method. The following source parameters were used in negative mode: 1,600 V for the ion spray voltage, 550°C for the temperature, 40 psi for the curtain gas, 40 psi for the nebulizer gas (GS1), 60 psi for the heating gas (GS2). In the negative mode, the linear gradient consisted of an initial composition of 5% B for 2.1 min with a flow rate of 0.2 $mL{\cdot}min^{-1}$, which was ramped up gradually to 95% B and a maximum flow rate of 0.46 mL/min over 14 min to keep a constant pressure, prior to being switched to 5% B for the final 4 minutes with a minimum flow rate of 0.175 $mL{\cdot}min^{-1}$. The following source parameters were used in positive mode: 2,000 V for the ion spray voltage, 550°C for the temperature, 40 psi for the curtain gas, 40 psi for the nebulizer gas (GS1), 60 psi for the heating gas (GS2). In the positive mode, the linear gradient consisted of an initial composition of 5% B for 2.1 min with a flow rate of 0.2 mL/min, which was ramped up gradually to 95% B and a maximum flow rate of 0.46 ml/min over 14 min to keep a constant pressure, prior to being switched to 5% B for the final 4 minutes with a minimum flow rate of 0.175 $mL \cdot min^{-1}$. In both modes, the cycling time in the scheduled method was set to 1000 msec and the dwell time ranged from 3 to 250 msec depending on the number of MRMs triggered. Double blank (100 % methanol) and blank internal standard samples ran every 20 samples for quality control purposes.

Data analysis

Sciex OS 3.1 software (AB Sciex, Framingham, MA) was used to acquire and analyze chromatographic data. Peaks were integrated using area-under-the-curve and normalization was performed using isotopically labeled standards to account for any loss of material during sample preparation. Unlabeled external standard mixes ran in parallel to the samples with known concentrations (ranging from 6250 nM to 0.38147 nM using a 1-fold serial dilution) to allow for the quantitation of compounds.

Statistical analysis

The results for greenhouse-grown plants were not analyzed statistically as it was an unreplicated study that was conducted for validation of field-grown species. All statistical analyses of the field study were done in SAS studio 3.81 (2024) using PROC GLIMMIX and a mixed model. All species, five months, and their interactions were included as fixed effects, and five replications, replication and species interaction, and month within each species and replication were included as random effects. Tukey-

Kramer's method was applied to test pairwise differences among treatment groups, adjusting for multiplicity. The level of significance was kept at 0.05. PROC PLM sliced the LSMeans by combinations of species and months to generate visual contrasts. The total concentration of CT and HT measured using spectroscopic assays were assigned to specific subunits of CT and HT detected in samples analyzed by UHPLC-MS/MS for both greenhouse and field study.

Further, MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/) was used for performing partial least squares discriminant analysis (PLS-DA) to reduce variability and produce strong and interpretable models. The scores plot used component 1 for the x-axis and component 2 for the y-axis, displaying 95% confidence intervals. In addition, a hierarchical, ranked clustering heatmap was created to provide visualization of variability among the top 50 compounds responsible for variability among species and over months. For the heatmap, Pearson distance measure and Ward clustering was performed, and data was reported using the PLS-DA variable importance in projection (VIP) score.

Results

Greenhouse study

Forage quality

Crude protein, NDF, ADF, and lignin, concentrations (Fig. 4.1) were determined by NIRS for 24 unreplicated plant species grown under well-watered conditions in the greenhouse, harvested repeatedly at the early flowering stage of growth, and composited over time. Samples were analyzed to evaluate the potential forage quality and secondary metabolites of the species under study. Additionally, since many of the plant species were not included in the calibration of the NIRS Consortium equations, which were developed

for forages, NDF concentrations were also evaluated chemically using an Ankom Fiber Analyzer (ANKOM, Macedon, NY). Global H and Neighborhood H values calculated by the NIRS software are used to assess the degree to which a sample falls within the calibration equation that was used. The more these values exceed 3, the less accurate the data may be. Interestingly, the value predicted by the legume hay equation was more divergent from the Ankom value for alfalfa aNDF than many of the other species.
Based on NIRS, Canadian milkvetch, birdsfoot trefoil, falcata alfalfa, Ladak alfalfa, white prairie clover, and crownvetch contained >20% crude protein. Crested wheatgrass, falcata alfalfa, leadplant, Maximillian sunflower, prairie aster, purple prairie clover, prairie coneflower, western yarrow, white sagebrush, and white prairie clover contained a higher fibrous fraction exceeding 25% NDF and 22% ADF). Even in the vegetative stage, crested wheatgrass contained nearly 45% NDF concentration, the greatest among plant species tested based on both NIRS and chemical assay. The comparison of NDF concentration obtained from chemical assay using the Ankom fiber analyzer and NIRS is shown in Figure. 4.2.

Condensed Tannins

Total CT concentrations and proportion of CT subunits in 24 unreplicated species grown in the greenhouse are shown in Figure 4.3A. Based on the greenhouse study, the species that have CT content of interest were birdsfoot trefoil, crownvetch, leadplant, sainfoin, Utah sweetvetch, and white prairie clover. Based on the literature, only leadplant and Utah sweetvetch have not been discussed as valuable due to their CT content. We are also aware of literature reports of purple prairie clover containing CT (Peng et al., 2018), but the plant material we tested had a low concentration when a birdsfoot trefoil standard was used. All CT-rich species contained PC as the major CT subunit, although a few species contained smaller fractions of PD-rich CT, including Utah sweetvetch (35.06% PD), sainfoin (17.04% PD), and white prairie clover (9.57% PD).

Hydrolysable Tannins

Total HT concentration and distribution of different HT in all 24 species grown in the greenhouse is illustrated in Figure. 4.3B. Species of interest for their HT concentration are small burnet, showy goldeneye, western yarrow, smooth blue aster and white sagebrush. Most of the species containing HT had higher concentrations of ellagic acid than gallic acid and methyl gallate. However, gallic acid was detected in leadplant, Rocky Mountain penstemon, cicer milkvetch, blanketflower, and purple prairie clover.

Metabolic profiling

A PLS-DA model captured 71.7% of the total biochemical variation (51.6% due to component 1 and 20.1% due to component 2) highlighting the strong metabolic differentiation among species (Fig. 4.4) The score plot illustrates the distribution of species based on secondary metabolites accumulation. The axes represent the variables (component 1 and component 2) responsible for causing the most variance in dataset (Fig. 4.4A). The biplot overlays the results of score plot with vectors, representing individual metabolites. This represents the ten most pivotal compounds in separating the species along component 1 and component 2 (Fig. 4.4B). The direction of arrows suggests the increasing concentration of particular metabolite in that direction and the length of arrows points toward its influence on PLS-DA components. Based on the score plot and biplot, it can be inferred that the grey eclipse indicates accumulation of ellagic acid and chlorogenic acid in small burnet. These are the major metabolites differentiating small burnet from other species. Likewise, the spread of the purple and red eclipses in same direction for prairie aster and prairie coneflower, respectively, indicates higher accumulation of quercetin in these species. The yellow eclipse likely indicates Schaftoside (a flavonoid C- glycoside) richness in blanketflower and leadplant. All other species are pulled tight and have overlapping metabolic profile.

Further, the heat map illustrates the hierarchical clustering of the top 50 metabolites differentiated by species (Fig. 4.5). Palmer's penstemon was found to be rich in flavonoids, phenolic acids, and coumarins. Small burnet was found to be rich in both condensed and hydrolysable tannins. Purple prairie clover contained a high concentration of condensed tannins (procyanidin B1, B2, and C1) and kaempferitin. Birdsfoot trefoil contained high concentration of phenolic acids and flavonol. Further, blanketflower, crownvetch, leadplant, white sagebrush, prairie aster, rocky mountain penstemon, and Utah sweetvetch accumulated higher overall concentrations of metabolites compared with other species.

Field Study

Climate data

Temperature, precipitation, and evapotranspiration data for Clarkston, UT for 2022 (planting year), 2023, and 2024 (Fig. 4.6) were provided by the Utah Climate Center, Climate Database Server, which reports daily evapotranspiration estimated by the ASCE standardized Penman-Monteith method (ASCE-EWRI, 2005).

The total annual precipitation for the two years when plants were sampled was 254 mm (9.99 inches) in 2023, and 185 mm (7.26 inches) for 2024. The average maximum temperatures were 13.18 ºC, and 16.41 ºC and the average minimum temperatures were -1.06 ºC and 0.64 ºC in 2023 and 2024, respectively.

2023

Forage quality

The panels in Figure. 4.7 show differences in a forage quality parameter among the four species within each month and among months for each species from June through October.

Crude Protein and Neutral Detergent Fiber

The results of chemical assay for crude protein using the Elementar Combustion Analyzer and NDF using the Ankom Fiber Analyzer are presented in Figure. 4.7A and Figure. 4.7B, respectively. For all four species, crude protein decreased and NDF increased as standing, unharvested plants continued to mature from June through October. The month-to-month pattern in crude protein differed among species as indicated by significant species x month interaction ($p = 0.0007$; Fig. 4.7A) Alfalfa contained the highest crude protein content among all the species throughout the season ($p < 0.0001$), exhibiting a peak at 15.6% during June. This concentration significantly declined by 24.36% in July ($p < 0.0001$) and a further 20.34% in August ($p = 0.01$), remaining stable thereafter at ~ 9%, surpassing the crude protein requirements of ruminants (7.5%) even during the late season when grass goes into dormancy. Crude protein concentration did not differ between birdsfoot trefoil and small burnet throughout the season. Sainfoin exhibited significantly lower crude protein compared to birdsfoot trefoil in August. Birdsfoot trefoil and small burnet exceed minimum crude protein threshold up to June, whereas sainfoin exceeded this threshold up to July. Although birdsfoot trefoil and small burnet are not statistically different from sainfoin in July (p = 0.09 and p = 0.1, respectively), sainfoin falls below this threshold in July. (Sainfoin started with 10.5% crude protein in June followed by a significant decrease of 45.88% in August, resulting in a crude protein concentration of only ~5% from August to October. Birdsfoot trefoil started with a concentration of 9.8% in June and exhibited a significant 37.76% decline in July, maintaining stable concentration thereafter. Small burnet exhibited a significant decline of 27.08% from 8.42% crude protein in June to 6.14% in July. All species met the minimum protein requirement (7.5%) for cattle during June, whereas only alfalfa exceeded this threshold through October.

The significant species x month interaction (p = 0.0014) indicated month-to-month pattern of NDF concentration differed among species (Fig. 4.7B). The concentration of NDF was greater in alfalfa and sainfoin as compared to birdsfoot trefoil from July through October. Further, alfalfa and sainfoin contained similar NDF concentrations, both exceeding 50% after July, while birdsfoot trefoil and small burnet maintained NDF levels around 40 - 42% from August onward. A significant increase of 51% in NDF concentration was observed in alfalfa from June to July. For sainfoin, a significantly increase was observed in July (71%) and in August (27%). In birdsfoot trefoil, the NDF concentration increased by 33% from July to August. Small burnet exhibited a gradual but significant increase from 32% in June to a peak of 42% in August.

ADF and Lignin

Statistical analysis of NIRS data for lignin and ADF revealed a highly significant increase in both parameters in all species with the advancement of the season (p < 0.0001; Fig. 4.7C & Fig. 4.7D, respectively). Alfalfa and sainfoin contained a high concentration of lignin during all months (Fig. 4.7C) and higher ADF as compared to birdsfoot trefoil during July ($p < 0.0001$), September ($p < 0.0001$) and October ($p = 0.03$ and $p = 0.0001$, respectively; Fig. 4.7D). In alfalfa maximum ADF and the lignin was recorded during September and in sainfoin during August. In alfalfa, the ADF and lignin concentrations significantly increased by 36% and 69%, respectively in July and by 28% and 48%, respectively in September. Similarly, in sainfoin, the ADF and lignin concentrations significantly increased by 45% and 77%, respectively in July. In small burnet the ADF and lignin concentration significantly increased by 44% and 48%, respectively in July. Birdsfoot trefoil maintained lower ADF and lignin levels among all species throughout the season, with a gradual but significant increase from June to August.

Condensed Tannin

Condensed tannin concentrations were determined by spectrophotometric assay, while metabolic profiling was used to determine the individual CT subunits. Condensed tannin subunits are expressed as relative proportions based on the CT concentration measured spectrophotometrically. A negligible concentration of total CT was found in alfalfa and moderate levels in birdsfoot trefoil ranging from 15.3 $mg \cdot g^{-1}$ to 20.9 $mg \cdot g^{-1}$, while sainfoin contained a high concentration of total CT, ranging from 46.9 $mg \cdot g^{-1}$ to 18.1 $mg \cdot g^{-1}$ from June to October, with highest concentration among all species during June and July ($p < 0.0001$; Fig. 4.9). Birdsfoot trefoil maintained a similar CT concentration from June to October ($p = 0.75$), whereas in sainfoin, CT concentration declined by ~10 $mg \cdot g^{-1}$ in July ($p = 0.0013$) and 11.7 $mg \cdot g^{-1}$ in August ($p < 0.0001$), maintaining similar concentration through October.

UPLC-MS polyphenolic profiling revealed the distribution of CT subunits in alfalfa, birdsfoot trefoil and sainfoin. For birdsfoot trefoil, CT was enriched in PC subunits. The PC proportion in birdsfoot trefoil increased significantly in July and remained constant through October. In contrast, the CT of sainfoin was enriched in the PD subunit early in the season, but the PD proportion in sainfoin decreased significantly in August and maintained a similar level through October (Fig. 4.8A).

Hydrolysable Tannin

Of the four species analyzed in the field study in 2023, only small burnet contained significant HT. Total HT concentration ranged from 28.4 $mg \cdot g^{-1}$ in June to 17.4 $mg \cdot g^{-1}$ by October. A significant decline was observed in both HT concentration and ellagic acid proportion, the primary HT identified by UHPLC-MS/MS. The HT concentration decreased by 10 $mg \cdot g^{-1}$ in July ($p = 0.02$) after which concentration was constant through October (Fig. 4.8B).

Metabolic profiling

The PLS-DA model captured 74.2% of the total metabolomic variation (41.3% in component 1 and 32.9% in component 2) highlighting the metabolic differences among alfalfa, sainfoin, birdsfoot trefoil, and small burnet. Each species clusters away from the others except for sainfoin and small burnet, which overlap in the 95% confidence interval maps. The spherical eclipse for small burnet indicates more variability within each month and the metabolic profile did not follow a clear time-based trend. While the narrow eclipse for alfalfa, birdsfoot trefoil, and sainfoin indicate less variability at each time point within each species. Further, these species follow a gradual and consistent change in metabolic profile over the months (Fig. 4.9A). The biplot overlays the results of score plot with vectors to represent the ten, most pivotal metabolites in separating the species along component 1 and component 2 (Fig. 4.9B) The heat map illustrates the hierarchical clustering of top 50 metabolites differentiating among species from June through October (Fig. 4.10).

Alfalfa is clearly separated along component 1, clustering in the negative space and exhibiting substantial temporal variability especially between July and the later months. This suggests that alfalfa's metabolite expression is highly responsive to seasonal changes. Based on biplot, key metabolites associated with this pattern include Quercetin 3-O-rutinoside and Luteoloside. Further the heat map shows a strong flavonoid-based metabolic profile in this species including flavones, flavonols, anthocyanins, isoflavones and phenolic acids in June.

Birdsfoot trefoil occupies a more central metabolomic space with tight clustering across months, indicating stable metabolite profiles. The high accumulation of CT (cyanidin, delphinidins, procyanidin), flavones, flavonols, phenolic acids (sinapic acid, vanillic acid, etc.) during June and July suggests that birdsfoot trefoil maintains a higher concentration of metabolites during earlier months. Whereas moderate levels of secondary metabolites were evident throughout the season.

Sainfoin shows clear distinction, particularly along component 2, especially during July. It can be attributed to seasonal fluctuations in metabolite abundance during July. The distinct pattern is likely due to isovitexin during July. In addition to isovextin, sainfoin has a high accumulation of phenolic acids including caffeoylquincic acids, cholorogenic acid in June, tannins such as epigallocatechin and delphinidin in July.

Besides, sainfoin maintains a moderate concentration of other flavonoids (e.g, quercetin derivatives, kaempferol derivatives, etc.) throughout the season.

Small burnet presents the overlapping metabolic profile with sainfoin. It is marked by elevated levels of anthocyanins, tannins and flavonoid glycosides. The abundance of ellagic acid is especially dominant during June, may serve as a biochemical marker for this species, distinguishing it from the legumes.

2024

Forage samples for species including Ladak alfalfa, falcata alfalfa, birdsfoot trefoil, crested wheatgrass, sainfoin, small burnet, showy goldeneye, and Utah sweetvetch samples were collected for forage quality assessment and yield estimation. Additional samples of Ladak alfalfa and sainfoin were collected for evaluation of leaf and stem ratio from June to October 2024. The panels in Figure. 4.11 show differences in a forage quality parameter among the eight species within each month and among months for each species from June through October.

Crude protein and aNDF

The month-to-month pattern in crude protein differed among species as indicated by significant species x month interaction ($p < 0.0001$; Fig. 4.11A). The average crude protein in all species remained above the minimum threshold in June, except for small burnet. Although, small burnet contained 7.27% crude protein in June, the standard error suggests the true mean might meet the threshold. In June, falcata alfalfa contained higher crude protein (16.3%) as compared to all species except Ladak falcata ($p = 0.92$), whereas small burnet contained the lowest.

Sainfoin and small burnet dropped below 7.5% as early as July (declined by 49.5% and 26.7%, respectively). Birdsfoot trefoil fell below the minimum threshold in August (declined by 19.7% and 29.6% during July and August, respectively). Crude protein in falcata alfalfa also declined below 7.5% during October, reaching 6.6%. Showy goldeneye (7.6 ± 0.8%), and Utah sweetvetch (8.5 ± 0.79%) maintained ~7.5% crude protein till October. Crested wheatgrass contained low crude protein concentrations throughout the growing season dropping from 3.7% in July to 2.5% in October.

Neutral detergent fiber concentration increased in all species from June to October, although the pattern differed among species as indicated by significant species x month interaction ($p < 0.0001$; Fig. 4.11B). Showy goldeneye and Utah sweetvetch consistently exhibited statistically lower NDF than falcata alfalfa, and sainfoin throughout the season. The largest increase in NDF concentration over months was observed in birdsfoot trefoil (~75%) followed by sainfoin (57.5%), falcata alfalfa (~47%), Ladak alfalfa (~37%), showy goldeneye (~32.6%), small burnet (~30%), and Utah sweetvetch. Despite exhibiting the largest increase, birdsfoot trefoil remained statistically lower than the highest NDF-containing species like sainfoin and crested wheatgrass. Crested wheatgrass started high in July (64.4%) and exhibited only ~7% increase throughout October (68.9%). This species contained the highest NDF concentration throughout the season. Since for this species, the samples were not collected from the replicated plots, statistical comparison between months and among species was not conducted for this species.

Condensed tannin

Condensed tannin concentration was higher in Utah sweetvetch compared to all species (ranging from 75 mg·g^{-1} to 60.5 mg·g^{-1}, across the season) except birdsfoot trefoil during July (p = 0.15) and August (p = 0.1013). The CT concentration in Showy goldeneye varied from 17.4 to 12.4 mg·g^{-1}. Both alfalfa species and crested wheatgrass contained low CT concentrations ranging from 1-2 mg·g^{-1} except Ladak alfalfa in June containing 4.4 mg·g^{-1} CT. The concentration did not vary significantly over the months in Ladak alfalfa (p = 0.08), showy goldeneye (p = 0.0622), and Utah sweetvetch (p = 0.61). Birdsfoot trefoil exhibited a gradual but significant decline from June (28.9 mg·g^{-1}) to October (13.6 mg·g^{-1}), $p < 0.0001$. Sainfoin contained lower CT in August as compared to June ($p < 0.0001$), however, no statistical differences were observed among other months (Fig. 4.12A).

Hydrolysable tannin

Based on the results from the greenhouse study, only showy goldeneye, small burnet, and Utah sweetvetch were tested for HT. Similar to CT, Utah sweetvetch contained a higher concentration of hydrolysable tannins compared to other species throughout the season except for June, where HT concentration was similar in showy goldeneye, small burnet, and Utah sweetvetch (p = 0.07). HT concentration in Utah sweetvetch ranged from 43.7 mg·g^{-1} to 35.9 mg·g^{-1}. In showy goldeneye, the concentration varied from 28.5 mg·g^{-1} to 22 mg·g^{-1} from June to October, without significant differences over months. Small burnet contained lower HT in August (15.8 mg·g^{-1}) as compared to June (22.7 mg·g^{-1}), p = 0.0110, however, no statistical differences were observed among other months (Fig. 4.12B).

Leaf to stem ratio

The change in leaf to stem ratio was evaluated only in Ladak alfalfa and sainfoin as the other species biomass production was insufficient to conduct this analysis. The leaf to stem ratio was higher in alfalfa compared to sainfoin, in June ($p < 0.0001$), July ($p = 0.002$), and August ($p = 0.009$), while remained similar during September and October, indicating predominance of leaves on the alfalfa. Further, in alfalfa, the ratio declined consistently from June to August whereas the ratio in sainfoin did not vary significantly from July onwards (Fig. 4.13).

Yield

The dry matter yield estimation in eight species selected for forage quality estimation in 2024 is presented in Figure. 4.14. Dry matter yield did not vary significantly over months in Ladak alfalfa (6963 to 2696 kg·ha^{-1}, $p = 0.22$) and falcata alfalfa (3811 to 2640 kg·ha^{-1}, $p = 0.78$). However, it varied significantly in showy goldeneye (3020 to 779 kg·ha^{-1}) and Utah sweetvetch (2306 to 844 kg·ha^{-1}) from June to July ($p = 0.0003$ and $p = 0.002$, respectively) remaining stable in later months. More consistent decline in dry matter yield was observed in sainfoin (7058 to 1485 kg·ha^{-1}) and small burnet (3655 to 671 kg·ha^{-1}) from June to October, exhibiting reduction yield production from June to August ($p = 0.004$ and $p = 0.03$, respectively) and August to October ($p = 0.01$ and $p = 0.007$). Crested wheatgrass maintained a relatively consistent dry matter yield ranging from 2147 to 1843 kg·ha^{-1} from July to October. From June to August, the yield of Ladak alfalfa and sainfoin was significantly greater than birdsfoot trefoil, showy goldeneye, and Utah sweetvetch. In September, the dry matter yield of Ladak alfalfa, falcata alfalfa, sainfoin, and small burnet was greater than birdsfoot trefoil and Utah sweetvetch. In October again, the yield production of Ladak alfalfa, falcata alfalfa, was greater than all other species.

Discussion

An understanding of forage quality dynamics on rangeland informs the likelihood of improving nutrient resources for grazing cattle on semi-arid rangelands. In late summer and early fall, cowherds require significantly greater protein concentrations than are available from mature rangeland grass species. Protected stands of legumes and non-legume forbs have the potential to provide needed supplementation. Further, the CT and HT provided by a subset of the tested legumes and forbs can partition more N to feces, reducing losses of N to the environment from urine, and slowing the nitrification of feces. This study evaluated the nutrient and tannin concentrations of plant material from 24 unreplicated perennial forage species grown in the greenhouse under well-watered conditions that were repeatedly harvested and allowed to regrow. They were planted to serve as validation plants but were used to demonstrate the potential forage nutritive value and secondary metabolite type and concentration of the species seeded in a small plot establishment study reported elsewhere. We also determined the nutritive value and tannin concentrations of four species growing in a replicated rangeland field study as they matured from July through October.

Greenhouse Preliminary Study

When the nutritive value components of well-watered, greenhouse-grown plants (Fig. 4.2) are examined, the species with the lowest crude protein values are the penstemons, the asters, and crested wheatgrass, all of which were repeatedly harvested in the vegetative stage. Species with less than 20% crude protein under these conditions included the five species above plus Utah sweetvetch, small burnet, showy goldeneye, sainfoin, Maximillian sunflower, purple prairie flower, prairie coneflower, Lewis flax, white sagebrush, leadplant, cicer milkvetch, and blanketflower. The species with the highest concentrations of protein were white prairie clover, western yarrow, crownvetch, Canadian milkvetch, birdsfoot trefoil, Ladak alfalfa, and falcata alfalfa. Table 4.3 presents the crude protein, NDF, ADF, and lignin concentrations of 7 out of 8 species selected for seasonal forage quality assessment from Clarkston, UT during 2023 and 2024. Forage quality parameters for these species are presented under both greenhouse and field conditions during June 2023 and 2024, as the greenhouse condition is most similar to field conditions in June. Crested wheatgrass is not included in the table as the sample collection for this species began only in July 2024. Under both conditions, Ladak alfalfa and falcata alfalfa contained high crude protein concentrations. Under greenhouse conditions, birdsfoot trefoil also contained similar concentrations as both alfalfa species. Along with high protein, both alfalfa species contained high fibrous fraction (NDF, ADF, and lignin content) that could be due to the tall stature of the plants. The high lignin content of small burnet under both conditions suggests reduced digestibility.

Most of these 24 plants were seeded at our Clarkston site, and initial establishment and information from the literature caused us to select Ladak alfalfa, birdsfoot trefoil, sainfoin, and small burnet for a replicated field study of changes in nutritive value and secondary metabolites from summer through fall. Data for CT and HT from greenhouse-grown plants confirmed that the cultivars and species we were planting in the field would either produce no tannin (Ladak alfalfa), low and high CT (birdsfoot trefoil and sainfoin, respectively) as well as PC-only CT (birdsfoot trefoil) or a mix of PD and PC (sainfoin), and HT (small burnet).

The production of secondary metabolites is influenced by a number of extrinsic factors including species, growth stage, environmental conditions and intrinsic factors such as metabolic networks, enzymatic pathways, gene expression, etc. (Li et al., 2020). The synthesis of secondary metabolites may, in some cases, serve as an energy escape valve (Hernández & Breusegem, 2010), while under limited resources in dryland conditions, plants may constrain the allocation of energy toward secondary metabolite production (Cella Pizarro & Bisigato, 2010). This is aligned with our observation of CT levels in sainfoin that, in spring, were comparable to those produced by greenhouse-grown plants, but decreased to less than half the spring level by fall, while the concentration of CT in birdsfoot trefoil was maintained at a spring, or greenhouse, level from June through October. This likely reflects the contrast in the growth habit of these two species, with erect plants of sainfoin shedding leaves as seed pods matured in late summer, while birdsfoot trefoil did not elongate stems but developed a rosette of leaves that did not appear to senesce. Intermediate to sainfoin and birdsfoot trefoil, the concentration of small burnet in greenhouse-grown plants was similar to that of plants harvested in June, at close to 30 mg g^{-1} of dry matter, which decreased to two-thirds that level for the balance of the season.

Further, the metabolic profiling of 24 legume and non-legume species provides insights into the nutritional potential of these species for ruminants. The overlapped clustering of most species indicates the alignment in their metabolic profile that could be due to similar biosynthetic pathways among these species. The species such as penstemons, prairie aster, blanket flower, leadplant, prairie coneflower, purple prairie clover, white prairie clover, small burnet, crownvetch, Utah sweetvetch and birdsfoot trefoil were found to be higher in flavonoids and phenolic acids than others. Although sainfoin contained fewer secondary metabolites in the greenhouse compared to the field (Fig. 4.5 & Fig. 4.10). All of these species with rich secondary metabolite profiles are considered suitable for grazing, as their secondary metabolites have the potential to be antimicrobial, anti-inflammatory or antioxidant properties that could improve overall ruminant health (Ku-Vera et al., 2020).

Field Study: Seasonal change in forage quality and secondary metabolites

The change in nutritive value of plants growing on rangeland is associated with increasing plant maturity and with higher temperatures as the season advances. According to Nelson & Moser (2015), the cell wall materials deposited at lower temperatures are less lignified and higher in protein, thus more digestible. This observation is in alignment with our results as lignin content increased (Fig. 4.7) with increasing temperature from June into July or August. Furthermore, leaves contain more cells with thin cell walls and cell solubles, while stems contain more fiber and xylem cells, with thick, lignified cell walls, at least in plants with upright stems (Nelson & Moser, 2015). Thus, an increasing proportion of stems compared with leaves as plants mature substantially decreases the leaf to stem ratio (Fig. 4.13) and decreases forage nutritive value as the season progresses. Under irrigation, these plants would be periodically grazed or harvested and would regrow, but without water, regrowth is not supported. The rangeland-grown plants in this study either maintained a rosette of leaves, as in birdsfoot trefoil and small burnet, remained short in stature throughout the period of assessment (Utah sweetvetch and showy goldeneye), had upright stems with ground-level fall regrowth (sainfoin) or produced new leaves along stems throughout the growing season (Ladak and falcata alfalfa).

Given that cattle require no less than 7.5% crude protein during late summer and fall to meet their nutritional needs, among the four field-grown plant species were assayed for forage nutritive value from summer through fall in 2023, only alfalfa maintained this critical crude protein threshold beyond July. Birdsfoot trefoil and small burnet maintained crude protein content above 5.5% from August to October, which was higher than crude protein levels in crested wheatgrass reported by Rumbaugh et al. (1982), who observed a decline from 10.9% in May to 3.3% in August. In contrast, during 2024, birdsfoot trefoil maintained the threshold upto July, whereas Utah sweetvetch exceeded the critical threshold through October. There was no established crested wheatgrass to sample at Clarkston until 2024. While replicated plots of crested wheatgrass at Clarkston, there was a large border area where crested wheatgrass was successfully established in 2023. Therefore, unreplicated samples were collected for crested wheatgrass in 2024. No statistical comparison could be performed, but the crude protein content in crested wheatgrass remained lower than all other species throughout the season. Biligetu et al. (2014) reported crude protein levels of 5.3% in crested wheatgrass during late summer. In our study, the crude protein levels in crested wheatgrass were 3.6% in July which further declined to 2.6% in October. In 2023, sainfoin's crude protein concentration declined sharply in August, when ADF, NDF, and lignin all increased sharply. In 2024 the crude protein of sainfoin declined below the minimum threshold and NDF increased significantly as early as July. Furthermore, CT concentration of sainfoin also declined with plant maturity, from June through August during both years. This finding is in alignment with a study by Li et al. (2014) showing that the decrease in secondary metabolite concentration with plant maturity in sainfoin could be explained by the growth-differentiation balance hypothesis (Cella Pizarro & Bisigato, 2010). According to this hypothesis, as the plant matures, it allocates more energy toward reproduction, thereby reducing the resource availability for secondary metabolite production. Furthermore, the warmer and drier conditions on rangeland with the progression of the season toward fall results in fewer leaves, especially on plants with upright growth habits, and leaves are enriched in tannins compared with stems. A significant loss of leaves was observed in sainfoin during July and in Ladak alfalfa during July and August, which potentially contributed to a decline in forage quality as the season progressed. Due to the rosette growth habit of birdsfoot trefoil and small burnet, no significant shift was observed in forage quality parameters of these species after July in 2023, while gradual decline was observed from June to August in 2024, suggesting a slower and smaller shift in the leaf-to-stem ratio as the season progressed in birdsfoot trefoil and small burnet. Accordingly, in 2023, birdsfoot trefoil showed no significant fluctuation in CT concentration, and small burnet showed no significant change in HT concentration after July. In 2024, a significant decline in CT concentration in birdsfoot trefoil was observed only in October relative to June CT values. Hydrolysable tannin in small burnet declined only in August relative to June but remained similar during all other months. Similarly, there was no significant difference in CT and HT concentration over the five months of the study in Utah sweetvetch and showy goldeneye in 2024. This is likely attributed to their leaf retention and succulent growth throughout the season.

Similar to our findings, Bassendowski et al., (1989) compared the tannin content in *Hedysarum alpinum* var. *americanum* (a closely related species to Utah sweetvetch) with birdsfoot trefoil and alfalfa and found higher concentration of tannins in *Hedysarum alpinum*. No published information was found on CT and HT in showy goldeneye in the current literature, but this species contained a high concentration of both CT and HT in our study.

Where plant protein exceeds ruminant needs, such as in a dairy ration, as much as 80% of N can be excreted as urea to the environment, causing greenhouse gas emissions (Getachew et al., 2006). Species having adequate tannin concentrations such as birdsfoot trefoil, small burnet, sainfoin (Hymes-Fecht et al., 2013; Kamalak et al., 2014; Finimundy et al., 2020), Utah sweetvetch, and showy goldeneye can protect rumen proteins from rapid degradation in the rumen by binding and forming complexes that precipitate in the rumen and are separated in the low pH of the abomasum. Proteins become available for absorption in the small intestine. The resulting improvement in nitrogen retention, and the associated partitioning of nitrogen from urine into feces decreases loss of nitrogen to the environment. Stewart et al. (2019) reported reduced methane emissions in heifers fed small burnet hay containing 4.5% HT, along with a shift in nitrogen excretion from urine to feces, whereas CT-rich sainfoin and birdsfoot trefoil hay increased the N retention capacity of the animal.

The CT-rich sainfoin would most efficiently benefit the cattle by reducing protein degradation in rumen, reducing ammonia production and support improved nutritional N utilization in late summer and fall (Wang et al., 2015). Furthermore, in alignment with previous studies (Malisch et al., 2015; Verma et al., 2021), the PD-rich CT found in sainfoin would have greater efficacy in reducing gastrointestinal nematodes in cattle and in mitigating methane emissions (Desrues et al., 2016), , as well as reducing methane emissions more effectively (Hatew et al., 2015).

The PD-PC balance of subunits in sainfoin CT results in stronger protein and enzyme binding and the formation of larger molecular complexes that inhibit fermentation pathways related to methane production (Malisch et al., 2015). Birdsfoot trefoil maintains moderate CT levels, lower than sainfoin but meets the threshold of the recommended CT range (1.5% - 2.1% DM) for effective protein digestion. As found in previous studies (Malisch et al., 2015; Verma et al., 2021), birdsfoot trefoil contained PC-rich CTs, which are less effective in protein precipitation than PD-rich CT.

Aboagye et al. (2018) similarly demonstrated reduced methane emissions with a combined 1.5% HT and CT in beef cattle diets. Our data show that showy goldeneye, small burnet and Utah sweetvetch maintain sufficient HT levels, from summer through fall, which can reduce methane emissions, minimize nitrogen loss as urea, and enhance soil nitrogen via fecal excretion instead of ammonia emissions from urine (Aboagye et al., 2018). This suggests that inclusion of sainfoin, birdsfoot trefoil, showy goldeneye, small burnet, and Utah sweetvetch in ruminant diets can improve ruminal protein efficiency by reducing rapid protein degradation and also reduce methane gas emission from beef cattle production, particularly when sources of CT and HT are combined in appropriate proportions. This will be an interesting subject for future research.

The metabolic profile of four forage species from June to October in 2023 provided a comprehensive overview of distinct metabolic profiles of each species. The presence of flavonols, anthocyanins and phenolic acids may prove beneficial for enhancing the antioxidant capacity but is not known to provide protection against protein degradation in rumen (Sinz et al., 2018). The flavones, especially luteolin and apigenin detected in alfalfa, have been shown to reduce methanogenesis (Sinz et al., 2018). Besides the accumulation of tannins in birdsfoot trefoil, sainfoin, and small burnet, the presence of other flavonoids such as flavones, isoflavones, anthocyanin and phenolic acids would offer benefits to ruminants by improving the microbial environment in rumen and antioxidant potential. Flavonoids such as daidzein, quercetin derivatives and kaempferide derivatives can alter the population of methanogens, bacteria, and protozoa to mitigate methane emission (Ku-Vera et al., 2020). Thus, the incorporation of species with rich secondary metabolite profiles can provide a plethora of benefits by improving animal health and productivity.

However, despite the remarkable forage quality demonstrated by birdsfoot trefoil, small burnet, showy goldeneye, and Utah sweetvetch, these species did not outperform the high yielding species such as both Ladak and falcata alfalfa, and sainfoin. This might be due to the allocation of more resources toward production of secondary metabolites by sainfoin, or due to the unelongated stems of birdsfoot trefoil growth, and of small burnet's fall regrowth, these plants with leaves remaining effectively at the level of the soil surface compared with the alfalfas and sainfoin. The result is much less yield determined on dry weight per unit area.

Grazing preference

Animal performance is influenced by forage quality factors including nutrient and energy concentration, intake, digestibility, and metabolism. Voluntary intake is important to consider since grazing ruminants have selective grazing behavior which is governed by factors such as palatability and species preference. Palatability is a measure of feed acceptance, influenced by sensory attributes such as taste, smell, and appearance. It affects feed preference and consumption rate. Selectivity can be defined as the preferential consumption of specific feed subcomponents such as leaves vs stems and immature plants (Mertens, 2015).

According to Buxton (1996), the intake of a mature beef cow is not negatively affected by NDF as high 70-75%. In our field study of eight relatively productive species, their NDF concentrations were less than 65% throughout all months during both years except crested wheat grass. Even when protein concentrations are not optimal during late season, the reduced concentration of NDF should benefit intake.

Methane emissions are inversely related to intake levels, as increased food intake is a response to accelerated rate of passage of digesta, reducing feed retention time in the rumen and consequently lowering methane production rates (Stewart et al., 2019). Thus, species retaining a higher leaf-stem ratio and less fibrous fraction should have reduced methane emissions, which supports environmental sustainability and reduced energy losses from rumen microbes.

To further demonstrate the ability of ruminants to select a nutritionally balanced diet when given the opportunity for voluntary intake (Provenza et al., 2003), a study was conducted by Lagrange and Villalba (2019), where, among the three species, lambs preferred alfalfa the most (53%), which was also the richest source of protein in our study, followed by sainfoin (33%), which remains a key component in balancing high protein diet, and birdsfoot trefoil (14%), which also contains stable forage quality and beneficial secondary metabolites to support nitrogen efficiency and digestibility in late-season grazing. Similarly, small burnet, showy goldeneye, and Utah sweetvetch can also support the balancing of cattle diet by maintaining moderate forage quality, CT and HT into late summer and fall.

Conclusions

This chapter includes unreplicated greenhouse data demonstrating the plant species' potential for primary and secondary nutritive concentrations of nearly all the plant species that were seeded in small plots at locations across Utah. Some of these did not become established in significant numbers in any locations even though they are represented in habitats used by grazing cattle in this region (Chapter 3). However, those data identified a few species, such as Utah sweetvetch and showy goldeneye, that have valuable secondary metabolites that were also able to establish relatively dense stands over a longer time frame than alfalfa or sainfoin. These plant species were subsequently included in the 2024 yield and quality evaluations as potential resource island candidates. The replicated study carried out at Clarkston with four plant species in 2023 and eight species in 2024 demonstrated that only Ladak alfalfa was able to maintain protein above 7.5% into October during 2023, joined by showy goldeneye and Utah sweetvetch in 2024. Further, the study demonstrated that NDF in all species remained below 65% over the same timeframe. We have documented the major constituents of sainfoin CT in the cultivar we used when plants are grown in dryland conditions, as well as the decrease in sainfoin CT concentration from June through October as well as observing that birdsfoot trefoil, showy goldeneye, small burnet, and Utah sweetvetch all maintain their late-summer concentrations of CT and HT into fall.

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Table 4.1

Schedule of sample collection for forage quality assessment at Clarkston, UT.

		Sampling dates	
Location	**Planting**	**2023**	**2024**
Clarkston	18-Nov-2021	16-Jun	11-Jun
	7-May-2022	12-Jul	9-Jul
		13-Aug	12-Aug
		7-Sep	10-Sep
		13-Oct	10-Oct

Table 4.2

Values used to judge NIRS prediction equation fit for the field-grown species

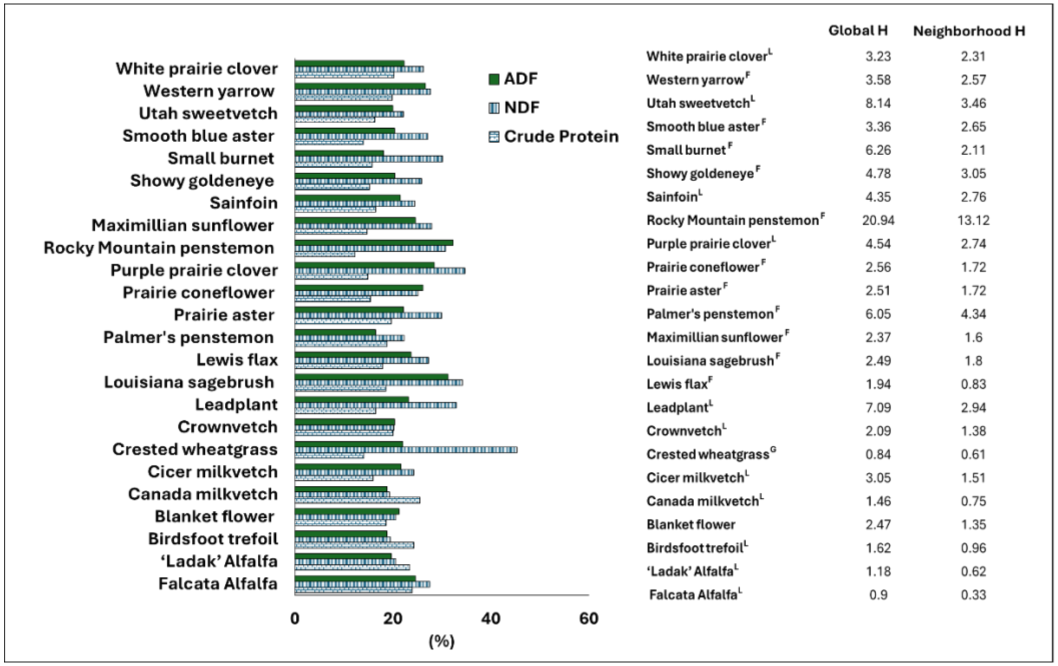
	June	**July**	**August**	**September**	**October**
			Neighborhood H[a]		
Alfalfa	0.67 ± 2.46	0.88 ± 0.52	8.53 ± 3.58	1.82 ± 0.68	1.96 ± 0.64
BFT*	1.96 ± 2.46	3.03 ± 0.52	3.54 ± 3.58	3.81 ± 0.68	4.24 ± 0.64
Sainfoin	6.70 ± 2.46	2.56 ± 0.52	1.95 ± 3.58	2.52 ± 0.68	2.17 ± 0.64
Small burnet	3.79 ± 2.46	6.93 ± 0.52	3.18 ± 3.58	3.53 ± 0.68	4.52 ± 0.64
			Global H[a]		
Alfalfa	1.09 ± 2.95	1.73 ± 0.81	10.76 ± 4.15	3.06 ± 1	3.72 ± 0.52
BFT	3.8 ± 2.95	4.74 ± 0.81	5.19 ± 4.15	5.73 ± 1	6.67 ± 0.52
Sainfoin	9.38 ± 2.95	4.47 ± 0.81	3.51 ± 4.15	4.20 ± 1	3.60 ± 0.52
Small burnet	8.58 ± 2.95	12.18 ± 0.81	6.09 ± 4.15	6.83 ± 1	5.96 ± 0.52

*BFT = birdsfoot trefoil, [a]The neighborhood H and global H values did not vary significantly within months for each species. The legume hay equation was used for alfalfa, BFT, and sainfoin, and the mixed hay equation was used for small burnet

Table 4.3

NIRS values for crude protein, neutral detergent fiber, acid detergent fiber, and lignin concentrations (%) in composited greenhouse-grown plants and field-grown plants (Clarkston, UT) collected in June for species assessed in 2023 and 2024

Crude protein			
	Greenhouse	**Field (June 2023)**	**Field (June 2024)**
Ladak alfalfa	23.44	15.6 ± 0.5	15.3 ± 0.6
Falcata alfalfa	24.01	--	16.3 ± 0.6
Birdsfoot trefoil	24.31	9.8 ± 0.5	10.4 ± 0.6
Sainfoin	16.49	10.5 ± 0.5	12.9 ± 0.6
Small burnet	15.76	8.4 ± 0.5	7.3 ± 0.6
Showy goldeneye	15.24	--	13.2 ± 0.6
Utah sweetvetch	16.29	--	13.6 ± 0.6
Neutral detergent fiber (Chemical assay)			
	Greenhouse	**Field (June 2023)**	**Field (June 2024)**
Ladak alfalfa	31.9	30.6 ± 1.4	40.0 ± 1.6
Falcata alfalfa	35.7	--	37.4 ± 1.6
Birdsfoot trefoil	19.0	28.4 ± 3.3	27.0 ± 1.6
Sainfoin	20.6	25.3 ± 2.0	37.7 ± 1.6
Small burnet	19.9	31.7 ± 2.9	34.2 ± 1.6
Showy goldeneye	19.1	--	27.2 ± 1.6
Utah sweetvetch	19.9	--	28.0 ± 1.6
Acid detergent fiber			
	Greenhouse	**Field (June 2023)**	**Field (June 2024)**
Ladak alfalfa	19.6	25.5 ± 2.3	--
Falcata alfalfa	24.6	--	--
Birdsfoot trefoil	18.8	18.8 ± 2.3	--
Sainfoin	21.5	24.3 ± 2.3	--
Small burnet	18.1	22.7 ± 2.3	--
Showy goldeneye	20.4	--	--
Utah sweetvetch	19.9	--	--
Lignin			
	Greenhouse	**Field (June 2023)**	**Field (June 2024)**
Ladak alfalfa	3.51	4.3 ± 0.8	--
Falcata alfalfa	4.6	--	--
Birdsfoot trefoil	2.8	1.9 ± 0.8	--
Sainfoin	2.9	3.6 ± 0.8	--
Small burnet	8.0	8.7 ± 0.8	--
Showy goldeneye	3.3	--	
Utah sweetvetch	2.1	--	



	Global H	Neighborhood H
White prairie clover^{L}	3.23	2.31
Western yarrow^{F}	3.58	2.57
Utah sweetvetch^{L}	8.14	3.46
Smooth blue aster^{F}	3.36	2.65
Small burnet^{F}	6.26	2.11
Showy goldeneye^{F}	4.78	3.05
Sainfoin^{L}	4.35	2.76
Rocky Mountain penstemon^{F}	20.94	13.12
Purple prairie clover^{L}	4.54	2.74
Prairie coneflower^{F}	2.56	1.72
Prairie aster^{F}	2.51	1.72
Palmer's penstemon^{F}	6.05	4.34
Maximillian sunflower^{F}	2.37	1.6
Louisiana sagebrush^{F}	2.49	1.8
Lewis flax^{F}	1.94	0.83
Leadplant^{L}	7.09	2.94
Crownvetch^{L}	2.09	1.38
Crested wheatgrass^{G}	0.84	0.61
Cicer milkvetch^{L}	3.05	1.51
Canada milkvetch^{L}	1.46	0.75
Blanket flower	2.47	1.35
Birdsfoot trefoil^{L}	1.62	0.96
'Ladak' Alfalfa^{L}	1.18	0.62
Falcata Alfalfa^{L}	0.9	0.33

Figure 4.1. Forage quality parameters for unreplicated greenhouse-grown plants collected using NIRS, including crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin concentrations (%) along with their global H and neighborhood H values. ^{L}The legume hay equation was used for all legume species, ^{F}mixed hay equation was used for all non-legume species, and ^{G} the grass hay equation was used for crested wheatgrass. Single pots were repeatedly harvested each time they flowered; plants that did not flower under greenhouse conditions (e.g., crested wheatgrass) were harvested in the vegetative stage.

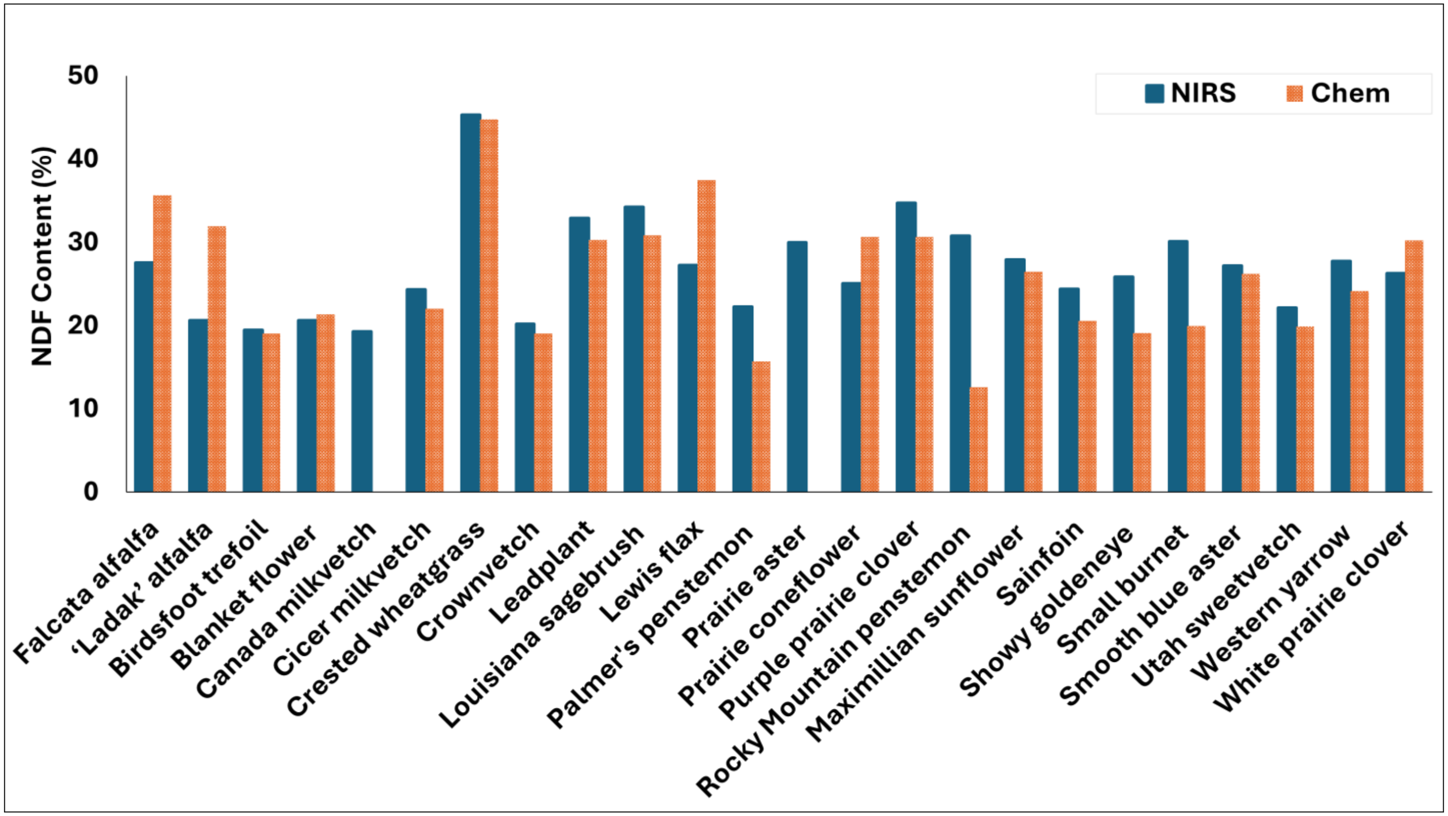


Figure 4.2. Comparison of NIRS prediction and chemical assay for concentration of NDF (%) right in species grown under greenhouse conditions

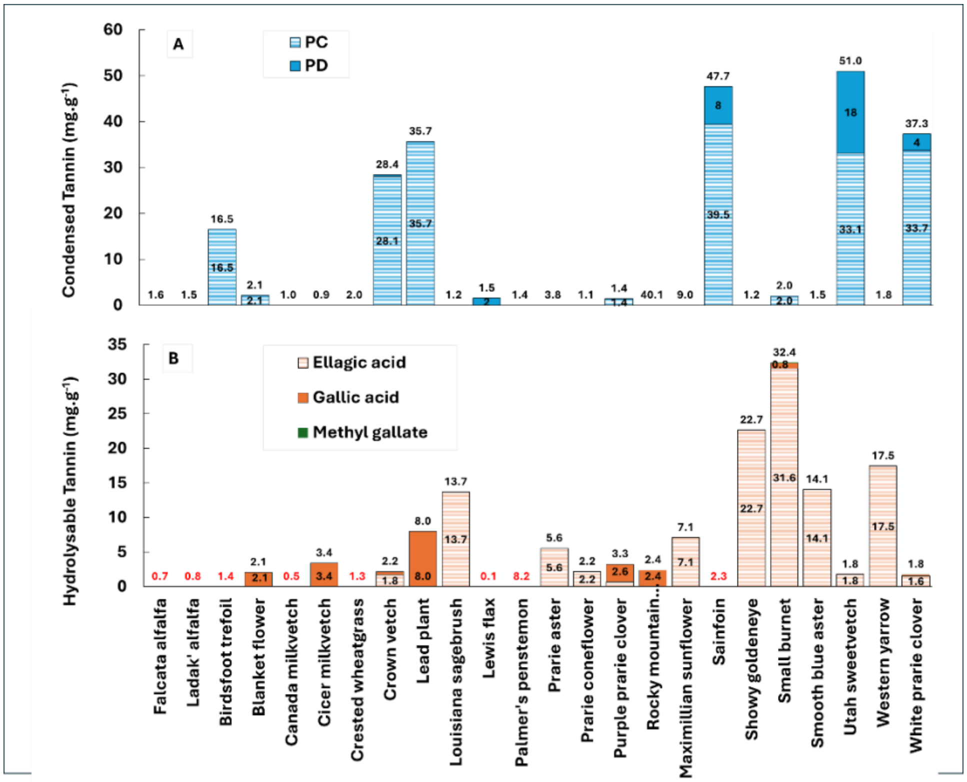


Figure 4.3. Proportion of condensed tannin (CT, A) and hydrolysable tannin (HT, B) subunits analyzed via UHPLC-MS/MS assigned to total CT and HT concentrations (mg·g^{-1}), respectively, from spectrometric assays of unreplicated samples of species grown under greenhouse conditions. The values on top of the bars represent the total CT and HT concentrations determined by spectrometric assays. For HT, none of the analyzed compounds—ellagic acid, gallic acid, and methyl gallate—were detected in some species (values in red), suggesting the presence of other HT derivatives beyond the tested compounds

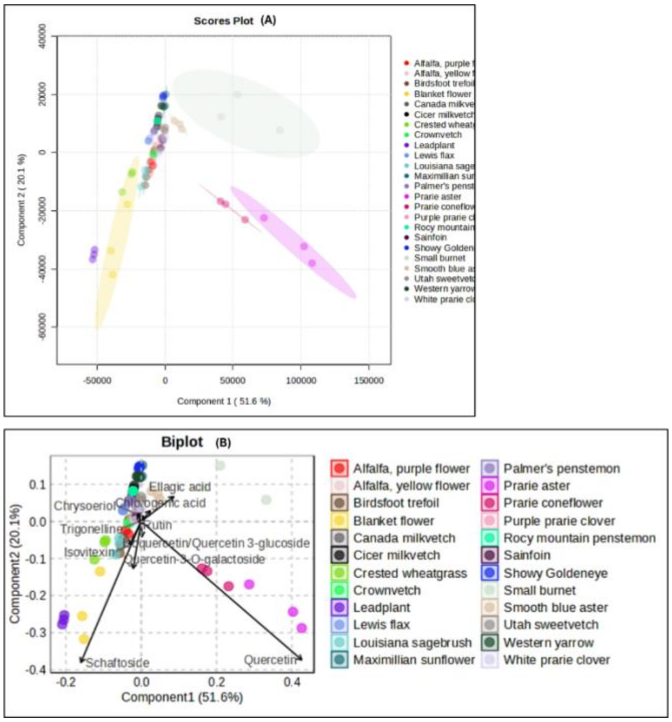


Figure 4.4. PLS-DA score plot (A) for mean polyphenol concentrations across species grown in the greenhouse. The mean concentrations were detected using LC-MS/MS technique. The species (colored dots) clustered together share similar metabolic profiles and species which are farther apart have distinct metabolic profiles. In the biplot (B), the direction of the arrows suggests the increasing concentration of a particular metabolite in that direction and the length of the arrow indicates its influence on PLS-DA components.

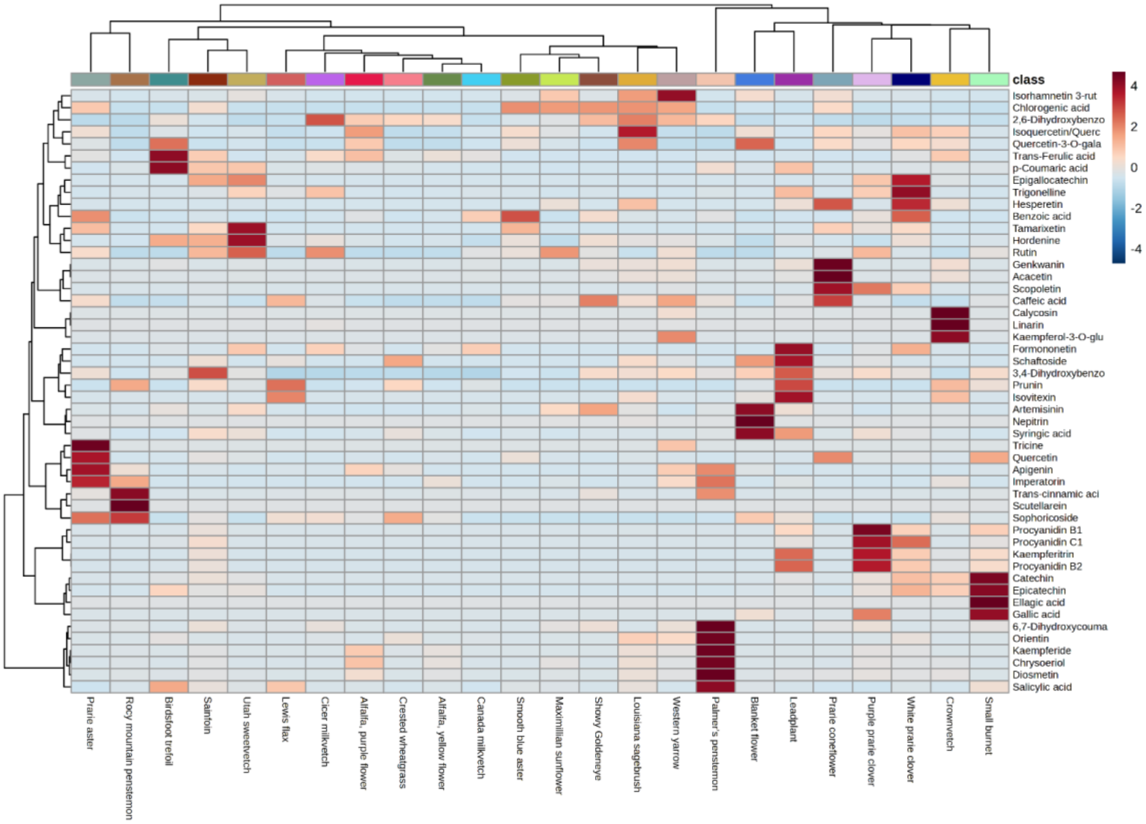


Figure 4.5. Heat map illustrating the hierarchical clustering of the top 50 metabolites differentiating among species

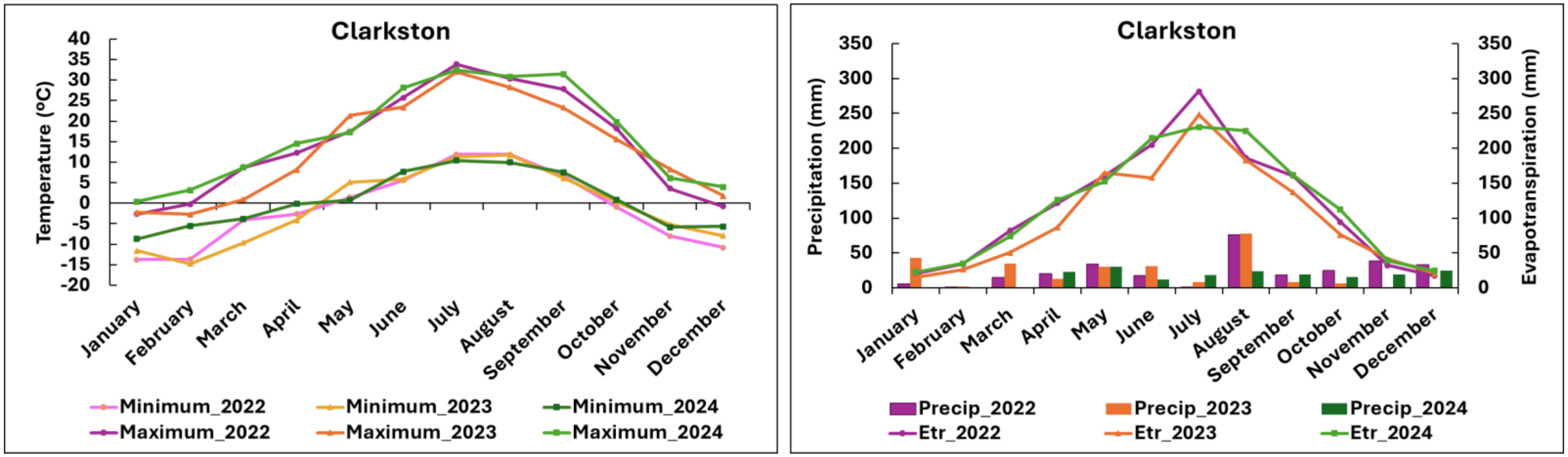


Figure 4.6. Comparison of daily maximum and minimum air temperatures (ºC), averaged over months (left) and daily precipitation and evapotranspiration (Etr), summed over months (right) at the Godfrey farm (Clarkston, UT) for the years 2023 and 2024, obtained from the Utah Climate Center

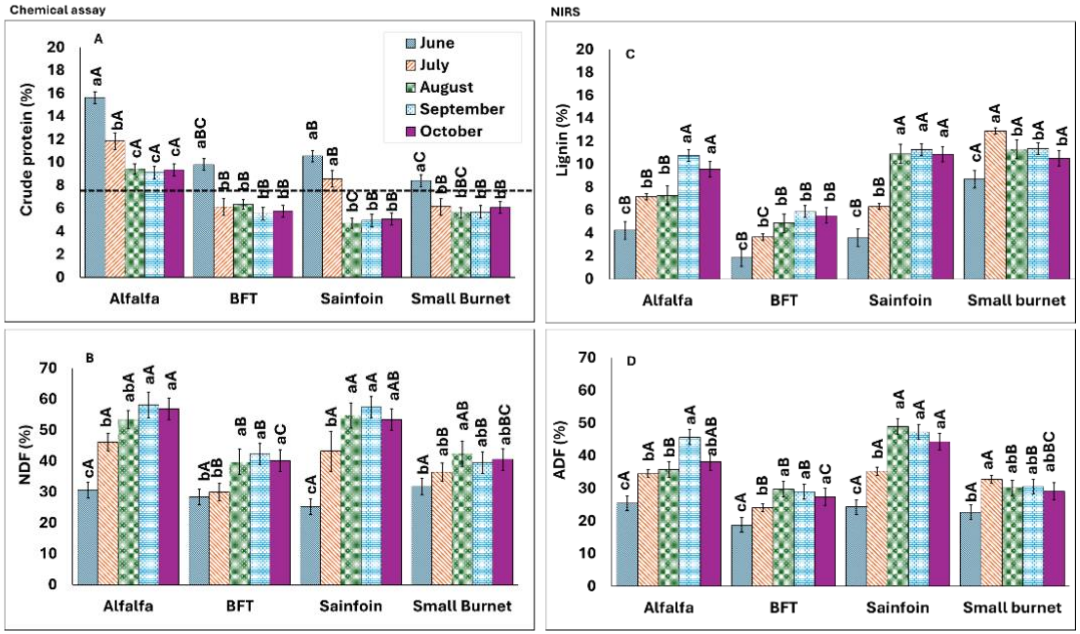


Figure 4.7. Concentration (%) of crude protein (A) and neutral detergent fiber (B) analyzed through chemical assay, and lignin (C), and acid detergent fiber (D) predicted using NIRS in species growing under field conditions at Clarkston, UT during 2023. Different lowercase letters above the bars indicate statistically significant differences in forage quality parameter concentration between months within each forage species. Different uppercase letters above the bars indicate statistically significant differences in forage quality parameter concentration between species within each month ($\alpha \leq 0.05$). The dashed line at 7.5% represents the minimum crude protein required by beef cows to maintain their physiological requirements

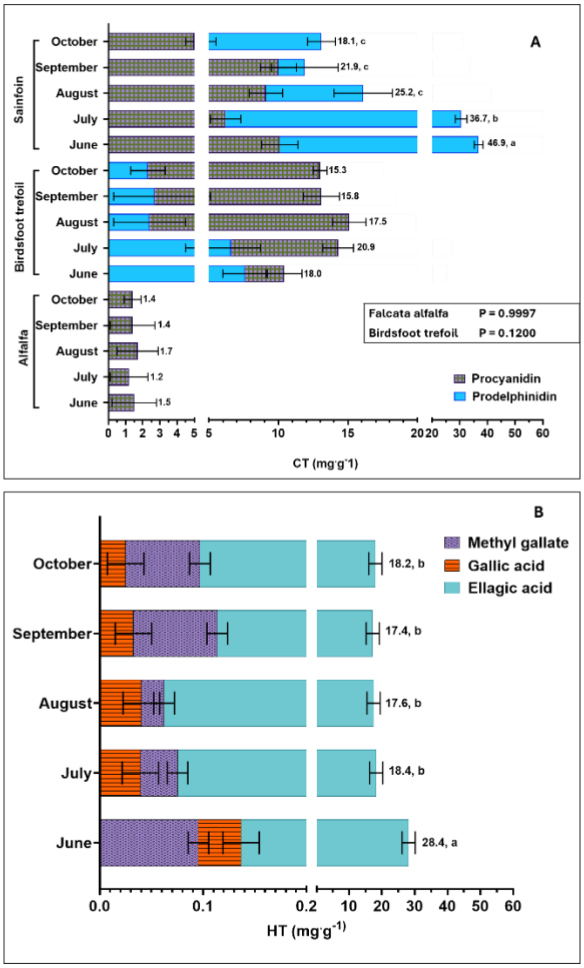


Figure 4.8. The concentration (mg·g^{-1}) of condensed tannin (CT, A) in alfalfa, birdsfoot trefoil, and sainfoin and hydrolysable tannin (HT, B) in small burnet grown under field conditions at Clarkston, UT during 2023 is presented as LSMeans ± SE. The proportion of CT and HT subunits analyzed via UHPLC-MS/MS were assigned to corresponding concentrations from spectrometric assays. Different letters above the bars indicate statistically significant differences in CT and HT concentration over the months ($\alpha \leq 0.05$). No significant difference was observed in alfalfa and birdsfoot trefoil over the months. The majority of HT was composed of ellagic acid, while gallic acid and methyl gallate were present in negligible proportions

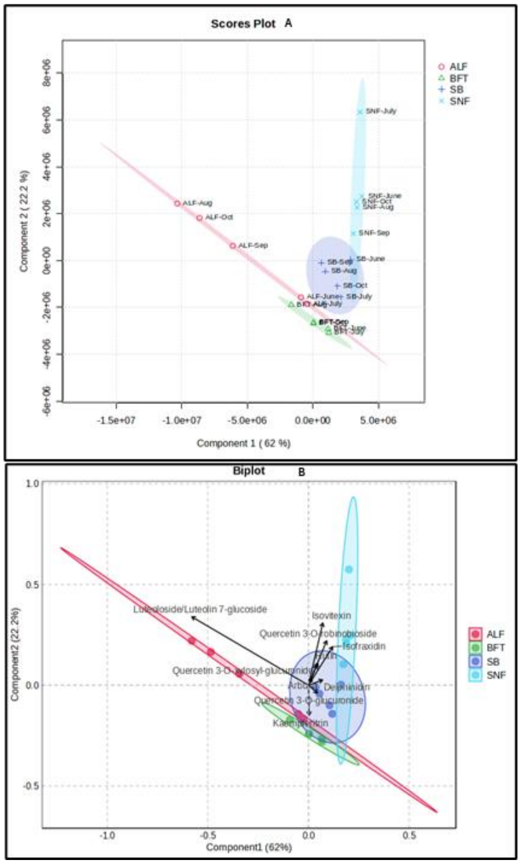


Figure 4.9. PLS-DA score plot (A) for mean polyphenols concentrations of species collected from Clarkston, UT, from June to October 2023. The mean concentrations were detected using LC-MS/MS technique. The species (colored dots) clustered together share similar metabolic profiles and species which are farther apart have distinct metabolic profiles. In the biplot (B), the direction of the arrows suggests the increasing concentration of a particular metabolite in that direction and the length of the arrow indicates its influence on PLS-DA components. ALF (Alfalfa), BFT (Birdsfoot trefoil), SB (Small burnet), SNF (Sainfoin).

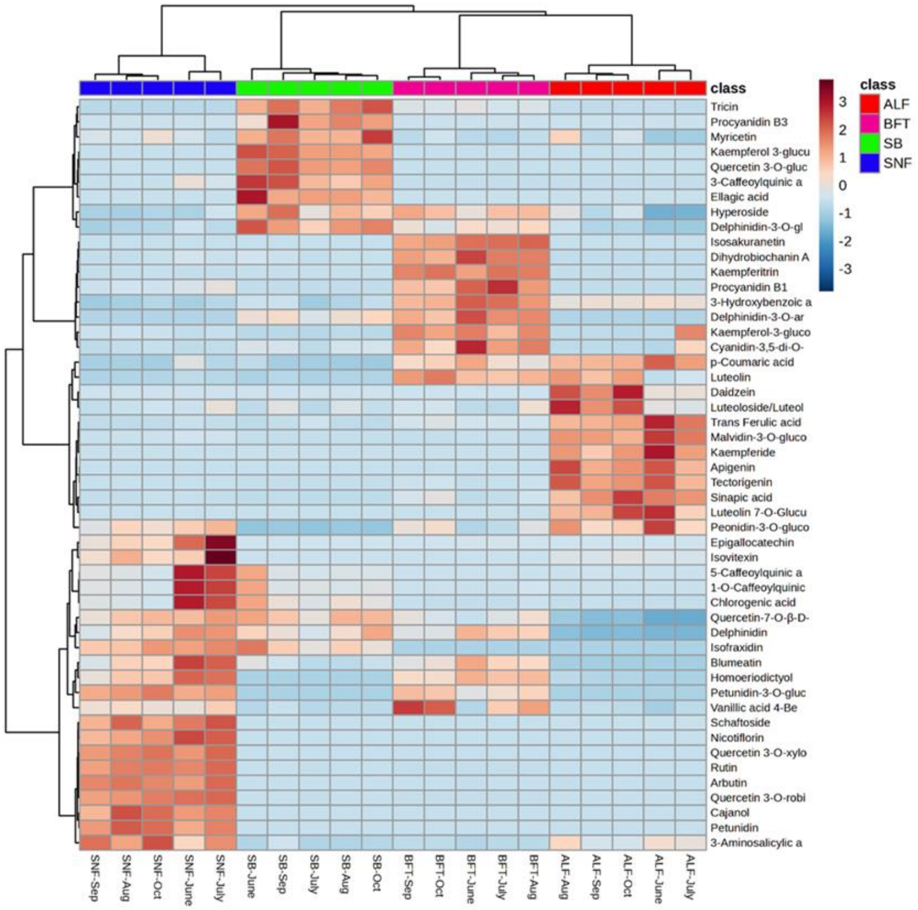


Figure 4.10. Heat map illustrating the hierarchical clustering of the top 50 metabolites, differentiating among species from June through October.

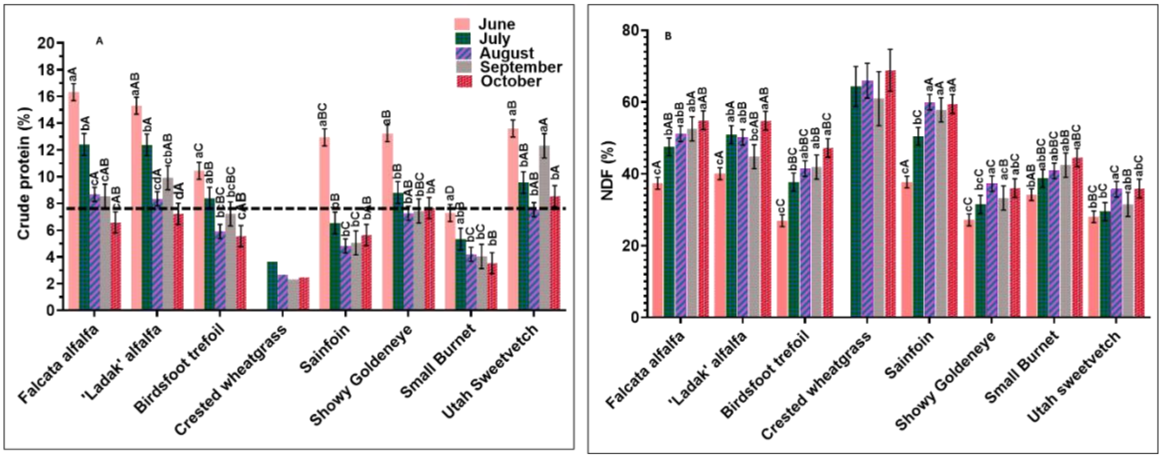


Figure 4.11. The concentration (%) of crude protein (A) and neutral detergent fiber (B) analyzed through chemical assay in species growing under field conditions at Clarkston, UT in 2024. Different lowercase letters above the bars indicate statistically significant differences in forage quality parameters concentration between months within each forage species. Different uppercase letters above the bars indicate statistically significant differences in forage quality parameters concentration between species within each month ($\alpha \leq 0.05$). The dashed line at 7.5% represents the minimum crude protein required by beef cows to maintain their physiological requirements.

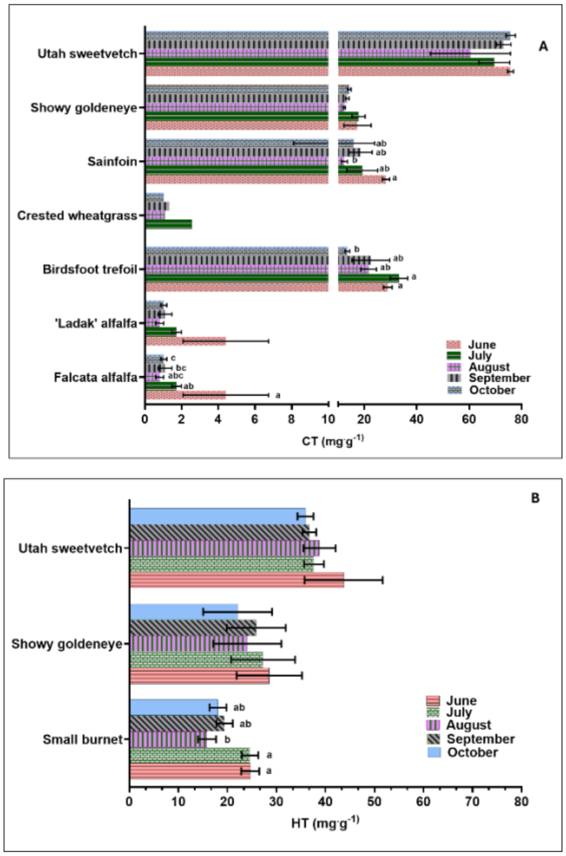


Figure 4.12. The concentration (mg·g^{-1}) of total condensed tannin (CT, A) and total hydrolysable tannin (HT, B) concentration analyzed via spectrometric assays in species grown under field conditions at Clarkston, UT during 2024, presented as LSMeans ± SE. Different letters on error bars indicate statistically significant differences in total condensed tannin concentration between months within each species. Over the months, no significant difference was observed in HT and CT concentration in showy goldeneye, Utah sweetvetch, or Ladak alfalfa (CT) ($\alpha \leq 0.05$)

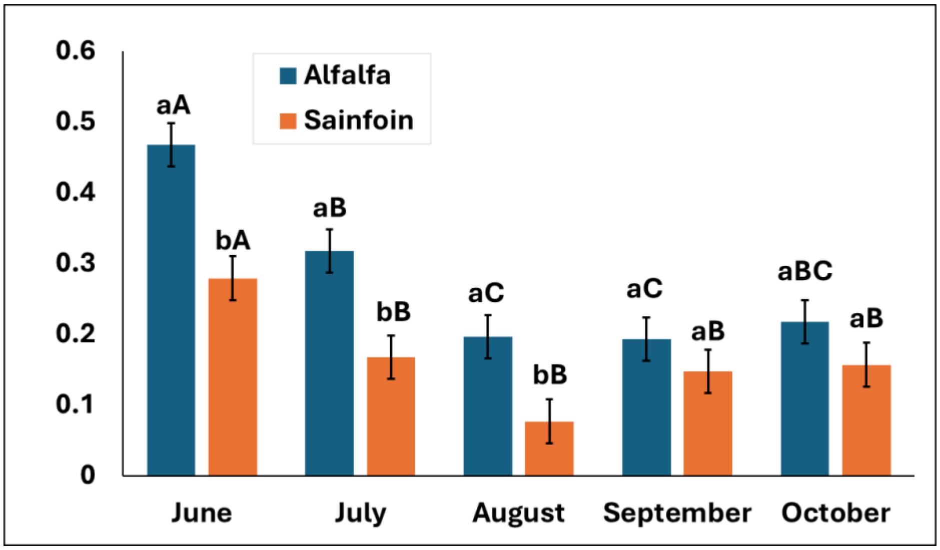


Figure 4.13. Comparison of monthly change in leaf-to-stem dry matter ratio between alfalfa and sainfoin growing at Clarkston, Utah in 2024 is presented as LSMeans ± standard error. The different lower-case letters indicate statistical differences in leaf-to-stem ratio between species within each month and different uppercase letters indicate statistical differences among months within each species ($\alpha \leq 0.05$)

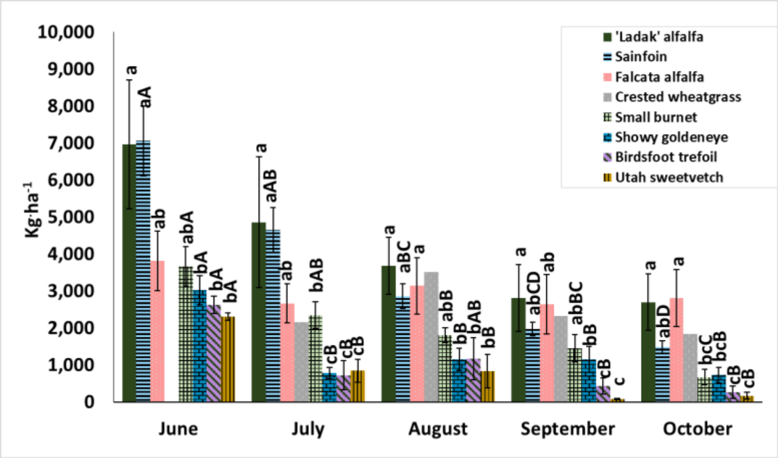


Figure 4.14. Comparison of standing dry matter yield (i.e., no previous harvest and regrowth; kg·ha^{-1}) among species growing at Clarkston, Utah in 2024 is presented as LSMeans ± standard error. The different lower-case letters indicate the statistical difference in yield production among species within each month and upper-case letters indicate a statistical difference in yield production among months within each species ($\alpha \leq 0.05$). Dry matter yield production did not vary significantly over months in Ladak alfalfa and falcata alfalfa

Chapter V

SUMMARY

This thesis reports the screening of legume and non-legume forage species to improve cattle nutrition on grass-dominated semi-arid rangelands in the western U.S., particularly during late season. The hot, dry conditions on rangeland in mid-summer led to a decline in forage quality that, for most species, did not meet the crude protein requirements of cattle. However, the neutral detergent fiber concentration of these forages was well below that of grasses, providing a better nitrogen-carbohydrate balance for microbial growth in the rumen.

This study included an evaluation of 27 perennial legumes, non-legume forbs, and grass species (with the addition of kura clover at the Panguitch field site) across five sites in Utah with the goal of selecting species capable of establishing and persisting under semi-arid conditions with minimal inputs. Although species performance varied due to the soil and climate at each location, a few plant species performed well overall. These included sainfoin, Ladak alfalfa, and small burnet, which quickly established dense plant stands at all locations and persisted for the two years they were studied. Utah sweetvetch, cicer milkvetch, falcata alfalfa, birdsfoot trefoil, Maximillian sunflower, crownvetch and showy goldeneye maintained moderate plant densities for two years after planting at most locations. Many species increased in plant density during the second year after planting, such as Palmer’s penstemon, Rocky Mountain penstemon, and Maximillian sunflower. Plants of species with longer development periods, such as arrowleaf balsamroot and fernleaf biscuitroot, were evident in May of 2023 and 2024 but began entering dormancy in June – the early establishment years are dedicated to root and crown development during mid-summer, not to shoot and seed production. These species will provide good quality, plentiful forage after 5-8 years and could be seeded into resource islands as a long-term investment. Other species either became established in very low density or did not establish at all. This is an expected outcome of a screening study incorporating plants valued on native rangeland that may need specialized niches to thrive. The plants noted above that persisted in significant numbers are the species that will be studied further for other characteristics such as persistence, long-term productivity, year-to-year variability of forage quality, including secondary metabolites.

The forage nutritive value and secondary metabolite profile of the four species Ladak alfalfa, birdsfoot trefoil, sainfoin, and small burnet were evaluated during 2023 for their ability to extend the grazing period until October with adequate protein and beneficial secondary metabolites with the potential to reduce nitrogen losses and methane emissions from beef cattle. Similarly, crested wheatgrass, Ladak alfalfa, showy goldeneye, and Utah sweetvetch were evaluated in 2024 in addition to the four species from 2023. Only Ladak alfalfa and Utah sweetvetch retained a crude protein concentration greater than 7.5%, the critical threshold required by cattle, from June through October in 2023 and 2024, respectively. However, all seven legume and non-legume forbs had comparatively less NDF concentrations than crested wheatgrass, remaining below 50% in 2023 and 60% in 2024, which would benefit rumen digestion and forage intake.

Future studies will examine the management of both Ladak and falcata alfalfa as well as sainfoin to determine the effect of partially defoliating these tall-growing species after flowering and before they expend energy on seed fill, with the goal of encouraging mid-summer regrowth that could increase protein concentration in late summer and early fall. We are further exploring the use of readily established shrubs and semi-shrubs, such as skunkbush sumac (*Rhus trilobata* Nutt.) and yellow rabbitbrush (*Chrysothamnus viscidiflorus* (Hook.) Nutt.), that can be established from seed, and of shrubs that would best be established from seed in the greenhouse and transplanted into resource islands, such as antelope bitterbrush (*Purshia tridentata* (Pursh) DC). Slow-establishing herbaceous forbs, including fernleaf biscuitroot, could be sown along with fast-establishing species and used as a second-generation grazing as resource islands evolve, gradually displacing first-generation, readily established herbaceous legumes and forbs such as sainfoin and small burnet.

We also determined the condensed tannin (CT) concentrations of birdsfoot trefoil, crested wheatgrass, falcata alfalfa, Ladak alfalfa, sainfoin, showy goldeneye, and Utah sweetvetch as the season progressed and plants matured from June through October. Neither crested wheatgrass nor alfalfa accumulate CT in their shoots, but when birdsfoot trefoil tannin is used as the standard, there can appear to be a small concentration of CT. For all three species, it was about 1 g mg^{-1}, or 0.1%, thought to be due to other phenolics. Late summer and early fall is the time period when resource islands were designed to be used, before heavy snow cover prevents grazing. While birdsfoot trefoil CT was dominated by procyanidin (PC) subunits, sainfoin CT was enriched in prodelphinidin (PD) subunits, especially early in the season. These results contrasted with greenhouse-grown plants of the same species and cultivars, where birdsfoot trefoil only contained PC and sainfoin was only about 17% enriched in PD, the opposite ratio to rangeland-grown, where birdsfoot trefoil contained about 44% and sainfoin contained about 79% PD.

Greenhouse-grown plants would be assumed to be most similar to early-season field-grown plants, but this is when the contrast between the two environments produced the greatest divergence. Condensed tannins enriched in PD are more effective in protein precipitation. The higher PD concentrations in field-grown birdsfoot trefoil and sainfoin in June as compared to greenhouse-grown plants suggest higher anti-herbivory (i.e., insect) responses in both plant species, as tannins are known to discourage plant consumption by precipitating the salivary enzymes of some herbivores. For ruminants, this increase in PD enrichment could improve tannin function in parasite inhibition and partitioning of nitrogen from urine to feces. Earlier hay-feeding studies suggest that sainfoin is more effective in partitioning nitrogen to feces and less effective, or less consistently effective, than PC-enriched birdsfoot trefoil in allowing cattle to retain nitrogen. In addition to birdsfoot trefoil and sainfoin, Utah sweetvetch exhibited high accumulation of CT, while birdsfoot trefoil and showy goldeneye had comparable CT concentrations. Hydrolyzable tannins (HT) produced by showy goldeneye, small burnet, and Utah sweetvetch not only partition waste nitrogen from urine to feces, they are more effective in reducing methane (CH_4) emissions, and small burnet was consistent in retaining a level through October that was effective as the sole diet in the same hay-feeding study. Many species grown in the greenhouse, including blanketflower, crownvetch, leadplant, white sagebrush, Palmer’s penstemon, small burnet, purple prairie clover, birdsfoot trefoil, prairie aster, Rocky Mountain penstemon, and Utah sweetvetch exhibited rich metabolic profiles comprising diverse flavonoids (e.g., tannins, flavonols, isoflavonols, anthocyanins) and phenolic acids. Birdsfoot trefoil, sainfoin, and small burnet growing under field condition during 2023 also exhibited rich secondary metabolite profiles. Sainfoin accumulated more secondary metabolites under field than greenhouse conditions. While Ladak alfalfa did not contain any tannin-related metabolites, it possessed other beneficial metabolites. The rich metabolic profile of the species imparts antioxidant, anti-inflammatory and anti-microbial ability to ruminants.

In 2024, all species except Ladak and falcata alfalfa declined in dry matter production as the season progressed. The plant species with high dry matter yield were crested wheatgrass, Ladak alfalfa, falcata alfalfa, and sainfoin, whereas birdsfoot trefoil, showy goldeneye, and Utah sweetvetch produced low dry matter throughout the season. Additionally, Ladak alfalfa maintained higher leaf to stem ration compared with sainfoin from June to August, likely to be with the source of high crude protein content seen in Ladak alfalfa in the fall.

These results of this research will help beef cattle producers on semiarid rangelands reduce their methane emissions by incorporating species such as small burnet, reduce dependence on costly protein supplements by including Ladak alfalfa as a productive, higher-protein foundation for resource islands, and including sainfoin to improve cattle health and nitrogen retention. Future research will identify plant species with the greatest short- and long-term productivity, and beneficial combinations of secondary metabolites, such as Utah sweetvetch and showy goldeneye, as well as long-term grazing resistant semi-shrubs and shrubs.

CURRICULUM VITAE

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EDUCATION

MS (Plant Sciences) *(August 2022- May 2025)*
Department of Plants, Soil, and Climate, College of Agriculture and Applied Sciences,
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Bachelor (B.Sc.) in Agriculture (Hons.) 6 Year Program *(2016-2022)*
College of Agriculture, Chaudhary Charan Singh Haryana Agricultural University
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EXPERIENCE

Graduate Research Assistant

- Effects of grazing monoculture versus polyculture pastures on epigenomic and metabolomic traits in US beef cattle *(January 2025-Present)*
- Creation of smart foodscapes to enhance the sustainability of western rangelands *(August 2022-December 2024)*

HONOURS, AWARDS, and PARTICIPATIONS

- Recipient of *Academic Opportunity fund*: Travel grant, USU *(2024)*
- Awarded the *H. Grant & Gayle P. Vest Scholarship,* USU *(2024)*
- Awarded the *John Shaw Welch Fellowship,* USU *(2024)*
- Oral presentation at Crop Science Society of America (CSSA) annual meeting on “Seasonal Change in Forage Nutritive Value and Secondary Metabolites in Forage Species Adapted to Western Rangeland” *(2024)*
- Oral presentation at USU at student symposium on “Temporal dynamics in forage value: Assessing the Seasonal Shift in Legumes and Non-Legume Forb During the Growing Period” *(2024)*
- Oral presentation at Crop Science Society of America (CSSA) annual meeting on “Germination and establishment of diverse legume and forb species for ruminant Supplementation on Rangeland” *(2023)*
- Poster presentation at USU at student symposium on “Overcoming seed dormancy: a critical challenge for rangeland species” *(2023)*
- CCS HAU Merit Gold Medal recipient for academic excellence during bachelor’s degree *(2016-2022)*
- Recipient of University merit scholarship during every semester of under-graduation *(2016-2022)*
- Poster presentation at an international e-conference on post-harvest disease

management and value addition in horticultural crops on “Mycotoxins and their effects on human beings” Division of Plant Pathology, Indian Council of Agricultural Research- Indian Agricultural Research Institute, New Delhi, India *(20 August 2021)*

LEADERSHIP EXPERIENCE

- Secretary for Indian Students Association at USU, UT, USA (*2023-24*)
- Organized and delivered training to farmers on soil and water testing, Mlkn, Haryana, India *(2021)*
- Organized women’s training on kitchen gardening, extraction, and preservation of pulps and juices *(2021)*