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Stem Development, Seeding Rate, and Establishment of Birdsfoot Trefoil (*Lotus corniculatus*) for Organic, Grazing-Based Dairies

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STEM DEVELOPMENT, SEEDING RATE, AND ESTABLISHMENT OF BIRDSFOOT

TREFOIL (*Lotus corniculatus*) FOR ORGANIC, GRAZING-BASED DAIRIES

by

Sara Hunt

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ABSTRACT

Stem Development, Seeding Rate, and Establishment of Birdsfoot Trefoil

(*Lotus corniculatus*) for Organic, Grazing-Based Dairies

by

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Three studies applicable to organic management and cultivation of birdsfoot trefoil (BFT, *Lotus corniculatus* L.) are presented here. The first is a histological analysis of lignification in BFT stems that supports recommendations to harvest BFT at approximately 6 weeks of regrowth, or early bloom. Lignification decreases digestibility and is correlated with high shear force required to break BFT stems. The sixth internodes from the base of 10 BFT and two alfalfa plants were sampled during 15 weeks of midsummer regrowth. Lignification occurred primarily in stem secondary xylem, and was apparent by 5 weeks of regrowth. The lignified xylary ring reached its maximum radial width by 7.5 weeks of regrowth in BFT. Flowering in BFT began at 5.5 weeks of regrowth, and full bloom was reached by 6.5 weeks of regrowth.

The second study evaluated establishment of BFT, which competes poorly with weeds and produces low yields during establishment. At an organic, irrigated site in northern Utah the effects of autumn vs. spring seeding, seeding rates of 3, 7, 20, and 34 kg pure live seed (PLS) ha^{-1} and use of a companion crop on 3 years of annual yields and foliar cover of mature stands were determined. First year yields increased linearly with seeding rate ($P \leq 0.05$). Use of a companion crop reduced first year yields, and did not reduce weed cover. Autumn seeding of BFT alone, following harvest of a summer crop, is recommended for irrigated production.

The final study evaluated BFT establishment on five organic dairy farms in southern Idaho and northern Utah. Participating producers broadcast seeded 4-ha BFT pastures in the fall at a rate of 25 kg PLS ha^{-1}. All farms achieved high BFT density, but only two farms had higher BFT than weed density. These two farms also had high BFT cover the spring following autumn seeding, and their pastures produced 6000 to 7600 kg of dry matter ha^{-1} by 20 June 2012 and supported grazing for the remainder of the summer. Establishment was enhanced by crop rotation and sprinkler irrigation.

(137 pages)

PUBLIC ABSTRACT

Stem Development, Seeding Rate, and Establishment of

Birdsfoot Trefoil (*Lotus corniculatus*) for Organic, Grazing-Based Dairies

Sara Hunt

Birdsfoot trefoil (BFT, *Lotus corniculatus* L.) is a non-bloating, productive forage legume well suited to grazing-based ruminant production. This thesis presents three studies conducted in the Mountain West applicable to organic management and cultivation of BFT. The first is an analysis of lignification in BFT stems that supports recommendations to harvest BFT at approximately 6 weeks of regrowth, or early bloom. Lignification decreases digestibility and is correlated with high shear force required for grazing animals to break BFT stems. The process of stem lignification was observed by harvesting increasingly mature stems; cutting thin sections of the sixth stem internode, which is located low on the plant at a recommendable harvest height; and then staining stem sections for lignin, viewing and photographing them under a microscope. Lignification was apparent in the 6th stem internode of BFT by 5 weeks of regrowth and reached maximum extent by 7.5 weeks of regrowth. Flowering in BFT began at 5.5 weeks of regrowth, and full bloom was reached by 6.5 weeks of regrowth.

The second study evaluated establishment of BFT, which often competes poorly with weeds and produces low yields during establishment. At an organic, irrigated site in northern Utah the effects of different establishment methods on 3 years of annual yields and foliar cover of mature stands were evaluated. The

establishment approaches tested were autumn and spring seeding, seeding rates of 3, 7, 20, and 34 kg pure live seed (PLS) ha^{-1} and seeding with or without a companion crop. Rates of 7 and 20 kg PLS ha^{-1} produced high first year yields and mature stands with low weed cover. Use of a companion crop reduced first year yields and did not reduce weed cover in mature stands, so is not recommendable. Autumn seeding of BFT at a rate between 14 and 18 kg PLS ha^{-1}, following harvest of a summer crop, is recommended for irrigated production.

The final study evaluated BFT establishment on five commercial organic dairy farms in southern Idaho and northern Utah. Participating producers broadcast seeded 4-ha BFT pastures in the fall at a rate of 25 kg PLS ha^{-1}. All farms established high BFT density, but only two farms had higher BFT than weed density. These two farms also had high BFT cover the spring following autumn seeding, and their pastures produced 6000 to 7600 kg of dry matter ha^{-1} by 20 June 2012 and supported grazing for the remainder of the summer. Establishment was enhanced by crop rotation and sprinkler irrigation.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Rising feed costs, fluctuating milk prices, and growing consumer interest in environmental and health benefits of grass-fed animal products are bringing pastures back to the attention of American dairy farmers. Perhaps the most widely recognized trend in the US dairy sector has been one of consolidation into large-scale confined feeding operations, a shift that has been driven by economies of scale (Mosheim and Lovell 2009) and resulted in the loss of many small dairy farms (Tauer and Mishra 2006). However, a parallel trend is occurring alongside the expansion of large-scale drylot dairy farming. Small to medium sized dairies are adopting alternative strategies to remain profitable, and consumer interest in organic and pasture-based dairy systems is growing, creating a market for their products. Although most milk produced in the US comes from larger dairies, 90% of dairies in the country have fewer than 200 cows (Brock and Barham 2008). These small dairy farms, which form an important part of rural American culture and economies, are increasingly turning to pasture as a low cost feed and a marketing strategy (Paine 2009). High quality pastures for dairy production are also receiving attention from the organic sector following the passage of the USDA National Organic Program pasture rule in 2010, which requires ruminants in organic systems to graze pasture for 120 days and receive a minimum of 30% of dry matter intake from pasture (Rinehart and Baier 2011). Dairy is one of the fastest growing sectors of the organic industry, demonstrating steady growth in both production and demand from 1997 to 2007 (Dimitri and Oberholtzer 2009).

Interest in pasture-based dairy products has been sparked by evidence that compared to cows fed a total mixed ration, milk from pasture-fed cows is higher in fatty acids beneficial to human health (Kay et al. 2005; Schroeder et al. 2005; Clancy 2006), that cows on pasture have lower rates of health problems like mastitis (Washburn et al. 2002), and reports that pasture-based production may lessen environmental impacts through lower energy expenditures and reduced acidification of soil and water compared with conventional systems (Koknaroglu et al. 2007; Arsenault et al. 2009; O'brien et al. 2012). In addition, there is evidence that grazing-based dairies can be more profitable on a small scale than those using confined feeding methods. Grazing systems tend to have lower operating expenses, lower feed costs, and higher net incomes per cow (Dartt et al. 1999; Hanson et al. 1998; Parker et al. 1992). However, milk production is lower from dairy cows on pasture-only diets than from those fed a total mixed ration (TMR), the mix of conserved forages, grains, protein and mineral supplements used in confined feeding systems (Kolver and Muller 1998; White et al. 2002). This reduced milk production has led some to question the sustainability of expanding grazing systems as it can result in greater land requirements and animal waste generation per unit of milk produced (Capper et al. 2009; Pimentel et al. 1980).

Providing pasture-fed cows with a high-energy supplement such as grain concentrate can increase milk production (Bargo et al. 2002; Reis and Combs 2000), but also results in lower levels of beneficial fatty acids in milk (Stockdale et al. 2003) and higher feed costs. McBride and Greene (2009) demonstrated that organic dairy producers who make the greatest use of pasture have feed costs that are 25% lower than organic producers who make the least use of pasture. However, these same grazing-intensive dairies have milk production levels that average 30% less than those using high-concentrate diets. In addition, a number of the environmental benefits associated with pasture-based production, including greater carbon sequestration in soils (Fisher et al. 1994) and increased biodiversity (Tilman et al. 1996) are achieved by replacing the annual cropping systems used to produce grains with perennial pastures. Maintaining high levels of milk production while minimizing grain supplementation of pasture-fed dairy cows can improve the sustainability of grazing-based dairy production.

Informed management of pastures and utilization of high quality forage species are important to the productivity of dairy cows on pasture. As early as the 1700s, strategies such as rotational grazing and planting legumes, members of the family *Fabaceae,* in mixture with grasses (*Poaceae*) were reported to improve dairy pastures (Fick and Clark 1998). In rotational grazing, cattle are moved regularly so that only one section of pasture is grazed at a time, allowing the rest to regrow and renew energy reserves (Undersander et al. 2002). As forages mature, they produce a greater quantity of feed and are better able to recover after grazing, but forage quality also decreases (Buxton 1996). Rotational grazing seeks to optimize the productivity and quality of forages over time. The practice has been well developed in New Zealand (Clark and Kanneganti 1998; Woodward and Wake 1995) and has more recently begun to receive research attention and wider use by producers in the United States (Conant et al. 2003; McCall et al. 1999; Ostrum and Jackson-Smith 2000; Paine et al. 1998). Incorporating forage legumes into grass pastures can increase productivity in a number of ways. Legumes incorporate nitrogen from the air into amino acids in root nodules, some of which is transferred to grasses (Ledgard and Steele 1992), and deep-rooted legumes are productive during hot, dry periods when the growth of cool-season grasses tends to slow, improving the seasonal distribution of yields (Berdahl et al. 2001; Lauriault et al. 2003; Sleugh et al. 2000). In addition, the forage quality of legumes does not decrease as rapidly with advancing maturity as does that of grasses (Waghorn and Clark 2004). However, many common legumes like alfalfa (*Medicago sativa*) and clover (*Trifolium* sp.) can cause bloat, a potentially lethal disorder in cattle, and should not constitute more than 50% of a pasture (Undersander et al. 2002). Birdsfoot trefoil (BFT; *Lotus corniculatus*) is a productive legume that does not cause bloat and is well suited to pasture-based dairy production due to the beneficial tannins it produces.

Birdsfoot Trefoil Literature Review

Importance and use

Birdsfoot trefoil is a tap-rooted perennial similar to alfalfa that can be found wild and in naturalized populations across temperate regions of Europe, Asia Minor, North and South America, Australia, and New Zealand (Steiner and Garcia de los Santos 2001). It is adapted to a wide range of conditions and exhibits high genetic variation, a trait important to further improvement of agronomic varieties (Steiner 1999). Although cultivated around the world, BFT is cultivated on less area than other legumes in most countries, and is only a major pasture species in Uruguay and Argentina where it is relied on for dairy production and crop-pasture rotations (Blumenthal and McGraw 1999). In the early 1900s, BFT was reported to be an important pasture crop in Northern regions of the US (Ahlgren 1956). More recently, the importance of BFT has declined with intensification of agriculture and the tendency among North American farmers to favor alfalfa for forage production (Blumenthal and McGraw 1999). In 1995, US cultivation of BFT was reported to be 1 million ha with greatest use in the Northeast, Midwest, and along the Pacific coast (Beuselinck and Grant 1995). In comparison, 10 to 11 million ha of alfalfa are cultivated in the US annually (Barnes and Sheaffer 1995).

Alfalfa, often referred to as the "Queen of Forages," has received the largest breeding effort of any forage species, and the commercial sector is directly involved in cultivar development (Brummer et al. 2009). As a result, alfalfa produces high yields of excellent quality and is relatively persistent under a range of conditions. However, BFT can be more productive than alfalfa on marginal sites (Seaney and Henson 1970) and is more tolerant of wet and waterlogged soils (Barta 1980). Comparison of BFT to alfalfa is useful due to similarities between the species as well as widespread familiarity among agricultural professionals and the vast body of research that has been done on alfalfa. Throughout this thesis comparisons will be drawn between BFT and alfalfa as well as clover, which has been widely used in mixed pasture plantings to improve pasture quality. Because of its wide adaptation and productivity, BFT has replaced much of the clover previously grown with grasses in the Northeast US (Blumenthal and McGraw 1999) and has received increasing research attention in New Zealand where grazing-based ruminant production is widespread and has traditionally relied heavily on mixed pastures of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) (Woodward et al. 2002). In the context of grazing based dairy production, BFT provides benefits due to its high digestibility and production of beneficial tannins.

Forage quality and tannins of birdsfoot trefoil

In confined feeding systems, cows are fed TMRs formulated to meet their needs and maximize production. Over time, dairy cow genetics has been selected to favor high levels of milk production on formulated diets, and pasture-based diets do not always provide the nutritional density and balance necessary for high producing dairy cows to perform up to their genetic ability (Kolver and Muller 1998). Dry matter intake, available energy and protein utilization tend to limit the productivity of dairy cows on pasture (Kolver 2003; Waghorn and Barry 1986). Fresh forages are high in water content and typically contain 12 to 30% DM (Waghorn and Clark 2004) as compared to 35 to 60% DM in a typical TMR (Robinson et al. 1990), so cows must have higher intake of fresh forages to consume a similar quantity of nutrients. Fresh forages also tend to be low in available energy, high in protein and high in fiber (measured as neutral detergent fiber; NDF) compared to a TMR (Bargo et al. 2003). High levels of NDF are generally correlated with low levels of digestibility in forages, and cattle intake of forages tends to decrease with high NDF and low digestibility (Allen 1996).

Birdsfoot trefoil fulfills the nutritional requirements of dairy cattle well in comparison to other forages. As a legume it maintains higher digestibility with maturity than grasses, due largely to lower fiber content. Measurements of forage NDF reflect concentrations of cellulose, hemicellulose, and lignin, the structural carbohydrates found in plant cell walls (Van Soest 1994). Cellulose and hemicellulose are potentially digestible by rumen microbes, while the complex structure of lignin inhibits its digestion. Lignin also crosslinks with other cell wall components, further decreasing digestibility. Plant stems become more lignified with maturity than leaves, and legumes maintain a higher leaf to stem ratio than grasses, causing legumes to maintain better digestibility with maturity than grasses (Waghorn and Barry 1986). The different chemical constituents (Buxton and Russell 1988) and patterns of lignification (Wilson and Hatfield 1997) further contribute to higher digestibility of legumes.

Within the legume family, there is evidence that lignification has less effect on digestibility in BFT than in alfalfa. At the early bloom stage Tomlin et al. (1965) demonstrated BFT cellulose to be digested more rapidly than alfalfa cellulose, despite similar lignin concentrations. McGraw and Marten (1986) reported higher digestibility of BFT stems than alfalfa stems, and while Mowat et al. (1969) found a highly significant negative correlation between cell wall lignin concentration and digestibility in alfalfa, BFT cell wall lignin concentration was not correlated with digestibility. High cell wall digestibility in BFT may lead to higher levels of DM intake in ruminants. Van Soest (1965) evaluated the correlation of voluntary intake with cell wall content for BFT, alfalfa and numerous grasses and considered BFT a statistical outlier because its voluntary intake (or digestibility of DM) was so much higher relative to its cell wall content than that of other forages. Douglas et al.

(1995) reported higher intake by lambs grazing BFT than those grazing alfalfa or an alfalfa/BFT mixture. However, in another study lambs on pasture did not consume BFT stems even under conditions of limited feed allowance due to stem resistance to shear force (Douglas et al. 1999), which is related to stem lignification.

In addition to having high digestibility, BFT also produces tannins that lead to more efficient utilization of protein by ruminants, which lowers nitrogen in waste, reducing the environmental impacts of grazing-based ruminant production. Tannins are an abundant secondary compound produced by many families of higher plants (Mole 1993). These polyphenolic compounds vary widely in chemical structure but exhibit the common characteristic of binding proteins. Although the function tannins serve in plant ecology has not been fully explained, there is evidence that they deter herbivory (Feeney 1976; Rhoades and Cates 1976), act as antimicrobial and antifungal agents (Scalbert 1991; Schultz et al. 1992), serve along with other flavonoids as a sink for excess carbon (Hernandez and Van Breusegem 2010; Bryant et al. 1983; Coley et al. 1985), and slow nutrient mineralization from organic matter (Kraus et al. 2003; Hattenschwiler and Vitousek 2000). It is likely that tannins serve a combination of functions, and it has been suggested that rather than search for a general ecological function, it may be more appropriate to consider the specific effects of unique tannins based on their chemical structures (Zucker 1983).

The effects tannins have on ruminants depend on their chemical structure as well as concentration, but in general, tannins in forages have negative effects at concentrations exceeding 4-5% DM and beneficial effects below this threshold

(Waghorn et al. 1998; Min et al. 2003). At high concentrations tannins tend to adversely affect ruminants by decreasing voluntary intake and limiting protein and dry matter (DM) digestibility (Kumar and Singh 1984), but they also benefit ruminants by preventing bloat (McMahon et al. 1999), reducing internal parasite numbers, and increasing the efficiency of protein metabolism when present in low to moderate concentrations (Aerts et al. 1999; Mueller-Harvey 2006; Waghorn 2008). Commonly available cultivars of BFT produce tannin concentrations below 4% (Roberts et al. 1993). However, BFT tannin concentrations from 2.5 to 8.5% DM have been found to reduce in vitro protein degradation without reducing dry matter digestibility (Miller and Ehlke 1994), indicating that within this range of concentrations BFT tannins may be beneficial for ruminants.

Fresh forages are high in proteins that are rapidly degraded by rumen microbes, resulting in a surplus of ammonia and inefficient utilization of protein. Ammonia is toxic at high concentrations so ruminants must expend energy to convert ammonia to urea, which is either recycled back to rumen microbes or excreted. Tannins reduce ruminal protein degradation and the associated release of ammonia both by binding protein in the rumen and by inhibiting activity of bacteria that break down protein (Patra and Saxena 2011). It has been demonstrated that BFT tannins bind to proteins at the pH of the rumen and then dissociate at the lower pH of the abomasum, releasing protein for digestion and absorption in the lower digestive tract (Waghorn et al. 1987). This mechanism can explain why BFT tannins increase milk yield in dairy cows (Turner et al. 2005; Woodward et al. 2000; Woodward et al. 2009), and divert dietary nitrogen from urine to feces, reducing the potential for volatilization of ammonia from dairy manure (Misselbrook et al. 2005; Woodward et al. 2009). BFT tannins also reduce enteric methane production (Woodward et al. 2004), possibly by directly inhibiting the activity of methanogenic bacteria (Tavendale et al. 2005). It has been suggested that tannins indirectly reduce methane production by reducing the number of ruminal protozoa (Patra and Saxena 2009), which are symbiotic with methanogenic bacteria, but BFT tannins have not been demonstrated to have this effect (Chiquette et al. 1989). Soil microbes are also affected by tannins, resulting in reduced mineralization of nitrogen from feces (Crush and Keogh 1998), which lowers the potential for nitrate leaching in soils. The tannins produced by BFT provide a number of benefits important to grazing-based ruminant production, and the high digestibility of BFT is also beneficial, but agronomic qualities such as yield, establishment and persistence must also be considered.

Cultivation and management

In addition to providing high quality feed, an ideal perennial forage should also be productive and persistent. BFT produces lower dry matter yields than alfalfa (Sleugh et al. 2000), but there is evidence that BFT can still provide similar or higher rates of daily gain when grazed by ruminants (Marten and Jordan 1979; Barker et al. 1999). Forage crops are generally harvested multiple times during the growing season to optimize DM production and forage quality. It is recommended that BFT should be harvested or grazed at the early bloom stage of growth (Rhykerd et al. 1981; Undersander et al. 1993; Hall and Cherney 2007). While alfalfa regrows from crown buds at or below the soil surface and accumulates root carbohydrate reserves throughout the growing season, new BFT stems develop from axillary buds on stubble (Nelson and Smith 1968) and storage of carbohydrates in the roots is minimal until autumn (Smith 1962). This means that BFT is dependent on photosynthesis for summer regrowth. Failure to leave sufficient leafy stubble for regrowth can decrease yields of BFT (Smith and Nelson 1967). Rotational grazing favors better persistence of BFT than continuous grazing, which can lead to limited persistence (Van Keuren and Davis 1968). Allowing BFT to flower and set seed can improve persistence (Taylor et al. 1973) and it has been suggested that BFT may be well suited to stockpiling summer forage because it maintains high quality with maturity (Collins 1982).

Although productive, BFT exhibits poor persistence in warm, humid regions where susceptibility to pathogens is high (Beuselinck et al. 1984; Emery et al. 1999; Murphy et al. 1985) and at low latitudes where day length is insufficient for full flowering (Beuselinck and McGraw, 1988). Work is being done to develop cultivars resistant to disease (Altier et al. 2000) and that will flower with shorter days (Ayres et al. 2007). In regions with adequate day length, BFT often performs better than other legumes in soils with poor drainage or low pH (Seaney and Henson 1970). Rhizomatous cultivars of BFT have been developed in an attempt to improve persistence. Beuselinck et al. (2005) compared the performance of rhizomatous cultivars ‘ARS-2620’ and ‘ARS-2424’ and non-rhizomatous ‘Norcen’ in five locations and found that both rhizomatous and non-rhizomatous plants grown in Logan, UT produced larger, heavier crowns than those in other locations. They attributed this difference to the effect of the environment, and suggested that the cool, arid conditions might discourage growth of pathogens. MacAdam and Griggs (2006) found that 'Norcen' BFT grown in Logan, UT was nearly as productive as alfalfa in mixtures under clipping, more productive than white clover under season-long grazing, and persistent under grazing from 2000 to 2006. Such evidence suggests that BFT, like alfalfa, may be particularly well suited for use under irrigation in the western US where cool, arid climates and moderately soils are common.

Slow establishment is commonly cited as a challenge in the cultivation of BFT (Ahlgren 1956; Seaney and Henson 1970). Due to its small seed size and low seedling vigor BFT competes poorly with weeds (Chapman et al. 2008; Henson and Taymon 1961) and often produces low yields during establishment. Chemical weed control reduces weed competition and produces higher yields of BFT during the establishment year (Scholl and Staniforth 1957) but does not clearly improve second year yields (Scholl and Brunk 1962; Wakefield and Skaland 1965). Studies have demonstrated increased DM production in the establishment year from BFT stands seeded with a companion crop (Scholl and Staniforth 1957; Wiersma et al. 1999) and at higher seeding rates (Wakefield and Skaland 1965). Laboratory studies have evaluated the temperature (Hur and Nelson 1985; McElgunn 1973) light intensity, and moisture (Gist and Mott 1957) requirements of BFT for germination and seedling growth, and numerous studies have assessed field establishment of BFT in the US (Buxton and Wedin 1970; Fribourg and Strand 1973; Strand and Fribourg 1973), Canada (Chapman et al. 2008), Australia (Ayres et al. 2006), and New Zealand (Douglas and Foote 1994). These studies indicate temperature, soil moisture, and seeding date as important factors influencing BFT seedling emergence and the establishment of a productive mature stand. Once established BFT can provide high quality, productive forage well suited to grazing-based dairy production.

Research Context and Objectives

The research presented here was done in support of a larger study comparing milk production of dairy cattle grazing BFT pastures with that of dairy cattle grazing grass pastures on organic farms. The overarching goals of these studies are to enhance understanding of BFT as a forage crop, spur further research, and encourage development of BFT cultivars with traits important to grazing-based ruminant production. Although the focus here is on dairy cattle, many of the benefits of BFT also apply to beef cattle production, particularly during finishing when high rates of weight gain are important. The objectives of the studies reported here are as follows:

1) To document the development and lignification of BFT stems cell walls by following cell wall accumulation and lignification in the sixth internode of BFT stems, and relate BFT stem development to recommendations for the timing of BFT harvest.
2) To assess the effects of seeding season, seeding rate and use of an annual oat companion crop on the organic establishment of BFT by measuring yields for three years following seeding as well as assessing foliar cover of mature stands.

3) To evaluate the on-farm establishment of pure BFT pastures under irrigation in the Mountain West using data for BFT and weed plant density and cover.

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CHAPTER 2

LIGNIFICATION AND TANNIN LOCALIZATION DURING THE DEVELOPMENT OF BIRDSFOOT TREFOIL STEMS

ABSTRACT

Stem dry matter accumulation is correlated with decline in digestibility in both birdsfoot trefoil (*Lotus corniculatus* L.; BFT) and alfalfa (*Medicago sativa* L.), and the shear force required to harvest BFT stems can inhibit BFT grazing by ruminants. Decline in digestibility and increase in shear force are both correlated with increase in stem lignin content. Alfalfa stem development has been well documented, and our objective was to analyze stem development, lignification and tannin in BFT stems using alfalfa as a benchmark. The sixth internode from the base of ten BFT and two alfalfa control plants was sampled from 3 to 15 weeks of stem regrowth in midsummer. Lignification occurred primarily in stem secondary xylem and was apparent in the xylary ring of Internode 6 by 5 weeks of regrowth. The xylary ring reached maximum thickness by 7.5 weeks of regrowth in both BFT and alfalfa, but developed at a more rapid rate and to a greater thickness in BFT. Flowering in BFT began at 5.5 weeks of regrowth, and full bloom was reached by 6.5 weeks of regrowth. The number of cells containing tannin remained constant with stem development, which would decrease stem tannin concentration with internode maturation. Internode 6 of BFT is located 60 to 120 mm from the base of stems, within the recommended cutting height of 75 mm, with sufficient leaves and branches at subtending nodes to support shoot regrowth. These data relate growth and lignification in lower stems of BFT to grazing management recommendations.

INTRODUCTION

Legumes are valuable forages because they provide higher nutritive value for ruminants than grasses (Ulyatt et al., 1977) and can synthesize the nitrogen needed to support their growth. However, many of the most commonly cultivated forage legumes, such as alfalfa, and the *Trifolium* (e.g. white, red) clovers should be limited to 50% or less of pasture mixtures to reduce the risk of ruminant bloat (Undersander et al., 2002; Majak et al., 2003). Birdsfoot trefoil (*Lotus corniculatus* L.; BFT) is a perennial legume that can be grazed in pure stands as well as in mixtures because it contains unique tannins that not only prevent bloat, but provide numerous other benefits for pasture-based ruminant production. The tannins synthesized by BFT are valuable because they increase the efficiency of plant protein utilization (Mueller-Harvey, 2006; Waghorn, 2008), reduce methane production (Woodward et al., 2004), increase milk yield in dairy cows (Woodward et al., 1999, 2000, 2004, Turner et al., 2005), and increase live weight gain in sheep (Douglas et al., 1995). Compared with other forages, feeding BFT reduces nitrogen excretion in urine (Woodward et al., 2009) and ammonia production from manure (Misselbrook et al., 2005), reduces the production of pastoral flavor compounds in lamb (Schreurs et al., 2007), and increases the rate of cattle gain (Marten et al., 1987; Wen et al., 2002; MacAdam et al., 2011). Because the market for sustainably produced pasture-fed livestock products continues to grow, there is increasing interest in the use of BFT both alone and in mixtures to support the efficient production of ruminants on pasture while reducing negative environmental impacts.

Compared with alfalfa, BFT is reported to produce more fine, less upright stems (Buxton et al., 1985). Birdsfoot trefoil and alfalfa both decline in nutritive value with advancing maturity because there is more fiber in stems than in leaves, and stem dry matter ha^{-1} accumulates longer and at a more rapid rate than leaf dry matter ha^{-1} relative to total dry matter accumulation ha^{-1} (McGraw and Marten, 1986). McGraw and Marten (1986) also found that in vitro digestible dry matter (IVDDM) kg^{-1} was greater in BFT than in alfalfa due to higher stem IVDDM in BFT, while leaf IVDDM between the two species did not differ. However, lambs refused to eat BFT stems even under conditions of limited feed allowance due to stem resistance to shear force (Douglas et al., 1999). We conclude that while BFT stems may be more digestible than alfalfa stems when forages are harvested on the same date, excessive maturation of BFT stems can limit ruminant grazing.

Understanding the timing and progression of lignification in stems can inform management that seeks to optimize ruminant gain ha^{-1} by balancing the desirable increase in forage dry matter accumulation with the undesirable decline in intake and nutritive value as forages mature. Engels and Jung (1998, 2005) and Jung and Engels (2001, 2002) thoroughly described the deposition of lignin in cell walls of the seventh internode from the base of alfalfa stems and the implications for rumen digestibility as plants matured during the growing season, but no similar histological examination of BFT stem development can be found in the literature. The objective of this study, therefore, was to document the growth of BFT Internode 6 and the accumulation of lignin in stem internode secondary xylem cell walls because lignification is not only negatively associated with fiber digestion (Jung,

1989) but positively associated with increase in shear strength of alfalfa stems (Iwaasa et al., 1995, 1999).

Since tannin in BFT prevents bloat and is beneficial to ruminant production, the localization of tannin in stems during internode development was also documented. The study was carried out in large pots to promote rapid plant establishment, allow for optimal fertilization and irrigation, and to minimize the confounding effects of suboptimal soil texture, nutrient or water availability on plant stem development. We assessed the development of BFT and alfalfa Internode 6 at the same stage of regrowth and under the same conditions to facilitate comparison of our data to those of Jung and Engels (2002).

MATERIALS AND METHODS

Plant material and sampling

Alfalfa and BFT plants were started from seed in a heated and wet air-cooled greenhouse at Utah State University in Logan, UT (41°74N, 111°83W) on 3 Apr. 2011. Ten 11.4-L pots were filled with a medium containing vermiculite, bark, peat moss, perlite and nutrients (Sunshine MetroMix 300, Sun Gro Horticulture, Bellevue, WA USA), and 10 g of bonemeal containing 44 g kg^{-1} P (Hi-Yield, Voluntary Purchasing Groups, Inc., Bonham, TX USA) was spread 5 cm below the surface. Three ‘Norcen’ BFT (Norfarm Seeds Inc., Bemidji, MN USA) seeds were inserted 0.6 cm below the surface. Two pots of ‘Viking 340M’ ALF (Albert Lea Seedhouse, Albert Lea, MN USA) were prepared in the same way.

Greenhouse temperature during seed germination and initial plant growth (3 Apr. to 7 June 2011) varied between 19 and 22 °C. Average natural integrated daily photosynthetic photon flux ranged from 4 to 56 mol m^{-2} day^{-1} between 6 AM and 9 PM. Plants were watered regularly, and on 16 May and 5 June, 1 g of fertilizer containing 150 g kg^{-1} N, 132 g kg^{-1} P and 125 g kg^{-1} K (Miracle-Gro 15-30-15 All-Purpose Water-Soluble Plant Food, ScottsMiracle-Gro, Marysville, OH USA) dissolved in 1 L of water was added to each pot. Plants were thinned to one per pot on 1 June. On 7 June all plants were cut back to 75 mm in height and moved outside to a 2.8 m^2 greenhouse bench, where they were irrigated to field capacity daily with water containing 0.5 g of 210 g kg^{-1} N, 22 g kg^{-1} P and 166 g kg^{-1} K (Peter's 21-5-20 Water-Soluble Fertilizer, JR Peters Inc., Allentown, PA USA) L^{-1}. Plants were not cut back for the remainder of the study. All plants were sprayed twice (6 and 12 July) with insecticidal soap (Safer, Woodstream Corp., Lititz, PA USA) for a minor aphid infestation, which responded to treatment.

Stem internode samples were first taken on 28 June 2011 after 3 weeks of regrowth, then on 6, 12, 15, 18, 21, 24, 27 July, 3, 10, 30 Aug., and 21 Sep., or at 4, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 12, and 15 weeks of regrowth, respectively. These 12 sampling dates were chosen to span a mid-summer regrowth period plus further stem maturation and to focus most intensively between 5 and 8 weeks of regrowth. On each sampling date one stem was removed from each plant at its point of initiation. Stems were chosen based on the total number of internodes present, beginning with stems with 7 internodes at the first harvest, then stems with 8, 10, 12, 14, 16, 18, 19-23, 20-24, 21-25, 22-26, and 23-27+ internodes, respectively, on successive sampling dates. Each harvest captured increasingly older stems with increasingly mature sixth internodes. Before stems were harvested, the longest stem on each plant was measured until 7.5 weeks of regrowth, after which the stems became too intertwined for accurate measurement. At 15 weeks, one additional measurement of the length of longest stems was made, and stems with 23-27+ internodes at that date were among the longest on each plant. Observations were also made at each harvest regarding the health of the plants and easily observable signs of maturity such as flowering and seed set.

At each sampling date, the length and diameter of the sixth internode from the base of each stem was measured, and sixth internodes were excised and stored temporarily in 0.1 M pH 7 potassium phosphate buffer. Diameters were measured using a digital caliper (VWR International, Radnor, PA) with 0.2-mm precision. Internodes were then fixed in 2.5% glutaraldehyde for 24 to 48 h, rinsed for 24 h in phosphate buffer, and dehydrated through an ethanol series to preserve them for histological analysis (Ruzin, 1999).

The potted alfalfa and BFT plants overwintered outside under straw mulch, and eight BFT and no alfalfa plants survived. The eight BFT plants were returned to the same greenhouse bench and watering system used the previous summer. On 6 June 2012 plants were cut back to 7.5 cm in height. Regrowth of these plants above 7.5 cm was harvested on 4 July, 18 July, 1 Aug., and 29 Aug. or at 4, 6, 8 and 12 weeks of regrowth, respectively. These harvests were taken from different quarters of each plant so that forage nutritive value could be assessed. Harvested plant material was oven dried for 48 h at 55 °C to constant weight and ground to pass the 1 mm screen of a UDY cyclone mill (UDY Corporation, Ft. Collins, CO).

Histological Analysis

Two internodes per genotype per sampling date allowed the detection of significant changes in internode growth and development variables over time and among genotypes in alfalfa stem histological studies (Jung and Engels, 2002). Therefore, a subset of three BFT internodes was randomly chosen for histological analysis for each BFT sampling date, and the two available alfalfa internodes were sectioned and stained for comparison.

Internodes were rehydrated overnight in a 1:4 ethanol:water (v/v) solution, and immediately prior to staining, internodes were hand sectioned and sections were fully rehydrated in distilled, deionized water. Unstained transverse stem sections were examined first under light microscopy, and then under fluorescence microscopy to assess the extent of lignification. For fluorescence microscopy an Olympus model BH-RFL fluorescence vertical illuminator (Tokyo, Japan) was used with an ultraviolet dichroic mirror, UG-1 exciter filter and Y-455 barrier filter. Lignin has been reported to fluoresce blue under ultraviolet light (Akin et al., 1985; Rudall and Caddick, 1994; Ruzin, 1999).

Successive sections from the same stem internode were stained for lignin with phloroglucinol-HCl according to Ruzin (1999) and with 0.05% toluidine blue O (TBO) according to O'Brien et al. (1964). Phloroglucinol-HCl reacts specifically with lignin, producing a red-pink coloration, and TBO is a polychromatic stain that colors many cell components, imparting a distinctive blue-green color to lignified cell walls. In addition, BFT sections were incubated for 1 h in 2% $FeSO_4$ prepared according to Ruzin (1999) to stain for tannins. Alfalfa does not express tannins in shoot tissue, but sections from the second sample date, 6 July, were also incubated with $FeSO_4$ for comparison with BFT. All sections were mounted in distilled water, viewed under a Model BHTU Olympus Biological Microscope (Tokyo, Japan) and photographed with an attached Nikon Coolpix 4500 (Tokyo, Japan) camera.

Internode radius and mean thickness of the xylary ring, including lignified primary xylem vessels, were measured using photographs of phloroglucinol-stained sections of three BFT and two alfalfa sixth internodes on each sampling date. These data are reported as the ratio of xylary ring thickness to stem radius.

Forage Quality Analysis

Forage nutritive value of ground shoot material from the eight overwintered BFT plants was determined with near-infrared reflectance spectroscopy (NIRS) using a scanning monochromator (Model 6500, FOSS NIRSystems, Inc., Laurel, MD) and chemometrics software (WinISI II, ver. 1.5, Infrasoft International LLC, State College, PA). Reflectance data were obtained at 2-nm increments between 1100 and 2498 nm. A legume hay equation developed according to procedures of Shenk and Westerhaus (1991) from a calibration set containing multiple species was used to predict sample composition. The distribution and boundaries of BFT sample spectra were well-represented by the population structure of spectra in the calibration set, so no additional wet chemistry was required. Digestibility of NDF (dNDF, as a proportion of DM) in calibration samples was determined by incubating samples in buffered rumen fluid for 48 h followed by refluxing of indigestible residues in neutral detergent solution (Goering and Van Soest, 1970). *In vitro* true DM digestibility (IVTDMD; Goering and Van Soest, 1970) was calculated from NDF and dNDF concentrations. Determination of ADF, CP, and amylase-treated NDF of calibration samples was according to AOAC International (2010) methods 973.18, 984.13, and 2004.04, respectively. For the sake of brevity, data for dNDF, CP and ADF are not reported for this study.

While it would have been ideal to analyze whole shoot material for forage nutritive value in the same year that stem internode development and lignification were investigated, harvesting of sufficient material for analysis from the same plants would have changed the environment and physiology of remaining stems. Therefore, the same plants were grown in the same location, under the same fertility and irrigation, and harvested at four of the same harvest dates in 2012 as in 2011. Fig. 1 illustrates that mean temperature data for Logan, UT in June, July and August of these two years was similarly variable, but that temperatures trended cooler by 1°C in 2011. The 2-wk period around 6 weeks of regrowth, which fell on 18 July in both years, is denoted with a grey bar.

Statistical Analysis

Changes in length of BFT and ALF stems, internode 6 length and diameter, and in the ratio of lignified xylary ring thickness to internode radius for internode 6 were described with linear regressions. Significance of and difference in the slopes of the lines was evaluated with PROC GLM using the least squares method in SAS version 9.3 (SAS Institute Inc. 2009, Cary, NC USA). Changes in BFT forage quality parameters over 12 weeks of regrowth (n=8) were evaluated using least-squares regression analysis in Excel version 14.3.1 (Microsoft 2011, Seattle, WA USA). No forage quality data were collected for alfalfa.

RESULTS

Growth and Development

For the regrowth period of 7 June through 21 Sept., mean daily temperature ranged from 11 to 28 °C and peaked on 26 Aug. (Fig. 2-1A), and daily photosynthetic flux ranged from 4 to 56 mol m^{-2} d^{-1} and peaked on 26 June (Fig. 2-1B). Stem length, based on the longest stem of each plant at each sampling date, increased linearly for the first 7.5 weeks of regrowth (Fig. 2-2A). Birdsfoot trefoil stems grew 80 mm $week^{-1}$ from 3 to 7.5 weeks of regrowth and reached 560 mm at 7.5 weeks, while alfalfa stems grew 160 mm $week^{-1}$ and reached 970 mm at 7.5 weeks. Stem growth rates from 3 to 7.5 weeks were significantly less for BFT than for alfalfa ($P < 0.05$). At Week 15 the longest stems of BFT averaged 600 mm, and the longest stems of ALF averaged 1060 mm, indicating that stem growth of both BFT and ALF had not continued at the same rate of growth after 7.5 weeks of regrowth (Fig. 2-2A).

To determine change in internode length, diameter and xylary ring thickness of BFT, internodes were harvested from stems having the same total number of internodes on each sampling date. Selecting a single stem from each plant was minimally disturbing to the microenvironment in which subsequent stems would develop. While stem length increased for 7.5 weeks (Fig. 2-2A), the length of BFT Internode 6 increased only through 5.5 weeks of regrowth (Fig. 2-2B) then appeared to decrease from 5.5 through 9 weeks of regrowth ($P = 0.06$). Between 3 and 5.5 weeks of regrowth, the elongation rate of BFT internode 6 was 8.7 mm $week^{-1}$ while that of ALF was 12.8 mm $week^{-1}$; however, these rates were not significantly different ($P = 0.17$). Elongation of BFT Internode 6 stopped at 5.5

weeks, but Internode 6 of alfalfa continued to elongate at 4.3 mm per week^{-} from 5.5 to 15 weeks of regrowth (Fig. 2-2B).

The average diameter of Internode 6 increased at similar rates of 0.41 mm week^{-1} for BFT and 0.39 mm week^{-1} for alfalfa (P = 0.63) between 3 and 7.5 weeks of regrowth (Fig. 2-2C). From 7.5 to 15 weeks, no significant change occurred in Internode 6 diameter (BFT slope P = 0.10, alfalfa slope P = 0.62). Increase in thickness of the lignified xylary ring is reported as a proportion of the radius of Internode 6 (Fig. 2-2D) to minimize the effect of plant-to-plant variation. The rate of increase in relative xylary ring thickness was greater for BFT than for alfalfa from 4 to 7.5 weeks of regrowth (P < 0.05); 0.13 µm µm^{-1} week^{-1} for BFT and 0.08 µm µm^{-1} week^{-1} for alfalfa. Maximum relative xylary ring thickness was reached at 7.5 weeks of regrowth in both BFT and alfalfa and was 0.45 µm µm^{-1} for BFT and 0.30 µm µm^{-1} for alfalfa. From 7.5 to 15 weeks of regrowth, the mature xylary ring of BFT occupied a larger proportion of the radius of the internode than the xylary ring of alfalfa. There was no change during this period (BFT slope P = 0.10, alfalfa slope P = 0.12).

Histological Analysis: Lignification

All methods of lignin visualization used showed localization of lignin in the same tissues at 6 weeks of regrowth (Fig. 2-3). In an unstained section (Fig. 2-3A), groups of large open xylem vessels can be seen in vascular bundles. Phloroglucinol-HCl stains specifically for lignin, and gave the clearest distinction of lignified tissues in young stems (Fig. 2-3B), while fluorescence (Fig. 2-3C) was transient and therefore significantly more difficult to photograph. Toluidine Blue O staining (Fig.

2-3D) more clearly elucidated a variety of different tissues and was especially helpful for differentiating structures within individual cells.

As both BFT and ALF stems matured and their diameter increased due to secondary growth (Fig. 2-4), their shape changed from angular (Fig. 2-4A and 2-4B) to round (Fig. 2-4G and 2-4H) although alfalfa retained distinct ridges on the perimeter of the stem. Secondary xylem was added to the primary xylem of discrete vascular bundles beginning at about 5 weeks (Fig. 2-4C and 2-4D) to form a ring of lignified secondary xylem (Fig. 2-4E to Fig. 2-4J). The xylary ring reached its mature proportion of radial width by about 7 weeks of regrowth (Fig. 2-4E and 2-4F), coinciding with cessation of increase in internode diameter (Fig. 2-2C). While stem diameter was similar in BFT and alfalfa at 7 weeks of regrowth, the lignified xylary ring can be seen to represent a larger proportion of the sixth internode in BFT than in alfalfa. Green staining by TBO indicates lignification, and in BFT, the pith did not become lignified with increasing maturity (Fig. 2-4G and 2-4I), but in alfalfa, a ring of thin-walled pith cells on the inner border of the xylary ring became lignified at later stages of stem maturation (Fig. 2-4H and 2-4J). Lignification of the pith of ALF was also noted by Engels and Jung (1998) and Jung and Engels (2002).

When the sixth internode was stained for lignin with phloroglucinol-HCl (Fig. 2-5), the definitive stain for lignin, lignification of secondary xylem tissue could be seen as early as 4 weeks of regrowth in BFT (Fig. 2-5A), but in alfalfa phloroglucinol-HCl staining was not observed between vascular bundles until 5 weeks of regrowth, when a lignified xylary ring was present in all sixth internodes from both BFT and ALF stems (Fig. 2-5C and 2-5D). The xylary ring assessed as a proportion of internode radius was fully expanded and lignified by 8 weeks of regrowth in both species (Fig. 2-5I and 2-5J).

In both BFT and alfalfa, lignification began in the cell walls of primary xylem vessel members, as observed in samples taken at 3 weeks of regrowth (Fig. 2-6A and 2-6B, respectively). Xylem is comprised of water-carrying elements as well as fiber cells that add strength and rigidity. After lignification was complete, some xylem fiber cells in BFT retained an inner secondary cell wall layer that failed to stain for lignin (Fig. 2-6C) while in alfalfa, the inner layer of some fiber cells became only lightly stained for lignin (Fig. 2-6D).

Phloem fibers of BFT were not lignified at 4 weeks of regrowth (circled in Fig. 2-7A, while alfalfa phloem fibers at the same age were partially lignified (Fig. 2-7B). At 12 weeks of regrowth, non-lignified phloem fiber cells in both BFT and alfalfa appear thick-walled but white, while lignified phloem fibers stained green. Fewer phloem fibers were lignified in fiber bundles of mature stems of BFT (Fig. 2-7C) than alfalfa (Fig. 2-7D).

Histological Analysis: Tannins

The $FeSO_4$ stain used to visualize BFT tannins (Ruzin, 1999) formed a blue precipitate in cortical cells bordering phloem fiber bundles, in a few primary xylem cells, and in pith cells near the lignified xylary ring of 4-week-old BFT sixth internodes (Fig. 2-8A). The $FeSO_4$ stain produced no similar precipitate in alfalfa sections at 4 weeks of regrowth (Fig. 2-8B). Tannin localization remained the same as BFT stems matured: tannins were not deposited in additional cells as the secondary xylem and phloem tissues grew between tannin-containing cells in the cortex, adjacent to phloem fiber bundles, and tannin-containing cells in the primary xylem and pith (Fig. 2-8C), resulting in a decrease in the density of tannins as stem secondary growth progressed. The same cells that reacted with $FeSO_4$ also stained a dark blue with TBO (Fig. 2-8D). The material that stained with TBO and $FeSO_4$ appeared red to dark brown in unstained stems (Fig. 2-8E). The dark blue TBO staining of tannin observed in BFT (Fig. 2-8D) was not seen in alfalfa sections from any sampling date (Fig. 2-4). This was expected because alfalfa does not produce tannins in its shoots.

Flowering

Flowers were present on some BFT plants as early as the first sampling date, at 3 weeks of regrowth, and most plants were in early bloom by 5.5 weeks of regrowth (Fig. 2-9A). By 6.5 weeks of regrowth, flowers were present on all plants of both species; most were in full flower (> 30 flowers per plant) (Fig. 2-9B), and some had seedpods. After 7.5 weeks of regrowth, flowers (Fig. 2-9C) were present on all harvested stems of both species, and after 9 weeks of regrowth seedpods (Fig. 2-9D) were present on all harvested stems of both species. By 15 weeks, seedpods had begun to shatter on all plants, and leaves in the lower canopy of BFT plants had begun to senesce and show signs of mildew. This was likely due to the decumbent growth pattern of BFT, which resulted in thick intertwined foliage. Alfalfa stems were longer and more upright, and plants did not show similar pathology.

Forage Quality Analysis

In order to link this histological study to the changes in forage nutritive value reported in the literature, whole BFT shoots from 4, 6, 8 and 12 weeks of regrowth were analyzed using NIRS analysis. The lignin (Fig. 2-10A) and fiber (aNDF) (Fig. 2-10B) concentration of shoots increased with maturity ($P < 0.05$) while digestibility (IVTDMD) (Fig. 2-10C) and RFQ (Fig. 2-10D) decreased with increasing maturity ($P < 0.05$). Rapid and significant change occurred in all parameters between 4 and 12 weeks of regrowth, including the period around the time of flowering at 5.5 to 6 weeks of regrowth, illustrating the loss of forage nutritive value that occurs with increasing BFT stem fiber and lignification. This analysis was carried out on forage harvested from the same plants used for histochemistry in their second year of growth. There is evidence that a primary difference between seedling year and established BFT plants is the number of stems produced, not the size or composition of individual stems. Scheffer-Basso et al. (2011) demonstrated in a greenhouse study that the total per-plant (root, crown and shoot) dry weight of eight BFT cultivars doubled between the first and second cutting in the seedling year, but the number of stems increased 13-fold. In both years of the current BFT study, the plant material used was in the second cycle of regrowth and had been exposed to the same growth conditions in the same location.

DISCUSSION

Stem Growth and Development

Following the harvest of initial growth on 7 June 2011, plants were moved outside from the greenhouse, and all regrowth occurred under natural light and temperatures. All stems sampled for this study began growth at approximately the same time and therefore added a similar number of internodes to their tips over the 15-week regrowth period. The sixth internodes of sampled BFT and alfalfa stems had formed by 3 weeks of regrowth and continued to develop and mature via elongation, increase in diameter, and lignification over the succeeding weeks of regrowth. This progression of growth and lignification of a basal stem internode has already been assessed for alfalfa (Engels and Jung, 1998; Jung and Engels, 2002), so in this study a minimal number of alfalfa plants were included for comparison. The cultivar Viking alfalfa was chosen because our current research is with legume pastures for organic dairy production, and organic seed of Viking alfalfa is commercially available. Norcen BFT was chosen because it is a widely studied and adapted cultivar for which seed is readily available. Our goal in this histological study was to document BFT stem development using a representative cultivar, and to demonstrate the broad differences in stem anatomy and internode development underlying the differing shoot morphologies of BFT and alfalfa.

Julier et al. (2008) studied the histology of two alfalfa genotypes that differed in lignin content, and examined internodes along whole stems from the base to the tip. They reported a decrease in both internode diameter and secondary xylem thickness that progressed linearly from the base to the tip of stems, and found that internodes located close to the base of the stem, such as Internode 6, had more lignified xylem tissue than internodes closer to the stem tip. Shear strength was also found to be greater in lower than upper stem segments of alfalfa, and was significantly correlated with lignin concentration (Ames et al., 1995; Iwaasa et al., 1995, 1999). The elevated level of shear strength that prevented sheep from consuming mature BFT stems as observed by Douglas et al. (1999) was likely due to thickening and lignification of the xylary ring of BFT that had progressed to stem internodes above a desirable grazing height.

Early or frequent harvesting of BFT can be as problematic as excessive stem maturity, causing decreased yields (Smith and Nelson, 1967). Compared with alfalfa, storage of carbohydrates in the roots and crowns of BFT is minimal during the growing season (Smith, 1962), meaning that BFT is dependent on the presence of leaves for photosynthesis to support summer regrowth. Furthermore, while alfalfa nodules are indeterminant and can recover function following harvest, BFT nodules are determinant and senesce following harvest. A new nodule population must form on BFT plants after each harvest, diverting more photosynthate to nodule maintenance in BFT than in alfalfa (Vance et al., 1982). Cutting or grazing recommendations for BFT must consider the implications for both forage nutritive value and persistence.

Grazing management recommendations for BFT in the Cooperative Extension Service literature range from continuous stocking (Hall and Cherney, 2007) to rotations of 3 to 4 weeks (Rhykerd et al., 1981), to harvest intervals of 6 weeks for improved yield and stand persistence (Undersander et al., 1993). However, in all cases, producers are cautioned to retain a stubble of at least 75 mm (Greub and Wedin, 1971) because new BFT stems develop from axillary buds on this stubble, rather than from crown buds at or below the soil surface as in alfalfa (Nelson and Smith, 1968), and current photosynthate rather than crown and root storage is the main source of carbohydrates for regrowth (Smith, 1962).

The current study has demonstrated that the sixth internode of BFT has a fully thickened and lignified ring of secondary xylem at 7.5 weeks of midsummer regrowth. The sixth internode is relatively low on the stem of BFT, 60 to 120 mm from the base, and was chosen for study because it is near the recommended stubble height for BFT of 75 mm. If grazing occurs after the sixth internode is lignified and resistant to shearing, there will be a sufficient number of subtending axillary buds or existing branches to support BFT regrowth. Herbage that would be desirable to remove by grazing (i.e., that above 75 mm) would be less lignified and easier to shear by grazing ruminants than the sixth and lower internodes. Grazing or harvesting BFT after flowering has begun and the xylary ring of the 6th internode is well-developed is consistent with the recommendations of Undersander et al. (1993) and should support the persistence of established BFT plants as well as a rapid rate of regrowth.

Internode Growth and Development

The length of the sixth internode of both BFT and alfalfa increased from 3 to 5.5 week of regrowth, then BFT internode length appeared to decrease while alfalfa sixth internodes continued elongating through 15 weeks. Engels and Jung (1998) reported that the length of alfalfa Internode 7 increased for only 3 weeks, then decreased slightly through 12 weeks of regrowth for the three alfalfa genotypes they assessed. While both studies were conducted under natural light and temperatures, the study reported in Engels and Jung (1998) was conducted in the field while the current study was conducted under non-limiting water and nutrient availability;

this difference in growth condition could have influenced the alfalfa internode elongation period. A decrease in length of basal stem internodes with time is counterintuitive, but could have resulted from change in whole plant light interception and plant carbohydrate status as canopies developed and matured over time.

While the length of the sixth internode and the overall length of stems was much less in Norcen BFT than in Viking alfalfa, the diameter of Internode 6 was not significantly less in BFT than in alfalfa. Although internode elongation ceased in BFT at 5.5 weeks, increase in internode diameter continued from 5.5 to 7.5 weeks of regrowth. This increase in diameter coincided with and is indicative of continuing deposition of secondary walls in fiber cells. Similar radial expansion of fiber cells following cessation of elongation was reported for tall fescue leaves by MacAdam and Nelson (2002).

Internode Lignification

The most unexpected finding, given the shorter and more decumbent stems of BFT, and literature reports of finer stems and greater stem digestibility for BFT than alfalfa, was that the thickness of the lignified xylary ring increased more rapidly in BFT than in alfalfa, and was approximately 50% greater at maturity in BFT than in alfalfa. In the alfalfa genotypes studied by Jung and Engels (2002) xylem tissue represented 25 to 35 percent of stem diameter at 87 days of 351 regrowth, which is consistent with our results for alfalfa of 30 percent of stem radius at 7.5 weeks of regrowth, and contrasts with the 40 to 45 percent of stem diameter occupied by lignified xylem tissue in BFT.

A greater level of lignification in BFT than alfalfa would seem to conflict with literature reports, such as the more rapid initial rate of dry matter disappearance for BFT than for alfalfa reported by Ingalls et al. (1966). Van Soest (1965) reported that voluntary intake regressed on cell wall content was so much greater for BFT compared with alfalfa and numerous grasses that it was considered a statistical outlier. Tomlin et al. (1965) demonstrated that, at the early bloom stage, BFT cellulose was digested more rapidly than alfalfa cellulose, even though lignin concentration was the same. Mowat et al. (1969) determined that at flowering, in vitro cell wall digestibility (IVCWD) of stems was 330 g kg-1 for alfalfa and 440 g kg-1 for BFT, while the correlation coefficients between IVCWD and cell wall lignin were -0.78 and highly significant for alfalfa but + 0.13 and nonsignificant for BFT. Since the ratio of cellulose and lignin concentrations was similar for BFT and alfalfa in the Mowat et al. (1969) study, these results collectively suggest that the effect of lignification on forage fiber digestibility is quite different for BFT and alfalfa.

Jung (1989) did not address differences between BFT and alfalfa lignification specifically, but provided examples from many species to demonstrate that the structure of lignin, the resulting linkages among lignin subunits within the cell wall, and the interaction of lignin with specific plant tissues, rather than the amount of lignin present, can significantly affect the digestion of forage cell walls. In a study by Buxton and Russell (1988), the ratio of the oxidation products of sinapyl:coniferyl alcohols was 0.79 in alfalfa and 0.14 in BFT, suggesting profound differences in the lignin composition of these two species.

The observation of an inner, non-lignified layer in the walls of many BFT xylem fiber cells is also of interest in seeking to explain how the presence of significantly more lignified tissue in BFT stems could be consistent with greater stem digestibility. Patten et al. (2007) reported increased formation of gelatinous layers in a transgenic line of alfalfa with elevated cellulose and reduced lignin that therefore had greater digestibility, and Jung and Engels (2001, 2002) reported that the fiber cell wall layers in alfalfa that stained less strongly for lignin were readily digestible in rumen fluid. MacAdam and Griggs (2013) reported significantly greater non-fibrous carbohydrates in 14 BFT cultivars than in two alfalfa check cultivars in forage harvested at three 6-week intervals (late spring, midsummer, and early autumn). Since digestion of fiber by rumen microbes is initiated in the cell lumen, microbial colonization of fiber would be aided by the presence of a significantly more digestible innermost layer in the cells that comprise the greatest portion of the dry matter of the stem.

Tannin

Tannins are water-soluble but are thought to be stored in vesicles called tannosomes within the vacuoles of cells, which sequesters them from vacuolar enzymes (Brillouet et al., 2013). The dark blue TBO stain and the $FeSO_4$ precipitate appeared to be localized around the periphery of vacuoles, or in vacuoles with a textured distribution that was also observed by Lees et al. (1993) in sainfoin. Both Lees et al. (1993) and Brillouet and Escoute (2012) reported that condensed tannins were transparent and colorless in unstained plant material. Howerver, in the BFT stems analyzed here, the material that stained with TBO and $FeSO_4$ appeared red to dark brown in unstained stems. The tannins found in BFT may be colored rather than transparent, considering the known variation in the chemical structure of tannins.

Lees et al. (1993) found similar localization of tannins alongside phloem fibers in the sainfoin (*Onobrychis viciifolia* Scop.) leaf rachis, and Maroso et al. (2009) found similar tannin localization in BFT stems harvested after 210 days of growth. Staining with FeSO4 and TBO indicated that the tannin content of BFT stems is minimal and does not increase with accumulation of stem dry matter as plants mature. No quantitative analysis of tannin concentration in stems was performed in this study, but Häring et al. (2007) found that most of the BFT tannin consumed by grazing is located in the foliage. They also determined that tannin concentration is relatively low in BFT stems, and that a non-significant decrease in tannin concentration occurs with stem maturation. Considering that BFT tannin promotes the sustainable production of milk and meat in ruminants (Ramírez-Restrepo and Barry, 2005), the accumulation of stem dry matter with BFT maturation would serve to dilute the desirable concentration of tannin in BFT herbage dry matter.

CONCLUSIONS

By providing a better understanding of the changes in stem composition that occur in concert with BFT stem growth and maturation, we hope to facilitate informed management, improved quality and persistence, and to support wider cultivation and use of this valuable non-bloating perennial legume for ruminant production. Further research on the chemical nature of BFT lignin and its effect on the kinetics of rumen digestion is needed to reconcile the seemingly contradictory results of greater lignified stem secondary xylem content in BFT than in alfalfa combined with greater stem cell wall digestibility reported for BFT than alfalfa.

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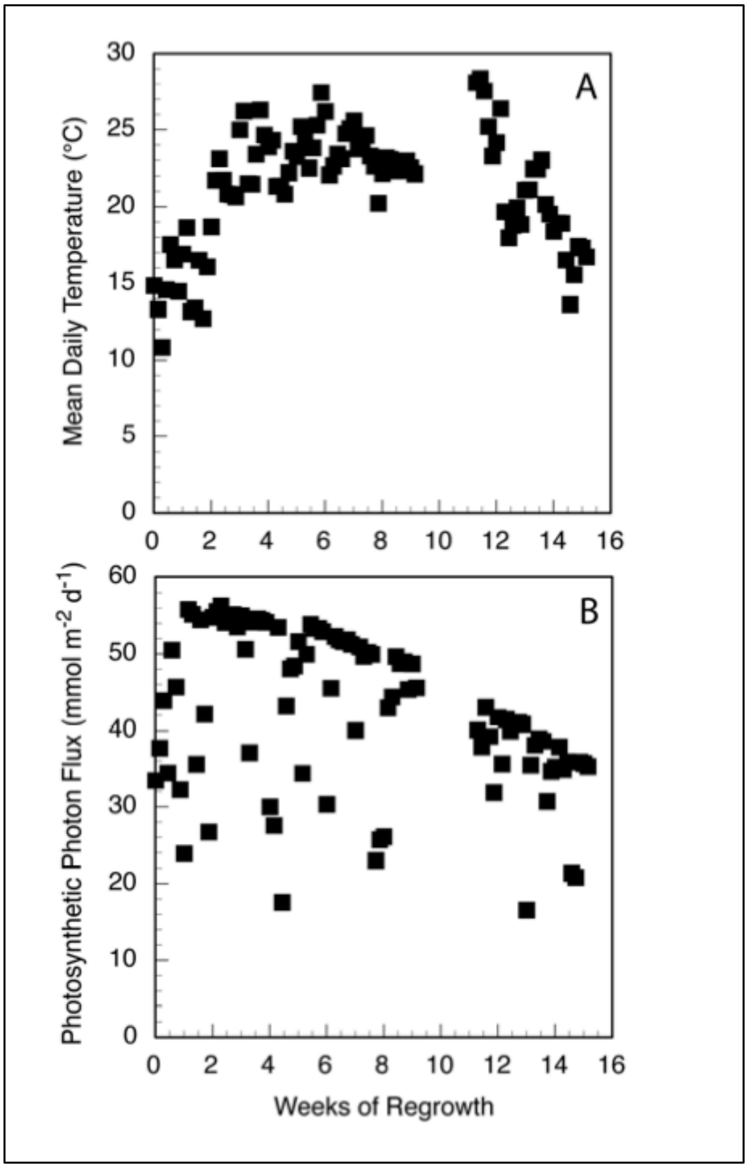

Figure 2-1. Mean daily air temperature (A) and photosynthetic photon flux (B) in Logan, Utah during BFT regrowth. Data were not recorded at this site between 11 and 24 Aug. 2011 (weeks 10 and 11 of regrowth).

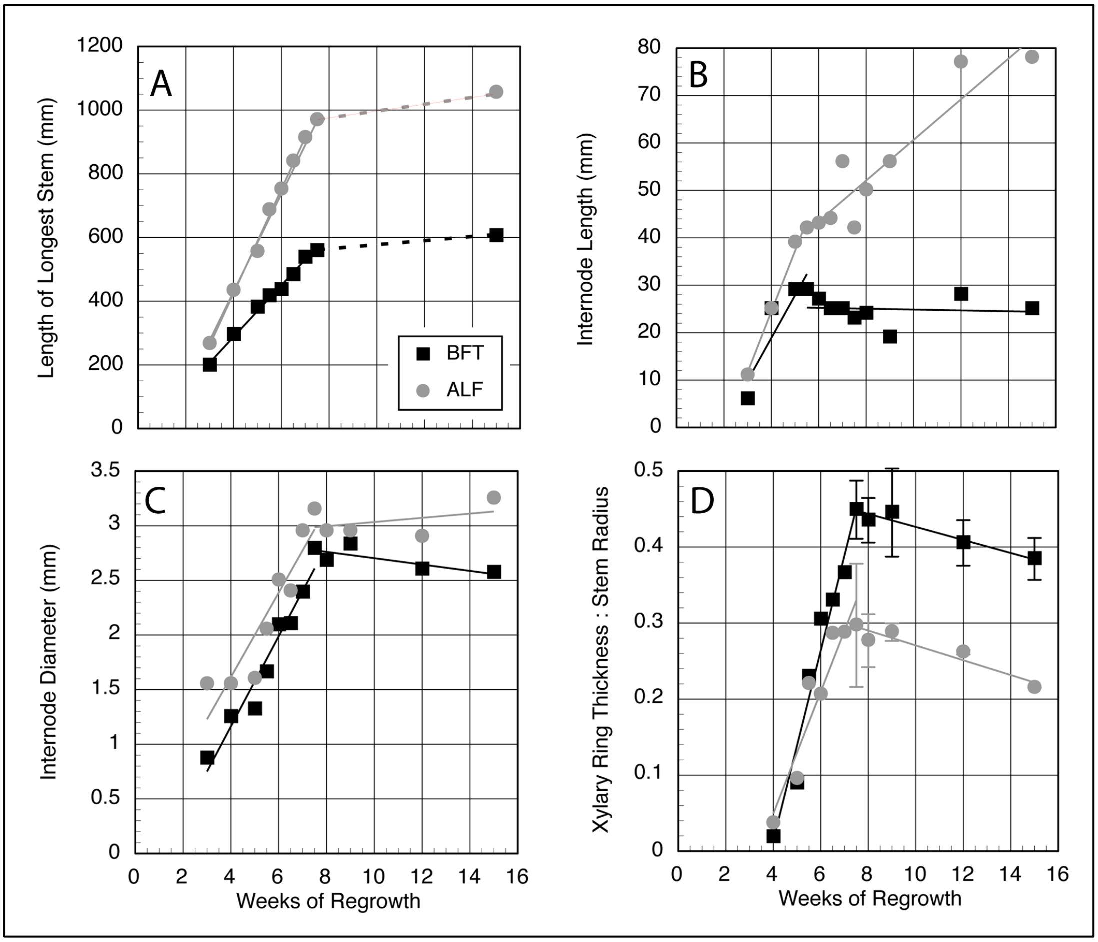

Figure 2-2. Change in length of longest stem (A) and development of Internode 6 (B, C, and D) in BFT and alfalfa. (B) Internode 6 elongation rate, (C) diameter, and (D) ratio of lignified xylary ring thickness to internode radius ± standard error.

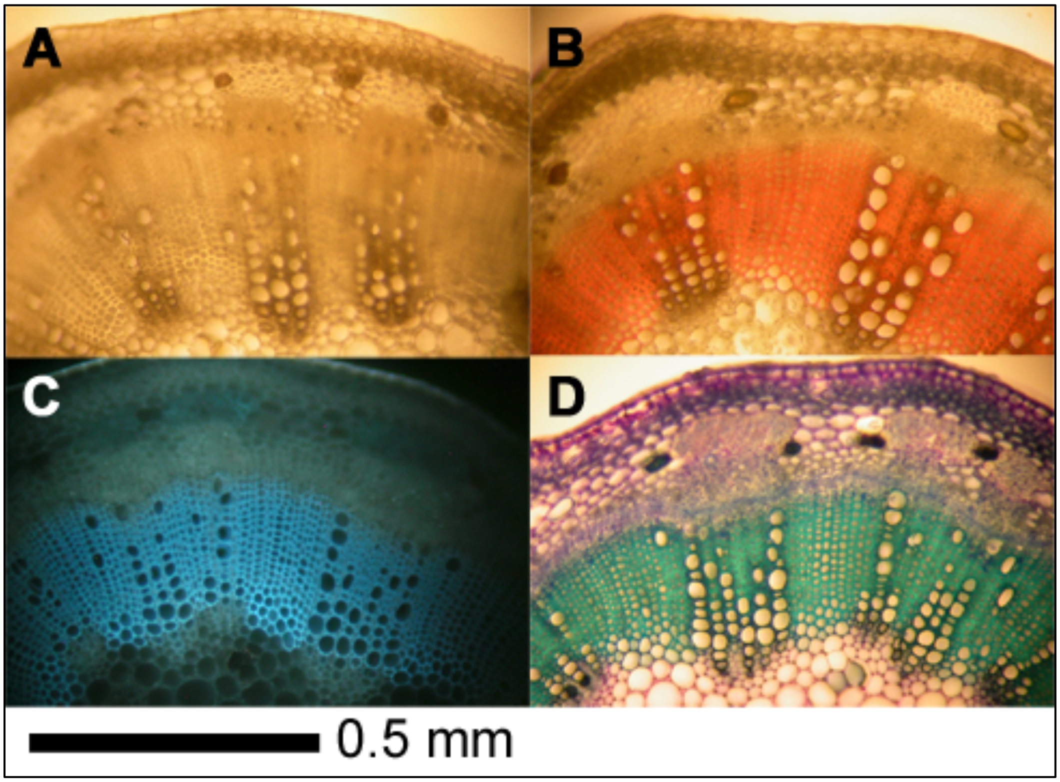

Figure 2-3. BFT internodes sectioned at 6 weeks of regrowth, showing the lignified xylary ring using different methods of visualization. Stem sections are unstained (A), stained with phloroglucinol-HCl coloring lignin pink (B), viewed under UV light showing blue fluorescence of lignin (C), and stained with TBO, which colors lignified tissues blue-green (D).

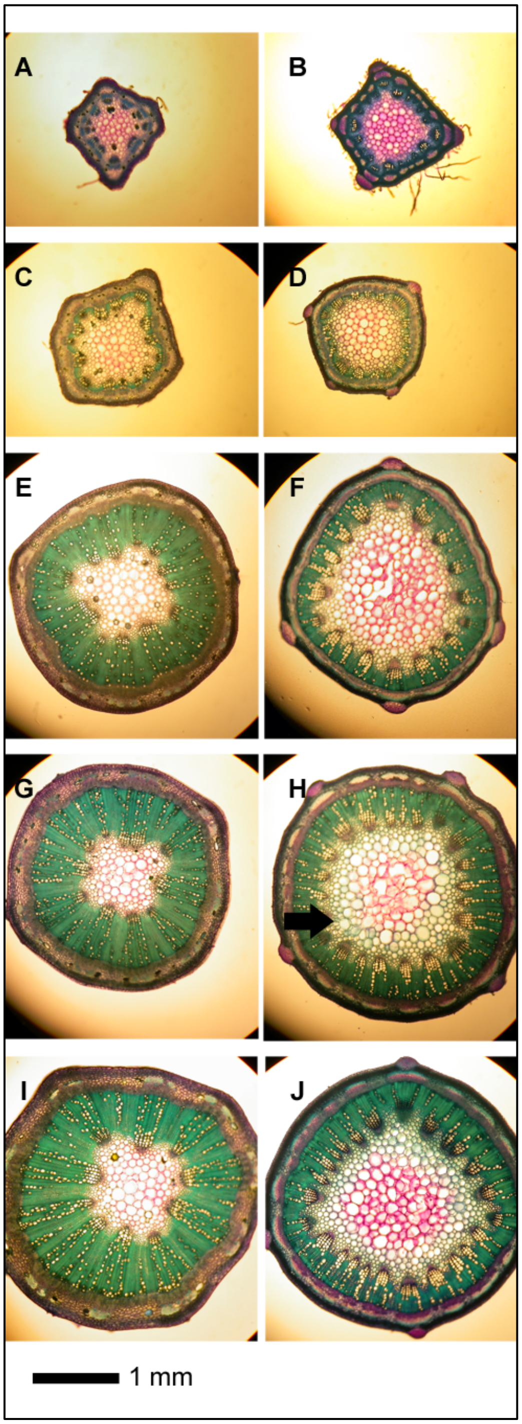

Figure 2-4. BFT (A, C, E, G, I) and alfalfa (B, D, F, H, J) Internode 6 stem sections at 3 (A, B), 5 (C, D), 7 (E, F), 9 (G, H) and 12 (I, J) weeks of regrowth, stained with TBO. By 7 weeks of regrowth, lignification expanded to thin-walled pith cells in alfalfa only (arrow, Fig. 4H).

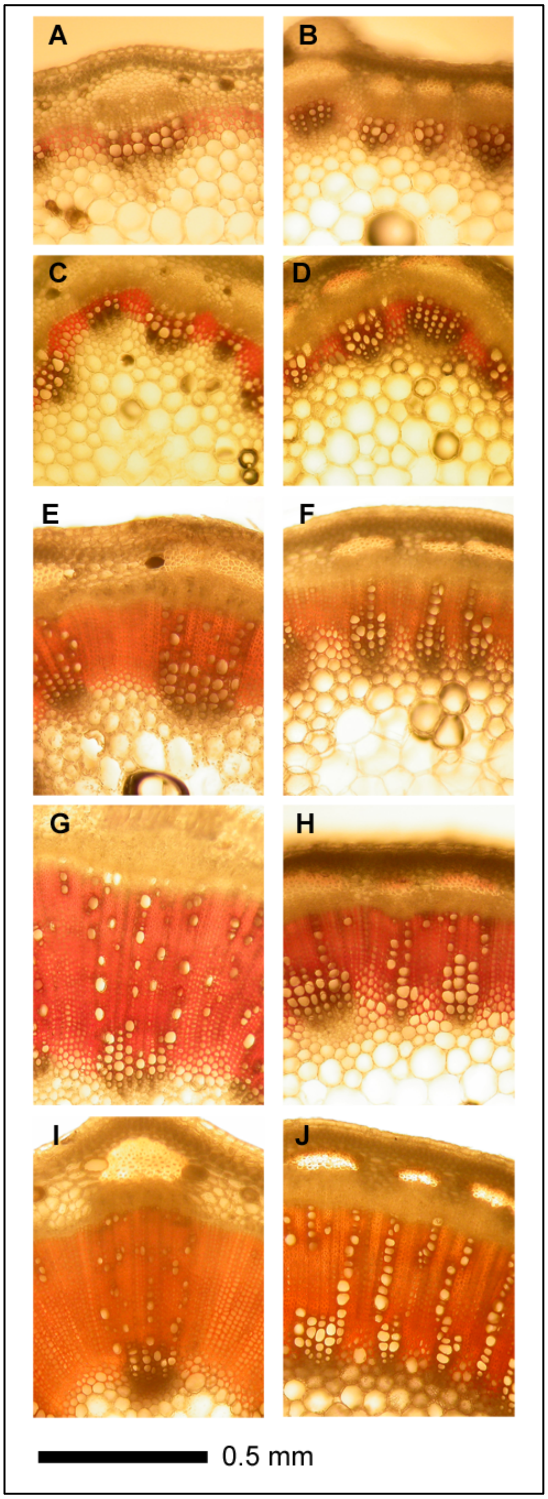

Figure 2-5. BFT (A, C, E, G, I) and alfalfa (B, D, F, H, J) Internode 6 stem sections at 4 (A, B), 5 (C, D), 6 (E, F), 7 (G, H) and 8 (I, J) weeks of regrowth, stained for lignin with phloroglucinol-HCl.

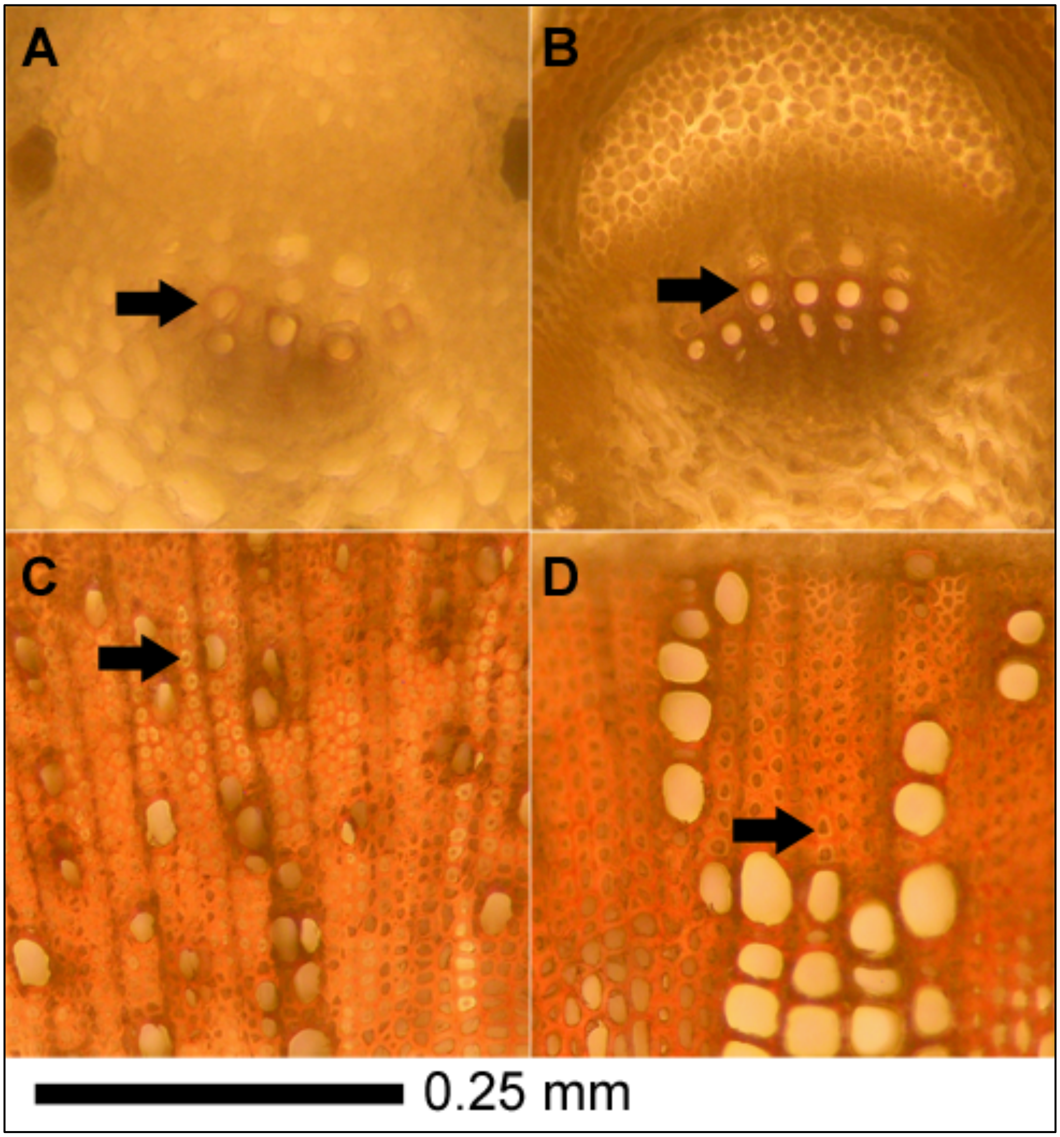

Figure 2-6. BFT (A, C) and alfalfa (B, D) Internode 6 stem sections after 3 (A, B) and 10 weeks of regrowth (C, D) stained for lignin with phloroglucinol-HCl. Arrows in A and B indicate early lignification in secondary walls of some xylem vessel members in young stems. Arrows in C and D indicate xylem fiber cells with an additional secondary cell wall layer that failed to in some cases to stain for lignin in BFT (white rings in lower right of Fig. 6C) or that stained less strongly than outer cell wall layers, as in alfalfa (Fig. 6D).

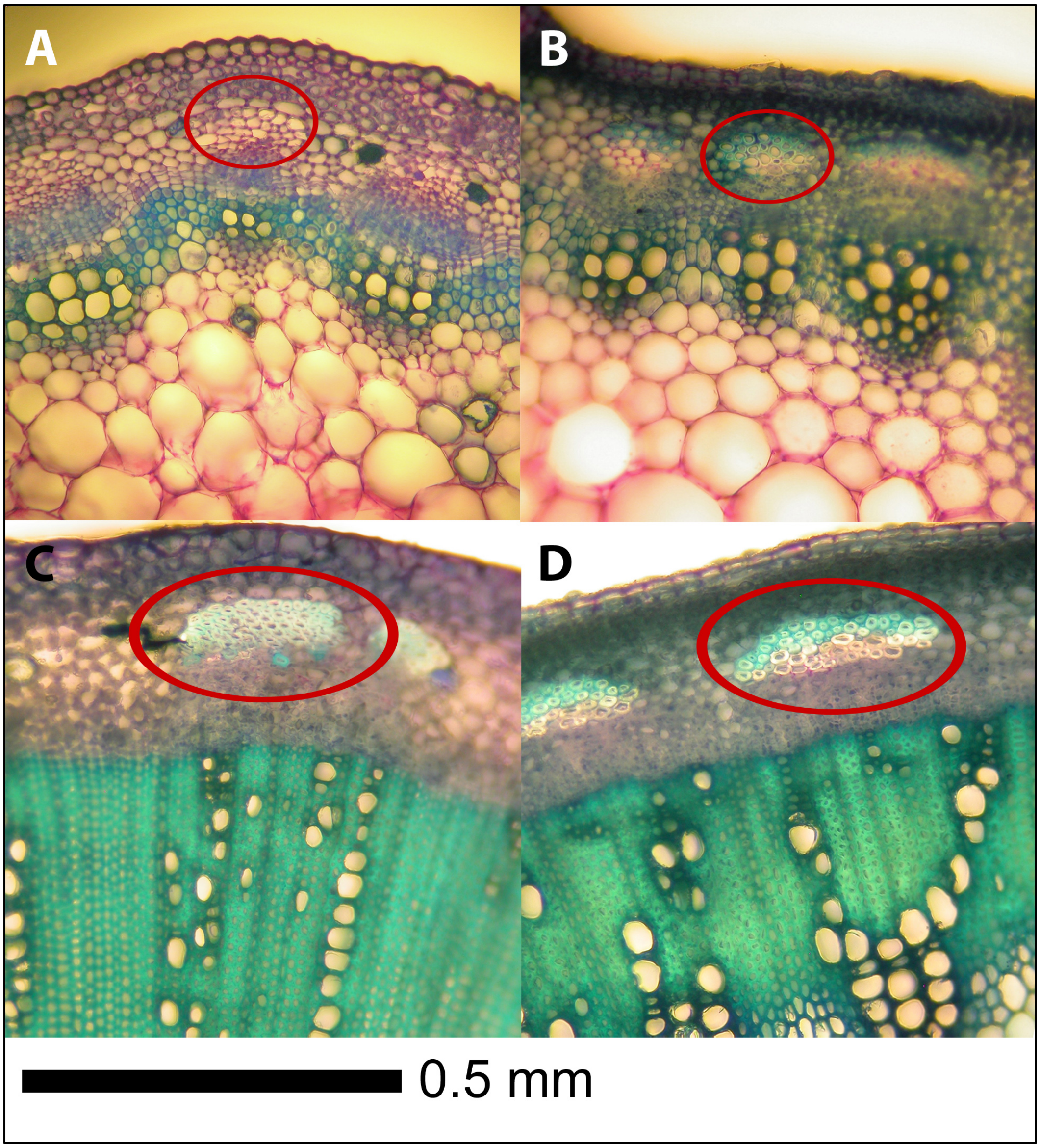

Figure 2-7. BFT (A, C) and alfalfa (B, D) sixth internode stem sections taken after 4 (A, B) and 12 (C, D) weeks of regrowth and stained with TBO to illustrate the lignification of phloem fibers, examples of which are circled in red..Alfalfa phloem fibers stained strongly in older, external cells (D), while BFT fibers did not stain as darkly (C).

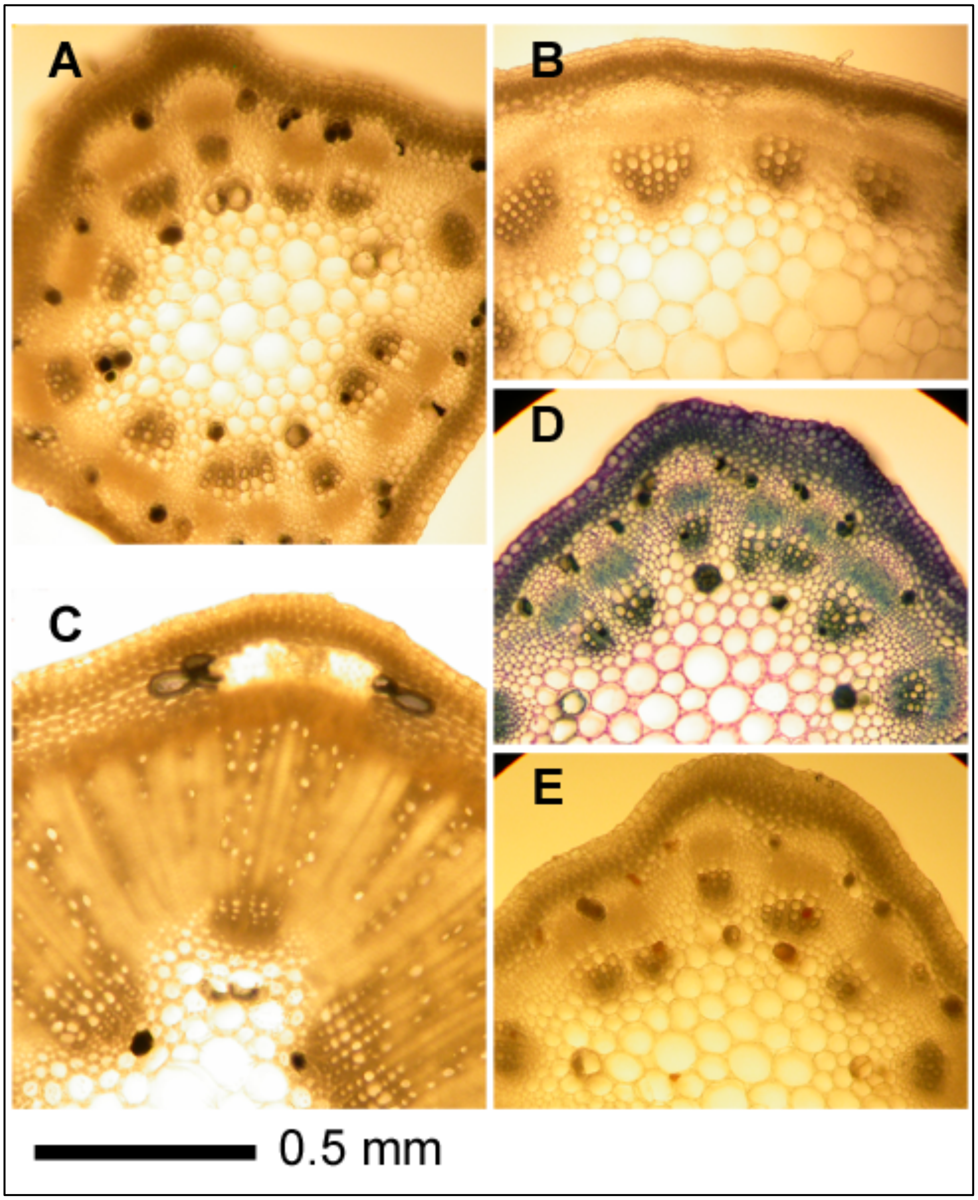

Figure 2-8. BFT (A) and alfalfa (B) stem sections after 4 weeks of regrowth stained with $FeSO_4$, which forms a dark blue-black precipitate with tannins. There are no cells in ALF that stain for tannins. The same localization of tannins is seen in immature BFT stems at 4 weeks of regrowth (A) and mature BFT stems at 9 weeks of regrowth (C). BFT stem sections after 4 weeks of regrowth stained with TBO (D) and unstained (E). TBO stains tannin containing-cells dark blue (D); in unstained stems, tannin-containing cells vary in color from bright red to dark brown (E).

Figure 2-9. Plants began to bloom after 5.5 weeks of regrowth (A) and were in full flower by 6.5 weeks of regrowth (B). Birdsfoot trefoil flowers before pollination and after seedpod initiation at 5.5 weeks of regrowth (C). Elongating seedpods after 6.5 weeks of regrowth (D).

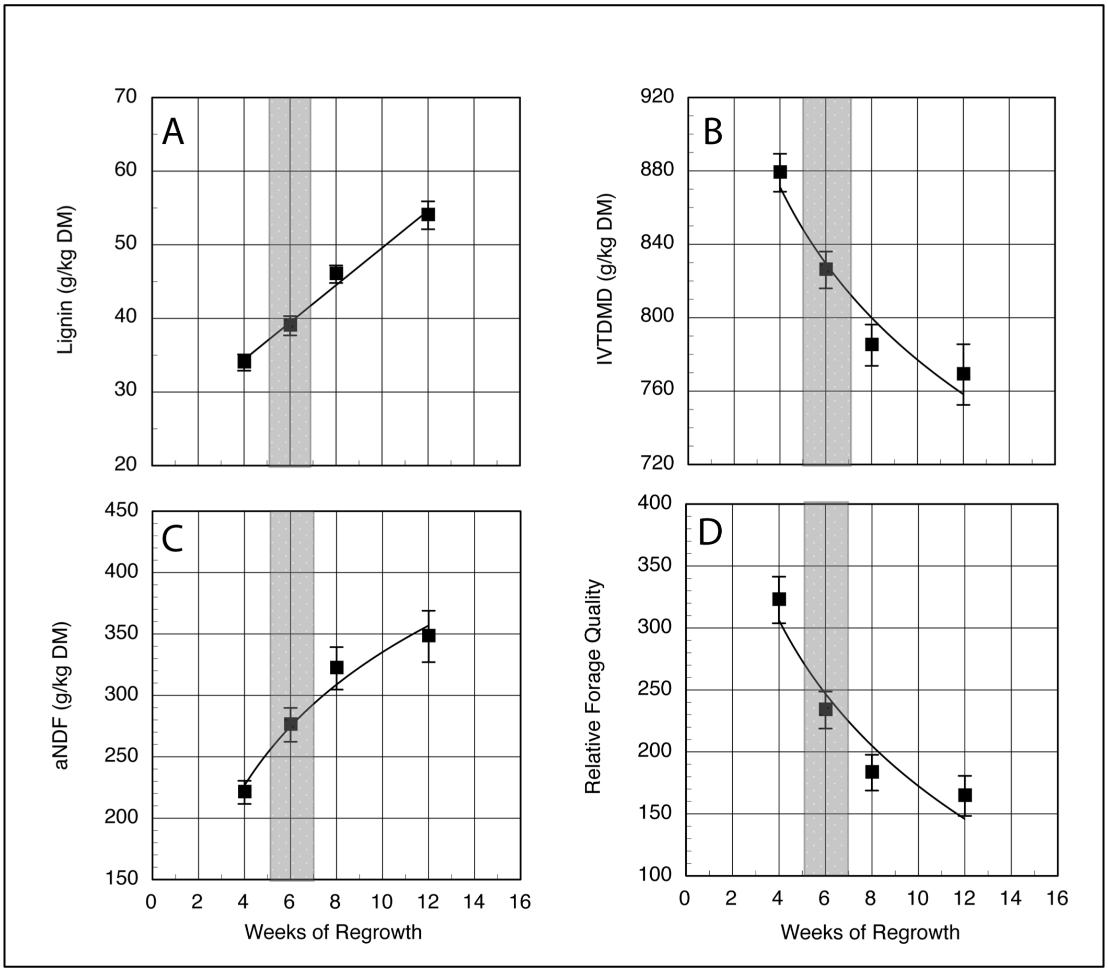

Figure 2-10. Forage nutritive value parameters for BFT between 4 and 12 weeks of regrowth. Of the plants sampled in 2011, eight BFT plants overwintered and were subjected to the same growing conditions and harvested at the same dates in 2012. No alfalfa plants overwintered. Grey bars indicate the period between 5 and 7 weeks of regrowth, and therefore near the recommended time of harvest. Data ± standard error; the slopes of all trend lines are significant at $P < 0.05$. A. Lignin; B. IVTDMD, in vitro true dry matter digestibility; C. aNDF, amylase-treated neutral detergent fiber; D. Relative forage quality (RFQ), a unitless index of potential daily digestible DM intake in which 100 equals full-bloom ALF hay.

CHAPTER 3

EFFECT OF SEEDING RATE, OAT COMPANION CROP, AND SEEDING DATE ON ORGANIC ESTABLISHMENT OF BIRDSFOOT TREFOIL (*Lotus corniculatus*)

Abstract

The non-bloating perennial legume birdsfoot trefoil (BFT, *Lotus corniculatus* L.) is well suited to ruminant production in pastures, supporting high levels of weight gain and milk production compared to other forages. However, BFT can be slow to establish, competing poorly with weeds and producing low yields in the first 1 to 2 years. This study evaluated the effects of organic BFT establishment methods on three years of annual yields and foliar cover of mature stands at an irrigated site in northern Utah. Autumn and spring seeding at seeding rates of 3, 7, 20, and 34 kg pure live seed (PLS) ha^{-1}, with and without an annual oat (*Avena sativa*) companion crop, were tested in a split-split plot design. Establishment method affected yields in the first year after seeding as well as BFT and weed foliar cover in the third year ($P \leq 0.05$), but did not affect second and third year yields, which were above 6000 kg DM ha^{-1} for all treatments. First year yields increased linearly with seeding rate. Quadratic trends of BFT and weed foliar cover predicted seeding rates of 18 and 14 kg PLS ha^{-1} would result in mature stands with greatest BFT and least weed cover, respectively. Use of a companion crop reduced first year yields, and did not reduce weed cover in mature stands. Autumn and spring seeding produced similar yields and BFT cover. Autumn seeding of BFT at a rate from 14 to 18 kg PLS ha^{-1}, following harvest of a summer crop, is recommended for irrigated production.

Introduction

There is a growing market for organic meat and dairy products in the United States. The number of organic beef and dairy cattle, as well as sales of organic milk and beef increased steadily and rapidly through the early 2000s (Dimitri and Oberholtzer 2009). In organic systems, cattle must graze pasture for a minimum of 120 days per year, obtaining at least 30% of their dry matter intake from pasture (Rinehart and Baier 2011). Deep-rooted perennial legumes have higher dry matter (DM) production in mid-summer than cool-season perennial grasses (Berdahl et al. 2001; Lauriault et al. 2003) supporting season-long ruminant production. Grain supplementation, which is permitted in organic production, tends to increase the productivity of cattle on pasture, but minimizing the use of grain can reduce feed costs (White et al*.* 2002) and increase the concentration of beneficial fatty acids in meat and milk (Alfaia et al. 2009; Stockdale et al*.* 2003). Cultivation of high quality forage legumes in organic pastures can help maintain cattle productivity while reducing grain supplementation.

The forage legume *Lotus corniculatus* L., commonly known as birdsfoot trefoil (BFT), has the potential to improve ruminant production on pastures. Studies have shown that BFT supports high weight gain relative to other forages when grazed by beef cattle (MacAdam et al. 2011; Marten et al. 1987; Wen et al. 2002) and lambs (Douglas et al. 1995; Marten and Jordan 1979). Additionally, the tannins produced by BFT provide a number of beneficial effects for ruminants, including prevention of bloat (McMahon et al. 1999), reduction of enteric methane emissions (Woodward et al*.* 2004), increased efficiency of protein utilization (Mueller-Harvey

2006; Waghorn 2008), and increased milk yield in dairy cows (Turner et al*.* 2005; Woodward et al. 1999; Woodward et al*.* 2009). Despite these advantages, BFT is less commonly cultivated in the United States than other perennial legumes such as alfalfa (*Medicago sativa* L.) and red or white clover (*Trifolium* spp.).

Slow establishment is one barrier that may discourage more widespread cultivation of BFT. Small seed size and low seedling vigor make early weed competition a significant challenge in the cultivation of BFT (Chapman et al. 2008; Henson and Taymon 1961). Herbicide use during establishment of BFT has been shown to reduce weed competition, increase yields during the seeding year (Wakefield and Skaland 1965; Scholl and Brunk 1962; Scholl and Staniforth 1957) and increase total yields over two years (Linscott and Hagin 1978), but chemical weed control is very limited in organic production, so other methods must be explored. It has been suggested that fall seedings tend to experience less weed competition than spring seedings (Vough et al. 1995). Additionally, use of a high seeding rate and establishment with a companion crop are methods that could be implemented in organic systems to improve the ability of BFT to compete with weeds and provide high yields in the seeding year.

The commonly recommended seeding rate for BFT is 6 to 11 kg pure live seed (PLS) ha^{-1} (Hall and Cherney 2007; Rhykerd et al*.* 1981; Undersander et al. 1993). Seed costs are a significant expense, but the cost of higher seeding rates may be justified if establishment is more rapid or more certain. Wakefield and Skaland (1965) compared BFT seeding rates of 4, 7, and 14 kg ha^{-1} and found that BFT yields increased with seeding rate, while weed yields decreased during the seeding year.

In establishment of alfalfa, it has been demonstrated that use of an oat (*Avena sativa* L.) companion crop can increase total first cutting yields, increase first year profits, and minimize weed competition without the use of herbicides, although competition from the companion crop can also decrease density and yields of alfalfa plants (Hansen and Krueger 1973; Lanini et al. 1991; Schmid and Behrens 1972). Use of a companion crop is not generally recommended with BFT but may be valuable in offsetting weed competition in the absence of herbicide use. Scholl and Brunk (1962) found that sowing a companion crop of oats (*Avena sativa*) with BFT increased total yields and decreased yields of weeds and BFT during the seeding and first years; by the second year after seeding there was no difference in yields between BFT established with a companion crop, with the use of herbicide, or with no herbicide or companion crop.

The objective of this study was to assess the effects of seeding season, seeding rate and use of an annual oat companion crop on the organic establishment of BFT by measuring yields for three years following seeding as well as assessing foliar cover of mature stands. Interactions between these factors were also assessed.

Methods

Field planting and experimental design

This study was conducted on irrigated certified organic acreage at Greenville Research Farm, North Logan, UT, 41°77N, 111°81W; 1413 m elevation. The soil is a Millville silt loam (coarse-silty, carbonatic, mesic Typic Haploxeroll). The study was designed as a split-split-plot with four replications in a randomized complete block with season as whole plot, companion crop as split plot, and seeding rate as split-

split plot (Fig. 3-1). Split-split plots measured 2.7 x 5.5 m. Seasons were spring (seeding on Jun 18, 2010) and fall (seeding on Sep 16, 2010). The companion crop was certified 'Jay' organic oats (Welter Seed, Onslow, IA). BFT seeding rates were 3, 7, 20, and 34 kg PLS ha^{-1}.

Raw 'Norcen' BFT seed (Agassiz Seed; West Fargo, ND) was inoculated with INTX BFT PreVail Inoculant (INTX Microbials; Kentland, IN) and broadcast seeded from east to west into a prepared seedbed. In companion crop split plots, oats were drill seeded from north to south over BFT at a rate of 54 kg ha^{-1} (equivalent to 1.5 bushels $acre^{-1}$) immediately following BFT seeding. A 0.9-m east-west BFT border was seeded on the north and south margins of the plot area, and 1.8-m-wide north-south grass alleys were seeded between spring and autumn whole plots and on the east and west margins of the plot area with certified organic 'Spring Green' festulolium (Welter Seed, Onslow, IA) on the spring seeding date. Plots were sprinkler-irrigated with approximately 50 mm of water applied every 7 days from mid-May through mid-September each year of the study. Plots were mowed to approximately 25 cm in height twice in 2010.

Weather data for 2010 to 2013 were provided by the Utah State University AgWeather Network and come from the Greenville Farm weather station. Thirty-year averages were provided by Western Regional Climate Center reports of National Climatic Data Center 1981-2010 monthly mean temperatures.

Yield and foliar cover

A 0.8-m wide 5.5-m long swath was harvested from the center of each split-split plot twice in 2011 (24 Jun and 26 Aug), and three times in 2012 (4 Jun, 16 July, and 28 Aug) and 2013 (3-4 Jun, 16 July, and 23 Aug) by cutting to a height of approximately 10 cm with a Swift Machine and Welding (Swift Current, SK) flail-type plot harvester with a hopper mounted on load cells that provided fresh weights. Grab samples were weighed immediately following harvest, dried to constant weight at 55 °C and weighed again to determine the moisture content of fresh samples. The harvested fresh weight of each split-split plot was used to determine yield in kg DM ha^{-1}. Dry matter yields from individual harvests were summed for each year.

Foliar cover of split-split plots was assessed on 15 May 2013 when growth from crown buds was approximately 15 to 20 cm in height. Visual estimates of BFT, weed and bare ground within a 30 x 30-cm quadrat were made to the nearest 10%. The two dominant cover types were estimated first and the remaining factor was calculated by difference with 100%. Two individuals made three estimates each along diagonal transects crossing split-split plots. Means of the six estimates were used to evaluate the effect of treatments on cover in the third year following seeding.

Statistical analysis

To evaluate annual yield differences between treatments, residual maximum likelihood estimation was conducted using SAS PROC MIXED (version 9.3, 2009; Cary, NC) with year, season of seeding, and companion crop as fixed effects; seeding rate as covariate; replication and its interactions with season and companion crop as random effects, and repeated measurement on years (n=192). Linear and quadratic trends were tested for rate, and quadratic trend was not significant so was removed from the model. There was a significant rate and year interaction, so rate slopes were evaluated separately for each year. Significance level is at 0.05.

Differences in 2013 BFT and weed foliar cover among treatments were assessed with the residual pseudo-likelihood estimation technique using SAS PROC GLIMMIX. The same model was used, but year was replaced with cover type, which has two levels for BFT or weeds (n=128), and a beta distribution was specified. Quadratic trend was significant for rate, allowing calculation of the rates that resulted in maximum BFT and minimum weed foliar cover in mature stands.

Results

Temperature, precipitation and relative humidity for Logan, UT from 2010 to 2013 are shown in Figure 3-2. In 2010 when plots were seeded and 2011, the establishment year, temperatures were near 30-year averages for Logan, UT. August average highs were 30°C in 2010 and 32°C in 2011, and lows were 10°C and 12°C, respectively. The first frost following 2010 seeding occurred on Oct 12 when minimum night temperatures dropped to -0.6°C for three nights in a row. Coldest temperatures in the winter following seeding occurred in January 2011 when lows averaged -9°C and highs -1°C.

Mean yields for each harvest, year, rate, season of seeding, and companion crop treatment are given in Table 3-1. Figure 3-3 shows mean annual yields for all treatment combinations. Significant fixed effects for yield were interactions between year and seeding rate and between year and companion crop. Season of seeding did not affect yields (P=0.79). Use of a companion crop resulted in significantly lower yields in 2011, but did not affect yields in 2012 or 2013.

Similarly, in 2011 there was a significant slope (56 kg DM per kg PLS ha^{-1}) for the linear trend of increasing yields with increasing seeding rate, but slope was not significant in 2012 or 2013.

Seeding rate affected foliar cover in mature stands, and no other fixed effects were significant. Table 3-2 gives mean BFT, weed, and bare ground cover by treatment; average standard errors of the means were 12.5, 11.3, and 7.4% for BFT, weed and bare ground, respectively. Figure 3-4 shows foliar cover of BFT and weeds by seeding rate. There was a highly significant (P=0.003) negative quadratic trend for BFT cover with increasing seeding rate, with maximum BFT cover predicted at a seeding rate of 18 kg PLS ha^{-1}. A weaker, but still significant (P=0.058) positive quadratic trend was present for weed cover, with minimum weed cover predicted at a seeding rate of 14 kg PLS ha^{-1}.

Discussion

We compared the effects of seeding rate, season of seeding and use of a companion crop on annual BFT yields for three years after the seeding year and on the foliar cover of mature stands in the third year after seeding to determine optimal establishment management. All establishment methods produced mean annual yields of 6000 kg DM ha^{-1} or more in the second and third years after seeding and had 70% or more BFT foliar cover in the third year. However, increasing seeding rate and seeding without a companion crop significantly increased first year yields and also tended to produce mature stands with high BFT and low weed foliar cover.

In establishing forage crops, higher seeding rates result in establishment of higher plant densities, smaller individual plants and greater total stand productivity up to a point (Hansen and Krueger 1973; Volenec et al. 1987). When very high initial plant densities are established, plant mortality is also high in the first year, meaning that a threshold density exists beyond which increasing seeding rate will not increase yields (Hall et al. 2004). We found that increasing seeding rate of BFT from 3 to 34 kg PLS ha^{-1} linearly increased first year yields across treatments, but yield differences were not significant in the second and third years after seeding (Fig. 3-2). Without a companion crop, we measured first yields of 3602 to 4248 kg ha^{-1} from seeding at a rate of 3 kg PLS ha^{-1} and yields of 5002 to 6113 kg ha^{-1} from a rate of 20 kg PLS ha^{-1}. Under management similar to that used in our study and with no companion crop Wakefield and Skaland (1965) reported total first year yields of 5830, 7041, and 6745 kg ha^{-1}produced by spring seeding BFT at rates of 4, 7, and 14 kg ha^{-1}, respectively. Scholl and Staniforth's (1957) unweeded check treatment seeded at a rate of 5 kg ha^{-1} yielded 2469 kg ha^{-1} in the year following seeding, while weeded treatments yields ranged from 2748 to 5385 kg ha^{-1}. Based on these results, increasing seeding rate can produce high first year yields of BFT without the use of herbicides.

In mature stands, weed cover tended to be higher and BFT cover lower in plots seeded at the highest and lowest rates (Fig. 3-3). Foliar cover trends indicated that seeding rates of 14 and 18 kg PLS ha^{-1} would result in mature stands with highest BFT and lowest weed cover, respectively. Wakefield and Skaland (1965) reported lower weed DM, higher BFT yields, greater number of BFT plants per square ft, and lower root-crown dry weight of BFT with a seeding rate increase from 4 to 14 kg ha^{-1}. Lower root-crown weights indicate the negative impact of intraspecific competition, although in this case the higher number of plants was able to compensate for smaller size of individual plants. At the high seeding rate of 34 kg ha^{-1} used in our study it is possible that BFT plant density was too high to allow for adequate seedling development, resulting in a less robust stand that was more susceptible to weeds at maturity. At the lowest seeding rate, BFT density was insufficient to prevent weed incursion.

As an alternative to the use of herbicides, perennial legumes can be seeded with a small grain companion crop to reduce weed competition and increase first year yields (Schmid and Behrens 1972; Wiersma et al. 1999). However, a companion crop can compete for light and moisture to the detriment of the legume being established (Buxton and Wedin 1970; Lanini et al. 1991; Wiersma et al. 1999). We found BFT yields to be higher without a companion crop across treatments in the first year after seeding. However, in the seeding year, when the companion crop was growing and may have contributed to DM production, plots were clipped only to remove immature seedheads, and yields were not assessed. Scholl and Staniforth (1957) assessed the effect of an oat companion crop on BFT in multiple experiments, demonstrating establishment of higher BFT density without a companion crop. They reported higher total seeding year yields with a companion crop when managed for hay or grain (cutting all forage once to a height of 4 cm in July or once to a height of 10 cm in Oct., respectively) but also found smaller BFT plant size, lower density and lower yields of BFT under this management. For Scholl and Staniforth (1957), pasture management of BFT seeded with a companion crop (mowing twice to a height of 30 cm) did not produce higher total seeding year yields than BFT seeded without a companion crop. We used similar pasture management, mowing twice to a height of 25 cm in the seeding year of our study to reduce competition and promote BFT establishment. Despite lower first year yields, use of a companion crop may be beneficial if it significantly decreases weed seed production. However, our measurements of BFT and weed foliar cover in mature stands indicated no long term beneficial effect from establishing BFT with a companion crop, indicating that the only potential benefit from use of a companion crop would be increased seeding year yields due to companion crop dry matter.

Season of seeding did not significantly affect annual yields or foliar cover in this study. Other studies have found BFT to be sensitive to seeding date and to establish poorly with late summer seedings in Iowa (Buxton and Wedin 1970) and Tennessee (Fribourg and Strand 1973). The authors attributed poor establishment to low moisture availability in late summer. In Utah, and generally throughout the Intermountain West, high temperatures, low relative humidity, and low rainfall during summer months (Fig. 3-4) make irrigation necessary for the cultivation of most crops. This study was irrigated, allowing adequate moisture levels to be maintained during autumn seeding. Also, by seeding in Sept., temperatures were more moderate and relative humidity was higher than it would have been during Aug. Buxton and Wedin (1970) recommend late summer seeding of alfalfa after harvesting an annual summer crop as a promising method for minimizing the loss of productivity that can occur during establishment of a new forage species. Based on the results presented here, this method may also work well for irrigated establishment of BFT.

Conclusions

Organic producers can achieve high first year yields and minimize weed encroachment during the establishment of BFT by seeding at a rate between 14 and 18 kg PLS ha^{-1}. The cost of seed relative to the value of increased yields and weed control is an important consideration in choosing a seeding rate, and should be balanced against the potential life of the stand. Good stands of BFT can be achieved even with very low seeding rates but will be weedier and take longer to establish. If stands of BFT are grazed, weeds will be avoided and will be more likely to proliferate than under clipping management, as in this study. Results of this study indicate that irrigated autumn seedings are as successful as spring seedings. Although not tested in this study, producers may further minimize yield losses during establishment by harvesting an annual crop prior to autumn seeding of BFT. If irrigation is not available for autumn seeding, it may be difficult to maintain adequate soil moisture and spring seeding would be preferable. Use of a companion crop is not recommended due to reduced first year yields and no evidence of a long-term reduction in weed cover in mature stands of BFT seeded with a companion crop.

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Table 3-1. Mean harvest and annual BFT yields by seeding season, companion crop, and seeding rate.

2011							2012							2013						
Seeding Season	Comp. Crop	Seeding Rate (kg PLS ha-1)	Harvest Date	Mean Harvest Yield (kg ha-1)	Mean Annual Yield (kg ha-1)	SEM	Seeding Season	Comp. Crop	Seeding Rate (kg PLS ha-1)	Harvest Date	Mean Harvest Yield (kg ha-1)	Mean Annual Yield (kg ha-1)	SEM	Seeding Season	Comp. Crop	Seeding Rate (kg PLS ha-1)	Harvest Date	Mean Harvest Yield (kg ha-1)	Mean Annual Yield (kg ha-1)	SEM
spring	no	3	Jun 24 Aug 26	1283 2318	3602	636	spring	no	3	Jun 24 Jul 16 Aug 28	2575 2230 1658	6463	817	spring	no	3	Jun 24 Jul 16 Aug 28	2846 1942 1707	6495	550
spring	yes	3	Jun 24 Aug 26	621 1959	2580	237	spring	yes	3	Jun 24 Jul 16 Aug 28	2974 2125 1713	6813	560	spring	yes	3	Jun 4 Jul 16 Aug 28	3029 1853 1496	6379	284
autumn	no	3	Jun 24 Aug 26	1369 2880	4248	504	autumn	no	3	Jun 24 Jul 16 Aug 28	3350 2236 1821	7407	1632	autumn	no	3	Jun 24 Jul 16 Aug 28	3090 1883 1593	6565	452
autumn	yes	3	Jun 24 Aug 26	558 1790	2348	312	autumn	yes	3	Jun 24 Jul 16 Aug 28	2673 2619 1451	6743	157	autumn	yes	3	Jun 4 Jul 16 Aug 28	3360 2041 1880	7280	332
spring	no	7	Jun 24 Aug 26	2453 2345	4798	730	spring	no	7	Jun 24 Jul 16 Aug 28	3151 2595 2217	7963	553	spring	no	7	Jun 24 Jul 16 Aug 28	3576 2018 2041	7635	625
spring	yes	7	Jun 24 Aug 26	969 2400	3369	950	spring	yes	7	Jun 24 Jul 16 Aug 28	2909 2258 2211	7378	1048	spring	yes	7	Jun 4 Jul 16 Aug 28	2773 1643 1804	6220	1004
autumn	no	7	Jun 24 Aug 26	2062 3020	5082	452	autumn	no	7	Jun 24 Jul 16 Aug 28	3334 2264 2050	7648	793	autumn	no	7	Jun 24 Jul 16 Aug 28	3525 2215 1985	7725	510
autumn	yes	7	Jun 24 Aug 26	472 1979	2451	322	autumn	yes	7	Jun 24 Jul 16 Aug 28	3051 2352 1597	7001	357	autumn	yes	7	Jun 4 Jul 16 Aug 28	3305 1625 1795	6725	763
spring	no	20	Jun 24 Aug 26	2087 2915	5002	764	spring	no	20	Jun 24 Jul 16 Aug 28	3257 2520 2110	7886	461	spring	no	20	Jun 24 Jul 16 Aug 28	2609 2315 2178	7101	336
spring	yes	20	Jun 24 Aug 26	1719 2121	3840	443	spring	yes	20	Jun 24 Jul 16 Aug 28	3270 2007 2242	7519	813	spring	yes	20	Jun 4 Jul 16 Aug 28	2965 1863 2206	7033	476
autumn	no	20	Jun 24 Aug 26	2550 3563	6113	235	autumn	no	20	Jun 24 Jul 16 Aug 28	3302 2394 1875	7571	1065	autumn	no	20	Jun 24 Jul 16 Aug 28	2859 2466 2387	7712	179
autumn	yes	20	Jun 24 Aug 26	918 2490	3408	378	autumn	yes	20	Jun 24 Jul 16 Aug 28	3024 2680 1504	7208	902	autumn	yes	20	Jun 4 Jul 16 Aug 28	3219 1703 2077	6999	445
spring	no	34	Jun 24 Aug 26	2598 2994	5592	740	spring	no	34	Jun 24 Jul 16 Aug 28	3571 2395 1898	7864	650	spring	no	34	Jun 4 Jul 16 Aug 28	3351 1807 1920	7078	512
spring	yes	34	Jun 24 Aug 26	1668 2441	4109	541	spring	yes	34	Jun 24 Jul 16 Aug 28	3436 2700 2037	8173	482	spring	yes	34	Jun 4 Jul 16 Aug 28	3325 1823 1848	6996	915
autumn	no	34	Jun 24 Aug 26	2305 3266	5571	533	autumn	no	34	Jun 24 Jul 16 Aug 28	3759 2098 1469	7326	1165	autumn	no	34	Jun 24 Jul 16 Aug 28	3268 2288 2286	7843	396
autumn	yes	34	Jun 24 Aug 26	1200 3014	4213	515	autumn	yes	34	Jun 24 Jul 16 Aug 28	3566 2555 1654	7776	287	autumn	yes	34	Jun 4 Jul 16 Aug 28	3239 1439 1615	6293	404

Table 3-2. Mean BFT, weed, and bare ground cover by treatment 15 May 2013.

Seeding Season	Companion Crop	Seeding Rate (kg PLS ha-1)	Mean BFT Cover	SEM	Mean Weed Cover	SEM	Mean Bare Ground	SEM
spring	no	3	72	3.5	21	1.3	7	2.7
spring	yes	3	72	4.4	22	3.9	6	1.8
autumn	no	3	77	5.3	11	3.6	12	2.1
autumn	yes	3	80	2.3	16	1.8	4	0.8
spring	no	7	78	4.6	18	4.5	4	1.1
spring	yes	7	77	4.5	15	3.8	8	3.2
autumn	no	7	85	2.5	12	1.4	4	1.3
autumn	yes	7	82	1.4	15	0.8	3	0.7
spring	no	20	84	2.6	13	3.0	3	0.4
spring	yes	20	81	1.6	17	0.7	2	1.6
autumn	no	20	85	2.5	13	2.3	2	1.0
autumn	yes	20	80	3.6	18	3.2	3	0.8
spring	no	34	77	1.4	21	0.5	3	1.4
spring	yes	34	83	1.8	14	1.6	3	1.0
autumn	no	34	81	3.1	15	3.0	4	2.1
autumn	yes	34	73	3.1	27	3.1	0	0.0

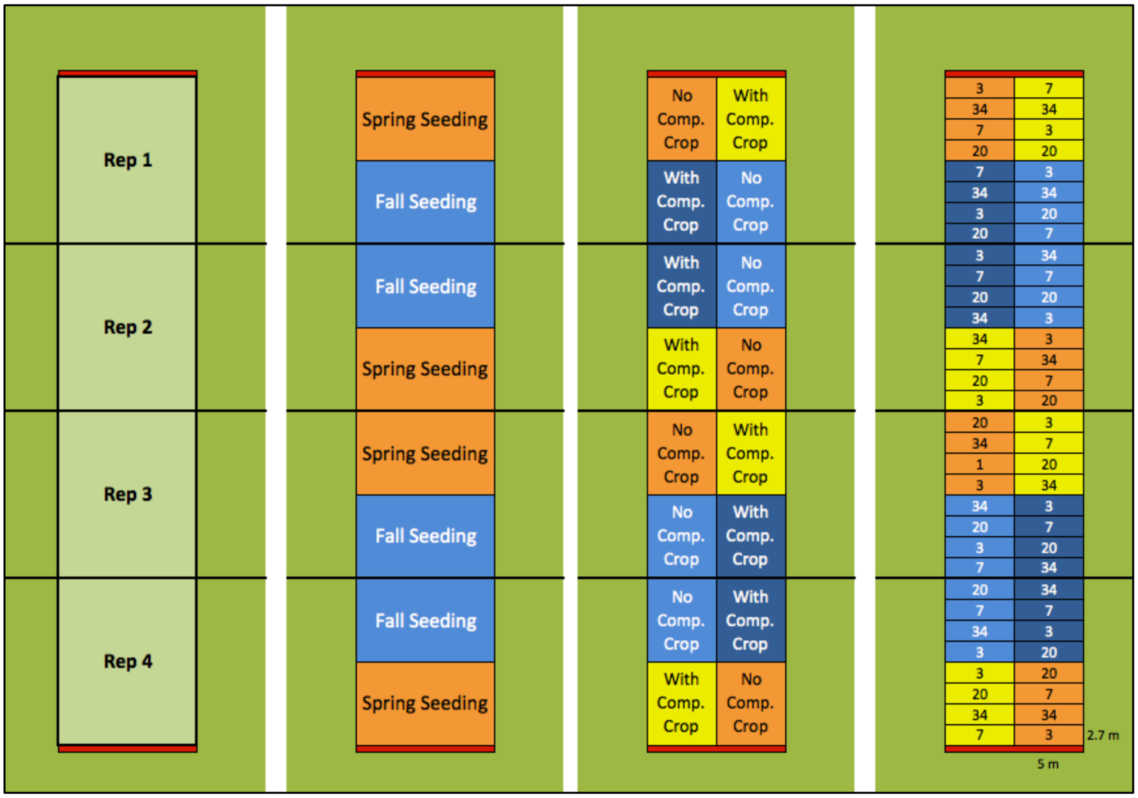

Fig. 3-1 Experimental design. From left to right: replications, whole plot seeding season treatments, split plot companion crop treatments, and split-split plot seeding rate treatments.

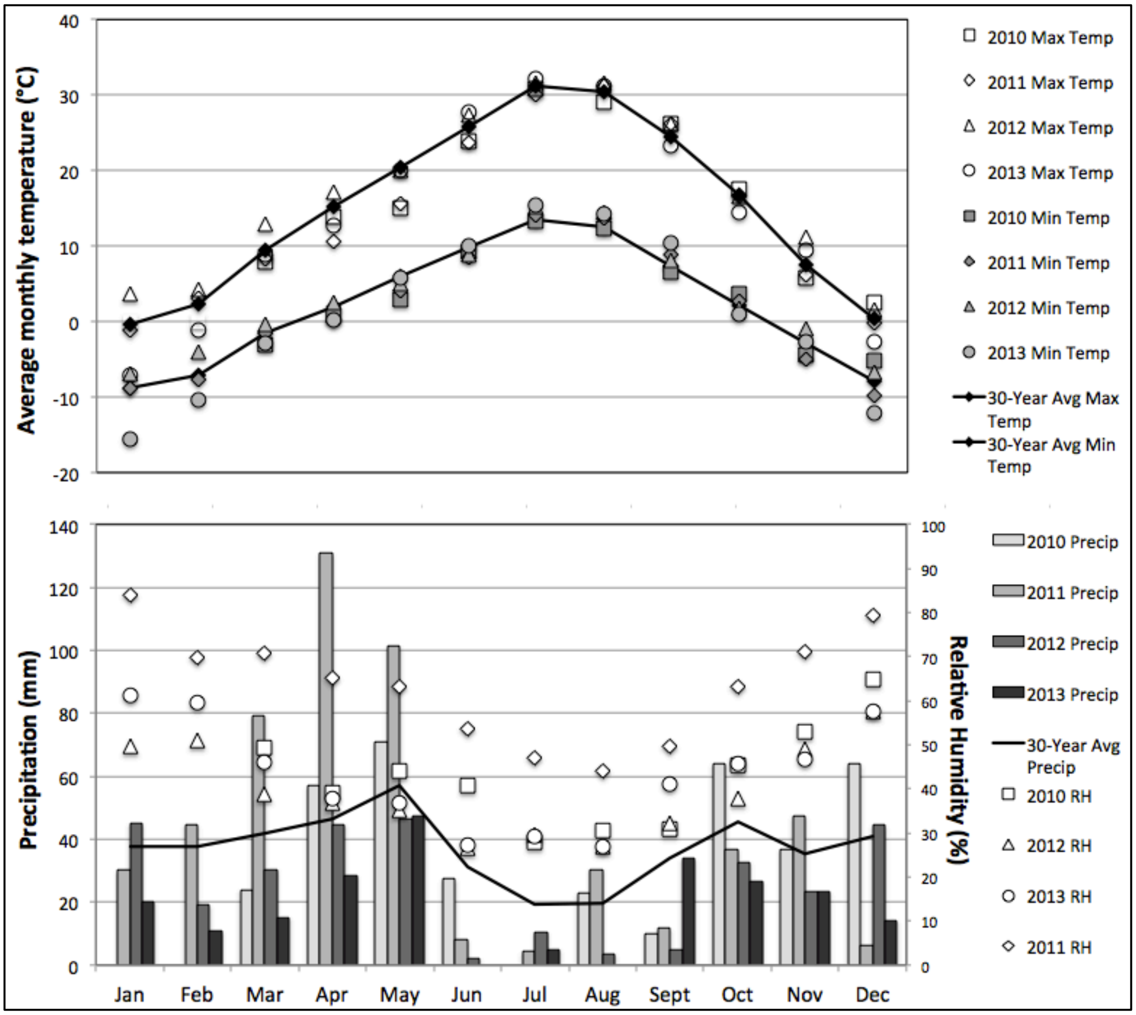

Fig. 3-2 Minimum and maximum average monthly temperatures for 2010 to 2013 and 30-year averages. Total monthly precipitation and relative humidity (RH) for 2010 to 2013 and 30-year average precipitation. Temperature and precipitation data for Jan and Feb 2010 were unavailable. Data from the Greenville Farm weather station, courtesy of Utah State University AgWeather Network, and Western Regional Climate Center reports of National Climatic Data Center 1981-2010 monthly mean temperatures.

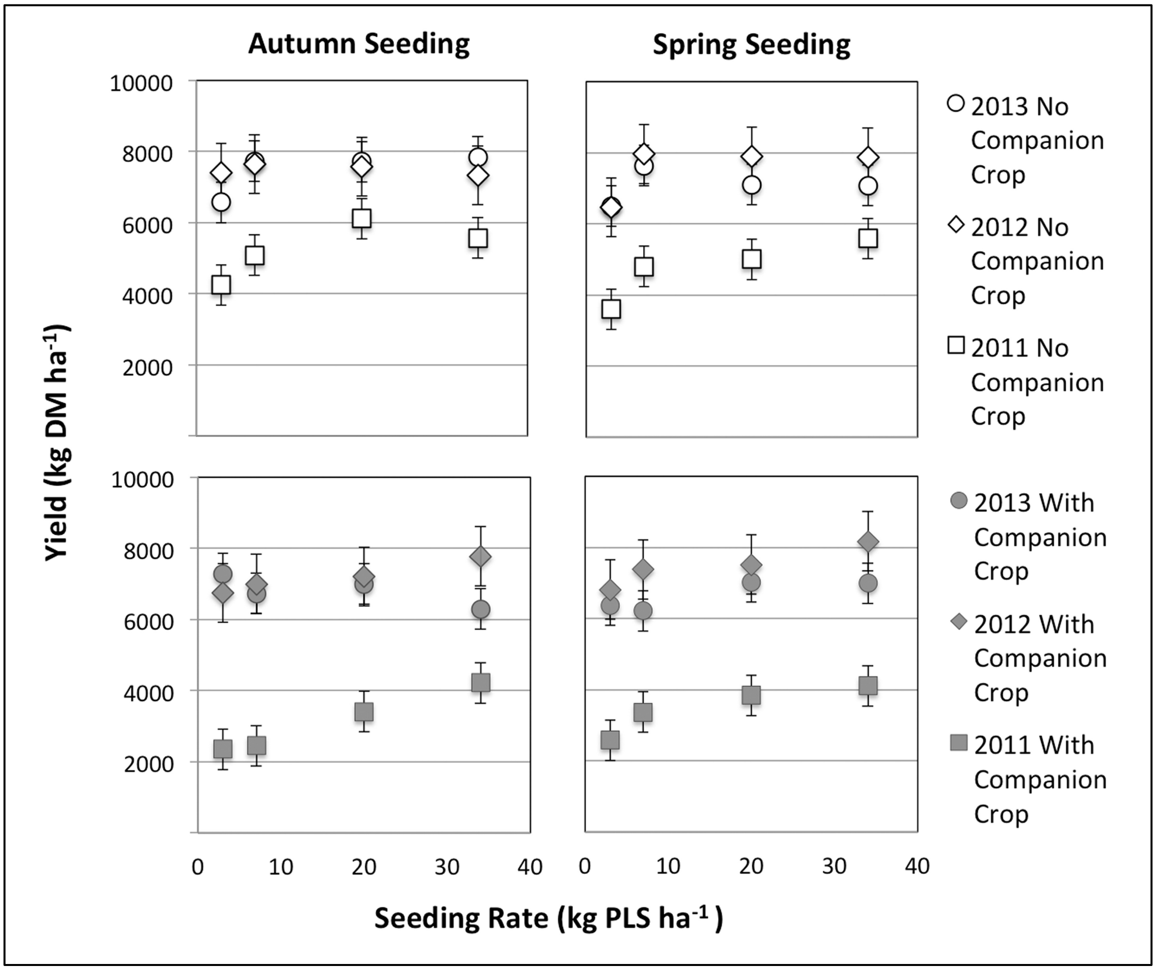

Fig. 3-3 Mean annual BFT stand yields (± standard error) of four replications by treatment. Stand yields include BFT and weeds.

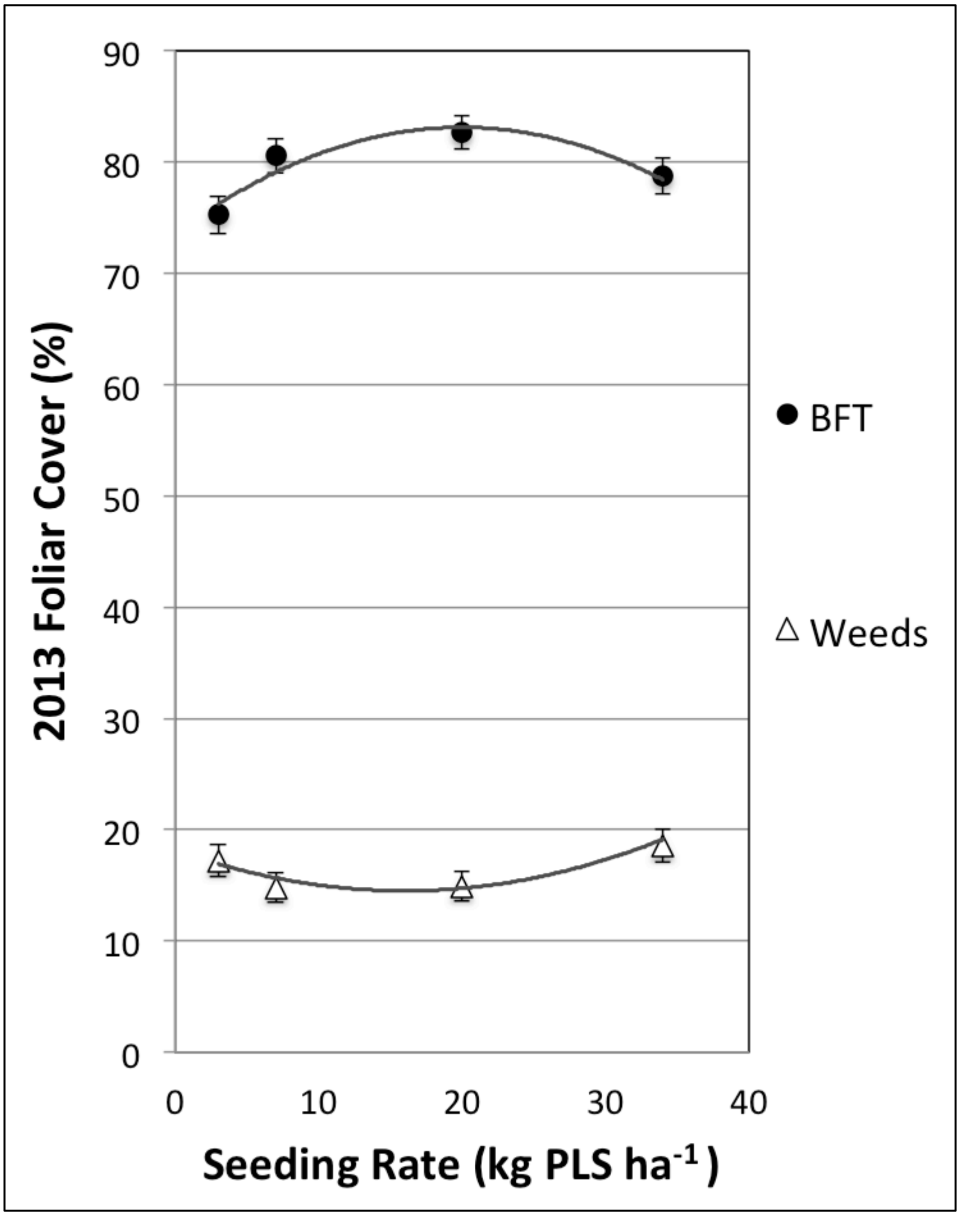

Fig. 3-4 Mean BFT and weed foliar cover (± standard error) of mature stands by seeding rate with quadratic trendlines.

CHAPTER 4

ESTABLISHMENT OF BIRDSFOOT TREFOIL (*Lotus corniculatus*) PASTURES ON ORGANIC DAIRY FARMS IN THE MOUNTAIN WEST USA

Abstract

Birdsfoot trefoil (BFT, *Lotus corniculatus* L.) is a non-bloating perennial forage legume well suited to ruminant production on pastures. It persists well under irrigation in cool, dry western climates, where it has been found to increase meat and milk production compared with other forages. The establishment of BFT pastures was evaluated on five commercial organic dairy farms in southern Idaho and northern Utah. Participating producers broadcast seeded 4-ha BFT pastures between 26 July and 8 September 2011 at a rate of 25 kg pure live seed ha^{-1}. Birdsfoot trefoil and weed densities were systematically sampled 17 to 23 days after first BFT emergence in fall 2011 and again in spring 2012. On all farms BFT seedling density was high, averaging 690 plants m^{-2} in the autumn and 340 plants m^{-2} the following spring. However, weed density varied between 130 and 750 plants m^{-2} in the autumn of 2011, and 240 and 410 plants m^{-2} in the spring of 2012. On the three farms where weed density was equal to or greater than BFT density in the spring of 2012, BFT cover averaged less than 10%. On the other two farms, weed density was less than BFT density and BFT cover was 48 to 66% in the spring following autumn seeding. These two pastures produced 6000 to 7600 kg of dry matter ha^{-1} by 20 June 2012 and were well enough established to support grazing for the remainder of the summer. Birdsfoot trefoil establishment was enhanced by crop rotation and sprinkler irrigation.

Introduction

There is growing interest in grass-fed and organic livestock production in the United States. Grazing-based beef and dairy systems have the potential to improve the fatty acid composition of meat and milk (Clancy 2006; Kay et al. 2005; Nuernberg et al. 2005; Realini et al. 2004; Schroeder et al. 2005), lessen environmental impacts through lower energy expenditures and reduced acidification of soil and water (Arsenault et al. 2009; Koknaroglu et al. 2007; O'brien et al. 2012), and increase profitability through reduced feed costs and niche marketing (Brock and Barham 2008; White et al. 2002). The forage legume birdsfoot trefoil (BFT; *Lotus corniculatus*) exhibits a number of characteristics that make it well suited for grazing-based ruminant production. Birdsfoot trefoil fixes the nitrogen it requires for growth, and unlike more commonly cultivated legumes such as alfalfa (*Medicago sativa* L.) and most true clovers (*Trifolium* sp.), the tannin content of BFT allows it to be grazed in pure stands without risk of bloat. The beneficial tannins produced by BFT reduce enteric methane production (Woodward et al. 2004), and increase milk yield in dairy cows (Turner et al. 2005; Woodward et al. 1999; Woodward et al. 2009). Birdsfoot trefoil reduces ammonia emissions from dairy manure compared with alfalfa (Misselbrook et al. 2005) by reducing the nitrogen content of urine (Woodward et al. 2009), and reduces the mineralization of nitrogen from feces (Crush and Keogh 1998), lowering the potential for nitrate leaching. Compared with other forages, BFT can support high weight gain in cattle (MacAdam et al. 2011; Marten et al. 1987; Wen et al. 2002) and lambs (Douglas et al. 1995; Marten and Jordan 1979). Despite these advantages, adoption of this legume is limited due to slow establishment and poor persistence in some climates (Ahlgren 1956; Seaney and Henson 1970).

In the cool, dry climates and moderately alkaline soils of mountain valleys in the western US, BFT grown under irrigation is productive and persistent (Beuselinck et al. 2005; MacAdam and Griggs 2006). However, slow establishment is problematic. Small seed size and low seedling vigor mean that weed competition can interfere with establishment of BFT pastures (Chapman et al. 2008; Henson and Taymon 1961), and it has been recommended that grazing during the seeding year be limited to that needed to control weeds (Ahlgren 1956). Chemical weed control has been demonstrated to improve first year yields, but by the second year BFT can yield equally well when established with no weed control (Scholl and Brunk 1962; Wakefield and Skaland 1965). Limited first year productivity and the potential for weeds to spread may dissuade organic producers from planting BFT despite the advantages the crop offers once established.

A number of studies have addressed BFT establishment under conventional management, but none under organic. This pasture establishment study was conducted on-farm, working within the constraints of producers' schedules and existing infrastructure. The five cooperating dairy producers are members of the Organic Valley® Cooperative who farm in southern Idaho and northern Utah. The objective of this study was to evaluate the seeding of BFT under irrigation on commercial organic dairy farms in the Mountain West, and relate management practices to differences in weed competition and the rate of BFT establishment. It is the first phase of a larger project comparing milk production of dairy cattle grazing

BFT pastures with that of dairy cattle grazing grass pastures; those milk production data will be reported separately.

Materials and Methods

Autumn seeding

Producers at three organic dairy farms in southern Idaho (ID-1, ID-2, ID-3) and two in northern Utah (UT-1 and UT-2) participated in this study. All farms were located within the Cache Valley, a remnant lake bed of Lake Bonneville, which covered 51,000 km^2 in the Great Basin 32,000-14,500 yr ago (Utah Geological Survey 2013). Irrigation is from rivers and reservoirs fed by autumn, winter and spring precipitation. In preparation for late summer-early autumn 2011 seeding of BFT, producers at all but ID-2 and ID-3 deep-plowed long-established grass pastures totaling at least 4 ha in autumn of 2010. An advising producer suggested planting a small grain crop to provide summer feed and allow additional soil cultivation before establishing BFT. In the fall of 2010, winter barley (*Hordeum vulare* L.) was planted on Farm UT-2, and in spring of 2011, spring oats was planted on Farms ID-1, ID-3, and UT-1. On Farm ID-2, the 4-ha field where BFT was to be planted had been cropped for alfalfa hay for 3 years, preceded by an annual crop of barley, so no annual crop was planted prior to BFT.

Beginning in July 2011, all producers cultivated their fields and prepared to seed BFT using a Brillion "Sure Stand" Seeder (Brillion Farm Equipment, Brillion, WI). Two farms (ID-1 and ID-2) were sprinkler irrigated, and on these seed was planted into the prepared seedbed and irrigated immediately following seeding. Producers on the three flood-irrigated farms (ID-3, UT-1 and UT-2) prepared a seedbed, irrigated, and then planted after the soil had dried sufficiently to be crossed with a tractor. On all farms the same seed lot of 'Norcen' BFT seed (Norfarm Seeds Inc., Bemidji, MN) coated with OMRI-certified Apex™ Green (Summit Seed Coatings LLC, Boise, ID) containing Nitragin K rhizobium inoculant (Novozymes BioAg Inc., Brookfield, WI) for BFT was broadcast at a rate of 25 kg pure live seed (PLS) ha^{-1}, including 3 kg ha^{-1} hard seed. Seeding dates were, in chronological order: 26 July at ID-1, 10 Aug at ID-2, 17-18 Aug at UT-1, 5 Sept. at ID-3, and 7-8 Sept. at UT-2.

Farms ID-1 and ID-2 applied sprinkler irrigation after seeding through the end of Sept.; ID-1 was equipped with a lateral roll sprinkler system, and reported applying approximately 50 mm of water every 8-9 days. ID-2 was equipped with an ATV-towed pod sprinkler system, and applied approximately 25 mm every 7 days. ID-3 and UT-2 flood irrigated once in the autumn after seedling emergence. UT-1 irrigated only the east 2-ha BFT field once after seeding; an autumn rain occurred before the second field could be irrigated. It is difficult to estimate the application rate of water with flood irrigation since the differential between the head and tail ends of the field depends on the soil infiltration rate. Farms UT-1 and UT-2 could not report how much water they applied; Farm ID-3 estimated applying approximately 75 mm. No farms resumed irrigation prior to spring sampling of plant densities. ID-1 and ID-2 clipped weeds that had grown taller than BFT once in the autumn by greenchopping (ID-1) or swathing (ID-2). Previous crop history, BFT seeding date, irrigation method, major soil type, soil texture and details of nutrient status of the BFT pastures on all farms are summarized in Table I.

Soil tests

Soil tests were carried out on all BFT pastures prior to seeding. Soils were sampled to a depth of 30 cm and a composite of 20 subsamples taken from each field was analyzed. Soil samples were passed through a 2-mm sieve and stored at 4ºC until analysis, which occurred within 2 weeks. The following properties were measured according to Gavlak et al. (2003): nitrate-nitrogen (NO_3-N) by the chromotropic acid method (Method 16 S3.30); Olsen extractable P and K (Method S4.10); water extractable calcium, magnesium, sodium, and sulfur were measured using the saturation paste method (Method S1.60), and soil pH (Method S2.20) and electrical conductivity (EC; Method S2.30) were measured in a 1:1 w/v water-saturated paste. Sodium absorption ratio (SAR) was calculated for Farm ID-3 as the proportion of sodium to calcium plus magnesium in the soil according to the formula given in Davis et al. (2012). Adequate phosphorus, potassium and sulfur concentrations for a deep-rooted perennial legume such as alfalfa are 15-30 ppm, 150-250 ppm and >8 ppm, respectively (Cardon et al. 2008).

Stand assessment

BFT and weed plant densities were systematically sampled on each farm in the autumn of 2011 and again in the spring of 2012. Each BFT field was divided into a grid of 30.5-m^2 subdivisions. Maps of the fields with the grid superimposed and a sampling point in the center of each subdivision were created using ArcGIS (ESRI, Redlands, CA 92373). These maps were uploaded to a handheld Archer GPS unit (Juniper Systems, Logan, UT 84321) so that the central point of each subdivision could be easily located in the field. Across each BFT field, one of every two subdivisions was sampled. A generic sampling schematic can be seen in Fig. 4-1a and the maps for each farm can be seen in Fig. 4-2a. BFT fields were not identical in shape or area, leading to minor differences in the number of subdivisions sampled on each farm.

Random sampling points were determined by standing at the center of each subdivision to be sampled and blindly tossing five different objects. Where each object landed (Fig. 4-1b) a 0.25-m^2 quadrat divided into 100-cm^2 sections was used to take two BFT and two weed counts (Fig. 4-1c). For each of the two counts, the number of weed or BFT seedlings rooted within one of the 100-cm^2 quadrat sections was determined. Quadrat design was adapted from Vogel and Masters (2001). When spring counts were performed, the center 900 cm^2 of the quadrat (Fig. 4-1c) was also used to make one visual estimate of foliar cover at each of the 5 random sampling points. Bare ground, BFT and weed foliar cover were estimated to the nearest 5% increment. The two dominant types of cover were estimated first, and the remaining type was calculated by difference with 100%.

In the autumn of 2011 following seeding, sampling occurred 17-23 days after first BFT emergence. Sampling dates were, in chronological order: 16 Aug. at ID-1, 3 Sept. at ID-2, 25 Sept. (east half) and 8 Oct. (west half) at UT-1, 9 Oct. at ID-3, and 10 Oct. at UT-2. Farm UT-1 was sampled on two dates due to earlier emergence on the east half of the field, which was irrigated prior to seedling emergence. In the spring of 2012, sampling occurred after the first observation of new BFT seedling emergence from hard seed. Dates of sampling were: 24 Apr. at UT-1, 28 Apr. at UT-2, 29 Apr. at ID-3, 6 May at ID-1, and 8 May at ID-2. Fig. 4-1d-f shows examples of autumn and spring quadrat observations.

To illustrate the distribution of BFT across each field, mean fall and spring plant densities and cover values from spring sampling dates were color coded and displayed on the field grids used for sampling (Fig. 4-2b-d). Values for unsampled subdivisions were calculated as the mean of all adjacent subdivisions sharing a full side. Dry matter (DM) production was measured on Farms ID-1 and ID-2 on 6, 13 and 20 June using a non-destructive FarmWorks F100 electronic rising plate meter (RPM; FarmWorks Systems, Fielding, New Zealand) calibrated to these two BFT fields. On each sampling date, four RPM readings were taken for each field. Fields were divided into four quadrants; NW, NE, SW and SE. One RPM reading, the mean of 30 measurements, was taken from each quadrant by walking in a 'W' pattern and taking one measurement every 12 steps. Each calibration sample was cut from the area under the RPM after taking a single reading for the forage at the same location. Calibration samples were oven-dried and calibration curves developed to predict the DM yield of grass or BFT, with more than 70 samples of each pasture type taken from each of the two farms throughout the summer.

Climate data

Average monthly maximum and minimum temperatures, and total monthly precipitation and evapotranspiration were calculated from data gathered by three weather stations (Preston, ID; Trenton, UT; and Cutler Dam UP&L, UT) in Cache Valley. Data were provided by the Utah State University Climate Center, Climate Database Server, which gives daily evapotranspiration estimated by the ASCE-standardized Penman-Monteith method (ASCE-EWRI, 2005). Thirty-year averages were calculated from data gathered by the same three weather stations, provided by Western Regional Climate Center reports of National Climatic Data Center 1981-2010 monthly mean temperatures.

Statistical analysis

For each farm, field means and standard errors of plant density and foliar cover were calculated from the means of sampled subdivisions (ID-1 n=20; ID-2 n=21; ID-3 n=30; UT-1 n=19; UT-2 n=24). Farms were treated as replications (n=5) to assess correlation between management parameters, plant density and plant cover across the variety of conditions present on different farms. Pearson correlations between spring BFT density, spring weed density, autumn BFT density, autumn weed density, spring BFT cover, spring weed cover, winter BFT mortality, irrigation method, and seeding date were evaluated using PROC CORR in SAS version 9.3 (SAS Institute Inc. 2009, Cary, NC USA). Winter BFT mortality was calculated as the difference between autumn and spring densities; planting dates were converted to day of year for analysis; and irrigation method was assessed categorically as either sprinkler or flood irrigation.

Results

A high BFT seedling density was established on all farms, averaging 690 (± 69 SEM; n=5 farms) plants m^{-2} in the autumn of 2011 and 340 (± 33 SEM; n=5) plants m^{-2} in the spring of 2012. The highest and lowest autumn BFT densities were 910 (± 88 SEM; n=20) plants m^{-2} at farm ID-1 and 540 (± 87 SEM; n=24) plants m^{-2}

at UT-2; the spring high and low were 410 (± 30 SEM; n=21) plants m^{-2} at ID-2 and 240 (± 40 SEM; n=24) plants m^{-2} at UT-2. Birdsfoot trefoil density was significantly higher than weed density on all three Idaho farms in the autumn after planting, but on only two (ID-1 and ID-2) of the five participating farms the following spring (Fig. 3). On the three farms where BFT density was equal to or lower than weed density in the spring of 2012 (ID-3, UT-1, UT-2), mean BFT cover was 10% or less on the same date.

Across farms (n=5), BFT density was not correlated with BFT cover (autumn density p=0.85; spring density p=0.36), but weed density was positively correlated with weed cover (fall r=0.97; spring r=0.88; p<0.05). Figure 4-4 shows spring foliar cover for each of the five farms. The most frequently observed weeds were common mallow (*Malva neglecta*), lambsquarters (*Chenopodium album*), redroot pigweed (*Amaranthus retroflexus*), annual mustards (*Brassica* spp.), prickly lettuce (*Lactuca serriola*), clover (*Trifolium* spp.), which may have been a crop contaminant in the BFT seed, and a number of annual and perennial grasses (Poaceae), including volunteer oats at ID-3 and volunteer barley at UT-2 in the autumn. At Farm ID-1 common mallow was the most frequently observed weed across most of the pasture, showing a strong presence in spring cover on the north end of the field (Fig. 4- 2d). The weed population at ID-2 was mixed, with no single species appearing more often than others, although annuals were more frequently observed than perennials. Grasses were the most frequently observed weeds at ID-3 and UT-2, although a mixture of annual and perennial forbs were also present. The weed population at UT-1 was dominated by annual mustards, lambsquarters, and prickly lettuce in autumn and spring, although common mallow was also present.

Farms ID-1, ID-2, and UT-1 exhibited even distribution of high BFT plant densities across their fields following autumn seeding (Fig. 4-2b) and into the spring (Fig. 4-2c). Despite overall high field averages, some subdivisions on Farms UT-2 and ID-3 showed BFT densities below 100 plants m^{-2} in the autumn of 2011, and by spring of 2012, seedling density had become more varied on these two farms (Fig. 4-2c). Percent foliar cover of BFT was uniformly low across these fields in the spring of 2012 (Fig. 4-2d). Although Farm UT-1 had high, evenly distributed BFT seedling density at both sampling dates (Fig. 4-2b and c), in the spring of 2012 BFT cover was low due to continuing weed competition; only Farms ID-1 and ID-2 had significant areas of BFT cover greater than 50% in the spring of 2012 (Fig. 4-2d).

Farms ID-1 and ID-2 had BFT stands well enough established to begin grazing in late June of 2012. Prior to grazing, these stands produced approximately 7600 and 6000 kg DM ha^{-1}, respectively, which was significantly more than a grass pasture at Farm ID-2 that was measured for comparison (Fig. 4-5). The grass pasture was a mixture of perennial ryegrass (*Lolium perenne*), orchardgrass (*Dactylis glomerata*), tall fescue (*Schedonorus arundinaceus*), quackgrass (*Elymus repens*) and also contained white clover (*Trifolium repens*).

Fig. 4-6 shows monthly temperature (a) and precipitation data (b) for 2011 and 2012 as well as means from 1981 to 2010 at weather stations with a similar geographical distribution in Cache Valley as the five farms. In July and Aug. of 2011, when BFT was planted on Farms ID-1, ID-2 and UT-1, maximum air temperatures averaged 30°C and minimum temperatures averaged 13°C. In Sept., when ID-3 and UT-2 pastures were seeded, maximum temperatures averaged 26°C and minimum temperatures averaged 8°C. In 2011 total monthly precipitation levels were 23 mm in July, 42 mm in Aug., and 8 mm in Sept. Evapotranspiration exceeded precipitation from May until Nov. of 2011 and from Feb. until Nov. of 2012.

Across farms (n=5) irrigation method was significantly correlated with spring BFT cover (r=0.97; $p<0.05$), but seeding date was not correlated with spring BFT cover ($p=0.13$). BFT density did not show a significant correlation with irrigation method or seeding date on either sampling date ($p>0.05$). Temperatures decreased rapidly in Sept. and Oct., and by the end of Oct. minimum nighttime temperatures began to drop below 0°C, which would effectively halt further establishment. Average minimum temperatures for Dec. 2011, Jan. and Feb. 2012 were -7.9, -8.8, and -7.1°C respectively. Winter mortality of BFT seedlings was higher on farms that had higher autumn BFT density, and was not correlated with seeding date ($p=0.50$, n=5).

Discussion

Producers participating in this study employed recommended strategies to promote rapid establishment and mitigate weed competition. All farms prepared firm seedbeds, seeded BFT into certified organic land at a rate of 25 kg ha^{-1} PLS, and planted with Brillion broadcast seeders. Two of the five farms participating in this study (ID-1 and ID-2) were able to establish pastures with sufficient BFT density, cover, and distribution to be grazed for most of 2012 after harvesting crops in late summer of 2011 and planting BFT in early autumn. On 6 June 2012, these two newly established BFT fields had produced approximately the same amount of dry matter as a long established grass pasture at ID-1; by June 20, these BFT pastures were significantly more productive than the grass pasture although none had been grazed. These results from farms ID-1 and ID-2 indicate it is possible to establish a productive BFT stand without sacrificing a full growing season.

The remaining three farms had a uniformly low BFT cover in the spring of 2012 due to aggressive weed competition, but BFT plant density was more than 100 plants m^{-2} in the majority of pasture subdivisions in all three BFT pastures in spring of 2012. Recommended management to support continued stand establishment would suppress weed competition while maintaining a high ratio of BFT leaf area to land area (leaf area index) by clipping or light grazing during the growing season following autumn planting. Although other studies have shown that early weed competition does not necessarily affect yields of BFT in the second year after planting (Scholl and Brunk 1962; Wakefield and Skaland 1965), the rapid establishment achieved on Farms ID-1 and ID-2 is clearly advantageous.

Seeding rate and method

The commonly recommended seeding rate for BFT is 6 to 11 kg ha^{-1} PLS (Hall and Cherney 2007; Rhykerd et al. 1981; Undersander et al. 1993). However, in north central Alberta, Canada, Chapman et al. (2008) found that BFT seeded in spring at a rate of 12 kg ha^{-1} competed poorly against weeds and experienced significant winterkill, resulting in poor stands. It has been demonstrated that increasing seeding rate can compensate for lack of weed control, producing higher plant populations and yields when no chemical weed control is used (Wakefield and

Skaland 1965). Results of the seeding rate study presented in the previous chapter demonstrated that increasing seeding rate up to 34 kg PLS ha^{-1} increased first year yields, but 18 kg PLS ha^{-1} resulted in greatest BFT cover in mature stands. Broadcast seeding provides better distribution of seed across the soil than drilling and can be more successful than band seeding (Fribourg and Strand 1973). However, soil-to-seed contact is variable in broadcast seeding, increasing seedling mortality (Barker et al. 2012), so producers participating in this study broadcast seeded BFT at a high rate.

Plant density, cover, and mortality

The minimum density of BFT seedlings necessary to establish a productive stand has been defined in several studies: in Minnesota, mature BFT plants were transplanted into the field and the optimal plant density for BFT forage yield was found to be 30 plants m^{-2} (McGraw et al. 1986), Ayres et al. (2006) considered BFT pastures with 57 plants m^{-2} successfully established, and Taylor et al. (1973) judged BFT hay plots with a density of 102 plants m^{-2} to have excellent stands. In our study, whole field averages were well above 100 plants m^{-2} on all farms by the spring of 2012, and only small patches on two farms (ID-3 and UT-2) had BFT plant densities lower than 100 plants m^{-2}. These two farms, and Farm UT-1, had uniformly low BFT cover despite high BFT seedling densities.

While BFT winter mortality was highest for Farm UT-1, which had the highest fall BFT seedling density, concern that poor BFT establishment might result from an excessively high seeding rate and intraspecies competition can be addressed by comparing autumn BFT plant density and spring BFT cover in Fig. 4-

2b and 4-2d, where it is apparent that lower BFT seedling densities have not resulted in higher BFT cover. While there was no statistical correlation of BFT plant density and BFT cover across farms, weed plant density and weed cover were positively correlated.

This lack of BFT cover (i.e., leaf area) in spite of sufficient BFT seedling density suggests a physiological basis for slow establishment and poor competition with weeds, such as low photosynthetic rate or assimilate partitioning that favors root growth over shoot growth. Gist and Mott (1957) found that after 45 days of growth under a range of moisture conditions, shoot dry matter of BFT seedlings was approximately half that of alfalfa, while the dry matter of BFT seedling roots was only about one-third that of alfalfa. In Iowa, Buxton and Wedin (1970b) found that while BFT root dry weights were no more than one-quarter those of alfalfa in the seeding year, by the end of the year following seeding, root dry weights of BFT were nearly equal to those of alfalfa. Low shoot growth rate in BFT seedlings accompanied by low initial root dry matter suggests that BFT seedlings may have a lower rate of photosynthesis (or a higher rate of respiration) than alfalfa seedlings.

Irrigation and soil moisture

While organic BFT establishment has not been studied previously, extensive work under conventional management identified seeding date, air temperature and moisture availability as influential in BFT establishment. In this study, only irrigation method was significantly correlated with BFT cover in spring 2012. Low soil moisture was found to be a limiting factor in the success of BFT emergence and establishment in a number of studies. Ayres et al. (2006) evaluated the performance of BFT pastures at 17 sites across Australia to gauge its adaptation to regional climate and soil conditions. They found that BFT was well adapted to a wide variety of soil conditions, but that drought conditions at the time of seeding caused BFT establishment to fail. Given sufficient moisture, broadcast seeding into a cultivated seedbed followed by rolling resulted in the highest seedling density. At Palmerston North, New Zealand, Douglas and Foote (1994) assessed spring establishment of BFT. Rainfall was less than evapotranspiration at the start of the study and no rain fell for 3 weeks prior to seeding. They measured 8.2% BFT emergence, occurring between 8 and 15 days after seeding, and 50% survival of emerged seedlings after 10 weeks.

Hur and Nelson (1985) demonstrated that optimal BFT germination occurs at 20°C when temperature is constant, and relative humidity is maintained at 90 to 100%. However, germination percentages of more than 50% occurred in their study in less than 20 days at 356 temperatures ranging from 12 to 30°C. In Cache Valley during the planting and germination period, from 26 July to 28 Sept. 2011, temperatures ranged between 4 and 35°C, but evapotranspiration was high (Aug. 175 mm; Sept. 122 mm) compared with precipitation (Aug. 42 mm; Sept. 8 mm). As a result, relative humidity ranged between 6 and 99% but averaged 44% during the planting period of this study (Source MesoWest Data, http://mesowest.utah.edu/).

These conditions indicate the difficulty of maintaining adequate moisture close to the soil surface during seedling establishment, even under sprinkler irrigation much less with flood irrigation, which saturates the soil and can therefore only be applied at longer intervals. Flood irrigation precedes sprinkler technology historically and is still widely used due to its simplicity and low cost, although flood irrigation is more prone to cause erosion and more difficult to control. The producer at Farm UT-2 reported that his BFT field may not have been adequately leveled prior to seeding due to time constraints. This could have contributed to patchy establishment (Fig. 4-2b), as less water would reach unevenly elevated areas of the field.

Seeding date and seedling mortality

Because high initial plant densities were achieved on each farm regardless of irrigation method, this study can provide useful information on the interaction of seeding date, soil characteristics, and management prior to seeding with differences in establishment success. After evaluating a wide range of seeding dates in Tennessee, Fribourg and Strand (1973) concluded that good establishment occurred for BFT planted mid- to late April in the spring, and mid-July to early August in the autumn; supplemental irrigation improved the success of autumn seedings. In Iowa, Buxton and Wedin (1970a) determined that a late summer planting following the harvest of an oat crop yielded more forage in the year following seeding than planting BFT in the spring with an oat companion crop. Late summer-early autumn planting was chosen over spring planting for the present study for these and other reasons: spring moisture commonly delays cultivation and planting and can result in the establishment of perennial seedlings under high summer temperatures; feed from winter or annual spring grain crops can be harvested prior to an autumn planting and allow further cultivation of sod-bound soils; competition from annual weeds is shorter-lived in autumn than in spring (Vough et al. 1995).

Autumn seedings can fail if an early frost occurs before BFT seedlings have matured sufficiently. In a greenhouse experiment, Rachie and Schmid (1955) showed that 14 days of growth is insufficient for BFT to survive 12h at -10°C, but after 19 days of growth 76% of seedlings with 4 to 5 true leaves and a height of 5 to 10 cm from a range of BFT cultivars survived the same sub-freezing temperatures. In the study reported here, all seedlings had more than 20 days of growth prior to the first frost, which occurred on Oct. 19th, and winter mortality was not correlated with seeding date.

Soil conditions

Soil properties such as texture and fertility can also influence establishment. The soils on the three Idaho farms were sandy loams while the soils on the two Utah farms were higher in silt or clay, but none of the soils were considered limiting in texture or fertility for the establishment of legumes (Cardon et al. 2008). Water extractable soil sulfur was somewhat low on farm ID-2; however, no plant sulfur deficiency symptoms were observed. BFT is reported to tolerate soil pH ranging from 4.5 to 8.2 (Duke 1981) and to be moderately tolerant of soil salinity, reaching a threshold EC at 5.0 dS m^{-1} before demonstrating a yield decline (Maas and Hoffman 1977). Soil pH was moderately to strongly alkaline and soil salinity (electrical conductivity or EC) was low on all farms (Table 4-1).

High sodium levels relative to other salts negatively impact soil structure and drainage, and Farm ID-3 had a higher concentration of sodium than other farms in this study. Sodium concentration was not high enough to classify the soil as sodic (Farm ID-3 SAR=8.3, sodic soil SAR >13; Davis et al. 2012); however, the soil was observed to form a hard crust when dry despite its sandy loam texture, a characteristic of sodic soils. The ID-3 field where BFT was planted was flood-irrigated, and the producer reported a history of poor drainage and a seasonally high water table. Although Farm ID-3 weed cover in spring 2012 was similar to Farm ID-1, BFT cover was lower and percent of bare ground was higher on ID-3 than ID-1. Therefore, we suspect that the unusually patchy distribution of BFT on ID-3 (Fig. 4-2c) resulted from these unfavorable soil conditions.

Crop rotation

Differences in previous field management are likely to have influenced BFT establishment and weed competition in this study. On Farm ID-2, alfalfa hay had been cultivated for 3 years preceded by an annual crop of barley, while the fields planted with BFT on all other farms had been managed under grazing rather than cropping. The use of crop rotations can reduce the weed seed bank (Liebman and Davis 2000), and this may have provided a competitive edge for BFT establishment on Farm ID-2, which had the lowest autumn weed density, lowest spring weed cover and highest spring BFT cover. Farm ID-1 also had low weed densities, but BFT cover was lower and weed cover was higher than on ID-2. Weed populations were also different between these two farms; the predominant weed at ID-1 was the perennial common mallow, which has a large horizontally oriented leaf, while more upright annual weeds were dominant at ID-2.

Mixtures vs. monocultures

Planting mixed cultures rather than monocultures can reduce weed competition in pastures (Sanderson et al. 2005). MacAdam and Griggs (2006) evaluated the performance of BFT in binary grass-legume mixtures under irrigation in the Intermountain West, finding it as productive in mixtures as alfalfa and white clover. However, voluntary intake is higher for legumes than grasses (Van Soest 1965), and many of the advantages BFT provides for ruminant production, such as the prevention of bloat and increased nitrogen use efficiency, have been attributed to the condensed tannins produced by the BFT (Mueller-Harvey 2006; Waghorn 2008). Growing BFT in mixtures reduces the overall concentration of condensed tannins in the forage consumed by grazing animals, diluting potentially beneficial effects (Woodward et al. 2009). The central objective of the multi-year organic milk production research that includes this establishment study was to evaluate the effect of the tannin-containing legume BFT on subsequent milk production in comparison with grass pastures.

Conclusions

A high seeding rate of BFT was used in the broadcast planting of pastures on five organic dairies, with the goal of grazing as soon as establishment was achieved. Birdsfoot trefoil was planted as a monoculture so that the full benefit of this highly digestible tannin-containing legume for milk production could be assessed. Planting dates that ranged over six weeks at the end of summer did not have a significant effect on establishment, and excellent germination and initial establishment was achieved on all five farms. Sprinkler irrigation positively influenced spring 2012 BFT cover, and the field on which crop rotation had occurred preceding planting (ID-2) had the highest BFT density and cover at the end of the seedling establishment period. Because this study was conducted as on-farm research with a relatively small sample size of five farms and management that inevitably varied from farm to farm, some influential factors may not have resulted in significant correlations with data for BFT establishment. The findings reported here can, however, identify factors associated with successful establishment of BFT, particularly in the West, and serve as a starting point for future studies. Domination of foliar cover by weeds was the main factor associated with slow establishment of BFT, although the dominant weed differed from farm to farm. Investigation of traits that could improve the competitiveness of BFT seedlings and hasten establishment, such as such as higher seedling photosynthetic rate or low seedling respiration rate, would increase the value of BFT as a forage crop, especially for grass-fed and organic ruminant production.

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Table 4-1. Birdsfoot trefoil field management, irrigation infrastructure, and soil descriptions.

Farm	Previous Crops	Crop Autumn 2010 Spring 2011	BFT Seeding Date 2011	Irrigation Method	Major Soil Type(s) [a]	Soil									
						Texture	pH	EC	Na	NO_3	P	K	Ca	Mg	S
								ds m^{-1}	mg kg^{-1}						
ID-1	Grass pasture (5+ years)	Summer oats	26 July	Sprinkler	Kidman (Coarse-loamy, mixed, mesic Calcic Haploxerolls)	Sandy loam	8.28	0.26	36	24	36	535	44	19	8
ID-2	Barley, Alfalfa (3 years)	Alfalfa	10 Aug.	Sprinkler	Kidman (Coarse-loamy, mixed, mesic Calcic Haploxerolls)	Sandy loam	8.29	0.12	12	4	24	300	33	7	3
ID-3	Grass pasture (5+ years)	Summer oats	5 Sept.	Flood	Windernot-Lewnot-Stinkcreek (Sandy-skeletal, mixed, mesic Pachic Calcixerolls)	Sandy loam	9.04	0.41	105	3	59	489	35	19	57
UT-1	Grass pasture (10+ years)	Summer oats	18 Aug.	Flood	Greenson (Fine-silty, mixed, mesic Aquic Calciustolls)	Silty loam	8.25[b] 8.28[c]	0.24 0.21	10 34	6 17	14 18	709 842	65 67	11 12	9 13
UT-2	Grass pasture (10+ years)	Winter barley	8 Sept.	Flood	Nibley (Fine, mixed, mesic Aquic Argiustolls)	Clay	8.21	0.26	19	6	44	>900	58	20	10

[a] Major soil types provided by NRCS Web Soil Survey.
[b] West field
[c] East field

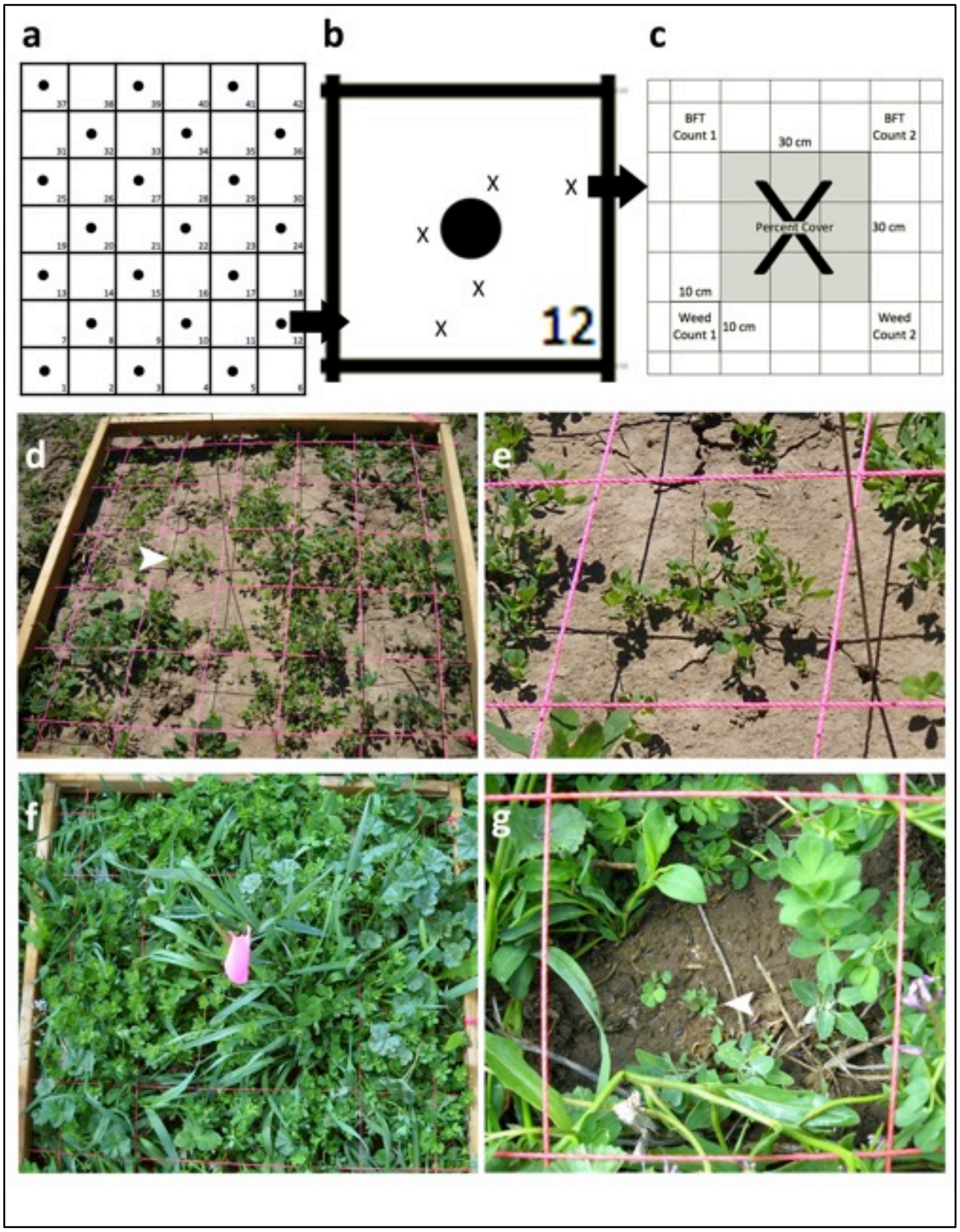

Fig. 4-1 Generic map grid (a), single subdivision with x's illustrating random sampling points (b), and schematic of quadrat used for spring plant density counts and cover estimates (c). Example plant density count in autumn 2011 showing whole quadrat (d), and a closeup of one 100-cm^2 subdivision (e); white arrowhead in (d) indicates BFT seedling shown in closeup in (e). Example of a 50% BFT, 50%

weeds foliar cover estimate (f) and closeup of one 100-cm^2 subdivision in spring 2012 showing a newly germinated BFT seedling (white arrowhead) next to a clover seedling (g). Seedling counts included only plants rooted within a given 100-cm^2 square. The center strings were removed from the quadrat in spring 2012 before cover sampling, creating a 900-cm^2 square.

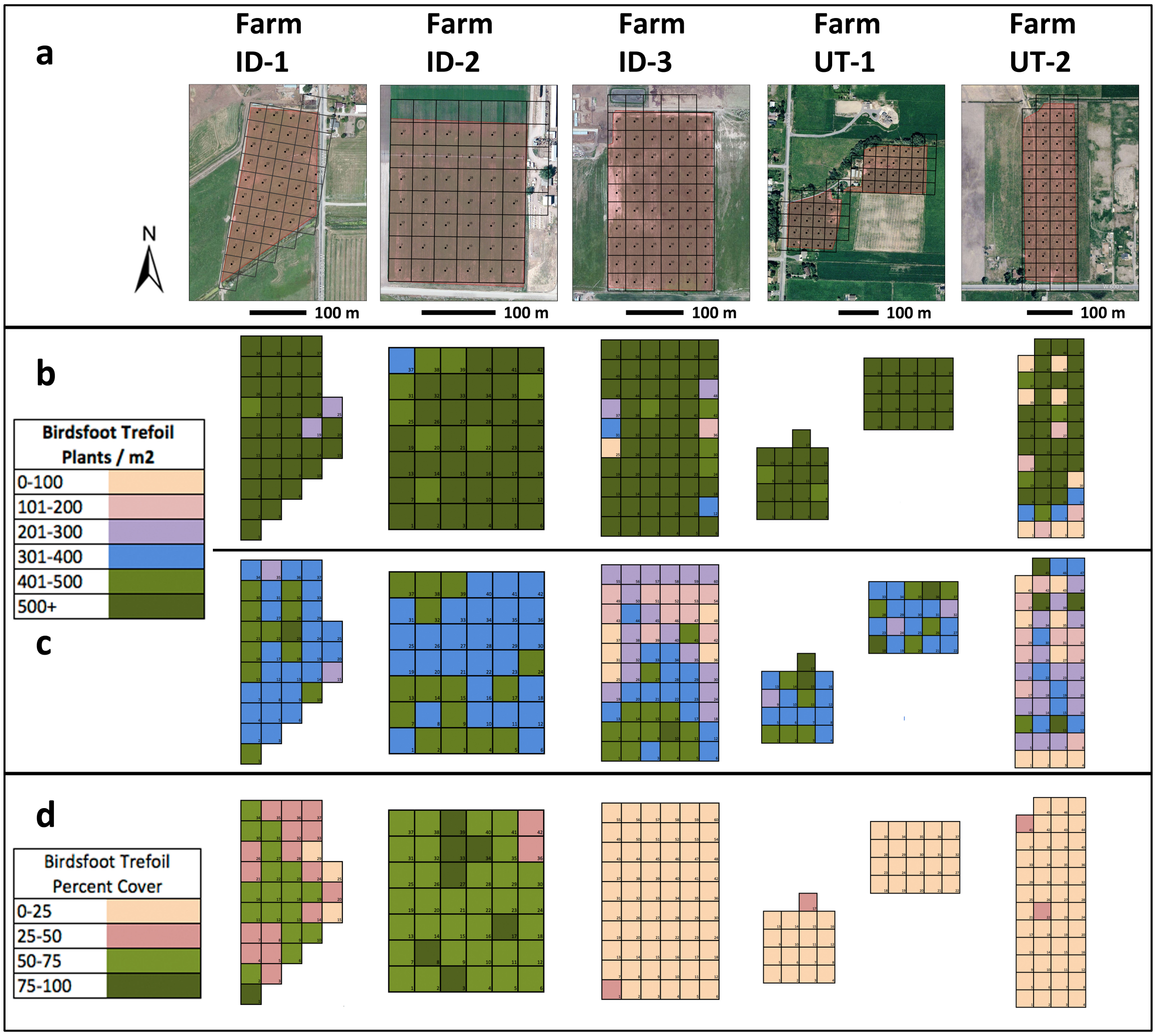

Fig. 4-2 Satellite images of farms with field subdivisions superimposed (a), schematics of BFT plant density in autumn 2011 (b) and spring 2012 (c), and percent BFT cover in spring 2012 (d). Plant density and cover maps are not drawn to scale.

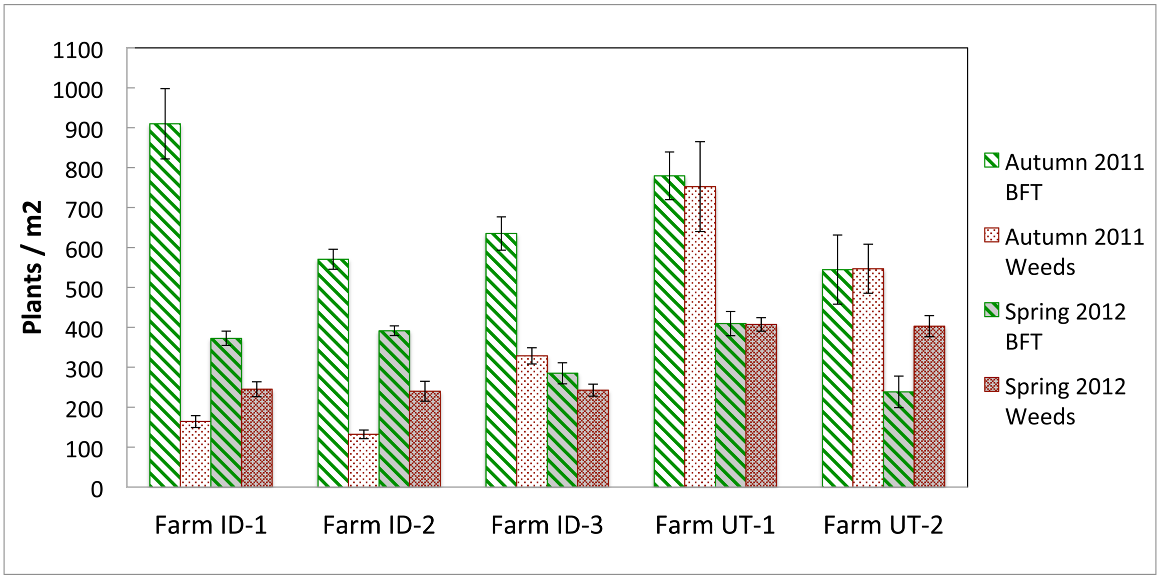

Fig. 4-3 Birdsfoot trefoil (BFT) and weed plant density following planting in autumn 2011 and in spring 2012 (± SEM) on five organic dairy farms. BFT fields were not identical in area, leading to variation in the number of subdivisions sampled (ID-1 n=20; ID-2 n=21; ID-3 n=30; UT-1 n=19; UT-2 n=24). Every other subdivision was sampled, and 10 BFT and 10 weed counts from a 100-cm^2 area were taken at random locations within sampled subdivisions.

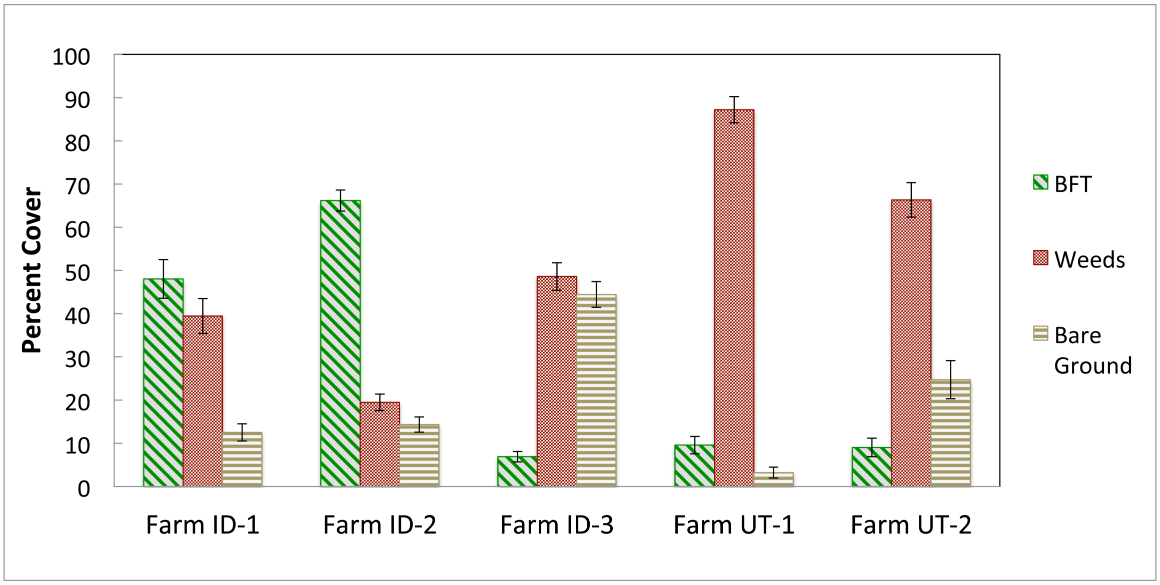

Fig. 4-4 Percent foliar cover of BFT, weeds and bare ground in spring 2012 (bars ± SEM) from subdivisions described in Fig. 3. In each sampled field subdivision (n), five 900-cm[2] cover estimates were taken at random locations using 5% increments for BFT and weed foliar cover and bare ground.

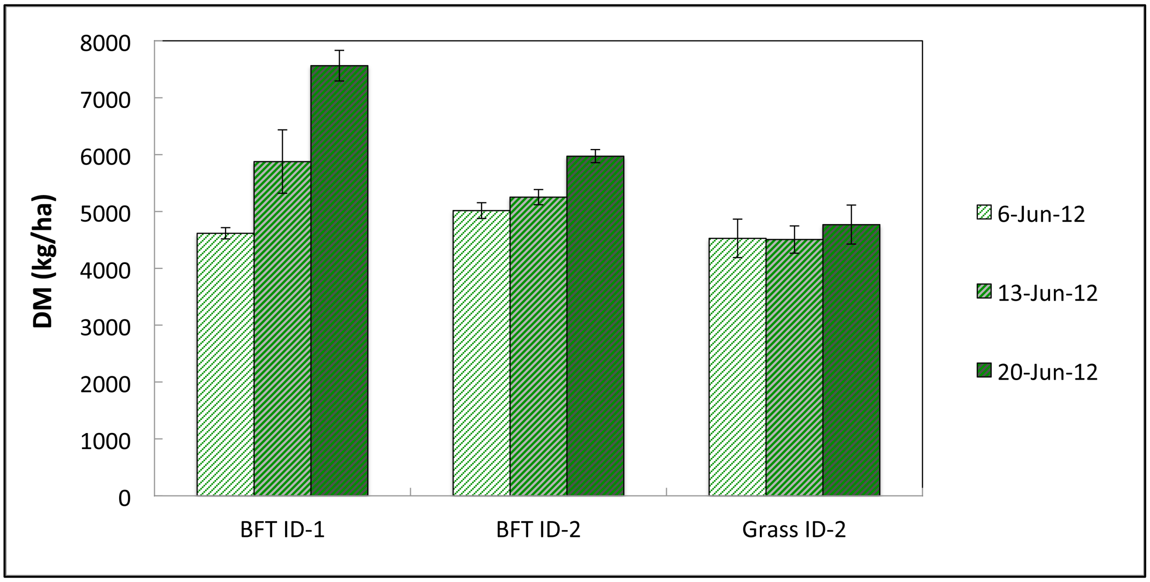

Fig. 4-5 Herbage dry matter accumulation of BFT pastures on the two farms with high BFT and low weed density and accumulation of grass on one of these farms evaluated at three dates prior to grazing in 2012.

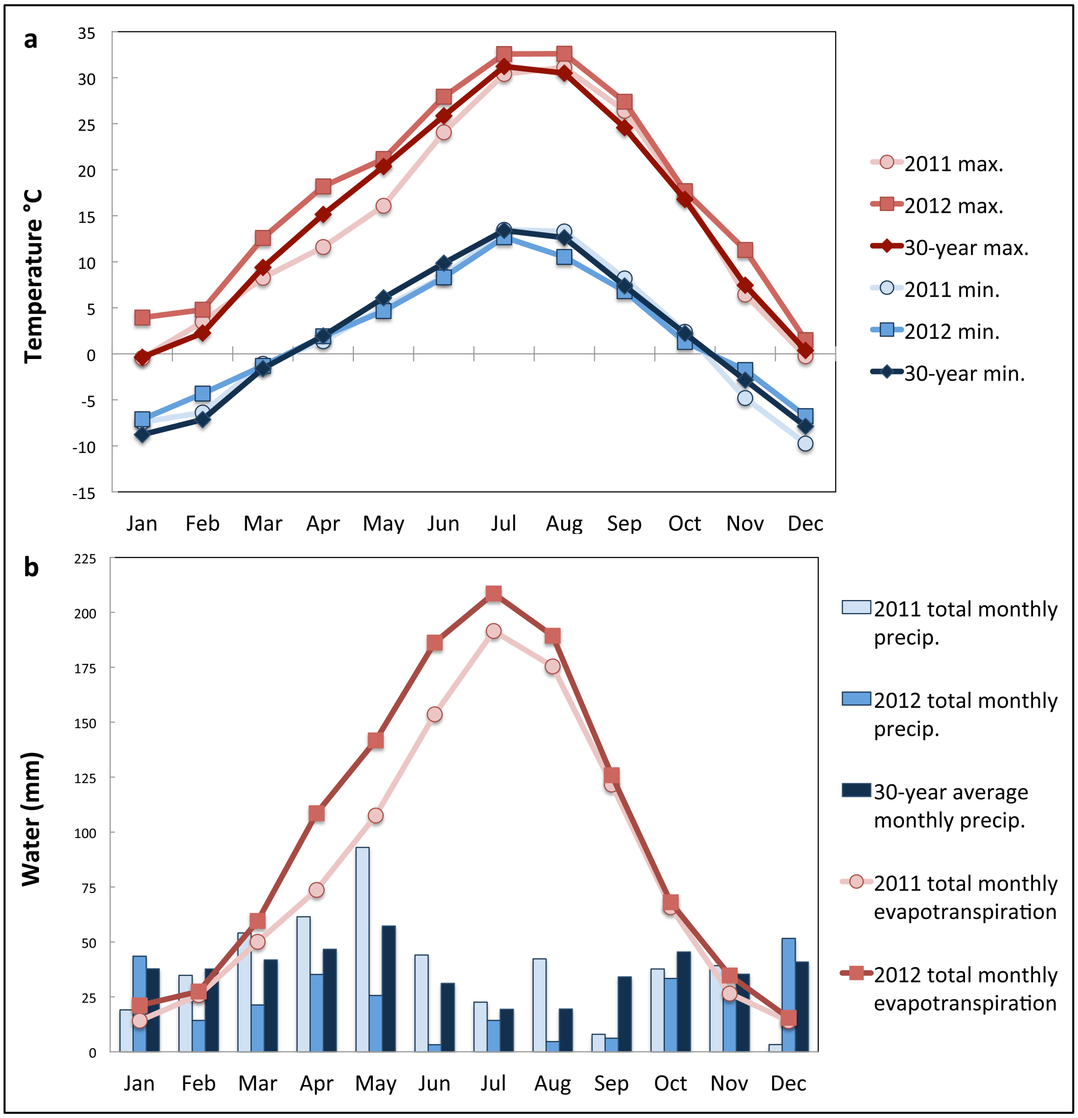

Fig. 4-6 Average monthly minimum and maximum temperatures for Cache Valley in 2011 and 2012 with 30-year average for comparison (a). Total monthly precipitation and evapotranspiration in 2011 and 2012, and 30-year average monthly precipitation for comparison (b). Calculated from data gathered by three weather stations (Preston, ID; Trenton, UT; and Cutler Dam UP&L, UT). Data provided by the Utah State University Climate Center, Climate Database Server and Western Regional Climate Center reports of National Climatic Data Center 1981-2010 monthly averages.

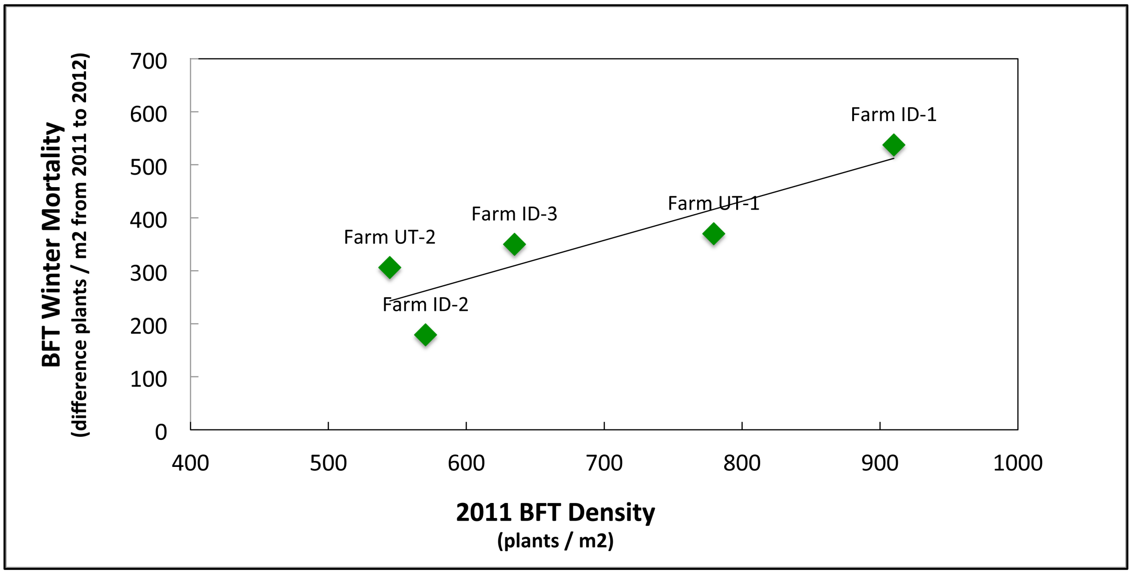

Fig. 4-7 Birdsfoot trefoil winter mortality calculated as the difference in BFT plant density between fall 2011 and spring 2012, and plotted as a function of fall 2011 BFT density. Winter mortality of BFT increased with fall 2011 BFT density (R^2 = 0.77; p = 0.05).

CHAPTER 5

SUMMARY AND CONCLUSIONS

Research presented here contributes to understanding of BFT cultivation and management for grazing-based dairy production. The histological analysis of lignification in BFT stem internodes supports recommendations to harvest BFT at approximately 6 weeks of regrowth, or early bloom. BFT stems showed similar patterns of lignification as alfalfa stems, but became more highly lignified. A higher ratio of lignified xylary ring to stem radius was observed in BFT than in alfalfa stems with more than 6 weeks of regrowth. Although other studies have found lignified tissues to be more digestible in BFT than in alfalfa (Mowat et al. 1969; Tomlin et al. 1965), excessively mature BFT stems may be difficult for grazing ruminants to harvest due to their high resistance to shear force (Douglas et al. 1999). Grazing or harvesting BFT at 6 weeks of regrowth gives time for sufficient DM production but prevents excessive lignification of BFT stems. Early flowering of BFT was also observed to occur at 6 weeks of regrowth and is an easily observable signal for harvest. Further research confirming the presence and exploring the role of gelatinous fibers in BFT could reconcile the seemingly contradictory results of high stem cell wall digestibility in BFT despite a higher proportion of lignified stem secondary xylem.

The recommendation to graze BFT at 6 weeks of regrowth is informative for rotational grazing systems in which grazing animals are limited to one section of pasture at a time and moved regularly. This style of management is used on many organic, grazing-based dairies and can be designed so that cows return to any given section of pasture every 6 weeks. More problematic for organic producers is the question of how to establish new BFT pastures successfully. BFT can be slow to establish, often competing poorly with weeds and producing low yields in the first 1 to 2 years. These are significant drawbacks for organic dairy producers, but the results of the second two studies presented here indicate that BFT can become well established and productive in the first season following an autumn seeding.

Measuring annual yields of BFT for three years after seeding and foliar cover in mature stands showed that seeding rate and use of a companion crop influence the success of establishment, but have greatest impact in the first year after seeding. First year yields increased linearly as seeding rate increased from 3 to 34 kg PLS ha^{-1}, but both high and low seeding rates resulted in mature stands with decreased BFT cover and increased weed cover. Organic producers can minimize yield losses and weed encroachment during the establishment of BFT by seeding at a rate between 14 and 18 kg PLS ha^{-1} in the autumn following harvest of another crop. Spring seeding is preferable if irrigation cannot be depended on to ensure adequate soil moisture levels in the fall. Use of a companion crop during establishment significantly decreased first year yields and did not lead to lower weed cover in mature stands, so is not recommended based on results of this study.

When organic on-farm establishment of BFT was evaluated, all farms had excellent germination and initial establishment in the fall, and on two out of five farms BFT stands were productive enough to support grazing the following summer. On the other three farms weeds dominated foliar cover in the summer following seeding, although density of BFT plants was still high. Planting dates that ranged over six weeks at the end of summer did not significantly affect establishment, but sprinkler irrigation positively influenced BFT cover. The farms that did not have sprinkler systems used flood irrigation, which is more difficult to control for maintaining surface soil moisture. In addition, the field on which crop rotation had occurred preceding planting had the highest BFT density and cover at the end of the seedling establishment period. In an on-farm setting, weed competition was the most significant barrier to BFT establishment. Selection for traits that could improve the competitiveness of BFT seedlings and hasten establishment, such as increased shoot growth rate, higher photosynthesis rate, or lower respiration rate would increase the value of BFT as a forage crop for organic grazing-based dairies.

Birdsfoot trefoil is a productive forage legume that provides high quality forage well suited to grazing-based dairy production. As discussed in the introduction, tannins produced by BFT are beneficial to ruminants, and a number of studies have shown BFT can increase milk production in dairy cattle (Woodward et al. 1999; 2000; 2004; Turner et al. 2005). Better understanding of BFT management and cultivation will facilitate more widespread use of this valuable forage legume to the benefit of organic grazing-based dairy production. The findings of research presented here inform BFT management by providing a physiological basis for the recommendation to harvest BFT after 6 weeks of regrowth, and inform BFT cultivation by demonstrating that with irrigation, autumn seeding of BFT can produce a stand ready to graze the following summer in climates like those of the Mountain West.

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APPENDIX

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