THESIS

ESTABLISHMENT AND MAINTENANCE OF LEGUMINOUS LIVING MULCHES FOR IRRIGATED SYSTEMS IN THE SEMI-ARID WEST

Submitted by

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ABSTRACT

ESTABLISHMENT AND MAINTENANCE OF LEGUMINOUS LIVING MULCHES FOR IRRIGATED SYSTEMS IN THE SEMI-ARID WEST

Management strategies for the establishment and maintenance of perennial leguminous living mulches were tested at irrigated sites in Colorado. Living mulches have been successfully integrated into corn (*Zea mays*) cropping systems in the upper Midwest of the United States. These studies focused on adapting the practice to irrigated environments in the semi-arid West through mulch and cash crop species selection and determination of appropriate suppression regimes. Different mulch/annual crop combinations were tested in both the establishment year and with previously established perennial legume stands. Spring herbicide regimes were tested on living mulches, and potential mulch species were screened for recovery from glyphosate [*N*-(phosphonomethyl)glycine] application.

The goal of the first study was to determine whether birdsfoot trefoil (*Lotus corniculatus*), white clover (*Trifolium repens*), and a mix of white clover, red clover (*Trifolium pratense*), and kura clover (*Trifolium ambiguum*) could be co-established with corn and oats (*Avena sativa*) for use as living mulches. Legumes were seeded with annual crops at two irrigated sites. Mulch crops did not have any effect on annual crop yield or quality. Yields of legumes established with corn averaged 276 kg ha^{-1} in spring of the following year while legumes established with oats cut at the boot and soft dough

stages yielded 951 and 611 kg ha^{-1}, respectively. Among legume treatments, the clover mix yielded the highest, averaging 869 kg ha^{-1} across annual crops followed by birdsfoot trefoil and white clover at 542 and 427 kg ha^{-1}, respectively.

The second study tested different living mulch and annual crops for performance and compatibility. Corn and soybeans (*Glycine max*) were planted into established legume stands. Fertility treatments of 0, 84, 168, and 225 kg ha^{-1} nitrogen (N) were also applied to corn without a living mulch and used to generate N response curves to quantify N inputs of living mulches, which received only 84 kg N ha^{-1}. Legume N contributions of living mulch treatments were 69, 46, 45, 32, and 23 kg ha^{-1} for alfalfa (*Medicago sativa*), white clover, birdsfoot trefoil, red clover, and a mix of birdsfoot trefoil/red clover, respectively, in corn silage. In corn grain, N contributions of legume treatments were 52, 43, 23, 20, and 18 kg ha^{-1} for white clover, alfalfa, birdsfoot trefoil/red clover mix, birdsfoot trefoil, and red clover, respectively. Soybean yields did not respond positively or negatively to the presence of living mulches. Birdsfoot trefoil had the greatest fall yield at 282 kg ha^{-1}.

The goal of the third study was to determine whether previously established birdsfoot trefoil, white clover, and a mix of white clover, red clover, and kura clover could be suppressed with paraquat (1,1'-dimethyl-4,4'-bipyridylium-dichloride) and glyphosate for use as living mulches in corn. Preplant treatments included: paraquat at 0.7 kg a.i. ha^{-1} and glyphosate at 1.0, 1.5, and 2.0 kg a.e. ha^{-1}. All of these were followed by a mid-season application of glyphosate at 1.0 kg a.e. ha^{-1}. Corn grain and legume yields were recorded in the fall. Legume by suppression treatment interactions occurred for both of these factors at the sprinkler irrigated site. At the furrow irrigated site, corn grain yields with birdsfoot trefoil averaged 11.2 Mg ha^{-1}, which was greater than with white clover and the clover mix that yielded 10.1 and 10.0 Mg ha^{-1}, respectively. Fall legume biomass yields of birdsfoot trefoil, white clover, and the clover mix were 11, 343, and 320 kg ha^{-1}, respectively. Suppression treatment did not have any effect on grain yield or legume biomass. Even modest recovery of the clovers during the growing season resulted in some corn yield reduction.

The fourth study evaluated persistence of legumes after glyphosate application by measuring biomass relative to an untreated control. Field tests included rates of 1.0, 1.5, 2.0, and 2.5 kg a.e. ha^{-1}, while trials of potted plants started in the greenhouse lacked the 2.5 kg a.e. ha^{-1} rate. White clover had the greatest recovery relative to the control in the field trial, with no glyphosate rate effect by sixteen weeks after application. Alfalfa and birdsfoot trefoil consistently recovered less than red and white clovers. In the potted plant trial, above ground biomass of kura clover, white clover, birdsfoot trefoil, red clover, and alfalfa averaged 19, 7, 5, 2, and 1% of the control, respectively across rates.

Preliminary results suggest that living mulch cropping systems may be a viable alternative under irrigation for producers in the western US. Mulch crops can be successfully co-established with corn or oats. White clover shows potential as a living mulch due to its positive effects on corn grain and silage yields when adequately suppressed. It also has high glyphosate tolerance. Leguminous living mulches can reduce nitrogen fertilizer needs, but adequately suppressing the mulch to minimize annual crop yield losses while maintaining the perennial legume stand remains a challenge.

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CHAPTER 1: CO-ESTABLISHMENT OF LEGUMINOUS LIVING MULCHES WITH ANNUAL CROPS

SUMMARY

Perennial legumes are excellent living mulches, but their establishment can be challenging. Given that producers may not be willing to take land out of annual crop production during the establishment year, co-establishment of the mulch with an annual crop could be a viable alternative. The goal of this study was to determine whether the perennial legumes birdsfoot trefoil (*Lotus corniculatus*), white clover (*Trifolium repens*), and a mix of white clover, red clover (*Trifolium pratense*), and kura clover (*Trifolium ambiguum*) could be co-established with corn (*Zea mays*) and oats (*Avena sativa*) for use as living mulches. Legumes were seeded with annual crops at two irrigated sites near Fort Collins, Colorado in 2009. Annual crop treatments included corn harvested for silage and oats harvested for hay at either the boot or soft dough stage. Silage and hay were analyzed for crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF). Perennial legumes were evaluated visually and by biomass sampling the following spring to determine success of establishment. Mulch crops did not have any effect on annual crop yield or quality when compared to a control with no living mulch. Early oats, late oats, and corn silage yielded 4.0, 6.3, and 17.6 Mg ha^{-1}, respectively. The following spring, legumes established under corn averaged 276 kg ha^{-1} while legumes established under oats cut at the boot and soft dough stages yielded 951 and 611 kg ha^{-1},

respectively. Among legume treatments, the clover mix yielded the highest, averaging 869 kg ha^{-1} across annual crops followed by birdsfoot trefoil and white clover at 542 and 427 kg ha^{-1}, respectively. Results indicate that these legumes can be successfully co-established with corn or oats without any adverse effects on the annual crop. Removal of the annual crop earlier in the growing season will result in superior establishment of the mulch.

INTRODUCTION

Increasing input costs along with environmental conservation issues have created the need for agricultural research in the area of low-input, sustainable cropping systems. Cover cropping, tillage reduction, and value-added crops have drawn a great deal of focus in addressing both environmental and economic concerns. One concept that embodies and expands upon such ideas is that of a living mulch.

Living mulches are cover crops grown in association with an annual cash crop (Paine and Harrison, 1993; SAN, 1998). These vegetative covers are unique in that they are not completely killed prior to planting of the annual crop like a traditional green manure or cover crop. Rather, growth is temporarily suppressed allowing eventual persistence and coexistence of the cover with the annual crop throughout the growing season and beyond (Echtenkamp and Moomaw, 1989; Singer and Pederson, 2005). These mulches can be annuals or perennials and can be interseeded with the cash crop or established before planting (Singer and Pederson, 2005). The use of a perennial cover offers many potential benefits including decreased wind and water erosion, increased water infiltration, weed suppression, reduced insect damage, and increased soil organic matter (Echtenkamp and Moomaw, 1989; White and Scott, 1991; Hartwig, 2004). Another advantage of living mulches is improved nutrient cycling. All covers provide some nitrogen retention by limiting nitrate leaching (Duiker and Hartwig 2004). However, leguminous living mulches offer the greatest fertility improvements through the biological fixation of atmospheric nitrogen.

Aside from their primary benefits as a mulch, legumes are highly palatable and increase the forage quality of grazed crop aftermath, such as corn stover, by

supplementing protein and energy (Zemenchik et al., 2000). This gives livestock producers the ability to substantially increase the feed value of forage they may already be grazing. In some cases, there is also potential for spring grazing or harvest before the cash crop is planted.

While living mulches have been tested extensively under rain-fed conditions (Eberlein et al., 1992, Affeldt et al., 2004; Duiker and Hartwig, 2004), there is a lack of published data on their use in semi-arid environments that require irrigation. Most of the research to date has come from the upper Midwest and Eastern United States. Soil types, climatic conditions, insects, weeds, and disease pressure in the semi-arid West differ from these humid regions. Thus, research on species selection and general management practices for living mulches must be conducted in the region if the system is to be adopted in the West.

One major obstacle to the adoption of living mulches by grain producers in the West is the economic cost of establishing the cover. Since land is a major input cost for most farmers, losing a year of production is often not a viable option. A potential solution to this problem is co-establishing the living mulch with an annual cash crop. The two crops can be planted at the same time, with the living mulch being allowed to persist for future use after harvest of the annual (Paine and Harrison, 1993). Of course, there are obvious drawbacks to this strategy. Competition with the annual crop will hinder establishment of the cover, particularly in the case of crops that form a thick canopy depriving the smaller cover crop of light. Another issue is the reduction in weed control options. Herbicide treatments may be severely limited when intercropping a grass with a legume. While established perennial legumes can be resilient to herbicide applications, they will be susceptible and easily killed during the establishment year. Thus, careful selection of both the cover crop species and the companion annual with which it is seeded are essential to the success of establishment. Accordingly, the objectives of this study were to:

1) Determine effects of interseeded legumes on yield and quality of corn silage and oat hay.
2) Evaluate relative success of establishment of various legumes seeded with different annual crops.

MATERIALS AND METHODS

Two fields were used for co-establishment studies in 2009. The study sites were located at the Colorado State University Agricultural Research, Development, and Education Center (ARDEC) about 6 km south of Wellington, CO (40^{o}39'N, 104^{o}59'W) at an elevation of 1554 m. Both were irrigated, one with a linear drive sprinkler system and the other by furrow irrigation from gated pipe. All subsequent references to the two sites will be made by the type of irrigation they received. The soil at the sprinkler irrigated site was a Fort Collins loam (fine loamy, mixed, mesic Aridic Haplustalf). Soil at the furrow irrigated site was classified as a Garrett loam (fine-loamy, mixed, superactive, mesic Pachic Argiustoll). ARDEC receives approximately 33 cm of precipitation annually. However, the study sites were irrigated on an as needed basis throughout the growing season. Average monthly temperatures range from 0^{o}C in January to 22^{o}C in July.

Field season: sprinkler irrigated site

The field was initially split into three sections to be used for three different annual crop treatments. These included corn, spring oats, and corn interseeded with spring oats. Each section was arranged in a randomized block design with four replications and included three legume treatments and a control with no living mulch (Table 1.1). Plot dimensions were 4.6 x 10.7 m. The field was clean tilled and fertilized with diammonium phosphate (DAP, 18-46-0) to achieve a rate of 90 kg ha^{-1} P_2O_5 in April 2009. 'Morton' spring oats were seeded on May 8, 2009 at 22.4 kg ha^{-1} using a no-till drill (Model 3P605NT, Great Plains Mfg., Inc., Salina, KS). Row spacing was set at 19 cm, and planting depth was 2.5 cm. Legumes were seeded the following day using

Table 1.1. Species, varieties, and seeding rates of legumes used in co-establishment studies at sprinkler and furrow irrigated sites in 2009.

Treatment	Scientific Name	Variety	Seeding Rate in Pure Live Seed (kg ha^{-1})
Treatment 1			
Birdsfoot trefoil	*Lotus corniculatus*	Leo	6.7
Treatment 2			
White clover	*Trifolium repens*	Kopu II	4.5
Treatment 3			
Kura clover	*Trifolium ambiguum*	Variety not stated	5.6
Red clover	*Trifolium pratense*	Starfire	3.4
White clover	*Trifolium repens*	Kopu II	2.2
Treatment 4			
Control	---	---	---

the same drill. Row spacing remained 19 cm, and seeding depth was adjusted to approximately 1 cm. Grand Valley Hybrids '22R77P' Roundup Ready hybrid silage corn was planted on May 15, 2009 using a 6-row John Deere corn planter (Maxemerge 7300, John Deere, Inc., Moline, IL). Row spacing was 76 cm, and planting depth was 3.8 cm. The planting population was 76,600 seeds ha^{-1}. Oats were seeded east to west, while legumes and corn were seeded north to south.

The study site was irrigated with a linear drive sprinkler system. Irrigation began on May 20, 2009 and continued throughout the growing season on a weekly basis. The quantity of water was adjusted to meet crop needs. Dates of irrigation events along with amounts applied are listed in Table 1.2.

The study site had several dense populations of Canada thistle (*Cirsium arvense*). These were hand clipped to ground level on June 16, 2009 to prevent their aggressive competition in the plot area. Their uneven distribution could have become a confounding

Table 1.2. Sprinkler irrigated site: irrigation timing and quantity in 2009.

Date	Irrigation (cm)
May 20	1.9
May 29	0.6
June 22	2.5
July 1	1.9
July 14	3.8
July 20	3.8
July 29	2.5
August 5	3.8
August 12	3.8
August 19	3.8
August 26	3.8
September 2	3.8
September 8	2.5
Total	38.5

factor, but the single clipping approximately one month after planting allowed the annual grasses to outcompete the thistle.

A hail storm on June 10, 2009 caused significant damage to the corn. Oats and legumes were also damaged to a lesser extent. The corn in the corn-only strip was able to recover, but the corn intercropped with oats was unable to compete with the oats and remained stunted. On June 29, 2009, the corn strip was side-dressed with 168 kg ha^{-1} nitrogen using 32-0-0. At that point, it was deemed unnecessary to side-dress the corn in the corn/oat strip due to its extremely poor performance. Instead, the strip was harvested for oat hay.

The corn/oat strip was harvested for hay at the boot stage of the oats on July 8, 2009. A self-propelled swather (Model 1469 Haybine, New Holland North America, Inc., New Holland, PA) set at a cutting height of 10 cm was used for harvest. A 2.8 m wide by 6.1 m length of windrow was collected onto a large tarp and weighed with a hanging scale to determine bulk yield. An 800 g subsample was taken and dried to determine percent moisture and adjust to dry matter (DM) yield. Another subsample of approximately 1000 g was taken to determine oat, weed, and corn biomass as percentages of total yield. The oat strip was harvested at the soft dough stage on July 27, 2009. The aforementioned harvest techniques were used.

The corn/oat strip was cut to a height of 10 cm again on August 18, 2009 to control oat regrowth and prevent seed dispersal. The strip was swathed and the residue removed. Hay yield was negligible and thus not recorded.

Biomass samples were collected from corn plots on September 27 and 28, 2009. A 76 x 76 cm frame was laid flat on the ground centered on a corn row, and all biomass within that frame was cut at a height of 10 cm. This sample was used to determine composition of yield in terms of corn, legume, and weeds. This technique was an alternative to the grab samples used for species composition analysis in the oat plots. It was chosen because the silage chopper would have shredded some plant material beyond recognition.

The corn strip was harvested on September 29, 2009 using a standard pull type two-row silage chopper (Model 717, New Holland North America, Inc., New Holland, PA). Chopped silage was blown into a silage truck with a weigh body. Weights were recorded for the middle two rows of each six-row corn plot. These weights were used to determine bulk yield. Subsamples were taken with a net by periodically putting it under the chute of the silage chopper as the plot was cut. A 600 g subsample was ensiled while another 750 g were dried and used to determine percent moisture in order to calculate yield on a DM basis.

Field season: furrow irrigated site

The field was leveled and set with irrigation furrows on 76 cm centers the previous year. This site had been furrow irrigated for numerous years prior to the current study. It was fertilized with diammonium phosphate (DAP) to achieve a rate of 90 kg ha^{-1} P_2O_5 on April 24, 2009. To control a large weed population of primarily Canada thistle, the field was mowed on May 1, 2009 and sprayed with glyphosate on May 7, 2009 and again on May 20, 2009. Furrows were cleaned immediately prior to planting.

Legume treatments and plot dimensions were identical to those described at the sprinkler irrigated site. Initially, three annual crop treatments: oats, corn, and a control with no annual crop were planted. However, herbicide carryover effects and non-uniform soil fertility led to extremely uneven legume establishment in the corn and legume-only blocks. Consequently, only the oat treatment could be used. The oat test was set up in a randomized block with four replications. Each block contained three plots of each legume treatment and only one control plot (no legume). This design was used in anticipation of a study to be conducted at the site the following year.

The legumes and oats were seeded on May 22, 2009 using a modified cone-seeder drill (Kincade Equipment Manufacturing, Haven KS). The planter was adjusted such that each bed had three rows of oats spaced at 16.5 cm between rows. ‘Morton’ spring oats were planted first at a rate of 22.4 kg ha^{-1}. Planting depth was 2.5 cm. Legumes were seeded using the same row spacing, but planting depth was adjusted to approximately 1 cm. The result was that oats and legumes were planted in the same row.

The site was furrow irrigated on an as needed basis. Dates and durations of irrigation events are listed in Table 1.3.

Table 1.3. Furrow irrigated site: irrigation timing and duration in 2009.

Date	Duration (hours)
May 29	6.0
July 14	10.0
July 27	5.5
August 12	5.0
August 24	5.0
September 4	4.5
September 28	4.0

Irrigation furrows were cleaned on July 1, 2009 using a three-row ditcher with shovels and trailing cans. This was done as a means of weed control and provided effective suppression of weeds in the furrows.

Oats were harvested for hay on July 24, 2009 at the soft dough stage. This was done using a Lacerator Green Chopper (Gruett’s, Potter, WI) with an attached weigh bin to determine bulk yield. Cutting height was set to 10 cm, and harvest data were obtained from the middle two beds of each 6-row plot. Two subsamples of approximately 550 g were taken from each plot by using a net to catch plant material as it was blown into the weigh bin. One was dried to determine percent moisture, which was used to calculate yield on a DM basis. The other was separated to determine percent weed, legume, and oat composition.

The study site was mowed twice after harvest (August 6, 2009 and September 14, 2009) to a height of 10 cm using a flail mower. This was done to control oat regrowth and prevent seed dispersal. Biomass in regrowth was minimal and was not removed from the field.

Sample Processing

Subsamples taken to determine percent moisture were weighed immediately after harvest in the field. They were placed in a forced air oven set to 55^{o}C for a minimum of 72 hours. After drying, samples were weighed again to calculate percent moisture, which was used to determine DM yield.

Oat composition samples were stored in a freezer until they could be separated. Weeds (and corn in the case of the oat/corn plots) were separated from oats, dried, and weighed to determine percent composition. There were no legumes found in the composition samples. Similarly, composition samples from the corn plots were separated in the field before being dried and weighed.

Corn samples were ensiled by putting 600 g into a 3.8 L plastic bag (FoodSaver Freezer Bags, Sunbeam Products, Inc., Boca Raton, FL). Air was evacuated from bags, and bags were then heat sealed using a kitchen-grade vacuum sealer kit (Vac Sealer V2220B, Sunbeam Products, Inc., Boca Raton, FL). Sample bags were stored in black plastic trash bags at room temperature (21^{o}C) for 90 days before being opened and dried. This procedure was proposed, tested, and verified by Cherney et al. (2004).

Dried samples from all three annual crop treatments (ensiled in the case of the corn) were ground using a Wiley mill (Wiley Model 4, Arthur H. Thomas Co., Philadelphia, PA) with a 2 mm screen and then a Cyclone mill (Cyclotec Model 1093, Foss Corp., Eden Prairie, MN) with a 2 mm screen. The second grinding was done to homogenize the particle size of the sample. No corn was included in the ground samples of early-cut oat hay.

Quality Analysis

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and total nitrogen content were determined for all oat hay and corn silage samples taken at time of harvest. NDF/ADF fiber analyses were performed according to the methods described by Van Soest et al. (1991) using the ANKOM filter bag technique. This method involved putting ground samples in filter bags which were sealed and digested in an ANKOM fiber analyzer (Model 200, ANKOM Technology, Macedon, NY). All samples were run in duplicate and re-run if the coefficient of variation between samples was greater than 5%.

Nitrogen concentration was determined via the Dumas combustion method (Etheridge et al. 1998) using a LECO carbon and nitrogen analyzer (Model CN 2000, Leco Corp., St. Joseph, MI). The percent nitrogen concentration was multiplied by 6.25 to estimate crude protein (CP).

Establishment Evaluation

Biomass samples were taken at the sprinkler irrigated site on May 3, 2010 to quantify success of legume establishment and overall stand health. This was done by randomly placing a 0.25 m^2 frame at two locations in the plot and clipping all plant biomass to ground level. Samples were separated between desired legume specie(s) and weeds, dried for 72 hours in a forced air oven at 55^oC, and weighed. Dry weights were used to calculate species abundance on a per hectare basis. Plots were also visually evaluated on a scale of 0 to 5 on the same day. The rating system is described in Table 1.4. It should be noted that this evaluation was not an estimation of legume biomass, but of percent plot area in which the legume was successfully established. Due to significant

Table 1.4. Rating scale for visual evaluation of legume establishment at sprinkler and furrow irrigated sites in spring 2010.

Rating	Description
0	no legume
1	very sparse, small plants
2	plants are either sparse or small
3	>50% establishment
4	>75% establishment
5	100% establishment

loss of clover to vole damage, the percent of the total plot area with vole damage was also recorded.

Biomass samples and visual evaluations were obtained at the furrow irrigated site on May 9, 2010 using the same methods described above for the sprinkler irrigated site. However, only one sample was taken from each plot due to the larger number of identical treatments compared to the sprinkler irrigated site.

Statistical analysis: sprinkler irrigated site

PROC MIXED in SAS (SAS Institute, 2009) was used to determine the effect of legume treatment on annual crop yield, percent weed biomass, NDF, ADF, and CP. Annual crop yield and quality factors were compared within each annual crop, but not between crops. These data were analyzed as a randomized block design with replication as the random effect and legume treatment as the fixed effect. Differences were recognized as significant at the $P \leq 0.05$ level. If legume treatment effect was found to be significant, treatment means were separated using LSMEANS (SAS Institute, 2009).

Establishment ratings, spring legume biomass, and spring weed biomass were compared within and between annual crops. These data were analyzed as a split-plot design where the whole plot was the annual crop, and the split plot was the legume treatment. The random effect was replication within annual crop. PROC MIXED was used to determine the main effects and interactions of legume treatment and annual crop. In the event of a significant effect, LSMEANS was used to separate means.

Transformations were performed when an examination of the residuals indicated the need. Contribution of weeds to total yield for both oat hay cuttings, spring 2010 legume and weed biomass, and vole damage ratings were square root transformed to homogenize variance. In these cases, the original data were reported, and the transformation was used to determine differences between treatments.

Statistical analysis: furrow irrigated site

Oat hay yield and percent weed composition in 2009 as well as establishment ratings, weed biomass, and legume biomass in 2010 were analyzed as a randomized block with subplots. Each legume treatment had three subplots, and the conventional (control) treatment had only one. Replication and replication by treatment were random effects, and treatment was the fixed effect. For NDF, ADF, and CP of oat hay, one plot of each treatment was randomly selected from each replication. These data were analyzed as a randomized block where replication was the random effect and legume treatment was the fixed effect.

All data were analyzed using PROC MIXED. If legume treatment effect was significant at the $P \leq 0.05$ level, treatment means were separated using LSMEANS. No transformations were required.

RESULTS AND DISCUSSION

Sprinkler irrigated site

Annual crop yield and quality

Legume treatment did not have an effect on the yield or quality of annual crops. This is not surprising as legumes were not well established at the time of harvest due to intense competition for light, nutrients, and water from the faster-growing annual grasses. In the case of the oats, both early and late cuttings, the legumes did not contribute to crop yield (Tables 1.5 and 1.6). While there were legumes present in both treatments at harvest, they did not have enough growth to be harvested at the 10 cm cutting height. In the case of the corn silage, biomass samples taken prior to harvest indicated that birdsfoot trefoil and the clover mix would contribute to biomass harvested at a 10 cm cutting height (Table 1.7). These legume treatments accounted for 0.8 and 0.6% of total silage dry matter, respectively. The two treatments were not significantly different, but were both found to be greater than the white clover treatment. Still, such a small contribution would not be expected to affect the feed value of the silage.

Table 1.5. Sprinkler irrigated site: effect of living mulches on yield and quality of early-cut oat hay (harvested at boot stage) in 2009.

Legume treatment	Dry matter yield ($Mg\ ha^{-1}$)	Avg. contribution of weeds to total yield (%)	Avg. contribution of legume(s) to total yield (%)	NDF (%)	ADF (%)	CP (%)
Birdsfoot trefoil	3.9 a §	0.0 a	0.0 a	60.5 a	35.6 a	11.5 a
Clover mix	4.0 a	4.2 b	0.0 a	61.0 a	35.6 a	11.6 a
White clover	4.1 a	0.0 a	0.0 a	55.5 a	35.3 a	11.1 a
Conventional (no legume)	4.1 a	0.1 a	n/a	54.7 a	35.2 a	12.0 a

§ Within columns, means followed by the same lowercase letter are not different at the 0.05 probability level.

Table 1.6. Sprinkler irrigated site: effect of living mulches on yield and quality of late-cut oat hay (harvested at soft dough stage) in 2009.

Legume treatment	Dry matter yield (Mg ha^{-1})	Avg. contribution of weeds to total yield (%)	Avg. contribution of legume(s) to total yield (%)	NDF (%)	ADF (%)	CP (%)
Birdsfoot trefoil	6.5 a §	0.6 a	0.0 a	61.6 a	38.5 a	7.4 a
Clover mix	5.9 a	4.4 a	0.0 a	59.3 a	37.2 a	7.1 a
White clover	6.6 a	0.2 a	0.0 a	61.5 a	37.4 a	6.7 a
Conventional (no legume)	6.0 a	2.0 a	n/a	60.7 a	37.1 a	7.5 a

§ Within columns, means followed by the same lowercase letter are not different at the 0.05 probability level.

Table 1.7. Sprinkler irrigated site: effect of living mulches on yield and quality of corn silage in 2009.

Legume treatment	Dry matter yield (Mg ha^{-1})	Avg. contribution of weeds to total yield (%)	Avg. contribution of legume(s) to total yield (%)	NDF (%)	ADF (%)	CP (%)
Birdsfoot trefoil	17.7 a §	6.7 a	0.8 a	42.2 a	24.4 a	6.6 a
Clover mix	17.6 a	5.4 a	0.6 a	42.8 a	25.1 a	6.8 a
White clover	17.9 a	10.5 a	0.0 b	42.0 a	23.8 a	6.6 a
Conventional (no legume)	17.3 a	7.4 a	n/a	41.4 a	25.0 a	6.7 a

§ Within columns, means followed by the same lowercase letter are not different at the 0.05 probability level.

While the soil nitrogen contribution of legumes was not quantified directly, there was no yield response or change in nitrogen concentration of plant tissue to suggest significant additions. Several reasons behind this can be attributed to the symbiotic relationship between the legume host and the *Rhizobium* bacteria that performs biological nitrogen fixation (BNF). The nature of this relationship dictates that the bacteria depend on its plant host for a continuous flow of carbohydrates. In alfalfa (*Medicago sativa*), the cost is 5.1 to 8.1 g C per g N fixed when the cost of all factors such as nodule growth are included (Twary and Heichel, 1991). While there is some variation between species and growth stages in carbon cost per unit N fixed, this illustrates the high energy cost of this process to the legume. As a result, the host plant will very quickly reduce or stop the outflow of carbohydrates when subjected to environmental stress.

In the case of this study, the main stress factor was reduced light due to shading by the annual crops. The rate of BNF in legumes is photosensitive and can be reduced after only a few days under low light (Tricot et al., 1990). Legumes established under oats were subjected to this type of stress very early in their establishment. When oats were harvested, the legumes were etiolated with very elongated stems. Such stress indicates a lack of excess carbohydrates to allocate for BNF.

Legumes established under corn did not experience this type of stress until later in the growing season. However, they likely suffered from reduced rates of BNF that occur after flowering. BNF reaches its maximum rate during the early reproductive stage of growth and then sharply declines as carbohydrate sink competition occurs (Marschner, 1995). In a pasture, the legumes are mowed or grazed at this stage, and once the reproductive sink is removed, the plant reallocates carbohydrates to the *Rhizobium*. However, since the living mulch was established under corn, it is likely that BNF was reduced after flowering. By this point, light competition had also become a factor. Another influence reducing the BNF potential of legumes seeded under corn was the application of 168 kg ha^{-1} N in the form of a urea ammonium nitrate (UAN) solution. This was done to ensure that N was not limiting to the corn, but elevated soil N levels will decrease rates of BNF as legumes allocate fewer carbohydrates to the process when N is not limiting (Marschner, 1995).

Finally, any N that was fixed by the legumes was not likely to be available to the annual crops during the growing season. While some of the fixed N can remain in the soil as root excretions (Havlin et al., 2005), residues, and nodules (Marschner, 1995), 75-80% of the plant's total N is generally found in the top growth (Jennings, 2010). This material must senesce and decompose before that N is converted to a plant available form through mineralization. Thus, significant N credits to the associated annual crop in the establishment year are not to be expected.

It should be noted that the primary goal in co-establishment of a living mulch with an annual crop is not to reap the full, long-term benefits of the mulch. Rather, it is to successfully establish the perennial without negatively affecting the annual cash crop. In this case, none of the legume treatments reduced productivity of the oats or corn when compared to the conventional treatment with no living mulch. This indicates that the only costs associated with establishing the perennial cover are those of seed and planting. That being the case, there is very little risk to the producer involved in establishing a living mulch.

The one notable challenge found in this study was that of weed control. Intercropping a grass with a broadleaf, especially in the establishment year, makes weed control difficult. This did not prove to be a major issue in the oat plots, which quickly formed a dense canopy and outcompeted the weeds. However, in the case of the early cut oats, the clover mix did have more weeds as a percent of total harvested biomass (Table1.5), which is surprising as good legume establishment should suppress weeds. Conversely, the corn was plagued with high weed populations that contributed significantly to total biomass at harvest time (Table 1.7). However, weed pressure did not differ between mulch treatments and the control.

This does raise an issue in the management of our control or "conventional" corn treatment in that a producer would likely spray when weed populations grew as high as they were in this study. We did not spray so as not to introduce another variable that would keep us from quantifying any weed suppression by the legume treatments. One recommendation from the 2009 results of this study is that a site with relatively low weed pressure should be chosen for establishment of the mulch. If such a field is not available, the use of an annual that provides rapid cover and vigorous growth is advisable.

Establishment of living mulches

There was an annual crop effect on the success of legume establishment. While there was no difference between the oat cuttings, they both outperformed the corn in the visual evaluations (Table 1.8) and the amount of legume biomass (Table 1.9). This is due to a much longer period of competition-free growth the previous year. However, there was one exception in the case of the visual ratings in which the clover mix ranked the same in the corn and late-cut oats. The clover mix established better than the white clover and birdsfoot trefoil under the corn. However, the trefoil established better than both the clovers under the late-cut oats. This can be attributed to damage that voles caused to the clover in the oat plots.

Vole damage was much greater in the oat plots compared to the corn silage (Table 1.10). This damage occurred between the fall of 2009 and early spring of 2010. Early and late-cut oats were harvested on July 8 and July 27, 2009, respectively, allowing ample time for competition-free clover growth after harvest. Conversely, corn was harvested on September 29, 2009 leaving very little time for clover regrowth before winter. The resulting superior clover growth after harvest in the oat plots likely provided an excellent source of food and shelter for the voles.

Table 1.8. Sprinkler irrigated site: establishment ratings of living mulches in spring 2010.

	Early-cut oats	Late-cut oats	Corn Silage	Avg.
	------------------------Rating--------------------			
Birdsfoot trefoil	3.9 aA §	4.0 aA	2.1 bB	3.3
Clover mix	4.0 aA	3.3 bB	3.3 aB	3.5
White clover	3.8 aA	3.9 abA	2.4 bB	3.3
Avg.	3.9	3.7	2.6	

* Establishment based on a scale of 0 = no legumes to 5 = 100% establishment.
§The interaction of legume treatment by annual crop was significant. Legume treatments followed by the same lowercase letter are not different. Annual crop treatments followed by the same uppercase letter are not different. Differences were declared significant at the 0.05 probability level.

Table 1.9. Sprinkler irrigated site: legume biomass of living mulches in spring 2010.

	Early-cut oats	Late-cut oats	Corn Silage	Avg.
	---------------DM yield (kg ha^{-1})---------------			
Birdsfoot trefoil	937 §	544	146	542 b †
Clover mix	1306	779	523	869 a
White clover	610	509	161	427 b
Avg.	951 A ‡	611 A	276 B	

§The interaction of legume treatment by annual crop was not significant at the 0.05 probability level.
†Legume treatment means followed by the same lowercase letter are not different at the 0.05 probability level.
‡ Annual crop means followed by the same uppercase letter are not different at the 0.05 probability level.

Table 1.10. Sprinkler irrigated site: vole damage to living mulches in spring 2010.

	Early-cut oats	Late-cut oats	Corn Silage	Avg.
	------Plot area damaged by voles (%)-------			
Birdsfoot trefoil	4 §	3	0	2 b †
Clover mix	16	29	4	16 a
White clover	13	11	0	8 b
Avg.	11 A ‡	14 A	1 B	

§The interaction of legume treatment by annual crop was not significant at the 0.05 probability level.
†Legume treatment means followed by the same lowercase letter are not different at the 0.05 probability level.
‡ Annual crop averages followed by the same uppercase letter are not different at the 0.05 probability level.

Legumes are known to be a preferred food source for voles (Thompson, 1965). Meadow voles (*Microtus pennsylvanicus*) in an enclosed environment favored legumes from among 30 potential food sources. Of these, white clover and red clover (both of which are in the clover mix) were ranked first and third, respectively. Aside from a food source, the cover offered by live clover in the fall and spring, as well as a thick mat of residue in the winter, would create ideal rodent habitat. Previous research has shown that cover crops are excellent rodent habitats that can increase pest pressure (Sullivan et al., 2001). Winman et al. (2009) found that living mulches containing legumes attracted larger vole populations than those without. The clover mix had the most biomass in the fall and consequently left more residue behind.

Thus, vole damage is the most likely cause of this annual crop by legume treatment interaction. Additionally, clover mix establishment in the oat strips tended to be very strong in areas not affected by voles. It is possible that a late fall harvest or grazing of the clover could have made for less enticing rodent habitat.

Legume biomass was highest under the oats, despite the vole damage, with no differences between cutting dates (Table 1.9). The clover mix produced the greatest biomass when averaged across annual crops, due in large part to the red clover with its quick establishment and high spring biomass production.

Spring weed biomass was highest in the early-cut oats (Table 1.11). This was somewhat surprising as legumes established well in this strip, and legume biomass was relatively high. One possible reason is the variable weed pressure in different areas of the field. The dominant weed was Canada thistle, which was very thick in some areas. No differences were found between legume species.

Table 1.11. Sprinkler irrigated site: weed biomass of living mulches in spring 2010.

	Early-cut oats	Late-cut oats	Corn Silage	Avg.
	-------------------DM yield (kg ha^{-1})-----------------			
Birdsfoot trefoil	327 §	46	100	158 a †
Clover mix	179	89	17	95 a
White clover	442	37	78	186 a
Conventional (no legume)	123	1	121	82 a
Avg.	267 A‡	43 B	79 B	

§The interaction of legume treatment by annual crop was not significant at the 0.05 probability level.
†Legume treatment means followed by the same lowercase letter are not different at the 0.05 probability level.
‡ Annual crop means followed by the same uppercase letter are not different at the 0.05 probability level.

Furrow irrigated site

Annual crop yield and quality

As was the case at the sprinkler irrigated site, legume treatment did not have any effect on the yield or quality of oat hay (Table 1.12). Similarly, legumes were still too small to contribute to crop yield at the 10 cm cutting height. The contribution of weeds to total yield on a percent basis did not differ between treatments suggesting that no weed suppression was provided by the mulch crop prior to harvest.

Table 1.12. Furrow irrigated site: effect of living mulches on yield and quality of oat hay (harvested at soft dough stage) in 2009.

Legume treatment	Dry matter yield (Mg ha^{-1})	Avg. contribution of weeds to total yield (%)	Avg. contribution of legume(s) to total yield (%)	NDF (%)	ADF (%)	CP (%)
Birdsfoot trefoil	3.6 a §	6.3 a	0.0 a	55.2 a	34.9 a	16.2 a
Clover mix	3.6 a	7.5 a	0.0 a	57.5 a	34.4 a	15.6 a
White clover	3.5 a	8.1 a	0.0 a	57.2 a	33.9 a	16.3 a
Conventional (no legume)	3.6 a	7.1 a	n/a	60.6 a	34.6 a	15.6 a

§ Within columns, means followed by the same lowercase letter are not different at the 0.05 probability level.

Establishment of living mulches

The clover mix was rated higher than white clover in the visual evaluations indicating superior establishment (Table 1.13). The trefoil did not differ from either the clover mix or the white clover. This is consistent with results for the late-cut oats at the sprinkler irrigated site, which were also harvested at the soft dough stage. Neither legume nor weed biomass differed among legume species. In the sprinkler irrigated field, the clover mix yielded the highest spring biomass across all annual crops. One possible reason for this difference in relative biomass production is the sampling date. Plots at the furrow irrigated site were sampled six days later than those at the sprinkler irrigated site at a time when the legumes were growing rapidly. It is conceivable that the white clover could have grown enough during that time to reduce the differences in biomass among species. Vole damage at the furrow irrigated site was negligible and only occurred in small sections of two of the 40 plots.

Table 1.13. Furrow irrigated site: establishment ratings, legume biomass, and weed biomass of living mulches in spring 2010.

	Establishment Rating *	Weed Biomass (kg ha^{-1})	Legume Biomass (kg ha^{-1})
Birdsfoot trefoil	4.6 ab §	14 a	1314 a
Clover mix	4.9 a	26 a	1223 a
White clover	4.2 b	5 a	954 a
Conventional (no legume)	n/a	20 a	n/a

* Establishment based on a scale of 0 = no legumes to 5 = 100% establishment.
§ Within columns, means followed by the same lowercase letter are not different at the 0.05 probability level.

CONCLUSION

Co-establishment of perennial leguminous living mulches with corn or oats can eliminate the cost of production loss in the establishment year. This would limit establishment costs to legume seed and planting operations. No yield or quality effects of living mulches were found on either annual crop, and all living mulches were successfully established. Legume establishment was superior with oats due to the earlier harvest of oat hay compared to corn silage. However, these results were obtained in the complete absence of chemical weed control resulting in high weed populations across conventional and mulch treatments in corn. Weed pressure should be taken into consideration when choosing a site for mulch establishment as weed control options are limited in a living mulch cropping system, particularly in the establishment year.

LITERATURE CITED

Affeldt, R.P., K.A. Albrecht, C.M. Boerboom, and E.J. Bures. 2004. Integrating herbicide-resistant corn technology in a kura clover living mulch system. Agron. J. 96:247-251.

Cherney, D.J.R., J.H. Cherney, and W.J. Cox. 2004. Fermentation characteristics of corn forage ensiled in mini-silos. J. Dairy Sci. 87: 4238-4246.

Duiker, S.W. and N.L. Hartwig. 2004. Living mulches of legumes in imidazolinone-resistant corn. Agron. J. 96:1-21-1028.

Eberlein, C.V., C.C. Sheaffer, and V.F. Oliveira. 1992. Corn growth and yield in an alfalfa living mulch system. J. Prod. Agric. 5:332-339.

Echtenkamp, G.W. and R.S. Moomaw. 1989. No-till corn production in a living mulch system. Weed Technology. 3:261-266.

Etheridge, R.D., G.M. Pesti, and E.H. Foster. 1998. A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco CNS 2000) on sample typical of an animal nutrition analytical laboratory. Animal Feed Science and Technology 73:21-28.

Hartwig, N.L. 2004. Soil and production enhancing cover crop and living mulch systems. Pennsylvania State University. Ext., State College, PA.

Havlin, J.L., S.L. Tisdale, J.D. Beaton, and W.L. Nelson. 2005. Soil fertility and fertilizers: an introduction to nutrient management. 7th ed. Pearson Education Inc., Upper Saddle River, NJ.

Jennings, J. 2010. Value of nitrogen fixation from clovers and other legumes. Univ. of Arkansas. Ext., Fayetteville, AR. FSA 2160.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Elsevier, San Diego, CA.

Paine, L.K., and Harrison. 1993. The historical roots of living mulches and related practices. Hort.Technology 3:137-143.

SAS Institute. 2009. SAS version 9.2. SAS Inst., Cary, NC.

Singer, J.W. and P. Pedersen. 2005. Legume living mulches in corn and soybean. PM-2006. Iowa State Univ. Ext., Ames, IA.

Sullivan, T.P., D.S. Sullivan, and E.J.Hogue. 2001. Reinvasion dynamics of northern pocket gopher (*Thomomys talpoides*) populations in removal areas. Crop Prot. 20:189–198.

Sustainable Agriculture Network. 1998. Managing cover crops profitably. San handbook. Ser. Book 3. SAN, Beltsville, Maryland.

Thompson, D.Q. 1965. Food preferences of the meadow vole (*Microtus pennsylvanicus*) in relation to habitat affinities. Amer. Midl. Nat. 74:76–85.

Tricot, F., Y. Crozat, F. Tardieu and M. Sebillotte. 1990. Establishment and distribution of pea primary root nodules (*Pisum sativum* L.) as affected by shading. Symbiosis 9:97-103.

Twary, S.N. and G.H. Heichel.1991. Carbon costs of dinitrogen fixation associated with dry matter accumulation in alfalfa. Crop Sci. 31: 985-992.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Symposium: Carbohydrate methodology, metabolism, and nutritional implication in dairy cattle. J. Dairy Sci. 74: 3583-3597.

White, J.G. and T.W. Scott. 1991. Effects of perennial forage-legume living mulches on no-till winter wheat and rye. Field Crops Res., 28:135-148.

Winman, M.R., E.M. Kirby, D.M. Granatstein and TP Sullivan. 2009. Cover crops influence meadow vole presence in organic orchards. Hort. Technology 19:55-62.

Zemenchik, R.A., K.A. Albrecht, C.M. Boerboom, and J.G. Lauer. 2000. Corn production with kura clover as a living mulch. Agron. J. 92:698-705.

CHAPTER 2: CORN AND SOYBEAN PRODUCTION IN A LEGUMINOUS LIVING MULCH SYSTEM

SUMMARY

Leguminous living mulches have been successfully integrated into corn cropping systems in the upper Midwest of the United States. This study evaluated the feasibility of adapting the practice to irrigated cropping systems in the semi-arid West. Specifically, different living mulch and annual crops were tested for performance and compatibility at Fruita, CO in 2009. Corn for grain, corn for silage, and soybeans were planted into stands of alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), white clover (*Trifolium repens*), birdsfoot trefoil (*Lotus corniculatus*), and a mix of red clover and birdsfoot trefoil that had been established for two years. Fertility treatments of 0, 84, 168, and 252 kg ha^{-1} nitrogen (N) without a living mulch were also applied to the corn crops. These were used to generate N response curves and quantify N inputs of living mulches. All living mulch plots received 84 kg ha^{-1} N. Legume N contributions of living mulch treatments were 69, 46, 45, 32, and 23 kg ha^{-1} for alfalfa, white clover, birdsfoot trefoil, red clover, and a mix of birdsfoot trefoil/red clover, respectively, in corn silage. In corn grain, N contributions of legume treatments were 52, 43, 23, 20, and 18 kg ha^{-1} for white clover, alfalfa, birdsfoot trefoil/red clover mix, birdsfoot trefoil, and red clover, respectively. Corn silage yields in legume plots ranged from 17.2 to 20.4 Mg ha^{-1} while grain yields ranged from 9.7 to 12.0 Mg ha^{-1} in legume plots. Soybean yields did not

respond positively or negatively to the presence of living mulches (average 3545 kg ha^{-1}). Corn stalk residue, which is commonly grazed, was similar among legume treatments in the corn grain plots, while birdsfoot trefoil had the greatest fall yield at 282 kg ha^{-1}. Living mulch cropping systems may be a viable alternative under irrigation for producers in the western US.

INTRODUCTION

Increasing input costs along with environmental conservation issues have created the need for agricultural research in the area of low-input, sustainable cropping systems. Cover cropping, tillage reduction, and value-added crops have drawn a great deal of focus in addressing both environmental and economic concerns. One concept that embodies and expands upon such ideas is that of a living mulch.

Living mulches are cover crops grown in association with an annual cash crop (Paine and Harrison, 1993; SAN, 1998; Hartwig and Ammon, 2002). These vegetative covers are unique in that they are not completely killed prior to planting of the annual crop like a traditional green manure or cover crop. Rather, growth is temporarily suppressed allowing eventual persistence and coexistence of the cover with the annual crop throughout the growing season and beyond (Echtenkamp and Moomaw, 1989; Singer and Pederson, 2005). These mulches can be annuals or perennials and can be interseeded with the cash crop or established before planting (Singer and Pederson, 2005). The use of a perennial cover offers many potential benefits including decreased wind and water erosion, increased water infiltration, weed suppression, reduced insect damage, and increased soil organic matter (Echtenkamp and Moomaw, 1989; White and Scott, 1991; Hartwig, 2004). Another advantage of living mulches is improved nutrient cycling. All covers provide some nitrogen retention by limiting nitrate leaching (Duiker and Hartwig, 2004). However, leguminous living mulches offer the greatest potential for fertility improvement through the biological fixation of atmospheric nitrogen (N).

Aside from their primary benefits as a mulch, legumes are highly palatable and increase the forage quality of grazed crop aftermath, such as corn stover, by

supplementing protein and energy (Zemenchik et al., 2000). This gives livestock producers the ability to substantially increase the feed value of crops they may already be grazing. In some cases, there is also potential for spring grazing or mechanical harvest before the cash crop is planted.

Several recent field studies on the use of leguminous living mulches for corn production in the upper Midwest of the US have yielded positive results showing significant nitrogen additions and subsequent corn yield responses. Albrecht et al. (2009) found that N additions beyond 22 kg ha^{-1} did not induce a yield response in corn grown with a kura clover (*Trifolium ambiguum*) living mulch. This would suggest that the majority of the N requirement was met by the clover. Studies by both Zemenchik et al. (2000) and Affeldt et al. (2004) indicated that corn planted into an established kura clover living mulch required little to no addition of N fertilizer and experienced no yield reduction.

Conversely, Sawyer et al. (2010) did not report any significant reduction in the N requirement of corn planted into kura clover in northeast Iowa. A lack of yield response to N fertilization was only found when corn growth was already unacceptably limited by competition with the living mulch. Duiker and Hartwig (2004) also found that N fertilizer could not be reduced in the presence of leguminous living mulches without decreasing corn yield. In this case, N contribution by the legumes was only observed at severely deficient levels when corn yield was already depressed by N deficiency. Their trials in southeastern Pennsylvania included crown vetch (*Coronilla varia*), flat pea (*Lathyrus sylvestris*), birdsfoot trefoil (*Lotus corniculatus*), hairy vetch (*Vicia villosa*), and galega (*Galaega officinalis*).

Though less research has been conducted on the potential of living mulches for soybean production, initial results indicate that seed yields are reduced in a living mulch system. Pederson et al. (2009) found that yields in living mulch systems under three different suppression regimes were lower than those in a clean-till system in which the kura clover cover crop was completely killed prior to planting of the annual.

These variable results highlight the need for additional research to determine optimal management practices for living mulches. Practices will vary based on the production goals of individual growers and the environments in which they are operating.

While living mulches have been tested extensively under rain-fed conditions (Eberlein et al., 1992, Affeldt et al., 2004; Duiker and Hartwig, 2004; Pederson et al., 2009), there is a lack of published data on their use in semi-arid environments that require irrigation. Most of the research to date has been in the upper Midwest and Eastern United States. Soil types, climatic conditions, insects, weeds, and disease pressure in the semi-arid West differ from these humid regions. Thus, research on species selection and general management practices for living mulches must be conducted in this region if the system is to be adopted by producers in the West. The objectives of this study were to:

1) Compare corn grain, corn silage, and soybean yields when seeded into different living mulches.
2) Determine yield effects of leguminous mulch treatments relative to various nitrogen fertilization rates (corn) and conventional management with no nitrogen additions (soybeans).
3) Evaluate the contribution of legumes to the forage value of grazed crop aftermath (corn harvested for grain).

MATERIALS AND METHODS

Field testing of established living mulches was conducted at the Colorado State University Western Colorado Research Center (WCRC) at Fruita, Colorado (39°10'N, 108°42'W). The WCRC is at an elevation of 1,375 m and gets approximately 21 cm of precipitation annually. The soil was classified as a Billings silty clay loam (fine-silty, mixed (calcareous), mesic Typic Torrifluvent). The field was irrigated with furrows set on 76 cm centers. Water was applied throughout the growing season to meet annual crop needs.

Experimental Design

The study site had small plots of well-established legumes that were planted in the spring of 2007. The field was composed of three annual crop strips, each of which was arranged in a randomized complete block with four replications. Strips were seeded with corn for grain, corn for silage, and soybeans in the spring of 2009. Treatments within each strip included five different living mulches composed of varying legume species/combinations and four plots without legumes that were used for fertility treatments. Legume treatments were 'Focus' alfalfa (*Medicago sativa*), 'Starfire' red clover (*Trifolium pratense*), 'Kopu II' white clover (*Trifolium repens*), 'Norcen' birdsfoot trefoil (*Lotus corniculatus*), and a mix of red clover and birdsfoot trefoil (Table 2.1). Fertility treatments included 0, 84, 168, and 252 kg N ha^{-1}, which was applied midseason. Variable N rates were not applied to the soybeans, since they do not commonly receive midseason nitrogen fertilization. Instead, this strip had four control plots with no mulch in each replication. Plot dimensions were 4.6 x 15.2 m.

Table 2.1. Living mulch/fertility treatments tested at Fruita, CO in 2009.

Treatment	Species/Fertility *
Living Mulch 1	Alfalfa
Living Mulch 2	Birdsfoot trefoil
Living Mulch 3	Birdsfoot trefoil + Red clover
Living Mulch 4	Red clover
Living Mulch 5	White clover
Fertility 1	0 kg N ha^{-1}
Fertility 2	84 kg N ha^{-1}
Fertility 3	168 kg N ha^{-1}
Fertility 4	252 kg N ha^{-1}

* Fertility treatments were not applied to soybeans.

Spring Sampling

All plots were evaluated on February 27, 2009 to ensure that legume stands were adequate and uniform across species. Plots were rated on a scale of 1 to 5 according to Table 2.2. Soil samples were taken from two of the three strips (soybean and corn for grain) on April 8, 2009 at depths of 0 to 15 cm, 15 to 53 cm, and 53 to 91 cm. This was done using a truck-mounted Giddings soil probe (Giddings Equipment Company, Inc., Windsor, CO). Cores were 75 mm in diameter. Legume aerial phytomass samples were obtained from the soybean strip on April 27, 2009 by randomly placing a circular hoop with a diameter of 67.3 cm into the center of each plot and clipping all plant material in that area to ground level. These samples were dried, weighed, and used to calculate above ground biomass on a per hectare basis.

Table 2.2. Rating scale for visual evaluation of legume stands at Fruita, CO in spring 2009.

Rating	Description
1	Poor
2	Good
3	Very good
4	Excellent
5	Superior

Growing Season

Pre-plant fertilization consisted of a broadcast application of mono-ammonium phosphate (MAP, 11-52-0) to achieve 116.5 kg P_2O_5 ha^{-1}. This was applied on April 14, 2009. The fertility plots, those without a living mulch, were sprayed with glyphosate [*N*-(phosphonomethyl)glycine] at a rate of 3.8 kg a.e. ha^{-1} on April 19, 2009 to ensure that they were free of any legumes. This was done using a CO_2 pressurized backpack sprayer. The following day, glyphosate was broadcast applied across all plots at a rate of 1.3 kg a.e. ha^{-1} using a tractor-mounted boom sprayer. This reduced rate of glyphosate was applied as a means of living mulch suppression prior to planting of annual crops. The furrows were cleaned the next day to ensure adequate flow of irrigation water. On May 4, 2009, the field was strip-tilled using an Orthman 1tRIPr (Orthman Manufacturing Inc., Lexington, NE) two-row model. Tilled swaths were 25 cm wide on 76 cm centers, leaving approximately 50 cm untilled between strips. Upon visual evaluation, it was decided that tillage was inadequate for planting of annual crops, particularly in the white clover plots which had formed a thick, stoloniferous mat. As a result, all plots were strip-tilled a second time the following day, with white clover plots being tilled a third time.

Surprisingly, the white and red clover appeared relatively unaffected by the earlier glyphosate application, while the alfalfa and birdsfoot trefoil had no visible green tissue. For this reason, a broadcast application of paraquat (1,1'-dimethyl-4,4'-bipyridylium-dichloride) at a rate of 0.8 kg a.i. ha^{-1} was made on May 5, 2009. This was intended to ensure adequate suppression of mulches and allow time for early growth of annual crops.

Varieties and planting dates of annual crops are listed in Table 2.3. Corn and soybeans were planted at 5 and 4 cm depths, respectively, using a Buffalo no-till planter (Model 7010-6-30, Fleischer Manufacturing, Inc., Columbus, NE).

Table 2.3. Varieties, planting dates, and seeding rates of annual crops tested at Fruita, CO in 2009.

Crop	Variety	Planting Date	Seeding rate (seeds ha^{-1})
Corn for Grain	Grand Valley Hybrids GVH 22R77P	May 7	88,200
Corn for Silage	Grand Valley Hybrids GVH 23T53P	May 7	88,200
Soybeans	Northrup King S-28-B4 02RM018047	May 29	379,000

Nitrogen fertility treatments were side-dressed when the corn was at the V6 to V7 growth stage. This was done by dribbling a urea ammonium nitrate (UAN, 32-0-0) solution on either side of each corn row through a rolling fluted coulter equipped with a fertilizer drop tube. All living mulch plots were fertilized at a rate of 84 kg N ha^{-1} while fertility plots received their designated rates (Table 2.1).

A midseason herbicide application was deemed necessary in the soybean plots due to competition from the living mulches. Glyphosate (0.6 kg a.e. ha^{-1}) mixed with Activator 90 surfactant (0.3% v/v) and UAN (0.6% v/v) was broadcast applied with a boom sprayer on July 14, 2009. No midseason herbicide applications were made on the corn grain or silage plots.

Harvest

Corn silage was harvested on September 9, 2009 with a standard pull-type, two-row silage chopper (Heston model 2000-150**,** AGCO, Duluth, GA). Chopped silage was blown into a silage truck with a weigh body. Weights were recorded for the middle two rows of each six-row corn plot. These weights were used to determine bulk yield. Samples were taken with a net by periodically putting it under the chute of the silage chopper as the plot was cut. A 600 g subsample was ensiled and later used to determine percent moisture to calculate yield on a dry matter (DM) basis.

Soybean plots were harvested on October 17, 2009 using a Hege plot combine. Weights were taken from the center two rows of each plot, and subsamples were used to determine moisture. All yields were adjusted to a seed moisture content of 120 g kg^{-1}.

Corn was harvested for grain on October 23, 2009 with a modified Gleaner plot combine. Plot yields for the center two rows were recorded and adjusted to a moisture content of 155 g kg^{-1}.

Corn residue and legume biomass were collected from corn grain plots on November 10, 2009. This was done by laying a 76 x 76 cm frame flat on the ground at random locations in the center each plot. The frame was centered on a bed so that it covered the area from one furrow to the next. All corn residue and legume biomass within this area was collected by cutting at the ground level. Material was cut at the edge of the frame using hand clippers to ensure that only residue within the frame was taken. Corn residue was later separated into leaf, stem, and cob components, dried, and weighed. Legume residue was also dried and weighed.

Sample Processing/Analysis

<u>Soil Samples</u>

Soil samples were air dried and ground in a soil grinder (Custom Laboratory Equipment Inc., Orange City, FL) before being analyzed for ammonium (NH_4^+), nitrate (NO_3^-), and soil organic matter content (SOM). NO_3^- and NH_4^+concentrations were determined using a 2M potassium chloride (KCl) extract (Keeney and Nelson, 1982). The method was modified such that 25 mL of potassium chloride and 5.0 g of soil were used, and NO_3^- was determined by reduction to nitrite (NO_2^-) using cadmium (Cd) reduction followed by a flow injection analyzer (FIA) measurement. NH_4^+ concentration was determined by FIA using the automated phenate method (Sparks, 1996). Percent organic matter was determined using the Walkley-Black method (Walkley and Black, 1934) as modified by Sparks (1996).

Plant Samples

Spring legume biomass samples were dried to a constant weight in a forced air oven at 55^{o}C for a minimum of 72 hours. Dry weights were used to calculate potential spring legume yield on a DM basis.

Corn silage samples were ensiled by putting 600 g of wet material into a 3.8 L plastic bag (FoodSaver Freezer bags, Sunbeam Products, Inc., Boca Raton, FL). Air was evacuated from bags, and bags were heat sealed using a kitchen-grade vacuum sealer kit (Vac Sealer V2220B, Sunbeam Products, Inc., Boca Raton, FL). Sample bags were stored in black plastic trash bags at room temperature (21^{o}C) for 90 days before being opened and dried. This procedure for ensiling small samples was proposed, tested, and verified by Cherney et al. (2004).

After removal from the sealed bags, silage samples were immediately dried in a forced air oven at 55^{o}C for a minimum of 72 hours. Samples were weighed to calculate percent moisture, which was used to determine DM yield.

Dried legume and corn silage samples were first ground through a Wiley mill (Wiley Model 4, Arthur H. Thomas Co., Philadelphia, PA) with a 2 mm screen and subsequently through a Cyclone mill (Cyclotec Model 1093, Foss Corp., Eden Prairie, MN) with a 2 mm screen. The second grinding was done to homogenize particle size of the sample.

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and total nitrogen content were determined for all legume and corn silage samples.

NDF/ADF fiber analyses were performed according to the methods described by Van Soest et al. (1991) using the ANKOM filter bag technique. This method involved putting ground samples in filter bags which were sealed and digested in an ANKOM fiber analyzer (Model 200, ANKOM Technology, Macedon, NY). All silage and legume samples were run in duplicate and re-run if the coefficient of variation between samples was greater than 5%.

Nitrogen concentration was determined via the Dumas combustion method (Etheridge et al., 1998) using a LECO carbon and nitrogen analyzer (Model CN 2000, Leco Corp., St. Joseph, MI). The percent nitrogen concentration was multiplied by 6.25 to estimate crude protein (CP).

Corn Grain/Soybean Samples

Moistures for corn grain and soybean samples were determined using a DICKEY-john seed analyzer (Model GAC2100B, Dickey-john, Inc., Springfield, IL). These were used to calculate yields by adjusting to the appropriate moisture content.

Legume Mortality Ratings

Due to an observed lack of recovery by many of the legumes, all plots were evaluated on April 27, 2010 to determine stand loss. Plots were rated on a scale of 0 to 5 with 0 indicating no remaining legumes and 5 being a full, healthy stand (Table 2.4).

2.4. Rating scale for visual evaluation of legume mortality at Fruita, CO in spring 2010.

Rating	Description
0	No remaining legumes
1	Very few stunted plants
2	Very little ground cover, but some healthy plants
3	Approximately 50% ground cover
4	Approximately 75% ground cover
5	Full ground cover

Statistical Analysis

Statistical analyses were performed using PROC MIXED in SAS (SAS Institute, 2009). Legume establishment and mortality ratings were compared within and among annual crops. These data were analyzed as a split-plot design where the whole plot was the annual crop, and the split plot was the legume treatment. The random effect was replication within annual crop. PROC MIXED was used to determine main effects and interactions of legume treatment and annual crop. All yield, quality, and residue data were analyzed as a randomized block design with replication as the random effect and legume/fertility treatment as the fixed effect. These comparisons were made within a given annual crop, not among crops. Soils data were also analyzed using PROC MIXED where replication, replication by annual crop, and replication by annual crop by legume/fertility treatment were random effects. Legume/fertility treatment, strip, and depth were fixed effects, and depth was a repeated measure. Differences were recognized as significant at the $P \leq 0.05$ level. When aforementioned effects were found to be significant, treatment means were separated using LSMEANS (SAS Institute, 2009).

Either a log or square root transformation was performed when an examination of the residuals indicated the need. Corn grain yields were log transformed while fall legume biomass and corn leaf residue were square root transformed. In these cases, the original data were reported, while the transformations were used to determine differences among treatments.

Nitrogen fertilizer equivalencies for legume treatments were determined by means of an inverse prediction based on crop yield response to nitrogen fertility treatments. This response was based on a linear regression generated in Microsoft Excel (Microsoft, 2007). Accordingly, confidence intervals for these estimates were calculated as described by Neter et al. (1990).

RESULTS AND DISCUSSION

Spring Soil Samples

A treatment effect was observed for NH_4^+ levels in the soil (Table 2.5). Of the legume treatments, red clover had a higher concentration than birdsfoot trefoil or the birdsfoot trefoil+red clover mix. NH_4^+ levels in alfalfa and white clover plots were not significantly different from those of the other treatments. It should be noted that while averages are reported for the fertility treatments, no variable rates of N fertilizer had been applied at the time of spring soil sampling. Thus, NH_4^+ differences in these treatments must be attributed to biological nitrogen fixation (BNF) by legumes established on these plots in previous years.

Table 2.5. Effect of living mulches on ammonium (NH_4^+), Nitrate (NO_3^-), and soil organic matter (SOM) levels from 0 to 91 cm at Fruita, CO in spring 2009.

Treatment *	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	SOM (%)
Alfalfa	3.57 ab §	1.85 a	2.34 a
Birdsfoot trefoil	3.32 b	2.10 a	2.38 a
Birdsfoot trefoil + Red clover	3.18 b	2.16 a	2.46 a
Red clover	3.96 a	1.95 a	2.54 a
White clover	3.60 ab	1.60 a	2.72 a
0 kg N ha^{-1}	3.43 b	2.29 a	2.48 a
84 kg N ha^{-1}	3.32 b	1.73 a	2.39 a
168 kg N ha^{-1}	3.52 ab	2.19 a	2.60 a
252 kg N ha^{-1}	3.87 a	2.19 a	2.45 a

§ Within columns, means followed by the same lowercase letter are not different at the 0.05 probability level.
* Variable nitrogen rates had not been applied to fertility treatments at time of soil sampling.

While there was no annual crop by treatment interaction, the soybean strip did have higher NH_4^+ levels than the corn grain strip. Averaged across annual crops, NH_4^+ concentrations were 4.01 and 3.05 mg kg^{-1} for the soybean and corn grain strips, respectively. Again, since the annual crops had not been planted at this point, differences must be attributed to some other unknown factor. No treatment effects were observed on NO_3^- or SOM levels.

For NH_4^+, NO_3^-, and SOM, there was both a depth effect and an annual crop by depth interaction. The general trend was a decrease in all three properties as depth increased with two exceptions. While NH_4^+ levels declined with increasing depth in the soybean strip, it was highest at the deepest depth in the corn grain strip (Figure 2.1). This can likely be attributed to a decreased rate of nitrification at the 53-91 cm depth rather than increased deposition of NH_4^+. Nitrification is carried out by aerobic bacteria which would be less populous and less active at lower depths where oxygen is limiting. The observed differences across annual crop strips could be due to the presence of shale bedrock that is approximately 1 m below the soil surface, but likely has some variability in depth across the field. Since the field is furrow irrigated, it is possible that soils in areas with shallow bedrock could remain saturated and almost devoid of oxygen for prolonged periods of time.

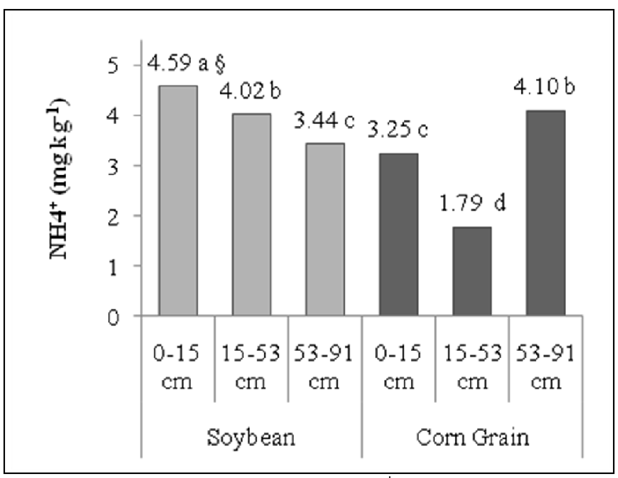

Figure 2.1. Soil ammonium (NH_4^+) concentrations at 0-15, 15-53, and 53-91 cm sampling depths in strips to be planted in corn for grain and soybeans at Fruita, CO in spring 2009.
§Means followed by the same lowercase letter are not different at the 0.05 probability level.

NO_3^- levels in both annual crop strips declined with depth, but the rate of that decline differed between strips (Figure 2.2). This could also be related to variable nitrification rates as a result of oxygen availability.

SOM declined with depth in the corn grain strip (Figure 2.3). However, the 15-53 cm depth had the highest level of organic matter in the soybean strip. This is surprising as minimal tillage was used for the previous two years. Based on knowledge of past management and available data, such an increase cannot be explained.

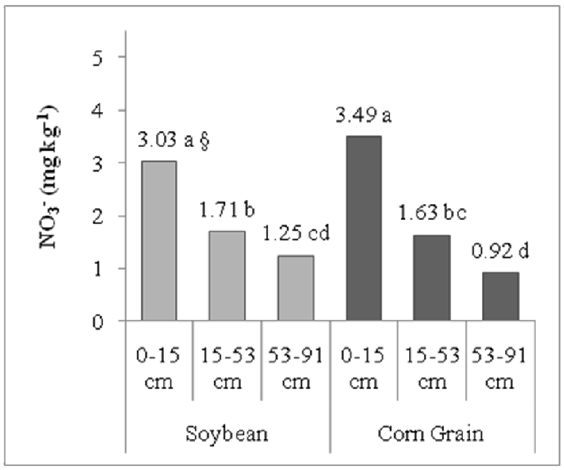

Figure 2.2. Soil nitrate (NO_3^-) concentrations at 0-15 , 15-53, and 53-91 cm sampling depths in strips to be planted in corn for grain and soybeans at Fruita, CO in spring 2009. §Means followed by the same lowercase letter are not different at the 0.05 probability level.

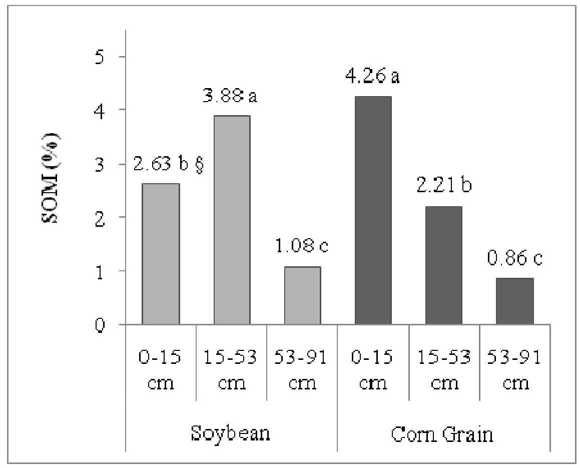

Figure 2.3. Soil organic matter (SOM) concentrations at 0-15, 15-53, and 53-91 cm sampling depths in strips to be planted in corn for grain and soybeans at Fruita, CO in spring 2009. §Means followed by the same lowercase letter are not different at the 0.05 probability level.

Based on visual evaluations, there were no differences in legume stand ratings across annual crop strips. White clover stands consistently received the highest possible rating due to their complete ground cover. This species formed a very thick, stoloniferous mat that proved difficult to cut through with the strip-tiller. These types of thick sod mats are desirable for erosion control and buildup of SOM. Alfalfa followed by red clover received the second and third highest ratings while treatments that included birdsfoot trefoil received the lowest ratings.

Table 2.6. Stand ratings for living mulches at Fruita, CO in spring 2009.

Treatment	Corn Silage	Soybean	Corn Grain	Avg.
	------------------------Rating*---------------------			
Alfalfa	4.1 §	4.0	4.0	4.0 b †
Birdsfoot trefoil	2.6	3.0	3.0	2.9 d
Birdsfoot trefoil + Red clover	2.5	2.9	3.1	2.8 d
Red clover	3.1	3.3	3.9	3.4 c
White clover	5.0	5.0	5.0	5.0 a
Avg.	3.5 A ‡	3.6 A	3.8 A	

* Ratings based on a scale of 1 = poor to 5 = superior.
§The interaction of legume treatment by annual crop was not significant at the 0.05 probability level.
†Legume treatment means followed by the same lowercase letter are not different at the 0.05 probability level.
‡ Annual crop means followed by the same uppercase letter are not different at the 0.05 probability level.

Spring Legumes

The clovers and alfalfa had the greatest biomass production with trefoil treatments the least (Table 2.7). Singer et al. (2009) also noted poor persistence of birdsfoot trefoil after the second year when used as a living mulch. There were no differences in ADF values among species, and only trefoil had significantly higher NDF than white clover, red clover, and the red clover/birdsfoot trefoil mix. CP was highest for alfalfa and lowest for birdsfoot trefoil with no differences among the other treatments.

Table 2.7. Yield and quality of living mulches at Fruita, CO in spring 2009.

Treatment	Dry matter yield (kg ha^{-1})	NDF (%)	ADF (%)	CP (%)
Alfalfa	1452 ab §	42.3 ab	32.4 a	23.1 a
Birdsfoot trefoil	1170 bc	46.4 b	36.9 a	15.3 c
Birdsfoot trefoil + red clover	916 c	40.6 a	32.7 a	19.5 b
Red clover	1584 a	40.1 a	31.9 a	20.0 b
White clover	1730 a	37.2 a	29.5 a	18.7 b

§ Within columns, means followed by the same lowercase letter are not different at the 0.05 probability level.

Yield Data

Soybean

No significant differences in soybean yields were observed as a result of legume treatment (Figure 2.4). This was not surprising as soybeans are also legumes and are thus capable of meeting most of their own nitrogen needs. While deficiencies can occur if the proper *Rhizobium* bacteria is not present or some environmental factor limits its activity (Jones, 2003), no deficiency symptoms were observed in the plot area and would not have been expected. Because nitrogen was not a yield-limiting factor, any nitrogen contributed by the legumes would not have significantly impacted soybean yield.

There was, however, the potential for reduced yields due to competition with the perennial. This risk is particularly great in soybeans which accumulate biomass slowly prior to flowering (Pederson and Lauer, 2004). Delayed biomass production along with a later planting date results in much later canopy formation compared to corn. This allowed the mulch more time to recover without competition from the annual. That is why a light application of glyphosate was made midseason. It seemed to adequately suppress the mulches as those treatments did not differ in yield from the conventional

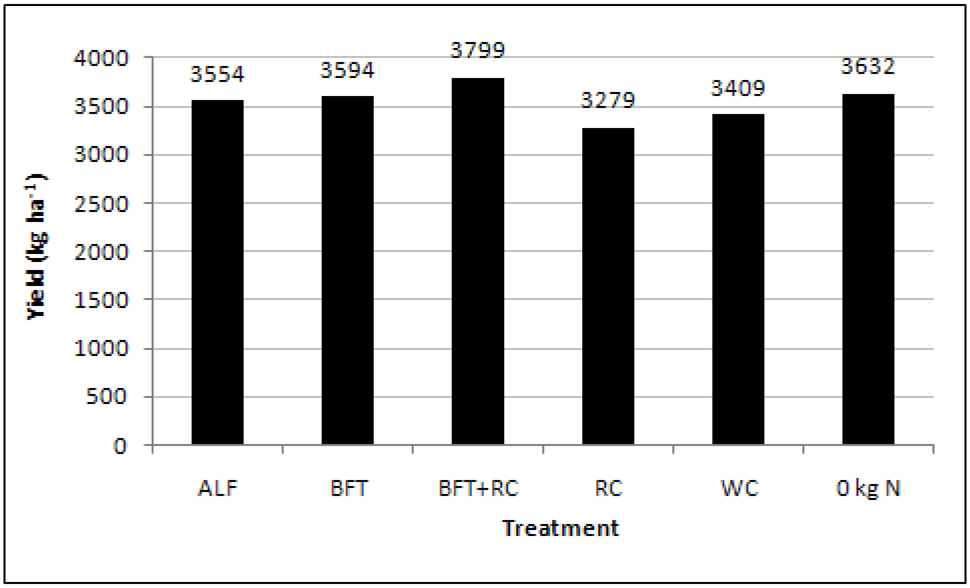

Figure 2.4. Effect of living mulch/fertility treatments on soybean yields at Fruita, CO in 2009.

treatment with no living mulch. This finding differed from Pederson et al. (2009) who saw no effect of number of glyphosate treatments on soybean yield. In their case, glyphosate applications of 0.75 kg a.e. ha^{-1} were made at planting and 1 week later; at planting, 1 week later, and 4 weeks later; and at planting, 1 week later, 4 weeks later, and 6 weeks later. None of these treatments performed as well as when the mulch was completely killed with a combination of tillage and glyphosate. Two key differences between Pederson et al. (2009) and the current one were that they used a different mulch crop, kura clover, which is known to be resilient to glyphosate application (Zemenchik et al., 2000), and less aggressive initial suppression.

The midseason glyphosate application was made 6 weeks after planting, which was determined based on observation of competition between the soybeans and mulch crops. This situation highlights the advantage, if not the necessity, of employing an herbicide-resistant annual cash crop so as to retain a post-emergence suppression option.

The advantage of using an herbicide resistant annual crop has been highlighted numerous times in the literature (Affeldt et al., 2004; Duiker and Hartwig, 2004).

Corn Silage

In the silage trial, the 252 kg ha^{-1} N rate yielded the highest followed by the 168 kg ha^{-1} N rate, though this treatment was not different from the yield of the alfalfa treatment (Table 2.8). All of the legume treatments, with the exception of the birdsfoot trefoil-red clover mix, yielded higher than the 84 kg ha^{-1} N treatment, indicating that there was at least some yield increase that resulted from nitrogen inputs by the living mulches. The alfalfa, birdsfoot trefoil, and white clover treatments, which did not differ from one another in yield, appear to be promising living mulches in terms of their positive effects on corn silage yield.

Table 2.8. Effect of living mulch/fertility treatments on yield and quality of corn silage at Fruita, CO in 2009.

Treatment	Dry matter yield (Mg ha^{-1})	NDF (%)	ADF (%)	CP (%)
Alfalfa	20.4 bc §	43.8 bc	26.2 abc	5.3 bc
Birdsfoot trefoil	18.7 cd	44.9 cd	26.9 bcd	4.8 c
Birdsfoot trefoil + red clover	17.2 de	47.1 ef	28.2 cde	4.6 c
Red clover	17.9 d	46.5 def	28.1 cde	4.8 c
White clover	18.8 cd	46.0 de	28.0 bcde	5.3 bc
0 kg ha^{-1} N	13.1 f	48.5 f	29.4 e	4.6 c
84 kg ha^{-1} N	15.6 e	48.3 f	28.7 de	4.6 c
168 kg ha^{-1} N	21.5 ab	42.7 ab	26.0 ab	5.6 b
252 kg ha^{-1} N	22.9 a	40.9 a	24.6 a	6.5 a

§ Within columns, means followed by the same lowercase letter are not different at the 0.05 probability level.

Treatments containing red clover may have been at a disadvantage in that this was the third year of perennial legume production. While red clover is botanically classified as a perennial, it is known to behave like a biennial (Taylor and Quesenberry, 1996). It is generally accepted that red clover will only be highly productive for two and sometimes three years (Frame et al., 1998). Recommendations for red clover in a mixed pasture involve interseeding every two to three years to maintain the population (Bran et al., 2000). Due to competition and physiological stress from chemical and cultural suppression, similar guidelines would likely need to be followed in a living mulch system. This is further supported when examining legume mortality in the spring of 2010.

Silage quality factors were influenced by legume and fertility treatments (Table 2.8). Crude protein was highest in the 252 kg ha^{-1} N treatment and fell as N fertility rate was reduced. The positive correlation between N fertility and CP is well established (Cox and Cherney, 2001; Sheaffer et al., 2006) and would suggest higher levels of plant available N in the alfalfa and white clover treatments. The silage produced in these two treatments had higher levels of CP than the other living mulches and also tended to yield high.

There were fewer differences in fiber concentration among treatments. The lowest fiber contents were found at the two highest N rates, while ADF of the silage produced in the alfalfa treatment did not differ from the 168 kg ha^{-1} rate. The NDF value of the alfalfa treatment was greater than all the other mulches except birdsfoot trefoil. None of the living mulch treatments differed from one another in ADF. These limited differences in fiber contents among mulch treatments are not surprising given that differences in nitrogen fertility have not been as clearly correlated to fiber content as CP. Thus, while N does play some role in carbohydrate partitioning within the plant (Sheaffer et al., 2006), it has a much greater impact on protein levels, of which nitrogen is a structural constituent (Jones, 2003).

Corn Grain

Among the living mulch treatments, alfalfa and white clover had the highest grain yields (Table 2.9). However, all living mulch treatment yields exceeded that of the conventional treatment with the same N fertilization rate showing the net positive effect of the living mulches. Total corn residue did not differ among mulch treatments. Only the alfalfa and birdsfoot trefoil treatments did not exceed the 84 kg ha^{-1} N rate in terms of total residue. No differences were observed in production of individual residue components among mulch treatments.

Table 2.9. Effect of living mulch/fertility treatments on corn grain yield and crop residue at Fruita, CO in fall 2009.

		Residue			
Treatment	Grain yield	Leaf	Stem	Cob	Total
	------------------------------Mg ha^{-1}------------------------------				
Alfalfa	11.4 b §	3.2 bc	5.0 ab	0.5 a	8.7 bc
Birdsfoot trefoil	9.8 c	3.2 bc	3.9 bc	1.3 a	8.4 bc
Birdsfoot trefoil + red clover	10.0 c	3.3 abc	4.6 ab	1.0 a	9.0 b
Red clover	9.7 c	3.3 abc	4.8 ab	0.8 a	8.9 b
White clover	12.0 b	2.9 c	4.6 ab	1.3 a	8.8 b
0 kg ha^{-1} N	6.4 e	2.1 d	3.0 c	0.4 a	5.5 d
84 kg ha^{-1} N	8.5 d	2.6 cd	3.3 c	0.6 a	6.6 cd
168 kg ha^{-1} N	14.2 a	3.9 ab	5.2 a	1.2 a	10.3 ab
252 kg ha^{-1} N	15.1 a	4.1 a	5.3 a	2.0 a	11.4 a

§ Within columns, means followed by the same lowercase letter are not different at the 0.05 probability level.

Birdsfoot trefoil biomass was significantly higher than all other legumes in the fall (Table 2.10). However, biomass yield of all legume species was low. Even the trefoil accounted for only 5% of the biomass yield recorded in the spring prior to suppression. This is far below the desired level of production one would hope to see by the end of the growing season. Zemenchik et al. (2000) observed an increase in growth of kura clover under corn during the ear-fill period. This eventually resulted in 60% ground cover in the winter. To achieve such a level of recovery and subsequent production with the legumes used in this study, a less aggressive suppression regime would have to be adopted.

Table 2.10. Legume biomass after corn grain harvest at Fruita, CO in fall 2009.

Treatment	Dry matter yield (kg ha^{-1})
Alfalfa	24 b §
Birdsfoot trefoil	293 a
Birdsfoot trefoil + red clover	28 b
Red clover	0 c
White clover	50 b

§ Means followed by the same lowercase letter are not different at the 0.05 probability level.

Nitrogen Equivalencies

Corn silage and grain yields relative to nitrogen fertility treatments were plotted to generate nitrogen response curves (Figures 2.5 and 2.6). In both cases, the relationship between N-rate and yield produced a classic nitrogen response curve with the greatest slope occurring between the middle two nitrogen fertility rates. The 84 and 168 kg N ha^{-1} rates were then fitted with a linear regression (Figures 2.7 and 2.8) that was used to make an inverse prediction of the nitrogen fertilizer equivalency of each legume treatment. The aforementioned N rates were used because the yield means of all living mulch treatments fell between the means of these two fertility treatments. This prediction cannot be used as a direct quantification of BNF due to other variables introduced by living mulches such as altered soil properties and nutrient competition between crops. Instead, it is meant to measure the net effect of the living mulch in terms of reduction in N fertilizer required. Nitrogen equivalencies of legume treatments are presented in Table 2.11. It should be noted that all legume treatments received 84 kg N ha^{-1} as a side-dress application.

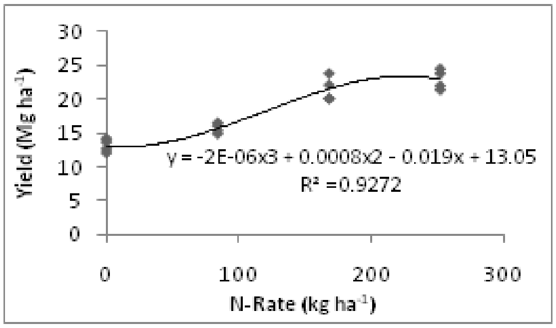

Figure 2.5. Corn silage yield nitrogen fertilizer response curve for Fruita, CO in 2009.

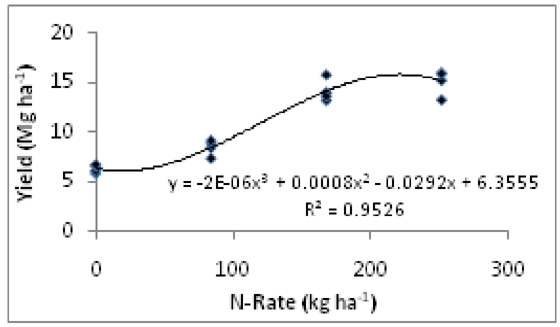

Figure 2.6. Corn grain yield nitrogen fertilizer response curve for Fruita, CO in 2009.

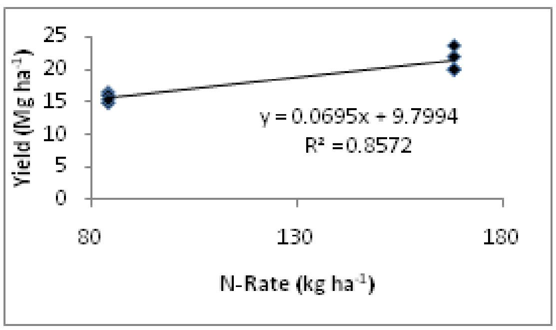

Figure 2.7. Linear regression of corn silage yield on nitrogen fertilizer rate for 84 and 168 kg ha^{-1} rates.

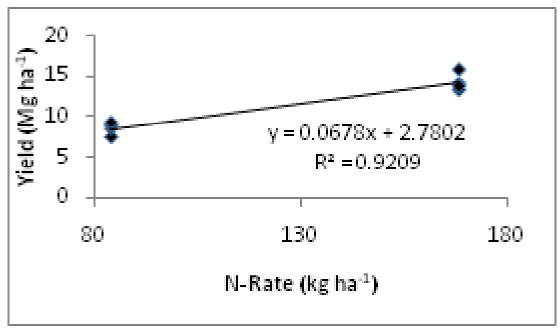

Figure 2.8. Linear regression of corn grain yield on nitrogen fertilizer rate for 84 and 168 kg ha^{-1} rates.

Table 2.11. Nitrogen fertilizer equivalencies of living mulch treatments at Fruita, CO in 2009 based on yield response to N fertilization.

Living Mulch Treatment	Corn Silage Nitrogen Fertilizer Equivalency		Corn Grain Nitrogen Fertilizer Equivalency	
	Including N fertilizer*	Legume Contribution	Including N fertilizer	Legume Contribution
	--------------------------(kg N ha^{-1})--------------------------			
Alfalfa	153 ± 51	69	127 ± 49	43
Birdsfoot trefoil	129 ± 49	45	104 ± 49	20
Birdsfoot trefoil + red clover	107 ± 49	23	107 ± 49	23
Red clover	116 ± 48	32	102 ± 49	18
White clover	130 ± 49	46	136 ± 49	52

* All living mulch treatments included 84 kg N ha^{-1} applied at the same time as the fertility treatments.

The confidence intervals surrounding estimates of nitrogen fertilizer equivalencies were relatively large (Table 2.11). This is the result of making an inverse prediction on a line with a relatively flat slope. The intervals on the y-axis (yield) are exaggerated on the x-axis (N-rate). As a result, no significant differences were found among living mulch treatments.

Legume Mortality

There was no interaction between legume treatment and annual crop in terms of legume mortality (Table 2.12). The soybean treatment had the least remaining legumes. This was likely due to the mid-season glyphosate application followed by the formation of a thick soybean canopy that outcompeted the perennial legumes for light. Alfalfa and birdsfoot trefoil had the most remaining plants, though even these averaged only about 50% ground cover. White clover had very few remaining plants, and no red clover plants persisted. The mix of birdsfoot trefoil and red clover had only trefoil at this point. Based on these evaluations, only the trefoil following corn and possibly the alfalfa following corn were deemed suitable for continued use as a living mulch without reseeding. Even these stands did not have as much regrowth as desired. Based on these results, it would appear that the broadcast application of glyphosate followed by paraquat was too aggressive to maintain any of these species, particularly the clovers, as a living mulch.

Table 2.12. Legume mortality ratings at Fruita, CO in spring 2010.

	Corn Silage	Soybean	Corn Grain	Avg.
	------------------------Rating *---------------------			
Alfalfa	3.3 §	2.0	3.0	2.8 a †
Birdsfoot trefoil	3.8	2.0	3.5	3.1 a
Birdsfoot trefoil + Red clover	1.5	1.0	1.3	1.3 b
Red clover	0.0	0.0	0.0	0.0 d
White clover	1.0	0.3	0.8	0.7 c
Avg.	1.9 A ‡	1.1 B	1.7 A	

* Mortality based on a scale of 0 = no legumes to 5 = full ground cover.
§The interaction of legume treatment by annual crop was not significant at the 0.05 probability level.
†Legume treatment means followed by the same lowercase letter are not different at the 0.05 probability level.
‡ Annual crop means followed by the same uppercase letter are not different at the 0.05 probability level.

CONCLUSION

This research demonstrated the potential for leguminous living mulches to reduce N fertilizer inputs to corn. Living mulches tended to increase corn yields (grain and silage) beyond those of conventional corn with the same rate of N fertilization. In soybeans, no yield effects (positive or negative) were observed from living mulch treatments. In this study, all legume residue was minimal and would not likely contribute significantly to the forage quality of grazed corn stover. Legume mortality was high and variable by species. If suppression methods could be determined that allow higher survival of legumes, greater fall grazing benefits may exist. While results suggest that living mulch cropping systems may be a viable alternative under irrigation for producers in the semi-arid West, additional trials are needed. Future research in the region should focus on spring suppression regimes to effectively reduce competition with the annual while maintaining the perennial mulch crop.

LITERATURE CITED

Affeldt, R.P., K.A. Albrecht, C.M. Boerboom, and E.J. Bures. 2004. Integrating herbicide-resistant corn technology in a kura clover living mulch system. Agron. J. 96:247-251.

Albrecht, K.A., T. Ochsner, and B. Berkevich. 2009. Farming for nitrogen: intercropping corn and kura clover. p. 163-166. *In* Proc. 2009 Wisconsin crop management conference, Madison, WI, 13-15 Jan. 2009.

Brann, D.E. and P.R. Peterson. 2000. Forage crops. p. 31-40. *In* Brann, D.E., D.L. Holshouser, and G.L. Mullins (eds.). Agronomy handbook. Virginia Cooperative Ext. Publication 424-100.

Cherney, D.J.R., J.H. Cherney, W.J. Cox. 2004. Fermentation characteristics of corn forage ensiled in mini-silos. J. Dairy Sci. 87: 4238-4246.

Cox, W.J. and D.L. Cherney. 2001. Row spacing, plant density, and nitrogen effects on corn silage. Agron. J. 93:597-602.

Duiker, S.W. and N.L. Hartwig. 2004. Living mulches of legumes in imidazolinone-resistant corn. Agron. J. 96:1-21-1028.

Eberlein, C.V., C.C. Sheaffer, and V.F. Oliveira. 1992. Corn growth and yield in an alfalfa living mulch system. J. Prod. Agric. 5:332-339.

Echtenkamp, G.W. and R.S. Moomaw. 1989. No-till corn production in a living mulch system. Weed Technology. 3:261-266.

Etheridge, R.D., G.M. Pesti, and E.H. Foster. 1998. A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco CNS 2000) on sample typical of an animal nutrition analytical laboratory. Animal Feed Science and Technology. 73:21-28.

Frame, J., J.F.L. Charlton, and A.S. Laidlaw. 1998. Temperate forage legumes. Cab International. New York, NY.

Hartwig, N.L. 2004. Soil and production enhancing cover crop and living mulch systems. Pennsylvania State University. Ext., State College, PA.

Hartwig, N.L. and H.U. Ammon. 2002. Cover crops and living mulches. Weed Science. 50:688-699.

Jones, J.B. Jr. 2003. Agronomic handbook: management of crops, soils, and their fertility. CRC Press, Boca Raton, FL.

Keeney, D.R. and D.W. Nelson. 1982. Nitrogen-inorganic forms. *In* Page, A.L., R.H. Miller and D.R. Keeney (eds.). Methods of soil analysis. Part 2. Chemical and microbiological properties. (2nd edition). Agronomy 9:643-698.

Neter, J., W. Wasserman, and M.H. Hunter. 1990. Applied linear statistical models: regression, analysis of variance, and experimental designs. (3rd edition). Richard D. Irwin Inc., Homewood, IL.

Paine, L.K., and Harrison. 1993. The historical roots of living mulches and related practices. Hort Technology. 3:137-143.

Pederson, P. and J.G. Lauer. 2004. Soybean growth and development in various management systems and planting dates. Crop Sci. 44:508-515.

Pederson, P., E. J. Bures, and K.A. Albrecht. 2009. Soybean production in a kura clover living mulch system. Agron. J. 101:653-656.

SAS Institute. 2009. SAS version 9.2. SAS Inst., Cary, NC.

Sawyer, J.E., P. Pedersen, D.W. Barker, D.A. Ruiz Diaz, and K.A. Albrecht. 2010. Intercropping corn and kura clover: response to nitrogen fertilization. Agron. J. 102:568-574.

Sheaffer, C.C., J.L. Halgerson, and H.G. Jung. 2006. Hybrid and N fertilization affect corn silage yield and quality. J. Agronomy and Crop Science. 192:278-283.

Singer, J.W., K.A. Kohler, K.J. Moore, and D.W. Meek. 2009. Living mulch forage yield and botanical composition in a corn-soybean-forage rotation. Agron. J. 101:1249-1257.

Singer, J.W. and P. Pedersen. 2006. Legume living mulches in corn and soybean. Iowa State Univ. Ext., Ames, IA.

Sparks, D.L., (ed). 1996. Methods of soil analysis: Part 3. Chemical methods. Soil Science Society of America, Inc, Madison, WI.

Sustainable Agriculture Network. 1998. Managing cover crops profitably. San handbook. Ser. Book 3. SAN, Beltsville, Maryland.

Taylor, N.L. and K.H. Quesenberry. 1996. Red clover science. Kluwer Academic Publishers. Dordrecht, The Netherlands.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Symposium: Carbohydrate methodology, metabolism, and nutritional implication in dairy cattle. J. Dairy Sci. 74: 3583-3597.

Walkley A. and I.A. Black. 1934. An Examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37: 29-38.

White, J.G. and T.W. Scott. 1991. Effects of perennial forage-legume living mulches on no-till winter wheat and rye. Field Crops Res. 28:135-148.

Zemenchik, R.A., K.A. Albrecht, C.M. Boerboom, and J.G. Lauer. 2000. Corn production with kura clover as a living mulch. Agron. J. 92:698-705.

CHAPTER 3: CHEMICAL SUPPRESSION OF ESTABLISHED LEGUMINOUS LIVING MULCHES

SUMMARY

One of the greatest challenges in maintaining a living mulch as a perennial cover crop is adequately suppressing the mulch to minimize annual cash crop losses while maintaining an acceptable stand of the perennial. The goal of this study was to determine whether established perennial legumes including birdsfoot trefoil (*Lotus corniculatus*), white clover (*Trifolium repens*), and a mix of white clover, red clover (*Trifolium pratense*), and kura clover (*Trifolium ambiguum*) could be suppressed with paraquat (1,1'-dimethyl-4,4'-bipyridylium-dichloride) or glyphosate [*N*-(phosphonomethyl)glycine] for use as living mulches in corn. Variable suppression treatments were applied prior to corn planting at two sites, one irrigated with a linear drive sprinkler system and the other furrow irrigated. Treatments included: paraquat at 0.7 kg a.i. ha^{-1} and glyphosate at 1.0, 1.5, and 2.0 kg a.e. ha^{-1}. All of these were followed by a mid-season application of glyphosate at 1.0 kg a.e. ha^{-1}. Corn grain yields, corn residue biomass, and legume biomass were collected in the fall. Legume by suppression treatment interactions occurred for all of these factors at the sprinkler irrigated site. At the furrow irrigated site, corn grain yields in the birdsfoot trefoil treatment averaged 13.2 Mg ha^{-1}, which was greater than white clover and the clover mix, yielding 11.9 and 11.8 Mg ha^{-1}, respectively. Fall legume biomass yields of birdsfoot trefoil, white clover, and clover mix were 11, 343, and 320 kg ha^{-1}, respectively.

No legume treatment effects were found on corn residue accumulation, and suppression treatment did not have any effect on grain yield, residue biomass, or legume biomass. These results indicate that birdsfoot trefoil was the least competitive with corn, but may be unsuitable for use as a living mulch due to its lack of tolerance to glyphosate. Further, it appears that even modest recovery of legumes during the growing season results in some corn yield reduction. Increasing the rate of glyphosate to 2.0 kg a.e. ha^{-1} did not appear to decrease clover biomass except for white clover at one site. Thus, the higher initial rates could likely be used for weed control without increasing injury to the clover living mulch.

INTRODUCTION

Increasing input costs along with environmental conservation issues have created the need for agricultural research in the area of low-input, sustainable cropping systems. Cover cropping, tillage reduction, and value-added crops have drawn a great deal of focus in addressing both environmental and economic concerns. One concept that embodies and expands upon such ideas is that of a living mulch.

Living mulches are cover crops grown in association with an annual cash crop (Paine and Harrison, 1993; SAN, 1998). These vegetative covers are unique in that they are not completely killed prior to planting of the annual crop like a traditional green manure or cover crop. Rather, growth is temporarily suppressed allowing eventual persistence and coexistence of the cover with the annual crop throughout the growing season and beyond (Echtenkamp and Moomaw, 1989; Singer and Pederson, 2005). These mulches can be annuals or perennials and can be interseeded with the cash crop or established before planting (Singer and Pederson, 2005). The use of a perennial cover offers many potential benefits including decreased wind and water erosion, increased water infiltration, weed suppression, reduced insect damage, and increased soil organic matter (Echtenkamp and Moomaw, 1989; White and Scott, 1991; Hartwig, 2004). Another advantage of living mulches is improved nutrient cycling. All covers provide some nitrogen retention by limiting nitrate leaching (Duiker and Hartwig 2004). However, leguminous living mulches offer the greatest fertility improvements through the biological fixation of atmospheric nitrogen.

Aside from their primary benefits as a mulch, legumes are highly palatable and increase the forage quality of grazed crop aftermath, such as corn stover, by

supplementing protein and energy (Zemenchik et al., 2000). This gives livestock producers the ability to substantially increase the feed value of crops they may already be grazing. In some cases, there is also potential for spring grazing or harvest before the cash crop is planted.

The most obvious and inherent obstacle in this system is the challenge of managing competition between two crops in the same field at the same time. The relative importance of the cash and mulch crops will be specific to the individual producer, but maintenance of cash crop yields will likely be a priority for most growers.

Suppression of living mulches can be achieved through cultural practices, sub lethal rates of herbicide, or a combination of the two (Singer and Pederson, 2005). Both Affeldt et al. (2004) and Zemenchik et al. (2000) grew corn in a kura clover (*Trifolium ambiguum*) living mulch with no whole plant or grain yield loss when adequate suppression was employed. Even with the necessary level of suppression, both studies found that the clover was able to recover to full production within 12 months of corn harvest. Affeldt et al. (2004) observed the highest corn yields when kura was suppressed using a preplant combination of glyphosate [*N*-(phosphonomethyl)glycine] and dicamba (3,6-dichloro-2-methoxybenzoic acid) followed by a postplant application of dicamba and clopyralid (3,6-dichlor-2-pyridinecarboxylic acid) in a 25 cm band. Later, glyphosate or glufosinate [2-amino-4-(hydroxymethylphosphinyl) butonic acid) was applied at the V3 to V5 growth stage of the corn. Zemenchik et al. (2000) were most successful with glyphosate band-applied in 61 cm strips over the corn row pre-emergence followed by a post-emergence band application of dicamba. More recent studies on the use of kura clover as a living mulch in corn have used glyphosate either as a component of their chemical suppression regime (Sawyer et al., 2010; Ochsner et al., 2010) or as the sole herbicide suppressant (Singer et al., 2010). Strip or minimum tillage has also been successfully employed as a means of additional mulch suppression in conjunction with atrazine (2-chloro-4-ethytlamino-6-isopropylamine-1,3,5-triazine) at planting (Fischer and Burrill, 1993).

While these results are encouraging, it must be emphasized that kura is ideal for use as a living mulch due to its resilience after stresses such as herbicide application (Zimenchik et al., 2000). However, poor seedling vigor (Singer and Pederson, 2005) and slow establishment (Speer and Allinson, 1985; Seguin et al., 2001) can be major obstacles to the adoption of this crop. Thus, other mulch crops, which may require different management strategies, should be tested.

Duiker and Hartwig (2004) evaluated several mulch crops including crown vetch (*Coronilla varia*), flatpea (*Lathyrus sylvestris*), birdsfoot trefoil (*Lotus corniculatus*), hairy vetch (*Vicia villosa*), and galega (*Galaega officinalis*) grown with corn. They found that crown vetch, birdsfoot trefoil, and flatpea could be suppressed sufficiently to maintain corn yields, but flatpea and birdsfoot trefoil required periodic reseeding. Thus, the lack of resilience in these crops prevented continued production of the mulches. While numerous chemical suppression regimes were investigated from 1994 to 2000, they concluded that crownvetch and birdsfoot trefoil can be managed with a program based on glyphosate.

Alfalfa (*Medicago sativa*) has also been tested as a living mulch in corn with several suppression regimes (Eberlein et al., 1992). Under irrigation, partial suppression of alfalfa at planting by applying atrazine either in 38 cm bands (lethal rate) over the corn row or as a broadcast application (sub-lethal rate) resulted in similar corn grain yields. Further, no yield losses were observed when compared to monoculture corn planted after killed alfalfa. Dry weight reduction of alfalfa when compared to an unsuppressed control treatment was less in the broadcast application than in the band treatment. The conclusion was that a uniform application provided better suppression than band killing over the corn row.

These findings on the timing, application methods, chemicals, and rates used for suppression of different mulch species can be used as the basis for continued research. It is particularly important to test management practices such as these under different environmental conditions. While living mulches have been tested extensively under rain-fed conditions (Eberlein et al., 1992; Affeldt et al., 2004; Duiker and Hartwig, 2004; Pederson et al., 2009), there is a lack of published data on their use in semi-arid environments that require irrigation. Most of the research to date has come from the upper Midwestern and Eastern US. Soil types, climatic conditions, insects, weeds, and disease pressure in the semi-arid West differ from these humid regions. Thus, research on species selection and general management practices for living mulches must be conducted in this region if the system is to be adopted by producers in the western US. The objectives of this study were to:

1) Evaluate different chemical suppression regimes in terms of their effects on the survival of leguminous living mulches.
2) Determine the effects of living mulches under variable rates of glyphosate and paraquat suppression on corn grain yield and residue composition.

MATERIALS AND METHODS

Two fields were used to test different chemical suppression regimes in the spring of 2010. Legumes at these sites were seeded in the spring of 2009 as part of the co-establishment studies discussed earlier in chapter 2. The study sites were located at the Colorado State University Agricultural Research, Development, and Education Center (ARDEC) about 6 km south of Wellington, CO (40^{o}39'N, 104^{o}59'W) at an elevation of 1554 m. Both were irrigated, one with a linear drive sprinkler system and the other with furrow irrigation from gated pipe. All subsequent references to the two sites will be made by the type of irrigation they received. The soil at the sprinkler irrigated site was classified as a Fort Collins loam (fine loamy, mixed, mesic Aridic Haplustalf) while at the furrow irrigated site, soil was classified as a Garrett loam (fine-loamy, mixed, superactive, mesic Pachic Argiustoll). ARDEC receives approximately 33 cm of precipitation annually. However, the study sites were irrigated according to annual crop needs throughout the growing season. Average monthly temperatures range from 0^{o}C in January to 22^{o}C in July.

Experimental design

Legumes tested at both sites included birdsfoot trefoil, white clover (*Trifolium repens*), and a mix of red clover (*Trifolium pratense*), kura clover, and white clover that were planted in spring 2009. Legumes are listed along with varieties and seeding rates (Table 3.1).

The sprinkler irrigated site consisted 4.6 x 10.7 m plots, which were split into three 16-plot blocks. These were used as the three replications for this study. Blocks had been seeded with corn, spring oats, and corn interseeded with spring oats the previous

Table 3.1. Species, varieties, and seeding rates of legumes used in suppression study at sprinkler and furrow irrigated sites in 2010.

Species	Variety	Seeding Rate-Pure Live Seed ($kg\ ha^{-1}$)
Birdsfoot trefoil	Leo	6.7
White clover	Kopu II	4.5
Kura clover (in mix)	Variety not stated	5.6
Red clover (in mix)	Starfire	3.4
White clover (in mix)	Kopu II	2.2

year, during which all legumes were successfully established. The field was laid out in a split plot design where the spray treatment was the whole plot and the legume treatment was the split plot. Variable rates of glyphosate and paraquat (1,1'-dimethyl-4,4'-bipyridylium-dichloride) were applied in the spring in order to suppress the mulches prior to corn planting (Table 3.2).

Table 3.2. Suppression treatments applied to living mulch plots at sprinkler and furrow irrigated sites in spring 2010.

Treatment	Herbicide	Rate *
1	Glyphosate	1.0 kg a.e. ha^{-1}
2	Glyphosate	1.5 kg a.e. ha^{-1}
3	Glyphosate	2.0 kg a.e. ha^{-1}
4	Paraquat	0.7 kg a.i. ha^{-1} **

* a.e. = acid equivalency
a.i. = active ingredient
** Paraquat was applied at the sprinkler irrigated site only.

Legume treatments and plot dimensions at the furrow irrigated site were identical to those described at the sprinkler irrigated site. However, the experimental design differed. In this case, the field was laid out in a randomized block design with four replications. The glyphosate rates were the same as the sprinkler irrigated site, but there was no paraquat treatment (Table 3.2). This site also differed in that it only had one treatment with no legumes, which was considered the control. This was treated with glyphosate at 2.0 kg a.e. ha^{-1} for weed control. Chemical suppression treatments were applied on the mornings of May 21 and 22, 2010 at the sprinkler and furrow irrigated sites, respectively. Herbicides were applied at approximately 200 kPa using a CO_2-pressurized backpack sprayer with wide angle flat spray tips (TT VP 11001, Teejet Technologies, Wheaton, IL). All glyphosate rates and paraquat (sprinkler irrigated site only) were applied with 94 L water ha^{-1}. Glyphosate concentrations were adjusted in mix bottles, and ground speed was kept constant. The ambient temperature was 13.1^{o}C with an average wind speed of 0.6 km hr^{-1} at the sprinkler irrigated site. At the furrow irrigated site, ambient temperature was 11.4^{o}C with an average wind speed of 3.7 km hr^{-1}. Spraying at both sites began between 6:30 and 7:00 am. The center 3.0 m of each plot was sprayed. After applying treatments to the middle of each plot, the outer edges were sprayed with glyphosate at a rate of 1.5 kg a.e. ha^{-1}. Birdsfoot trefoil, white clover, and the clover mix were approximately 10, 12, and 25 cm tall, respectively.

Both sites were strip-tilled on May 24, 2010. An Orthman 1tRIPr (Orthman Manufacturing Inc., Lexington, NE) six-row model was used to till 33 cm strips on 76 cm centers at the sprinkler irrigated site. An Orthman 1tRIPr two-row model was used at the furrow irrigated site to till 25 cm strips on 76 cm centers. A narrower tilled strip was required at the furrow-irrigated site in order to maintain some legume growth on the edges of the raised beds. Producers Hybrids '5004VT3' glyphosate resistant corn and Croplan Genetics '421RR2' glyphosate resistant corn were planted at the sprinkler and furrow irrigated sites, respectively, immediately after tilling. Corn was planted to a depth of 3.8 cm at a population of 80,300 seeds ha^{-1} using a 6-row no-till corn planter (Monosem NG+ Mounted Planter, Monosem Inc., Edwardsville, KS). Planting into the tilled strips ensured that six rows of corn were planted in the width of each plot with the center four of those falling in the treated area.

Irrigation at the sprinkler irrigated site began on June 2, 2010 and continued throughout the growing season on a weekly basis. The quantity of water was adjusted to meet crop needs (Table 3.3).

Table 3.3. Sprinkler irrigated site: irrigation timing and quantity in 2010.

Date	Irrigation (cm)
June 2	2.5
June 10	2.5
June 24	3.2
July 1	3.8
July 8	3.8
July 16	3.8
July 22	3.2
July 29	3.8
August 5	3.2
August 12	3.8
August 19	3.2
August 26	3.2
September 2	3.2
September 9	3.2
Total	43.2

The furrow site was irrigated on an as needed basis beginning on June 4, 2010. Dates and durations of irrigation events are listed in Table 3.4. Irrigation furrows were cleaned on June 3 and July 2, 2010 using a three-row ditcher with shovels and trailing cans. This was done both as a means of weed control and to ensure proper movement of water during irrigation.

Corn was side-dressed with 112 kg N ha^{-1} in the form of 32-0-0 on June 21, 2010. A midseason herbicide application was also deemed necessary due to weed pressure. This was made on July 6, 2010 as a broadcast application over all plots. Glyphosate was applied at a rate of 1.0 kg a.e. ha^{-1} with 150 L water ha^{-1} using a tractor-mounted boom sprayer.

Table 3.4. Furrow irrigated site: 2010 irrigation timing and duration in 2010.

Date	Duration (hours)
June 4	6.0
June 24	7.0
July 2	5.0
July 16	6.0
July 30	6.0
August 16	5.0
August 30	5.0
September 16	7.0

Harvest

Due to severe bird damage that occurred in some areas of both study sites late in the season, all plots were rated for percent loss to bird damage. This was done by randomly selecting ten ears from the middle two rows of each plot and estimating the percent of total grain lost from each ear. All the percentages for a given plot were then averaged and used to adjust plot yields to estimate full yield potential in the absence of bird damage. While field averages for bird damage were relatively low (16 and 8% at the sprinkler and furrow irrigated sites, respectively), this was deemed necessary due to variability in the extent of damage across the plot areas. Bird damage ratings were recorded immediately prior to corn harvest.

Corn was harvested for grain on October 27, 2010 using a combine (JD 3300, John Deere, Inc., Moline, IL) equipped with a yield monitor. Three of the center four rows were harvested from each plot. Ag Leader 10.50 (Ag Leader Technology, Ames, IA) software was used to record moisture and weight averages. Coordinates for the corners of the plot area at each site were recorded, and MapInfo Professional 7.0

(MapInfo Corp., Troy, NY) was used to generate a plot map that was overlaid on the yield map to calculate yield averages for each plot. Plot yields were recorded and adjusted to a moisture content of 155 g kg^{-1}.

Residue and legume biomass collection

Residue was collected from corn grain plots on October 28-30, 2010 and November 4-6, 2010 at the furrow and the sprinkler irrigated sites, respectively. This was done by laying a 76 x 76 cm frame flat on the ground in the center of each plot. The frame was centered on a bed so that it covered the area from one furrow to the next. All corn residue, legume, and green weed biomass within this area was collected. Desiccated or partially decomposed legume and weed material was discarded. Material was cut at the edge of the frame using hand clippers to ensure that only residue within the frame was collected. Legumes and corn stubble were cut at ground level using hand clippers. Corn residue was later separated into leaf, stem, and cob components. Corn components, legumes, and weeds were dried to a constant weight in a forced air oven at 55^{o}C for a minimum of 72 hours. Dry weights were then used to calculate corn residue, legume biomass, and weed biomass on a per hectare basis.

Statistical analysis: sprinkler irrigated site

PROC MIXED in SAS (SAS Institute, 2009) was used to determine the effect of legume and spray treatments on annual crop yield, corn residue components, and fall legume and weed biomass. Data were analyzed as a randomized block design with a split plot treatment structure where spray treatment was the whole plot, and legume treatment was the split plot. Block and block interactions were random effects. Legume and suppression treatments were considered fixed effects. Treatments with no legume were omitted from comparisons of legume residue. Differences were recognized as significant at the $P \leq 0.05$ level. If legume or suppression treatment effects were found to be significant, treatment means were separated using LSMEANS (SAS Institute, 2009).

Transformations were performed when an examination of the residuals indicated the need. Square root transformations were required to homogenize variances of fall legume and weed biomass. In these cases, the original data were reported, and the transformation was used to determine differences among treatments.

Statistical analysis: furrow irrigated site

PROC MIXED in SAS (SAS Institute, 2009) was used to determine the effects of legume and spray treatments on annual crop yield, corn residue components, and fall legume and weed biomass. Data were analyzed as a randomized block design. The control treatment (with no legume) was omitted. Block was the random effect, and legume and suppression treatments were considered fixed effects.

Dunnett's Test was then used to compare each individual treatment (legume/suppression combination) to the control. Differences were recognized as significant at the $P \leq 0.05$ level. If legume or suppression treatment effects were found to be significant, treatment means were separated using LSMEANS (SAS Institute, 2009). Either log or square root transformations were performed when necessary. Corn stem residue, legume biomass, and weed biomass were square root transformed while total corn residue was log transformed. The appropriate transformation was determined based on the spread of the residuals. Transformations were used to determine treatment differences, but original data were reported.

RESULTS AND DISCUSSION

Sprinkler irrigated site

An interaction between legume species and suppression treatment occurred for corn grain yield (Table 3.5). No legume treatment effects were found at the lower two rates of glyphosate. At the highest rate of glyphosate, corn yield was lower in white clover mulch than in birdsfoot trefoil mulch and the conventional treatment. The conventional treatment out-yielded the clover mix when treated with paraquat. Suppression treatment had no effect on the white clover and conventional treatments. However, the clover mix yielded lowest in the paraquat treatment. The trefoil yield tended to decline with decreasing glyphosate rate and was low in the paraquat treatment.

Table 3.5. Sprinkler irrigated site: effect of living mulches on corn grain yields in 2010.

	Glyphosate (rate in kg a.e. ha^{-1})			Paraquat (rate in kg a.i ha^{-1})	
	1.0	1.5	2.0	0.7	Avg.
	--------------------------DM yield (Mg ha^{-1})--------------------------				
Birdsfoot trefoil	13.0 aB §	15.3 aAB	16.4 aA	12.9 abB	14.4
Clover mix	15.4 aA	15.1 aA	14.0 abA	10.4 bB	13.7
White clover	15.6 aA	13.6 aA	13.1 bA	12.9 abA	13.8
Conventional (no legume)	15.1 aA	15.6 aA	16.3 aA	15.6 aA	15.6
Avg.	14.8	14.9	14.9	13.0	

§The interaction of legume treatment by annual crop was significant. Legume treatments followed by the same lowercase letter are not different while spray treatments followed by the same uppercase letter are not different. All differences were declared significant at the 0.05 probability level.

Based on visual evaluations prior to the July 6th broadcast glyphosate application, the burn-down treatment appeared to be ineffective at suppressing any of the mulch species. It is likely that the legumes had too much biomass accumulation at the time of spring suppression resulting in incomplete spray coverage and the loss of only the upper part of the canopy. Recovery of both the clover treatments and birdsfoot trefoil were very rapid, and the corn appeared stunted and chlorotic soon after emergence. The later glyphosate application was effective at not only controlling weed competition, but also the competition from inadequately suppressed legumes. This early season competition could easily explain yield losses in the burn-down treatments for the clover mix and trefoil.

Legume by suppression treatment interactions also occurred for both total corn residue and corn leaf residue (the component with the greatest forage quality). While the interaction for total residue cannot be completely explained, it can be noted that for both clover treatments, burn-down suppression resulted in the least amount of corn residue (Table 3.6). This trend was also evident to a lesser extent in leaf residue (Table 3.7). No treatment effects or interactions were found for corn stem (Table 3.8) or cob (data not shown) residue.

Table 3.6. Sprinkler irrigated site: effect of living mulches on total corn residue in fall 2010.

	Glyphosate (rate in kg a.e. ha^{-1})			Paraquat (rate in kg a.i ha^{-1})	
	1.0	1.5	2.0	0.7	Avg.
	--------------------------DM yield (Mg ha^{-1})--------------------------				
Birdsfoot trefoil	9.1 bA §	9.2 bA	11.2 aA	10.6 aA	10.0
Clover mix	9.2 abAB	9.8 bA	10.6 aA	6.9 bB	9.2
White clover	11.4 aA	8.8 bBC	11.1 aAB	8.1 bD	9.9
Conventional (no legume)	8.9 bB	12.3 aA	9.5 aB	10.5 aAB	10.3
Avg.	9.6	10.1	10.6	9.0	

§The interaction of legume treatment by annual crop was significant. Legume treatments followed by the same lowercase letter are not different while spray treatments followed by the same uppercase letter are not different. All differences were declared significant at the 0.05 probability level.

Table 3.7. Sprinkler irrigated site: effect of living mulches on corn leaf residue in fall 2010.

	Glyphosate (rate in kg a.e. ha^{-1})			Paraquat (rate in kg a.i ha^{-1})	
	1.0	1.5	2.0	0.7	Avg.
	------------------------DM yield (Mg ha^{-1})------------------------				
Birdsfoot trefoil	3.7aA §	3.6 bA	4.3 abA	3.9 abA	3.9
Clover mix	3.5 aAB	4.1 abA	4.2 bA	2.8 dB	3.7
White clover	4.0 aB	3.9 bB	5.2 aA	3.3 bcB	4.1
Conventional (no legume)	3.7 aB	5.1 aA	4.1 bAB	4.3 aAB	4.3
Avg.	3.7	4.2	4.4	3.6	

§The interaction of legume treatment by annual crop was significant. Legume treatments followed by the same lowercase letter are not different while spray treatments followed by the same uppercase letter are not different. All differences were declared significant at the 0.05 probability level.

Table 3.8. Sprinkler irrigated site: effect of living mulches on corn stem residue in fall 2010.

	Glyphosate (rate in kg a.e. ha^{-1})			Paraquat (rate in kg a.i ha^{-1})	
	1.0	1.5	2.0	0.7	Avg.
	------------------------DM yield (Mg ha^{-1})------------------------				
Birdsfoot trefoil	3.4 §	3.4	4.1	3.7	3.7 †
Clover mix	3.3	4.0	3.9	2.6	3.5
White clover	3.7	3.4	3.7	2.5	3.3
Conventional (no legume)	3.2	4.2	3.6	3.8	3.7
Avg.	3.4 ‡	3.8	3.8	3.1	

§The interaction of legume treatment by spray rate was not significant at the 0.05 probability level.
† The effect of legume treatment was not significant at the 0.05 probability level.
‡ The effect of spray treatment was not significant at the 0.05 probability level.

Fall legume biomass was also influenced by the interaction of legume species and herbicide treatment (Table 3.9). Trefoil survival was only apparent in the paraquat treatment suggesting that trefoil's tolerance to glyphosate in a living mulch system is still very low in the second year after establishment. However, Boerboom et al. (1990) noted a wide range of glyphosate tolerance among birdsfoot trefoil selections from a recurrent selection breeding program that included 'Leo' birdsfoot trefoil. A threefold difference in the rate of glyphosate required to reduce fresh weight by 50% (I_{50}) was found among

Table 3.9. Sprinkler irrigated site: legume biomass after corn grain harvest in fall 2010.

	Glyphosate (rate in kg a.e. ha^{-1})			Paraquat (rate in kg a.i ha^{-1})	
	1.0	1.5	2.0	0.7	Avg.
	-------------------------DM yield (kg ha^{-1})-------------------------				
Birdsfoot trefoil	0 bB §	0 bB	0 bB	50 aA	12
Clover mix	70 aA	39 aA	47 aA	47 aA	51
White clover	37 aA	43 aA	1 bB	1 bB	20
Avg.	36	27	16	33	

§The interaction of legume treatment by annual crop was significant. Legume treatments followed by the same lowercase letter are not different while spray treatments followed by the same uppercase letter are not different. All differences were declared significant at the 0.05 probability level.

nine selections previously tested in the field (Boerboom, 1989). The biomass of these selections ranged from 16 to 54% of an untreated control when evaluated as ramets 14 days after treatment with 0.5 kg ha^{-1} of glyphosate. Even the two selections of 'Leo' tested ranged from 26 to 45% making them the 2nd and 6th most tolerant among all selections tested. This tolerance was attributed to the specific activity of 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS). Thus, suitability of trefoil for use as a living mulch suppressed with glyphosate would likely differ among cultivars.

No treatment effect was found on fall legume biomass of the clover mix, while white clover biomass was lower at the highest rate of glyphosate and in the burn-down treatment (Table 3.9). The very limited survival of white clover treated with paraquat was surprising as it recovered significantly less than both the clover mix and trefoil in that same suppression treatment. This would not likely be attributed to the mid-season glyphosate application as white clover appears to have much greater glyphosate tolerance compared to birdsfoot trefoil.

Fall weed biomass was greater in the birdsfoot trefoil treatment than the clover mix and conventional treatments (Table 3.10). Suppression treatment had no effect on fall weed biomass.

Table 3.10. Sprinkler irrigated site: weed biomass after corn grain harvest in fall 2010.

	Glyphosate (rate in kg a.e. ha^{-1})			Paraquat (rate in kg a.i ha^{-1})	
	1.0	1.5	2.0	0.7	Avg.
	------------------------DM yield (kg ha^{-1})-----------------------				
Birdsfoot trefoil	3 §	11	6	5	6 a †
Clover mix	1	2	2	1	2 b
White clover	3	14	6	0	6 ab
Conventional (no legume)	3	0	3	2	2 b
Avg.	3 ‡	7	4	2	

§The interaction of legume treatment by spray rate was not significant at the 0.05 probability level.
†Legume treatment means followed by the same lowercase letter are not different at the 0.05 probability level.
‡ The effect of spray rate was not significant at the 0.05 probability level.

Furrow irrigated site

An effect of legume treatment on corn grain yield was found at the furrow irrigated site with corn grown in birdsfoot trefoil yielding significantly higher than in both clover treatments (Table 3.11). This was not true of total corn (Table 3.12), stem (Table 3.13), leaf (Table 3.14), or cob (data not shown) residue, in which there were no effects of either legume or suppression treatments on biomass production. The effect of legume species on grain yield can likely be related to its inverse relationship with fall biomass production. Legume biomass was significantly greater in the clover treatments (Table 3.15) with trefoil averaging only 3% of the white clover and clover mix biomass. Since competition between the crops is a likely cause of yield reductions, the higher grain yield in the trefoil treatment, which was completely killed, is not surprising. There was no effect of either legume or suppression treatment on fall weed biomass (Table 3.16).

Table 3.11. Furrow irrigated site: effect of living mulches on corn grain yields in 2010.

	Glyphosate (rate in kg a.e. ha^{-1})			
	1.0	1.5	2.0	Avg.
	---------------------DM yield (Mg ha^{-1})--------------------			
Birdsfoot trefoil	12.5 §	13.8	13.4	13.2 a †
Clover mix	11.0	12.1	12.4	11.8 b
White clover	11.3	12.1	12.4	11.9 b
Avg.	11.6 ‡	12.7	12.7	

§The interaction of legume treatment by spray rate was not significant at the 0.05 probability level.
†Legume treatment means followed by the same lowercase letter are not different at the 0.05 probability level.
‡ The effect of spray rate was not significant at the 0.05 probability level.

Table 3.12. Furrow irrigated site: effect of living mulches on total corn residue in fall 2010.

	Glyphosate (rate in kg a.e. ha^{-1})			
	1.0	1.5	2.0	Avg.
	---------------------DM yield (Mg ha^{-1})--------------------			
Birdsfoot trefoil	8.2 §	10.7	9.4	9.4 †
Clover mix	7.8	8.1	10.5	8.8
White clover	8.3	9.3	10.3	9.3
Avg.	8.1 ‡	9.3	10.0	

§The interaction of legume treatment by spray rate was not significant at the 0.05 probability level.
† The effect of legume treatment was not significant at the 0.05 probability level.
‡ The effect of spray rate was not significant at the 0.05 probability level.

Table 3.13. Furrow irrigated site: effect of living mulches on corn stem residue in fall 2010.

	Glyphosate (rate in kg a.e. ha^{-1})			
	1.0	1.5	2.0	Avg.
	---------------------DM yield (Mg ha^{-1})--------------------			
Birdsfoot trefoil	3.4 §	3.6	3.6	3.5 †
Clover mix	3.2	3.4	3.8	3.5
White clover	2.9	3.3	3.4	3.2
Avg.	3.2 ‡	3.4	3.6	

§The interaction of legume treatment by spray rate was not significant at the 0.05 probability level.
† The effect of legume treatment was not significant at the 0.05 probability level.
‡ The effect of spray rate was not significant at the 0.05 probability level.

Table 3.14. Furrow irrigated site: effect of living mulches on corn leaf residue in fall 2010.

	Glyphosate (rate in kg a.e. ha^{-1})			
	1.0	1.5	2.0	Avg.
	---------------------DM yield (Mg ha^{-1})--------------------			
Birdsfoot trefoil	3.4 §	3.5	3.5	3.4 †
Clover mix	3.0	3.3	3.9	3.4
White clover	3.2	3.3	3.6	3.4
Avg.	3.2 ‡	3.4	3.7	

§The interaction of legume treatment by spray rate was not significant at the 0.05 probability level.
† The effect of legume treatment was not significant at the 0.05 probability level.
‡ The effect of spray rate was not significant at the 0.05 probability level.

Table 3.15. Furrow irrigated site: legume biomass after corn grain harvest in fall 2010.

	Glyphosate (rate in kg a.e. ha^{-1})			
	1.0	1.5	2.0	Avg.
	---------------------DM yield (kg ha^{-1})--------------------			
Birdsfoot trefoil	3 §	1	28	11 b †
Clover mix	420	339	201	320 a
White clover	320	416	293	343 a
Avg.	248 ‡	252	174	

§The interaction of legume treatment by spray rate was not significant at the 0.05 probability level.
† Legume treatment means followed by the same lowercase letter are not different at the 0.05 probability level.
‡ The effect of spray rate was not significant at the 0.05 probability level.

Table 3.16. Furrow irrigated site: weed biomass after corn grain harvest in fall 2010.

	Glyphosate (rate in kg a.e. ha^{-1})			
	1.0	1.5	2.0	Avg.
	---------------------DM yield (kg ha^{-1})--------------------			
Birdsfoot trefoil	5 §	10	35	17 †
Clover mix	4	3	28	11
White clover	30	2	24	19
Avg.	13 ‡	5	29	

§The interaction of legume treatment by spray rate was not significant at the 0.05 probability level.
† The effect of legume treatment was not significant at the 0.05 probability level.
‡ The effect of spray rate was not significant at the 0.05 probability level.

Both the white clover and clover mix treatments with glyphosate applied at 1.0 kg a.e. ha^{-1} yielded significantly lower than the control treatment with no living mulch. Again, this was likely due to competition from the clovers which were inadequately suppressed at the lowest application rate. No other treatments differed from the control indicating that there was no yield loss due to presence of the living mulches.

It should be noted that all treatments at both sites were followed by a mid-season glyphosate application of 1.0 kg a.e. ha^{-1}, which likely reduced legume recovery below what it would have been with only the pre-plant suppression treatments. This was deemed necessary because of intense weed pressure and not to provide additional legume suppression, but it likely served to reduce treatment effects. Based on visual evaluation, additional mulch suppression would only have been required in the paraquat treatment. A marked reduction in weed control options is a key drawback to living mulch systems. The need for multiple herbicide applications has been well documented (Zimenchik et al., 2000; Affeldt et al., 2004; Duiker and Hartwig 2004; Sawyer et al., 2010) and should be taken into account when selecting a mulch species.

Fall legume biomass in all treatments was below levels that would significantly add to the diet of grazing beef cattle and help to meet their protein needs. White clover hay averages 22% CP, while red clover and birdsfoot trefoil hay average 16% (NRC, 1984). Kura clover hay harvested in October averaged 19% CP over four years (Singer et al., 2010). It is well-established that cattle will graze corn leaves/husks selectively when pastured on corn stover (Lamm and Ward 1981; Fernandez-Rivera and Klopfenstein, 1989). Leaves/husks averaged approximately 4.8% CP when corn was grown with a kura clover living mulch (unpublished data). Based on these percentages and corn leaf/husk biomass averaged across all treatments at the furrow irrigated site, the approximate legume biomass required to raise the CP of the grazed crop aftermath to a given percent can be calculated. To raise CP to 7%, an approximate protein requirement for dry beef cows (NRC, 1996), 623 and 498 kg ha^{-1} of kura and white clover, respectively or 830 kg ha^{-1} of red clover or trefoil residue would be required. While the levels of clover residue found in this study would reduce the need for supplemental protein, they could not meet total protein requirements for dry beef cattle.

CONCLUSION

Based on results of these studies, there was no clear effect of glyphosate application rate on living mulch recovery, corn grain yield, or corn residue biomass. Among legume species, birdsfoot trefoil was the least resilient to glyphosate application, but appeared to make some recovery after treatment with paraquat. Such limited recovery suggests its poor suitability as a living mulch, but testing with other cultivars may potentially yield different results. Clovers tended to recover more when treated with glyphosate, but also reduced corn grain yields compared to trefoil at the furrow irrigated site. Based on this finding, it would appear that even a modest recovery of legumes during the growing season results in some corn yield reduction.

LITERATURE CITED

Affeldt, R.P., K.A. Albrecht, C.M. Boerboom, and E.J. Bures. 2004. Integrating herbicide-resistant corn technology in a kura clover living mulch system. Agron. J. 96:247-251.

Boerboom, C.M., D.L. Wyse, and D.A. Somers. 1990. Mechanism of Glyphosate Tolerance in Birdsfoot Trefoil (*Lotus corniculatus*). Weed Sci. 38:463-467.

Boerboom, C. M. 1989. Selection and characterization of glyphosate tolerance in birdsfoot trefoil (*Lotus corniculatus*). PhD. Thesis, Univ. Minnesota. 67 pp.

Duiker, S.W. and N.L. Hartwig. 2004. Living mulches of legumes in imidazolinone-resistant corn. Agron. J. 96:1-21-1028.

Eberlein, C.V., C.C. Sheaffer, and V.F. Oliveira. 1992. Corn growth and yield in an alfalfa living mulch system. J. Prod. Agric. 5:332-339.

Echtenkamp, G.W. and R.S. Moomaw. 1989. No-till corn production in a living mulch system. Weed Technology. 3:261-266.

Fernandez-Rivera, S. and T.J. Klopfenstein. 1989. Yield and quality components of corn crop residues and utilization of these residues by grazing cattle. J Anim. Sci. 67:597-605.

Fischer, A. and L. Burrill. 1993. Managing interference in a sweet corn-white clover living mulch system. American Journal of Alternative Agriculture. 8:51-56.

Hartwig, N.L. 2004. Soil and production enhancing cover crop and living mulch systems. Pennsylvania State University. Ext., State College, PA.

Lamm, W.D. and J.K. Ward. 1981. Compositional changes in corn crop residues grazed by gestating beef cows. J Anim. Sci. 52:954-958.

NRC. 1984. Nutrient requirements for beef cattle (6th Ed.). National Academy Press, Washington, DC.

NRC. 1996. Nutrient requirements for beef cattle (7th Ed.). National Academy Press, Washington, DC.

Ochsner, T.E., K.A. Albrecht, T.W. Schumacher, J.M. Baker, and R.J. Berkevich. 2010. Water balance and nitrate leaching under corn in a kura clover living mulch. Agron. J. 102:1169-1178.

Paine, L.K., and Harrison. 1993. The historical roots of living mulches and related practices. Hort.Technology 3:137-143.

SAS Institute. 2009. SAS version 9.2. SAS Inst., Cary, NC.

Sawyer, J.E., P. Pedersen, D.W. Barker, D.A. Ruiz Diaz, and K.A. Albrecht. Intercropping corn and kura clover: response to nitrogen fertilization. Agron. J. 102:568-574.

Seguin, P., C.C. Schaeffer, N.J. Ehlke, M.P. Russelle, and P.H. Graham. 2001. Nitrogen fixation and rhizobial inoculation effects on kura clover growth. Agron J. 93:1262-1268.

Singer, J.W. and K.J. Moore. 2010. Living mulch nutritive value in a corn-soybean-forage-rotation. Agron. J. 102:282-288.

Singer, J.W., K.A. Kohler, K.J. Moore, and D.W. Meek. 2010. Living mulch forage yield and botanical composition in a corn-soybean-forage-rotation. Agron. J. 101:1249-1257.

Singer, J.W. and P. Pedersen. 2005. Legume living mulches in corn and soybean. PM-2006. Iowa State Univ. Ext., Ames, IA.

Speer G.S. and G.W. Allinson. 1985. Kura clover (*Trifolium ambiguum*): legume for forage and soil conservation. Econ. Bot. 39:165-176.

Sustainable Agriculture Network. 1998. Managing cover crops profitably. San handbook. Ser. Book 3. SAN, Beltsville, Maryland.

White, J.G. and T.W. Scott. 1991. Effects of perennial forage-legume living mulches on no-till winter wheat and rye. Field Crops Res., 28:135-148.

Zemenchik, R.A., K.A. Albrecht, C.M. Boerboom, and J.G. Lauer. 2000. Corn production with kura clover as a living mulch. Agron. J. 92:698-705.

CHAPTER 4: RECOVERY OF POTENTIAL LEGUMINOUS LIVING MULCHES AFTER SPRING GLYPHOSATE APPLICATION

SUMMARY

An important criterion in selecting legume species for use as living mulches is their ability to recover from suppressing herbicide applications. Herbicides are a key component of most successful mulch suppression regimes and may also be necessary for weed control. Recovery of living mulches is necessary for benefits such as erosion control, weed suppression, and biological nitrogen fixation to occur. Two studies, one conducted in the field and the other using potted plants, evaluated the persistence of legume species including alfalfa (*Medicago sativa*), birdsfoot trefoil (*Lotus corniculatus*), kura clover (*Trifolium ambiguum*), red clover (*Trifolium pratense*), and white clover (*Trifolium repense*) after glyphosate application by measuring their biomass relative to an untreated control. Field tests included rates of 1.0, 1.5, 2.0, and 2.5 kg a.e. ha^{-1}, while trials of potted plants started in the greenhouse lacked the 2.5 kg a.e. ha^{-1} rate. Shoot biomass was collected at 8 and 16 weeks after application in the field and at 8 weeks only from potted plants. Root/crown biomass was also collected from potted plants. White clover recovered the most relative to the control in the field trial with no differences among glyphosate treatments and averaging 101% of the control biomass by the 16-week sampling. No differences in kura clover biomass were found among glyphosate treatments at either sampling date, but these could not be compared with the control due

to the use of contaminated seed that reduced kura biomass in the control treatment. Alfalfa and birdsfoot trefoil consistently recovered less than the clover species. In the pot trial, above ground biomass of kura clover, white clover, birdsfoot trefoil, red clover, and alfalfa recovered to 19, 7, 5, 2, and 1% of the control, respectively. The 1.0 kg a.e. ha^{-1} rate yielded highest across species averaging an 11% recovery while the 1.5 and 2.0 kg a.e. ha^{-1} treatments averaged 5 and 4%, respectively. Root biomass averaged 26% of the control for the 1.0 kg a.e. ha^{-1} treatment and 17% for both the 1.5 and 2.0 kg a.e. ha^{-1} treatments. No species effects were found on root biomass, although this could be due to rooting limitations by the pots. Both pot and field trials had some degree of biomass reduction in response to increasing glyphosate application rate. The results of this study indicate that white and kura clovers have the greatest potential for use as living mulches in a glyphosate-based suppression regime due to their ability to recover when treated with high rates of glyphosate.

INTRODUCTION

Increasing input costs along with environmental conservation issues have created the need for agricultural research in the area of low-input, sustainable cropping systems. Cover cropping, tillage reduction, and value-added crops have drawn a great deal of focus in addressing both environmental and economic concerns. One concept that embodies and expands upon such ideas is that of a living mulch.

Living mulches are cover crops grown in association with an annual cash crop (Paine and Harrison, 1993; SAN, 1998; Hartwig and Ammon, 2002). These vegetative covers are unique in that they are not completely killed prior to planting of the annual crop like a traditional green manure or cover crop. Rather, growth is temporarily suppressed (if necessary) allowing eventual persistence and coexistence of the cover with the annual crop throughout the growing season and beyond (Echtenkamp and Moomaw, 1989; Singer and Pederson, 2005). These mulches can be annuals or perennials and can be interseeded with the cash crop or established before planting (Singer and Pederson, 2005). The use of a perennial cover offers many potential benefits including decreased wind and water erosion, increased water infiltration, weed suppression, reduced insect damage, and increased soil organic matter (Echtenkamp and Moomaw, 1989; White and Scott, 1991; Hartwig, 2004). Another advantage of living mulches is improved nutrient cycling. All covers provide some nitrogen retention by limiting nitrate leaching (Duiker and Hartwig 2004). However, leguminous living mulches offer the greatest fertility improvements through the biological fixation of atmospheric nitrogen.

The most challenging component of a living mulch cropping system is determining the appropriate mulch suppression regime. While mowing, grazing, and zone tillage are all options, the perennial mulch generally requires a sub-lethal herbicide application prior to planting the annual. This must be potent enough to prevent competition with the cash crop without completely killing the mulch.

Both Affeldt et al. (2004) and Zemenchik et al. (2000) grew corn in a kura clover living mulch with no whole plant or grain yield loss when adequate suppression was employed. Even with the necessary level of suppression, both studies found that the clover was able to recover to full production within 12 months of corn harvest. Affeldt et al. (2004) observed the highest corn yields when kura was suppressed using a preplant combination of glyphosate [*N*-(phosphonomethyl)glycine] and dicamba (3,6-dichloro-2-methoxybenzoic acid) followed by a postplant application of dicamba and clopyralid (3,6-dichlor-2-pyridinecarboxylic acid) in a 25 cm band. Later, glyphosate or glufosinate [2-amino-4-(hydroxymethylphosphinyl) butonic acid] was applied at the V3 to V5 growth stage of the corn. Zemenchik et al. (2000) were most successful with glyphosate band-applied in 61 cm strips over the corn row pre-emergence and a post-emergence band application of dicamba. More recent studies on the use of kura clover as a living mulch in corn have used glyphosate either as a component of their chemical suppression regime (Sawyer et al., 2010; Ochsner et al., 2010) or as the sole herbicide suppressant (Singer et al., 2010). The extensive use of glyphosate can likely be attributed to the fact that a postplant herbicide application is generally required making the use of herbicide-resistant corn cultivars a necessity.

While results with kura clover are encouraging, it must be emphasized that this clover species is ideal for use as a living mulch due to its resilience after stresses such as herbicide application (Zemenchik et al., 2000). However, poor seedling vigor (Pederson and Singer, 2005) and slow establishment (Speer and Allinson, 1985; Seguin et al., 2001) can be major obstacles to the adoption of this crop. Thus, other mulch crops, which may require different management strategies, need to be tested for recovery after glyphosate application. An ideal mulch crop would suffer significant biomass reduction after glyphosate application so as not to compete with the annual, yet recover in the later part of the growing season. It must also be able to survive rates of glyphosate required for weed control. If adequate suppression cannot be accomplished with glyphosate alone, other herbicides can be used was well, but the common use of glyphosate makes recovery at moderate application rates essential.

The objective of this study was to evaluate the relative recovery of perennial legumes after variable rates of glyphosate application to determine their suitability as living mulches in a glyphosate-based suppression regime.

MATERIALS AND METHODS

Glyphosate rate studies were conducted in the field and using potted plants started in the greenhouse. Both studies tested the same five legume species, which are listed in Table 4.1 along with varieties and seeding rates for the field study. All legumes were treated with the proper *Rhizobium* inoculants prior to planting. The field study subjected each legume to four rates of glyphosate, while the pot study included three rates.

Table 4.1. Legume species and varieties used in the pot and field studies and seeding rates used in the field study.

Legume	Scientific Name	Variety	Seeding Rate (kg pure live seed ha^{-1})
Alfalfa	*Medicago sativa*	WL 343HQ	13.5
Birdsfoot trefoil	*Lotus corniculatus*	Leo	6.7
Kura clover	*Trifolium ambiguum*	Variety not stated	11.2
Red clover	*Trifolium pratense*	Starfire	9.0
White clover	*Trifolium repens*	Kopu II	4.5

Field Study

The field study site was located at Colorado State University's Agricultural Research, Development, and Education Center (ARDEC). ARDEC is located about 6 km south of Wellington, CO (40^{o}39'N, 104^{o}59'W) at an elevation of 1554 m. The soil was classified as a Fort Collins loam (fine loamy, mixed, mesic Aridic Haplustalf). While ARDEC receives 33 cm of precipitation annually, the site was irrigated with a linear drive sprinkler system on an as-needed basis in both the fall of 2009 and spring of 2010. Average monthly temperatures range from 0^{o}C in January to 22^{o}C in July.

The field was previously planted in teff (*Eragrostis tef*) that was sprayed with glyphosate at 1.3 kg a.e. ha^{-1} and mowed on August 2, 2009. It was then sprayed with glyphosate at the same rate a second time one day prior to planting to ensure that the teff and any weeds were completely killed. Legumes were seeded on August 18, 2009 using a no-till drill (Model 3P605NT, Great Plains Mfg., Inc., Salina, KS). Row spacing was set at 19 cm and planting depth was approximately 1 cm. Legumes were irrigated weekly through September 16, 2009.

The site was laid out in a strip plot design with four replications. Legume species were randomized within each replication, and spray treatments were applied perpendicular to the direction of legume strips. Each plot was 2.1 x 1.5 m. The herbicide treatments were applied on the morning of May 18, 2010 using a CO_2-pressurized backpack sprayer. The boom was fitted with wide angle flat spray tips (TT VP 11001, Teejet Technologies, Wheaton, IL), and pressure was adjusted to approximately 210 kPa. Wind speed and ambient temperature were 6.4 km hr^{-1} and 11.0^{o}C, respectively. Spray treatments included glyphosate applied at 1.0, 1.5, 2.0, and 2.5 kg a.e. ha^{-1}. All rates were applied with 94 L water ha^{-1}. Glyphosate concentrations were adjusted in mix bottles, and ground speed was kept constant.

Weeds were controlled by hand clipping for eight weeks following herbicide application after which mowing was used for weed control. The predominant weeds in the plot area were redroot pigweed (*Amaranthus retroflexus*) and field bindweed (*Convolvulus arvensis).* Weeds were clipped for the first eight weeks to ensure that weed competition did not influence legume recovery. At that point, many treatments had enough legume recovery to suppress weed growth, and it was decided to control weeds only by mowing. This was also done to keep legumes from entering the reproductive stage. The entire plot area was mowed to a cutting height of 10 cm on July 28th and August 11th. Plots were irrigated on a weekly basis starting on June 2, 2010 and continuing for the duration of the study. Dates of irrigation events along with irrigation quantities are listed in table 4.2 below.

Table 4.2. Field study: irrigation timing and quantity in 2009 and 2010.

Date	Irrigation (cm)
2009	
August 19	3.8
August 26	3.8
September 2	3.8
September 8	2.5
Total	13.9
2010	
June 2	2.5
June 10	2.5
June 24	3.2
July 1	3.8
July 8	3.8
July 16	3.8
July 22	3.2
July 29	3.8
August 5	3.2
August 12	3.8
August 19	3.2
August 26	3.2
September 2	3.2
September 9	3.2
Total	46.4

Above ground biomass samples were taken eight weeks after herbicide application. Plot yields were determined by taking a 0.25 m^2 frame from the center of each plot. All plants were clipped to the ground level, weeds were removed, and samples were dried to a constant weight for 72 hours in a forced air oven at 55^{o}C. Dry weights were then recorded. Plot yields were taken again 16 weeks after glyphosate applications using the aforementioned technique. In this case, weeds were separated and dried as well.

Pot Study

The pot study was initially housed in the Colorado State University Greenhouse Facility where daytime and nighttime temperatures were maintained at 24 and 19^{o}C, respectively, and photoperiod was adjusted to 16 hours light and 8 hours dark. Relative humidity was maintained at 67%. Legumes were seeded on November 7, 2009 into 9 x 9 x 13 cm plastic pots filled with potting soil. They were thinned to five plants per pot on November 18, 2009. All plants were checked daily and watered on an as needed basis. On February 4, 2010, legumes were transplanted into larger round pots (16 cm diameter by 16.5 cm deep) filled with field soil. This soil was collected from ARDEC in a field adjacent to the one in which the field study was conducted. Excess potting soil was removed by shaking plants and gently pulling roots apart. Plants were transferred to field soil due to documented differences in efficacy of glyphosate in sterile potting soil vs. unsterile field soil (Schafer et al., 2009). It also offered a greater degree of continuity between the field and pot studies. All legumes were fertilized with 1 g of triple superphosphate per pot on February 15, 2010.

To simulate winter dormancy that these perennial species would undergo in the field, plants were gradually hardened off and moved outside. On February 25, all legumes were clipped to a height of 10 cm and moved from the main greenhouse bay to a ventilation corridor where temperatures varied from 5 to 19^{o}C depending on when ventilation fans were running. One week later, they were moved to a walk-in cooler and maintained at a temperature slightly above freezing (1 to 3^{o}C) with no light. On March 11, plants were moved to a fenced off area adjacent to the university greenhouses where they were exposed to outdoor temperatures and weather conditions. In this area, they were under 50% shade and were allowed to break dormancy under natural seasonal climate change.

Herbicide treatments were applied using a moving nozzle spray chamber equipped with a single nozzle (8001 E Teejet Technologies, Wheaton, IL). Glyphosate was applied at rates of 1.0, 1.5, and 2.0 kg a.e. ha^{-1}. There were three replications of each treatment and three untreated control pots for a total of 12 pots of each species per set. There were two sets of plants with the first set being sprayed on April 20th and the second on April 22nd. Set one was moved outside where ambient temperature was 11.7^{o}C immediately following application. Set two had to be moved back into the greenhouse for 24 hours after spraying due to rain. After this, both sets continued to be kept outside under 50% shade until May 3rd when the shade was reduced to 25%.

Pots were fertilized with 1 g of triple superphosphate on April 26th. Plants were treated with carbaryl (1-naphthyl methylcarbamate) for insect control on June 5th. All actions taken are listed in Table 4.3 below.

Approximately eight weeks after herbicide application, all plants were clipped to ground level, dried to a constant weight for 72 hours in a forced air oven at 55^{o}C, and weighed. Remaining plant roots (and crowns) were washed over a 6.35 mm screen, dried, and weighed. All rhizomes and stolons were included with root/crown material.

Table 4.3. Pot study: actions taken and days after planting.

Days After Planting	Action Taken
0	Planted legumes
11	Thinned to five plants per pot
89	Transplanted into field soil
100	Fertilized plants with triple superphosphate
110	Clipped plants to height of 10 cm and moved to ventilation corridor
117	Moved plants to cooler
124	Moved plants outside (50% shade)
164	Sprayed set 1
166	Sprayed set 2
170	Fertilized plants with triple superphosphate
177	Moved plants to 25% shade
210	Applied carbaryl
226	Harvested set 1
228	Harvested set 2

Statistical Analysis: Field Study

Above ground legume biomass dry weights for the different glyphosate treatments were analyzed as a percent of the untreated control allowing for a comparison between the two sampling dates. Data were analyzed as a strip plot design. PROC MIXED in SAS (SAS Institute, 2009) was first used to determine main effects and interactions of legume species, spray rate, and sampling date. Replication, replication x date, replication x legume, and replication x spray rate were considered to be random. In the event of a three-way interaction, data were separated by sampling date and analyzed to determine main effects and interactions of legume species and spray rate. Differences were recognized as significant at the $P \leq 0.05$ level. When aforementioned effects were found to be significant, treatment means were separated using LSMEANS (SAS Institute, 2009).

Kura clover had to be analyzed separately due to an irregularity in the control treatment. The kura seed was contaminated with red clover seed. The red clover, which has greater seedling vigor (Frame 2005), outcompeted the kura clover in the control treatment causing very low kura clover yields. However, the kura proved to be much more glyphosate tolerant and outcompeted the red clover in all of the spray treatments resulting in little to no red clover contamination. Thus, the four kura treatments were compared on an actual biomass basis rather than as a percent of the untreated control and could not be compared with the other species.

Transformations were performed when an examination of the residuals indicated the need. Square root transformations were performed on both the 8 and 16 week biomass data. The original data were reported, and the transformations were used to determine the differences between treatments.

Within each species for a given sampling date, legume biomass was regressed linearly against spray rate using Microsoft Excel (Microsoft 2007). PROC MIXED was used to determine whether the correlation between legume and spray rate was significant ($p \leq 0.05$).

Statistical Analysis: Pot Study

Final above ground and root/crown biomass for all spray treatments were converted to a percent of the untreated control and analyzed using PROC MIXED to determine main effects of legume species and spray rate. Spray set (one or two depending on the application date), set x legume, and set x legume x spray rate were random effects. Differences were recognized as significant at the $P \leq 0.05$ level. When aforementioned effects were found to be significant, treatment means were separated using LSMEANS. On examination of the legume above ground biomass residuals, a square root transformation was deemed necessary, while the root biomass data did not need require a transformation. Regression analyses were performed on root and shoot biomass using the same methodology as in the field trial.

RESULTS AND DISCUSSION

Field Study

Due to a legume x spray rate x sampling date interaction, the two sampling dates were analyzed separately. The 3-way interaction can be attributed to variable rates of recovery by different legume species with white clover recovering the fastest resulting in no spray rate effect 16 weeks after application. Spray rate effects were present in all other species at both 8 and 16 week sampling dates. Differences among spray treatments in birdsfoot trefoil varied across sampling dates with the 1.5 kg a.e. ha^{-1} rate producing the highest biomass at 8 weeks and the 1.0 kg a.e. ha^{-1} rate yielding the highest at 16 weeks.

Eight weeks after glyphosate application, white clover had the greatest recovery at all rates (Table 4.4). Red clover followed, and alfalfa had the least recovery with all treatments yielding less than 3% of the untreated control biomass. Birdsfoot trefoil also recovered very little averaging 5% of the untreated control across all treatments. However, at the 1.5 and 2.5 kg a.e. ha^{-1} rates, biomass of birdsfoot trefoil did not differ from red clover. The apparent greater recovery of birdsfoot trefoil at some of the higher herbicide rates can be explained by survival of a small number of plants that were randomly distributed within the plot area. This could be due to variable glyphosate tolerance within the population of trefoil plants (Boerboom et al., 1990).

There was a linear decline in biomass of the clover species as spray rate increased (Figures 4.1 and 4.2) with plants recovering more at the lowest application rate than at the two highest rates (Table 4.4). Responses of the two clover species did not significantly differ from one another. No linear relationships were found for trefoil (p=0.1966) or alfalfa (p=0.4619).

Table 4.4 Field study: above-ground legume biomass eight weeks after herbicide application in spring 2010.

Glyphosate application rate (kg a.e. ha^{-1})	Alfalfa	Birdsfoot trefoil	Red clover	White clover	Avg.
	------------------- Biomass relative to control (%) --------------				
1.0	2.7 aC §	5.3 bC	26.5 aB	78.4 aA	28.3
1.5	2.0 abC	11.1 aB	16.5 bB	65.6 abA	23.8
2.0	0.0 bC	0.7 cC	11.5 bB	58.7 bA	17.7
2.5	0.0 bC	2.9 bcB	4.9 cB	49.4 bA	14.3
Avg.	1.2	5.0	14.9	63.0	

§The interaction of legume treatment by annual crop was significant. Herbicide treatment means followed by the same lowercase letter are not different. Legume means followed by the same uppercase letter are not different. Differences were declared significant at the 0.05 probability level.

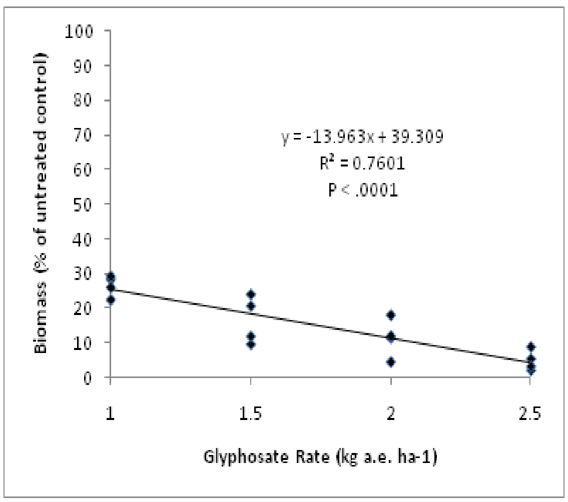

Figure 4.1. Field study: response of red clover biomass (percent of control) to increasing rates of glyphosate eight weeks after application in spring 2010.

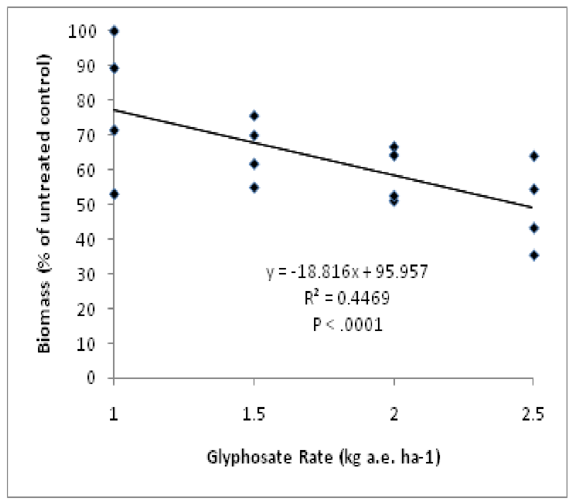

Figure 4.2. Field study: response of white clover biomass (percent of control) to increasing rates of glyphosate eight weeks after application in spring 2010.

An interaction of legume species by spray rate was also present at the 16-week sampling date (Table 4.5). The smallest biomass reductions were found in the clovers with no differences among spray rates for white clover. All treatments were close to 100% of the control indicating full recovery. There was no biomass decline for red clover with increasing spray rate except at the highest rate. Birdsfoot trefoil recovered to about 55% of the control at the lowest rate, but all other treatments were significantly lower.

The response of red clover to increasing glyphosate rate remained linear (Figure 4.3), while white clover had no response (Figure 4.4). Trefoil had a linear response to glyphosate rate ($p<0.0001$) at 16 weeks, which did not differ from that of red clover. There was still no response found for alfalfa ($p=0.1720$).

Table 4.5 Field study: above-ground legume biomass sixteen weeks after herbicide application in spring 2010.

Glyphosate application rate (kg a.e. ha^{-1})	Alfalfa	Birdsfoot trefoil	Red clover	White clover	Avg.
	---------------- Biomass relative to control (%) --------------				
1.0	15.5 aC §	54.7 aB	69.4 aAB	98.1 aA	59.4
1.5	1.0 bC	22.2 bB	66.7 aA	106.3 aA	49.1
2.0	1.4 bD	15.6 bC	57.9 aB	101.6 aA	44.1
2.5	0.1 bC	6.3 cC	29.5 bB	99.5 aA	33.9
Avg.	4.5	24.7	55.9	101.4	

§The interaction of legume treatment by annual crop was significant. Herbicide treatment means followed by the same lowercase letter are not different. Legume means followed by the same uppercase letter are not different. Differences were declared significant at the 0.05 probability level.

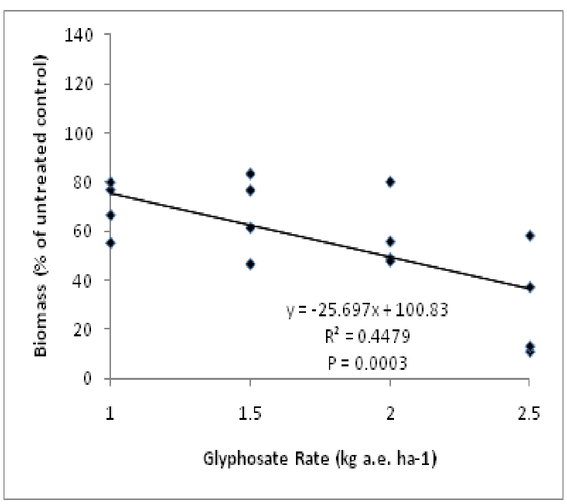

Figure 4.3. Field study: response of red clover biomass (percent of control) to increasing rates of glyphosate sixteen weeks after application in spring 2010.

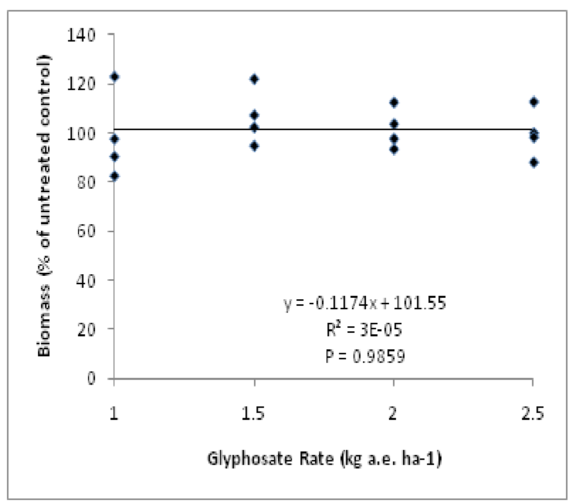

Figure 4.4. Field study: response of white clover biomass (percent of control) to increasing rates of glyphosate sixteen weeks after application in spring 2010.

Based on these results, white clover has great potential as a living mulch that can be suppressed with glyphosate. It was extremely resilient with full recovery at all rates after 16 weeks. Red clover also performed well recovering at the 1.0 and 1.5 kg a.e. ha^{-1} rates to the same extent as white clover. Birdsfoot trefoil appears to have some tolerance, but may not survive higher rates or repeated applications necessary for weed control. However, Boerboom et al. (1990) noted a wide range of glyphosate tolerance among birdsfoot trefoil selections from a recurrent selection breeding program that included 'Leo' birdsfoot trefoil. A threefold difference in the rate of glyphosate required to reduce fresh weight by 50% (I_{50}) was found among nine selections previously tested in the field (Boerboom, 1989). The biomass of these selections ranged from 16 to 54% of an untreated control when evaluated as ramets 14 days after treatment with 0.5 kg ha^{-1} of glyphosate. The two selections of 'Leo' tested ranged from 26 to 45% making them the 2^{nd} and 6^{th} most tolerant among all selections tested. This variable tolerance was attributed to the specific activity of 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS), with which it was positively correlated. Further, Duiker and Hartwig (2004) noted that birdsfoot trefoil tended to respond less to competition with corn than other legume species tested as living mulches indicating that it may have the advantage of greater shade tolerance. Thus, other cultivars of birdsfoot trefoil should be tested as they may be better suited for use as living mulches. Alfalfa has little potential since it recovered to only about 16% of the control at the lowest rate after 16 weeks, while at the higher rates, it yielded less than 2% of the control.

Kura clover is also worth noting in that none of the spray treatments differed in actual biomass accumulation at either the 8 or 16 week sampling dates, indicating no yield loss with increasing rates up to 2.5 kg a.e. ha^{-1}. While there were problems with plant growth in the control that prevented comparisons among other species, the average biomass of kura clover across treatments at 16 weeks was 1,827 kg ha^{-1}. White clover averaged 3,736 kg ha^{-1} across treatments at the same date. Poor yields of kura clover in the first one to two years are well-documented (Frame, 2005) and seedling vigor of kura clover has been shown to be less than that of white clover (Speer and Allinson, 1984). Difficult establishment is the main deterrent in the use of kura clover as a living mulch and may give preference to white clover, a more vigorous species that appears to share kura's tolerance for high rates of glyphosate.

Weeds were controlled only by mowing after the first biomass sampling, and there was an effect of both species and spray rate on weed biomass by the time of the second sampling (Table 4.6). The higher two spray rates had significantly greater weed biomass than the lower two. The legume species with the greatest recovery tended to have fewer weeds with white clover containing less weed biomass than any of the other species. All clover plots had fewer weeds than trefoil and alfalfa. Both the spray rate and species effects can be attributed to an increase in weed biomass as legume biomass decreased. This weed suppressing effect is a commonly cited benefit of living mulches (Enache and Ilnicki, 1990; SAN, 1998) and is illustrated in Figure 4.5. Combined poor legume recovery and higher weed biomass among alfalfa and trefoil, indicate that the clovers are the preferred candidates for a glyphosate-based suppression regime.

When interpreting these findings, one must consider that the use of these species as living mulches will introduce the added stress of competition for light, water, and in some cases, nutrients from the annual crop. Thus, lesser degrees of recovery would be expected than those found in this study.

Table 4.6 Field study: above-ground weed biomass sixteen weeks after herbicide application in spring 2010.

Glyphosate application rate (kg a.e. ha^{-1})	Alfalfa	Birdsfoot trefoil	Kura clover	Red clover	White clover	Avg.
	------------------------DM yield (kg ha^{-1})------------------------					
1.0	2722 §	2059	368	609	50	1201 a †
1.5	2710	2686	680	817	10	1380 a
2.0	3656	3290	959	955	37	1779 b
2.5	4002	3161	1462	1890	29	2109 b
Avg.	3273 C ‡	2799 C	867 B	1068 B	32 A	

§The interaction of legume species by herbicide rate was not significant at the 0.05 probability level.
†Herbicide rate means followed by the same lowercase letter are not different at the 0.05 probability level.
‡ Legume species means followed by the same uppercase letter are not different at the 0.05 probability level.

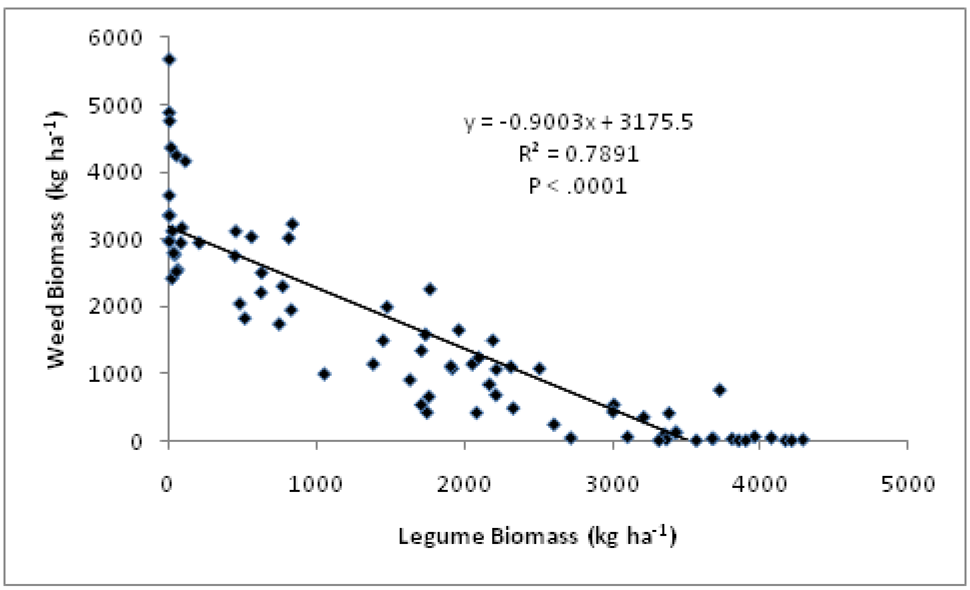

Figure 4.5. Field study: relationship of weed biomass to legume biomass sixteen weeks after herbicide application in spring 2010.

Pot Study

The effects of both legume species and spray rate were found to be significant in the pot study. All species recovered to a greater degree at the 1.0 kg a.e. ha^{-1} rate than the 1.5 and 2.0 kg a.e. ha^{-1} rates in terms of both shoot and root/crown biomass (Tables 4.7

and 4.8). Among legume species, shoot biomass accumulation relative to the untreated control was greatest for kura clover followed by white clover. Alfalfa was again the least resilient averaging 1% of the untreated control biomass across all rates. In this case, red clover recovered very little and did not differ from alfalfa or trefoil.

Table 4.7 Pot study: above-ground legume biomass eight weeks after herbicide application in spring 2010.

Glyphosate application rate (kg a.e. ha^{-1})	Alfalfa	Birdsfoot trefoil	Kura clover	Red clover	White clover	Avg.
	------------------- Biomass relative to control (%) -----------------					
1.0	2.2 §	9.4	26.3	3.1	13.5	10.9 a †
1.5	0.4	2.1	18.0	1.0	4.2	5.1 b
2.0	0.5	2.9	12.6	1.2	2.2	3.9 b
Avg.	1.1 D ‡	4.8 C	19.0 A	1.8 CD	6.7 B	

§The interaction of legume species by herbicide rate was not significant at the 0.05 probability level.
†Herbicide rate means followed by the same lowercase letter are not different at the 0.05 probability level.
‡ Legume species means followed by the same uppercase letter are not different at the 0.05 probability level.

Table 4.8 Pot study: legume root/crown biomass eight weeks after herbicide application in spring 2010.

Glyphosate application rate (kg a.e. ha^{-1})	Alfalfa	Birdsfoot trefoil	Kura clover	Red clover	White clover	Avg.
	------------------ Biomass relative to control (%) ----------------					
1.0	22.4 §	27.7	35.4	23.9	21.2	26.1 a †
1.5	13.8	18.6	29.8	15.3	9.3	17.4 b
2.0	17.3	17.4	26.7	16.6	6.2	16.8 b
Avg.	17.8 ‡	21.2	30.6	18.6	12.2	

§The interaction of legume species by herbicide rate was not significant at the 0.05 probability level.
†Herbicide rate means followed by the same lowercase letter are not different at the 0.05 probability level.
‡ The effect of legume species was not significant at the 0.05 probability level (p=0.1225).

While the effect of spray rate on root development was similar to the effect on shoot biomass, there were no differences found in root biomass among species (Table 4.8). This could be a result of the limited rooting area provided in the pots. Upon washing legume roots, it was noted that the control treatments of some of the species, particularly red clover, appeared to be severely root-bound. This limitation to rooting area could help explain the poor performance of red clover in the pot relative to the field.

The results of the pot study confirmed the resilience of white and kura clover relative to the other species tested. The poor performance of red clover must be balanced against its superior recovery in the field. Birdsfoot trefoil recovery relative to other species was better in the pot study. It was superior to that of alfalfa and did not differ from red clover. As in the field study, alfalfa appeared to have very little potential for use as a living mulch managed with glyphosate due to its lack of recovery at all rates.

The effect of spray rate was much more consistent across species in the pot study, most likely due to limitations in root development. Thus, the sharp drop in biomass at the 1.5 kg a.e. ha^{-1} cannot be used as an indication of appropriate spray rate. More relevant is the consistency between field and pot above ground biomass results (with the exception of red clover) in terms of relative species recovery. Based on results from both studies, white and kura clover show the greatest potential for use as living mulches.

CONCLUSION

Most living mulch suppression regimes include herbicide(s) applied at a sub-lethal rate. This is necessary to reduce competition with the annual crop from both the mulch crop and weeds. Recovery after herbicide application is essential to the persistence of living mulches. White clover was the most tolerant to glyphosate in the field. Although kura clover could not be compared among species, no effects of glyphosate rate were found on recovery indicating a very high level of tolerance. In the pot study, kura clover had the greatest tolerance followed by white clover. Birdsfoot trefoil and alfalfa recovery was poor in both the field and pot trials. Red clover recovered well in the field, but did not differ from trefoil or alfalfa in the pot study. While recovery is important to the success of living mulches, exceptionally high herbicide tolerance can lead to inadequate suppression and subsequent annual crop yield losses. Based on visual evaluations, none of the white clover treatments had fallen below 80% of the control by the third week after application (Figure D-3.5), which would be insufficient for planting of an annual crop. Thus, while the focus of this study was to determine relative glyphosate tolerance, additional research is needed to test clover species, particularly white clover, as mulches with various annual crops. Adequate suppression may require the inclusion of herbicides other than glyphosate.

LITERATURE CITED

Affeldt, R.P., K.A. Albrecht, C.M. Boerboom, and E.J. Bures. 2004. Integrating herbicide-resistant corn technology in a kura clover living mulch system. Agron. J. 96:247-251.

Boerboom, C. M. 1989. Selection and characterization of glyphosate tolerance in birdsfoot trefoil (*Lotus corniculatus*). PhD. Thesis, Univ. Minnesota. 67 pp.

Boerboom, C.M., D.L. Wyse, and D.A. Somers. 1990. Mechanism of glyphosate tolerance in birdsfoot trefoil (*Lotus corniculatus*). Weed Sci. 38:463-467.

Echtenkamp, G.W. and R.S. Moomaw. 1989. No-till corn production in a living mulch system. Weed Technology. 3:261-266.

Duiker, S.W. and N.L. Hartwig. 2004. Living mulches of legumes in imidazolinone-resistant corn. Agron. J. 96:1-21-1028.

Enache, A.J. and R.D. Ilnicki. 1990. Weed control by subterranean clover (*Trifolium subterranium*) used as a living mulch. Weed Technol. 4:534-538.

Frame, J. 2005. Forage legumes for temperate grasslands. Science Publishers, Inc., Enfield, NH.

Hartwig, N.L. 2004. Soil and production enhancing cover crop and living mulch systems. Pennsylvania State University. Ext., State College, PA.

Hartwig, N.L. and H.U. Ammon. 2002. Cover crops and living mulches. Weed Science. 50:688-699.

Ochsner, T.E., K.A. Albrecht, T.W. Schumacher, J.M. Baker, and R.J. Berkevich. 2010. Water balance and nitrate leaching under corn in a kura clover living mulch. Agron. J. 102:1169-1178.

Paine, L.K., and Harrison. 1993. The historical roots of living mulches and related practices. Hort.Technology 3:137-143.

SAS Institute. 2009. SAS version 9.2. SAS Inst., Cary, NC.

Sawyer, J.E., P. Pedersen, D.W. Barker, D.A. Ruiz Diaz, and K.A. Albrecht. 2010 Intercropping corn and kura clover: response to nitrogen fertilization. Agron. J. 102:568-574.

Schafer, J.R., A.M. Westhoven, G.R. Kruger, V.M. Davis, S.G. Hallett and W.G. Johnson, 2009. Effect of growth media on common lambsquarters and giant ragweed biotypes response to glyphosate. 2009 North Central Weed Science Society Proceedings 64: 102.

Seguin, P., C.C. Schaeffer, N.J. Ehlke, M.P. Russelle, and P.H. Graham. 2001. Nitrogen fixation and rhizobial inoculation effects on kura clover growth. Agron J. 93:1262-1268.

Singer, J.W., K.A. Kohler, K.J. Moore, and D.W. Meek. 2010. Living mulch forage yield and botanical composition in a corn-soybean-forage-rotation. Agron. J. 101:1249-1257.

Singer, J.W. and P. Pedersen. 2005. Legume living mulches in corn and soybean. PM-2006. Iowa State Univ. Ext., Ames, IA.

Speer G.S. and G.W. Allinson. 1985. Kura clover (*Trifolium ambiguum*): legume for forage and soil conservation. Econ. Bot. 39:165-176.

Sustainable Agriculture Network. 1998. Managing cover crops profitably. San handbook. Ser. Book 3. SAN, Beltsville, Maryland.

White, J.G. and T.W. Scott. 1991. Effects of perennial forage-legume living mulches on no-till winter wheat and rye. Field Crops Res., 28:135-148

Zemenchik, R.A., K.A. Albrecht, C.M. Boerboom, and J.G. Lauer. 2000. Corn production with kura clover as a living mulch. Agron. J. 92:698-705.

APPENDIX A-1: CO-ESTABLISHMENT OF LEGUMINOUS LIVING MULCHES WITH ANNUAL CROPS-FIELD MAPS

Rep 1	Rep2	Rep3	Rep 4
Plot 4 Oat (SD) WC	Plot 5 Oat (SD) BFT	Plot 12 Oat (SD) BFT	Plot 13 Oat (SD) WC
Plot 3 Oat (SD) MIX	Plot 6 Oat (SD) MIX	Plot 11 Oat (SD) WC	Plot 14 Oat (SD) BFT
Plot 2 Oat (SD) BFT	Plot 7 Oat (SD) CLEAN	Plot 10 Oat (SD) CLEAN	Plot 15 Oat (SD) MIX
Plot 1 Oat (SD) CLEAN	Plot 8 Oat (SD) WC	Plot 9 Oat (SD) MIX	Plot 16 Oat (SD) CLEAN

Rep 1	Rep2	Rep3	Rep 4
Plot 20 Oat (B) CLEAN	Plot 21 Oat (B) WC	Plot 28 Oat (B) CLEAN	Plot 29 Oat (B) MIX
Plot 19 Oat (B) MIX	Plot 22 Oat (B) MIX	Plot 27 Oat (B) BFT	Plot 30 Oat (B) BFT
Plot 18 Oat (B) BFT	Plot 23 Oat (B) BFT	Plot 26 Oat (B) WC	Plot 31 Oat (B) WC
Plot 17 Oat (B) WC	Plot 24 Oat (B) CLEAN	Plot 25 Oat (B) MIX	Plot 32 Oat (B) CLEAN

Rep 1	Rep2	Rep3	Rep 4
Plot 36 Corn BFT	Plot 37 Corn BFT	Plot 44 Corn MIX	Plot 45 Corn CLEAN
Plot 35 Corn MIX	Plot 38 Corn MIX	Plot 43 Corn WC	Plot 46 Corn WC
Plot 34 Corn CLEAN	Plot 39 Corn WC	Plot 42 Corn BFT	Plot 47 Corn MIX
Plot 33 Corn WC	Plot 40 Corn CLEAN	Plot 41 Corn CLEAN	Plot 48 Corn BFT

Abbrieviations	
WC	White Clover
MIX	Red Clover, White Clover, Kura Clover
BFT	Birdsfoot Trefoil
Clean	No Legume
(B)	Oats harvested at the boot stage
(SD)	Oats harvested at the soft dough stage

	N	
W		E
	S	

Plot Dimensions: 4.6m x 10.7 m

Figure A-1.1 Sprinkler site: 2009 field map.

Rep 1	Plot 8 Oats BFT	Plot 9 Oats MIX	Plot 24 Oats CLEAN	Plot 25 Oats WC	Plot 40 Oats MIX
	Plot 7 Oats WC	Plot 10 Oats BFT	Plot 23 Oats MIX	Plot 26 Oats BFT	Plot 39 Oats WC
Rep 2	Plot 6 Oats BFT	Plot 11 Oats WC	Plot 22 Oats BFT	Plot 27 Oats CLEAN	Plot 38 Oats WC
	Plot 5 Oats MIX	Plot 12 Oats MIX	Plot 21 Oats BFT	Plot 28 Oats WC	Plot 37 Oats MIX
Rep 3	Plot 4 Oats BFT	Plot 13 Oats MIX	Plot 20 Oats MIX	Plot 29 Oats WC	Plot 36 Oats BFT
	Plot 3 Oats BFT	Plot 14 Oats MIX	Plot 19 Oats WC	Plot 30 Oats WC	Plot 35 Oats CLEAN
Rep 4	Plot 2 Oats MIX	Plot 15 Oats WC	Plot 18 Oats BFT	Plot 31 Oats BFT	Plot 34 Oats WC
	Plot 1 Oats CLEAN	Plot 16 Oats MIX	Plot 17 Oats WC	Plot 32 Oats MIX	Plot 33 Oats BFT

Abrieviations	
WC	White Clover
MIX	Red Clover, White Clover, Kura Clover
BFT	Birdsfoot Trefoil
Clean	No Legume
(SD)	Oats harvested at the soft dough stage

N
W E
S

Plot Dimensions: 4.6m x 10.7 m

Figure A-1.2 Furrow irrigated site: 2009 field map.

APPENDIX A-2: CO-ESTABLISHMENT OF LEGUMINOUS LIVING MULCHES WITH ANNUAL CROPS-ANALYSIS OF VARIANCE TABLES

Table A-2.1. Analysis of variance for dry matter yield of early-cut oat hay at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	0.06	0.9782

Table A-2.2. Analysis of variance for NDF of early-cut oat hay at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	0.85	0.4999

Table A-2.3. Analysis of variance for ADF of early-cut oat hay at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	0.13	0.9421

Table A-2.4. Analysis of variance for CP of early-cut oat hay at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	0.93	0.4642

Table A-2.5. Analysis of variance for contribution of weeds to total yield of early-cut oat hay at the sprinkler irrigated site in 2009: square root transformed.

	Degrees of freedom	F value	P value
legume	3	6.04	0.0154

Table A-2.6. Analysis of variance for dry matter yield of late-cut oat hay at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	2.74	0.1057

Table A-2.7. Analysis of variance for NDF of late-cut oat hay at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	1.66	0.2433

Table A-2.8. Analysis of variance for ADF of late-cut oat hay at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	1.54	0.2708

Table A-2.9. Analysis of variance for CP of late-cut oat hay at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	1.11	0.3949

Table A-2.10. Analysis of variance for contribution of weeds to total yield of late-cut oat hay at the sprinkler irrigated site in 2009: square root transformed.

	Degrees of freedom	F value	P value
legume	3	2.21	0.2078

Table A-2.11. Analysis of variance for dry matter yield of corn silage at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	0.11	0.9506

Table A-2.12. Analysis of variance for NDF of corn silage at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	0.26	0.8529

Table A-2.13. Analysis of variance for ADF of corn silage at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	0.59	0.6361

Table A-2.14. Analysis of variance for CP of corn silage at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	0.47	0.7131

Table A-2.15. Analysis of variance for contribution of weeds to total yield of corn silage at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	0.92	0.4694

Table A-2.16. Analysis of variance for contribution of legumes to total yield of corn silage at the sprinkler irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	8.61	0.0173

Table A-2.17. Analysis of variance for legume establishment ratings at sprinkler irrigated site in spring 2010.

	Degrees of freedom	F value	P value
crop	2	29.08	<.0001
legume	2	0.55	0.5858
crop * legume	4	4.98	0.0043

Table A-2.18. Analysis of variance for spring legume biomass at sprinkler irrigated site in spring of 2010: square root transformed.

	Degrees of freedom	F value	P value
crop	2	11.73	0.0031
legume	2	9.09	0.0019
crop * legume	4	1.20	0.3446

Table A-2.19. Analysis of variance for vole damage at sprinkler irrigated site in spring 2010: square root transformed.

	Degrees of freedom	F value	P value
crop	2	9.80	0.0006
legume	2	7.03	0.0035
crop * legume	4	0.95	0.4480

Table A-2.20. Analysis of variance for weed biomass at sprinkler irrigated site in spring 2010: square root transformed.

	Degrees of freedom	F value	P value
crop	2	20.96	<.0001
legume	3	2.70	0.0600
crop * legume	4	2.72	0.0278

Table A-2.21. Analysis of variance for dry matter yield of oats at the furrow irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	0.26	0.8518

Table A-2.22. Analysis of variance for NDF of oats at the furrow irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	0.72	0.5659

Table A-2.23. Analysis of variance for ADF of oats at the furrow irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	1.31	0.3295

Table A-2.24. Analysis of variance for CP of oats at the furrow irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	0.60	0.6298

Table A-2.25. Analysis of variance for contribution of weeds to total yield of oats at the furrow irrigated site in 2009.

	Degrees of freedom	F value	P value
legume	3	0.45	0.7230

Table A-2.26 Analysis of variance for legume establishment ratings at furrow irrigated site in spring 2010.

	Degrees of freedom	F value	P value
legume	2	9.72	0.0131

Table A-2.27. Analysis of variance for legume biomass at furrow irrigated site in spring 2010.

	Degrees of freedom	F value	P value
legume	2	3.03	0.1233

Table A-2.28. Analysis of variance for weed biomass at furrow irrigated site in spring 2010.

	Degrees of freedom	F value	P value
legume	3	0.47	0.7074

APPENDIX B-1: CORN AND SOYBEAN PRODUCTION IN A LEGUMINOUS LIVING MULCH SYSTEM-FIELD MAPS

	Corn Silage			Soybeans			Corn Grain		
REP 4	Plot 409 ALF 84 kg N ha-1	Plot 408 BFT+RC 84 kg N ha-1	Plot 407 BFT 84 kg N ha-1	Plot 409 WC 0 kg N ha-1	Plot 408 0 kg N ha-1	Plot 407 RC 0 kg N ha-1	Plot 409 84 kg N ha-1	Plot 408 168 kg N ha-1	Plot 407 252 kg N ha-1
	Plot 406 252 kg N ha-1	Plot 405 84 kg N ha-1	Plot 404 0 kg N ha-1	Plot 406 0 kg N ha-1	Plot 405 0 kg N ha-1	Plot 404 0 kg N ha-1	Plot 406 RC 84 kg N ha-1	Plot 405 0 kg N ha-1	Plot 404 BFT 84 kg N ha-1
	Plot 403 WC 84 kg N ha-1	Plot 402 RC 84 kg N ha-1	Plot 401 168 kg N ha-1	Plot 403 BFT+RC 0 kg N ha-1	Plot 402 ALF 0 kg N ha-1	Plot 401 BFT 0 kg N ha-1	Plot 403 BFT+RC 84 kg N ha-1	Plot 402 ALF 84 kg N ha-1	Plot 401 WC 84 kg N ha-1
REP 3	Plot 309 168 kg N ha-1	Plot 308 WC 84 kg N ha-1	Plot 307 BFT 84 kg N ha-1	Plot 309 0 kg N ha-1	Plot 308 0 kg N ha-1	Plot 307 0 kg N ha-1	Plot 309 168 kg N ha-1	Plot 308 0 kg N ha-1	Plot 307 BFT+RC 84 kg N ha-1
	Plot 306 RC 84 kg N ha-1	Plot 305 0 kg N ha-1	Plot 304 252 kg N ha-1	Plot 306 0 kg N ha-1	Plot 305 BFT+RC 0 kg N ha-1	Plot 304 RC 0 kg N ha-1	Plot 306 BFT 84 kg N ha-1	Plot 305 ALF 84 kg N ha-1	Plot 304 84 kg N ha-1
	Plot 303 84 kg N ha-1	Plot 302 BFT+RC 84 kg N ha-1	Plot 301 ALF 84 kg N ha-1	Plot 303 BFT 0 kg N ha-1	Plot 302 ALF 0 kg N ha-1	Plot 301 WC 0 kg N ha-1	Plot 303 252 kg N ha-1	Plot 302 WC 84 kg N ha-1	Plot 301 RC 84 kg N ha-1
REP 2	Plot 209 84 kg N ha-1	Plot 208 BFT+RC 84 kg N ha-1	Plot 207 BFT 84 kg N ha-1	Plot 209 0 kg N ha-1	Plot 208 BFT+RC 0 kg N ha-1	Plot 207 ALF 0 kg N ha-1	Plot 209 252 kg N ha-1	Plot 208 84 kg N ha-1	Plot 207 168 kg N ha-1
	Plot 206 168 kg N ha-1	Plot 205 WC 84 kg N ha-1	Plot 204 0 kg N ha-1	Plot 206 BFT 0 kg N ha-1	Plot 205 WC 0 kg N ha-1	Plot 204 0 kg N ha-1	Plot 206 RC 84 kg N ha-1	Plot 205 BFT+RC 84 kg N ha-1	Plot 204 BFT 84 kg N ha-1
	Plot 203 ALF 84 kg N ha-1	Plot 202 RC 84 kg N ha-1	Plot 201 252 kg N ha-1	Plot 203 0 kg N ha-1	Plot 202 0 kg N ha-1	Plot 201 RC 0 kg N ha-1	Plot 203 ALF 84 kg N ha-1	Plot 202 0 kg N ha-1	Plot 201 WC 84 kg N ha-1
REP 1	Plot 109 BFT+RC 84 kg N ha-1	Plot 108 252 kg N ha-1	Plot 107 BFT 84 kg N ha-1	Plot 109 BFT 0 kg N ha-1	Plot 108 RC 0 kg N ha-1	Plot 107 0 kg N ha-1	Plot 109 168 kg N ha-1	Plot 108 0 kg N ha-1	Plot 107 WC 84 kg N ha-1
	Plot 106 84 kg N ha-1	Plot 105 RC 84 kg N ha-1	Plot 104 168 kg N ha-1	Plot 106 ALF 0 kg N ha-1	Plot 105 0 kg N ha-1	Plot 104 0 kg N ha-1	Plot 106 BFT 84 kg N ha-1	Plot 105 ALF 84 kg N ha-1	Plot 104 252 kg N ha-1
	Plot 103 0 kg N ha-1	Plot 102 WC 84 kg N ha-1	Plot 101 ALF 84 kg N ha-1	Plot 103 0 kg N ha-1	Plot 102 BFT+RC 0 kg N ha-1	Plot 101 WC 0 kg N ha-1	Plot 103 RC 84 kg N ha-1	Plot 102 84 kg N ha-1	Plot 101 BFT+RC 84 kg N ha-1

Abbreviations	
ALF	Alfalfa
WC	White Clover
RC	Red Clover
BFT	Birdsfoot Trefoil

White: Legume treatment
Grey: Fertility treatment

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Plot Dimensions: 4.6 m x 15.2 m

Figure B-1.1 Fruita: 2009 Field map.

APPENDIX B-2: CORN AND SOYBEAN PRODUCTION IN A LEGUMINOUS LIVING MULCH SYSTEM-ANALYSIS OF VARIANCE TABLES

Table B-2.1. Analysis of variance for soil NH_4^+ concentrations from 0 to 91 cm at Fruita, CO in spring 2009.

	Degrees of freedom	F value	P value
treat	8	2.58	0.0200
crop	1	43.67	0.0071
crop*treat	8	0.84	0.5764
depth	2	40.46	<.0001
treat*depth	16	1.06	0.4069
crop*depth	2	72.09	<.0001
crop*treat*depth	16	0.75	0.7392

Table B-2.2. Analysis of variance for soil NO_3^- concentrations from 0 to 91 cm at Fruita, CO in spring 2009.

	Degrees of freedom	F value	P value
treat	8	1.42	0.2139
crop	1	0.01	0.9232
crop*treat	8	0.76	0.6359
depth	2	133.22	<.0001
treat*depth	16	1.36	0.1776
crop*depth	2	4.17	0.0180
crop*treat*depth	16	0.46	0.9600

Table B-2.3. Analysis of variance for SOM concentrations from 0 to 91 cm at Fruita, CO in spring 2009.

	Degrees of freedom	F value	P value
treat	8	1.17	0.3379
crop	1	0.10	0.7694
crop*treat	8	0.41	0.9115
depth	2	344.35	<.0001
treat*depth	16	0.86	0.6166
crop*depth	2	92.11	<.0001
crop*treat*depth	16	0.44	0.9694

Table B-2.4. Analysis of variance for living mulch stand ratings at Fruita, CO in spring 2009.

	Degrees of freedom	F value	P value
crop	2	1.78	0.2231
legume	4	57.42	<.0001
crop*legume	8	1.05	0.4209

Table B-2.5. Analysis of variance for living mulch biomass at Fruita, CO in spring 2009.

	Degrees of freedom	F value	P value
legume	4	7.69	0.0026

Table B-2.6. Analysis of variance for living mulch NDF at Fruita, CO in spring 2009.

	Degrees of freedom	F value	P value
legume	4	3.44	0.0430

Table B-2.7. Analysis of variance for living mulch ADF at Fruita, CO in spring 2009.

	Degrees of freedom	F value	P value
legume	4	0.97	0.4588

Table B-2.8. Analysis of variance for living mulch CP at Fruita, CO in spring 2009.

	Degrees of freedom	F value	P value
legume	4	15.38	0.0001

Table B-2.9. Analysis of variance for soybean yields at Fruita, CO in 2009.

	Degrees of freedom	F value	P value
treat	5	1.29	0.2983

Table B-2.10. Analysis of variance for corn silage yields at Fruita, CO in 2009.

	Degrees of freedom	F value	P value
treat	8	17.56	<.0001

Table B-2.11. Analysis of variance for corn silage NDF at Fruita, CO in 2009.

	Degrees of freedom	F value	P value
treat	8	12.33	<.0001

Table B-2.12. Analysis of variance for corn silage ADF at Fruita, CO in 2009.

	Degrees of freedom	F value	P value
treat	8	4.62	0.0016

Table B-2.13. Analysis of variance for corn silage CP at Fruita, CO in 2009.

	Degrees of freedom	F value	P value
treat	8	6.63	0.0001

Table B-2.14. Analysis of variance for corn grain yields at Fruita, CO in 2009: log transformed.

	Degrees of freedom	F value	P value
treat	8	58.45	<.0001

Table B-2.15. Analysis of variance for corn grain leaf residue at Fruita, CO in fall 2009: square root transformed.

	Degrees of freedom	F value	P value
treat	8	5.45	0.0006

Table B-2.16. Analysis of variance for stem residue after corn grain harvest at Fruita, CO in fall 2009.

	Degrees of freedom	F value	P value
treat	8	5.25	0.0007

Table B-2.17. Analysis of variance for cob residue after corn grain harvest at Fruita, CO in fall 2009.

	Degrees of freedom	F value	P value
treat	8	2.16	0.0696

Table B-2.18. Analysis of variance for total corn residue after corn grain harvest at Fruita, CO in fall 2009.

	Degrees of freedom	F value	P value
treat	8	5.73	0.0004

Table B-2.19. Analysis of variance for legume residue after corn grain harvest at Fruita, CO in fall 2009: square root transformed.

	Degrees of freedom	F value	P value
treat	8	7.19	0.0034

Table B-2.20. Analysis of variance for legume mortality ratings at Fruita, CO in spring 2010.

	Degrees of freedom	F value	P value
crop	2	11.29	0.0001
legume	4	60.55	<.0001
crop * legume	8	1.58	0.1568

APPENDIX C-1: CHEMICAL SUPPRESSION OF ESTABLISHED LEGUMINOUS LIVING MULCHES-FIELD MAPS

Rep 1			
Plot 4 WC Gly Rate 3	Plot 5 BFT Gly Rate 1	Plot 12 BFT BD	Plot 13 WC Gly Rate 2
Plot 3 MIX Gly Rate 3	Plot 6 MIX Gly Rate 1	Plot 11 WC BD	Plot 14 BFT Gly Rate 2
Plot 2 BFT Gly Rate 3	Plot 7 CLEAN Gly Rate 1	Plot 10 CLEAN BD	Plot 15 MIX Gly Rate 2
Plot 1 CLEAN Gly Rate 3	Plot 8 WC Gly Rate 1	Plot 9 MIX BD	Plot 16 CLEAN Gly Rate 2

Rep 2			
Plot 20 CLEAN Gly Rate 2	Plot 21 WC BD	Plot 28 CLEAN Gly Rate 3	Plot 29 MIX Gly Rate 1
Plot 19 MIX Gly Rate 2	Plot 22 MIX BD	Plot 27 BFT Gly Rate 3	Plot 30 BFT Gly Rate 1
Plot 18 BFT Gly Rate 2	Plot 23 BFT BD	Plot 26 WC Gly Rate 3	Plot 31 WC Gly Rate 1
Plot 17 WC Gly Rate 2	Plot 24 CLEAN BD	Plot 25 MIX Gly Rate 3	Plot 32 CLEAN Gly Rate 1

Rep 3			
Plot 36 BFT BD	Plot 37 BFT Gly Rate 3	Plot 44 MIX Gly Rate 2	Plot 45 CLEAN Gly Rate 1
Plot 35 MIX BD	Plot 38 MIX Gly Rate 3	Plot 43 WC Gly Rate 2	Plot 46 WC Gly Rate 1
Plot 34 CLEAN BD	Plot 39 WC Gly Rate 3	Plot 42 BFT Gly Rate 2	Plot 47 MIX Gly Rate 1
Plot 33 WC BD	Plot 40 CLEAN Gly Rate 3	Plot 41 CLEAN Gly Rate 2	Plot 48 BFT Gly Rate 1

Abbrieviations	
WC	White Clover
MIX	Red Clover, White Clover, Kura Clover
BFT	Birdsfoot Trefoil
Clean	No Legume
Gly Rate 1	Glyphosate: 1.0 kg a.e. ha-1
Gly Rate 2	Glyphosate: 1.5 kg a.e. ha-1
Gly Rate 3	Glyphosate: 2.0 kg a.e. ha-1
BD	Burn Down-Paraquat: 0.7 kg a.i ha-1

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Plot Dimensions: 4.6m x 10.7 m

Figure C-1.1 Sprinkler irrigated site: 2010 field map.

Rep 1	Plot 8 BFT Gly Rate 3	Plot 9 MIX Gly Rate 1	Plot24 CLEAN Gly Rate 3	Plot 25 WC Gly Rate 1	Plot 40 MIX Gly Rate 3
	Plot 7 WC Gly Rate 2	Plot 10 BFT Gly Rate 1	Plot 23 MIX Gly Rate 2	Plot 26 BFT Gly Rate 2	Plot 39 WC Gly Rate 3
Rep 2	Plot 6 BFT Gly Rate 2	Plot 11 WC Gly Rate 3	Plot 22 BFT Gly Rate 1	Plot 27 CLEAN	Plot 38 WC Gly Rate 1
	Plot 5 MIX Gly Rate 2	Plot 12 MIX Gly Rate 1	Plot 21 BFT Gly Rate 3	Plot 28 WC Gly Rate 2	Plot 37 MIX Gly Rate 3
Rep 3	Plot 4 BFT Gly Rate 2	Plot 13 MIX Gly Rate 2	Plot 20 MIX Gly Rate 3	Plot 29 WC Gly Rate 2	Plot 36 BFT Gly Rate 1
	Plot 3 BFT Gly Rate 3	Plot 14 MIX Gly Rate 1	Plot 19 WC Gly Rate 1	Plot 30 WC Gly Rate 3	Plot 35 CLEAN Gly Rate 3
Rep 4	Plot 2 MIX Gly Rate 2	Plot 15 WC Gly Rate 1	Plot 18 BFT Gly Rate 1	Plot 31 BFT Gly Rate 2	Plot 34 WC Gly Rate 3
	Plot 1 CLEAN Gly Rate 3	Plot 16 MIX Gly Rate 3	Plot 17 WC Gly Rate 2	Plot 32 MIX Gly Rate 1	Plot 33 BFT Gly Rate 3

Abbrieviations	
WC	White Clover
MIX	Red Clover, White Clover, Kura Clover
BFT	Birdsfoot Trefoil
Clean	No Legume
Gly Rate 1	Glyphosate: 1.0 kg a.e. ha-1
Gly Rate 2	Glyphosate: 1.5 kg a.e. ha-1
Gly Rate 3	Glyphosate: 2.0 kg a.e. ha-1

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Plot Dimensions: 4.6m x 10.7 m

Figure C-1.2 Furrow irrigated site: 2010 field map.

APPENDIX C-2: CHEMICAL SUPPRESSION OF ESTABLISHED LEGUMINOUS LIVING MULCHES-ANALYSIS OF VARIANCE TABLES

Table C-2.1. Analysis of variance for corn grain yield at the sprinkler irrigated site in 2010.

	Degrees of freedom	F value	P value
suppression	3	3.30	0.0993
legume	3	3.50	0.0309
suppression * legume	9	2.31	0.0490

Table C-2.2. Analysis of variance for corn leaf residue at the sprinkler irrigated site in fall 2010.

	Degrees of freedom	F value	P value
suppression	3	1.86	0.2370
legume	3	2.57	0.0782
suppression * legume	9	2.50	0.0353

Table C-2.3. Analysis of variance for corn stem residue at the sprinkler irrigated site in fall 2010.

	Degrees of freedom	F value	P value
suppression	3	1.58	0.2891
legume	3	1.25	0.3136
suppression * legume	9	2.25	0.0549

Table C-2.4. Analysis of variance for total corn residue at the sprinkler irrigated site in fall 2010.

	Degrees of freedom	F value	P value
suppression	3	1.64	0.2763
legume	3	1.61	0.2132
suppression * legume	9	3.82	0.0041

Table C-2.5. Analysis of variance legume biomass at the sprinkler irrigated site in fall 2010: square root transformed.

	Degrees of freedom	F value	P value
suppression	3	1.62	0.2111
legume	3	17.51	<.0001
suppression * legume	9	4.80	0.0024

Table C-2.6. Analysis of variance for weed biomass at the sprinkler irrigated site in fall 2010: square root transformed.

	Degrees of freedom	F value	P value
suppression	3	1.89	0.1511
legume	3	3.84	0.0187
suppression * legume	9	1.95	0.0799

Table C-2.7. Analysis of variance for corn grain yields at the furrow irrigated site in 2010.

	Degrees of freedom	F value	P value
suppression	2	2.94	0.0719
legume	2	4.71	0.0188
suppression * legume	4	0.12	0.9752

Table C-2.8. Analysis of variance for corn grain yields at the furrow irrigated site in 2010-Dunnett's test.

	Degrees of freedom	F value	P value
treatment	9	2.57	0.0281

Table C-2.9. Analysis of variance for corn leaf residue at the furrow irrigated site in fall 2010.

	Degrees of freedom	F value	P value
suppression	2	1.73	0.1994
legume	2	0.06	0.9464
suppression * legume	4	0.43	0.7857

Table C-2.10. Analysis of variance for corn leaf residue at the furrow irrigated site in fall 2010-Dunnett's test.

	Degrees of freedom	F value	P value
treatment	9	0.57	0.8070

Table C-2.11. Analysis of variance for corn stem residue at the furrow irrigated site in fall 2010: square root transformed.

	Degrees of freedom	F value	P value
suppression	2	1.32	0.2864
legume	2	0.93	0.4078
suppression * legume	4	0.24	0.9143

Table C-2.12. Analysis of variance for corn stem residue at the furrow irrigated site in fall 2010: square root transformed-Dunnett's test.

	Degrees of freedom	F value	P value
treatment	9	0.63	0.7643

Table C-2.13. Analysis of variance for total corn residue at the furrow irrigated site in fall 2010: log transformed.

	Degrees of freedom	F value	P value
suppression	2	3.03	0.0669
legume	2	0.34	0.7145
suppression * legume	4	0.96	0.4490

Table C-2.14. Analysis of variance for total corn residue at the furrow irrigated site in fall 2010: log transformed-Dunnett's test.

	Degrees of freedom	F value	P value
treatment	9	1.03	0.4405

Table C-2.15. Analysis of variance for legume biomass at the furrow irrigated site in fall 2010: square root transformed.

	Degrees of freedom	F value	P value
suppression	2	0.38	0.6905
legume	2	45.17	<.0001
suppression * legume	4	0.98	0.4392

Table C-2.16. Analysis of variance for weed biomass at the furrow irrigated site in fall 2010: square root transformed.

	Degrees of freedom	F value	P value
suppression	2	1.61	0.2200
legume	2	0.38	0.6883
suppression * legume	4	0.48	0.7477

Table C-2.17. Analysis of variance for weed biomass at the furrow irrigated site in fall 2010: square root transformed -Dunnett's test.

	Degrees of freedom	F value	P value
treatment	9	0.61	0.7671

APPENDIX D-1: RECOVERY OF POTENTIAL LEGUMINOUS LIVING MULCHES AFTER SPRING GLYPHOSATE APPLICATION-FIELD MAP

Rep 1	Plot 1 ALF	Plot 40 KC	Plot 41 BFT	Plot 80 WC	Plot 81 RC	Glyphosate Rate 4
	Plot 2 ALF	Plot 39 KC	Plot 42 BFT	Plot 79 WC	Plot 82 RC	Glyphosate Rate 1
	Plot 3 ALF	Plot 38 KC	Plot 43 BFT	Plot 78 WC	Plot 83 RC	0
	Plot 4 ALF	Plot 37 KC	Plot 44 BFT	Plot 77 WC	Plot 84 RC	Glyphosate Rate 2
	Plot 5 ALF	Plot 36 KC	Plot 45 BFT	Plot 76 WC	Plot 85 RC	Glyphosate Rate 3
Rep 2	Plot 6 WC	Plot 35 ALF	Plot 46 BFT	Plot 75 KC	Plot 86 RC	Glyphosate Rate 3
	Plot 7 WC	Plot 34 ALF	Plot 47 BFT	Plot 74 KC	Plot 87 RC	Glyphosate Rate 4
	Plot 8 WC	Plot 33 ALF	Plot 48 BFT	Plot 73 KC	Plot 88 RC	Glyphosate Rate 2
	Plot 9 WC	Plot 32 ALF	Plot 49 BFT	Plot 72 KC	Plot 89 RC	0
	Plot 10 WC	Plot 31 ALF	Plot 50 BFT	Plot 71 KC	Plot 90 RC	Glyphosate Rate 1
Rep 3	Plot 11 KC	Plot 30 RC	Plot 51 WC	Plot 70 BFT	Plot 91 ALF	0
	Plot 12 KC	Plot 29 RC	Plot 52 WC	Plot 69 BFT	Plot 92 ALF	Glyphosate Rate 4
	Plot 13 KC	Plot 28 RC	Plot 53 WC	Plot 68 BFT	Plot 93 ALF	Glyphosate Rate 2
	Plot 14 KC	Plot 27 RC	Plot 54 WC	Plot 67 BFT	Plot 94 ALF	Glyphosate Rate 3
	Plot 15 KC	Plot 26 RC	Plot 55 WC	Plot 66 BFT	Plot 95 ALF	Glyphosate Rate 1
Rep 4	Plot 16 BFT	Plot 25 RC	Plot 56 KC	Plot 65 WC	Plot 96 ALF	0
	Plot 17 BFT	Plot 24 RC	Plot 57 KC	Plot 64 WC	Plot 97 ALF	Glyphosate Rate 3
	Plot 18 BFT	Plot 23 RC	Plot 58 KC	Plot 63 WC	Plot 98 ALF	Glyphosate Rate 2
	Plot 19 BFT	Plot 22 RC	Plot 59 KC	Plot 62 WC	Plot 99 ALF	Glyphosate Rate 4
	Plot 20 BFT	Plot 21 RC	Plot 60 KC	Plot 61 WC	Plot 100 ALF	Glyphosate Rate 1

Abbreviation	
WC	White Clover
RC	Red Clover
BFT	Birdsfoot Trefoil
KC	Kura Clover
ALF	Alfalfa
Glyphosate Rate 1	1.0 kg a.e. ha-1
Glyphosate Rate 2	1.5 kg a.e. ha-1
Glyphosate Rate 3	2.0 kg a.e. ha-1
Glyphosate Rate 4	2.5 kg a.e. ha-1

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Plot Dimensions: 2.1 m x 1.5 m

Figure D-1.1 Field study: 2010 field map.

APPENDIX D-2: RECOVERY OF POTENTIAL LEGUMINOUS LIVING MULCHES AFTER SPRING GLYPHOSATE APPLICATION-ANALYSIS OF VARIANCE TABLES

Table D-2.1. Analysis of variance for above-ground legume biomass (percent of control) of field herbicide rate study sampled eight and sixteen weeks after herbicide application in spring 2010: square root transformed.

	Degrees of freedom	F value	P value
date	1	33.35	0.0019
legume	3	93.30	<.0001
date *legume	3	10.34	<.0001
sprayRate	3	29.49	<.0001
date * sprayRate	3	3.15	0.0293
legume * sprayRate	9	2.25	0.0270
date * legume * sprayRate	9	3.45	0.0012

Table D-2.2. Analysis of variance for above-ground legume biomass (percent of control) of field herbicide rate study sampled eight weeks after herbicide application in spring 2010: square root transformed.

	Degrees of freedom	F value	P value
legume	3	164.63	<.0001
sprayRate	3	15.68	0.0004
legume * sprayRate	9	2.55	0.0285

Table D-2.3. Analysis of variance for above-ground legume biomass (percent of control) of field herbicide rate study sampled sixteen weeks after herbicide application in spring 2010: square root transformed.

	Degrees of freedom	F value	P value
legume	3	43.02	<.0001
sprayRate	3	16.90	<.0001
legume * sprayRate	9	3.35	0.0045

Table D-2.4. Analysis of variance for above-ground kura clover biomass of field herbicide rate study sampled eight and sixteen weeks after herbicide application in spring 2010.

	Degrees of freedom	F value	P value
date	1	66.32	<.0001
sprayRate	3	1.99	0.1855
date * sprayRate	3	0.22	0.8812

Table D-2.5. Analysis of variance for above-ground weed biomass of field herbicide rate study sampled sixteen weeks after herbicide application in spring 2010.

	Degrees of freedom	F value	P value
legume	4	66.27	<.0001
sprayRate	3	9.29	<.0001
legume * sprayRate	12	1.05	0.4208

Table D-2.6. Analysis of variance for above-ground legume biomass (percent of control) of pot herbicide rate study sampled eight weeks after herbicide application in spring 2010: square root transformed.

	Degrees of freedom	F value	P value
Legume	4	52.95	<.0001
sprayRate	2	31.82	<.0001
legume * sprayRate	8	1.46	0.1859

Table D-2.7. Analysis of variance for legume root/crown biomass (percent of control) of pot herbicide rate study sampled eight weeks after herbicide application in spring 2010.

	Degrees of freedom	F value	P value
Legume	4	3.11	0.1225
sprayRate	2	16.62	<.0001
legume * sprayRate	8	0.54	0.8187

APPENDIX D-3: RECOVERY OF POTENTIAL LEGUMINOUS LIVING MULCHES AFTER SPRING GLYPHOSATE APPLICATION-VISUAL EVALUATIONS OF HERBICIDE TREATMENTS

Table D-3.1. Rating scale for visual evaluations of legumes in pot and field studies in 2010.

Rating	Description
100	No visible damage
95	Slight wilting or chlorosis
90	Severe wilting, >50% of leaves are chlorotic OR 10% loss of green biomass relative to a untreated control
80	Most leaf tissue is chlorotic, severe desiccation, some necrosis OR 20% loss of green biomass relative to a untreated control
70	30% loss of green biomass relative to a untreated control
60	40% loss of green biomass relative to a untreated control
50	50% loss of green biomass relative to a untreated control
40	60% loss of green biomass relative to a untreated control
30	70% loss of green biomass relative to a untreated control
20	80% loss of green biomass relative to a untreated control
10	90% loss of green biomass relative to a untreated control
0	Plant has no green biomass and appears completely dead.

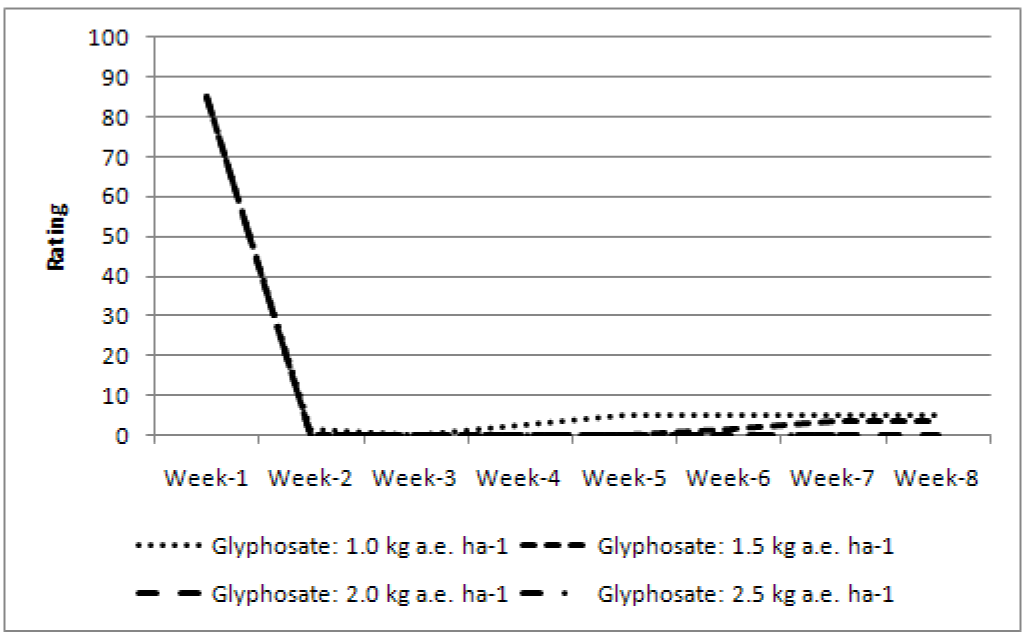

Figure D-3.1 Field herbicide rate study: visual ratings of alfalfa over eight weeks following glyphosate application in spring 2010.

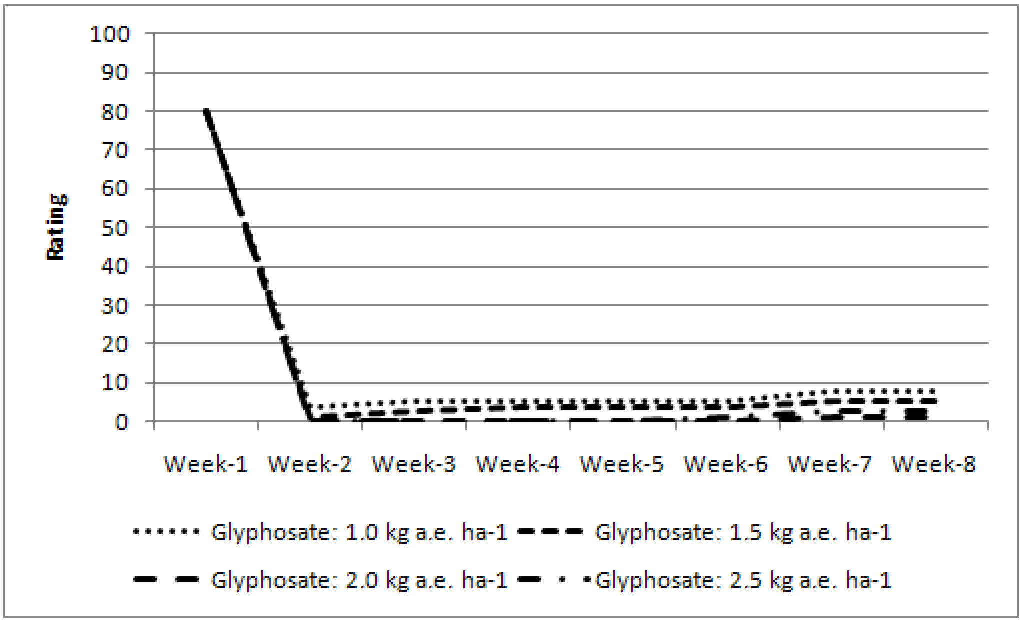

Figure D-3.2 Field herbicide rate study: visual ratings of birdsfoot trefoil over eight weeks following glyphosate application in spring 2010.

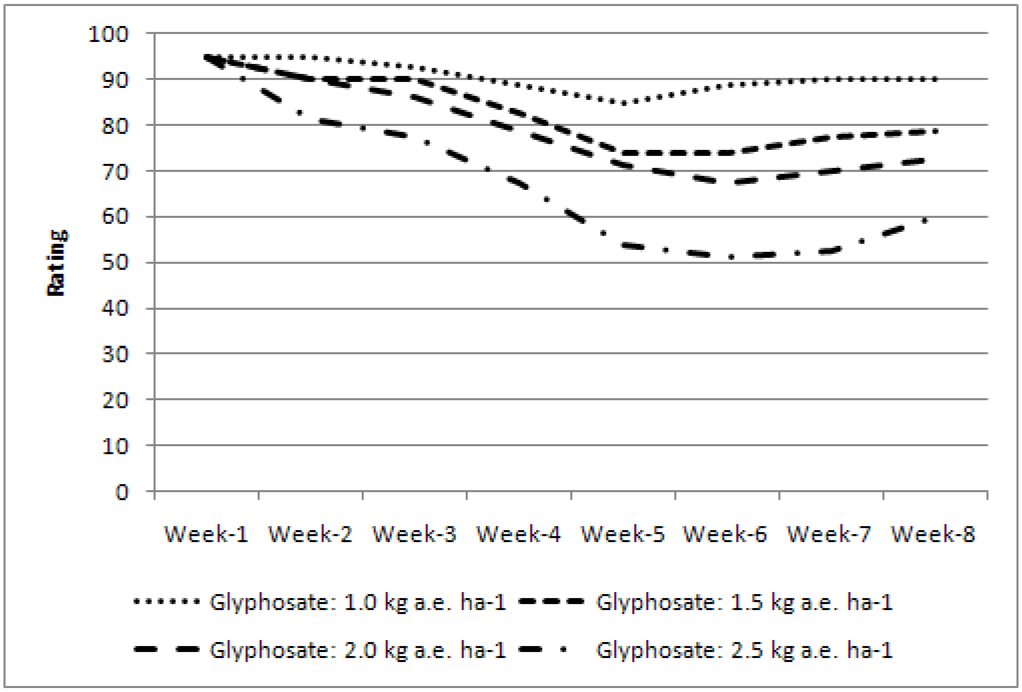

Figure D-3.3 Field herbicide rate study: visual ratings of kura clover over eight weeks following glyphosate application in spring 2010.

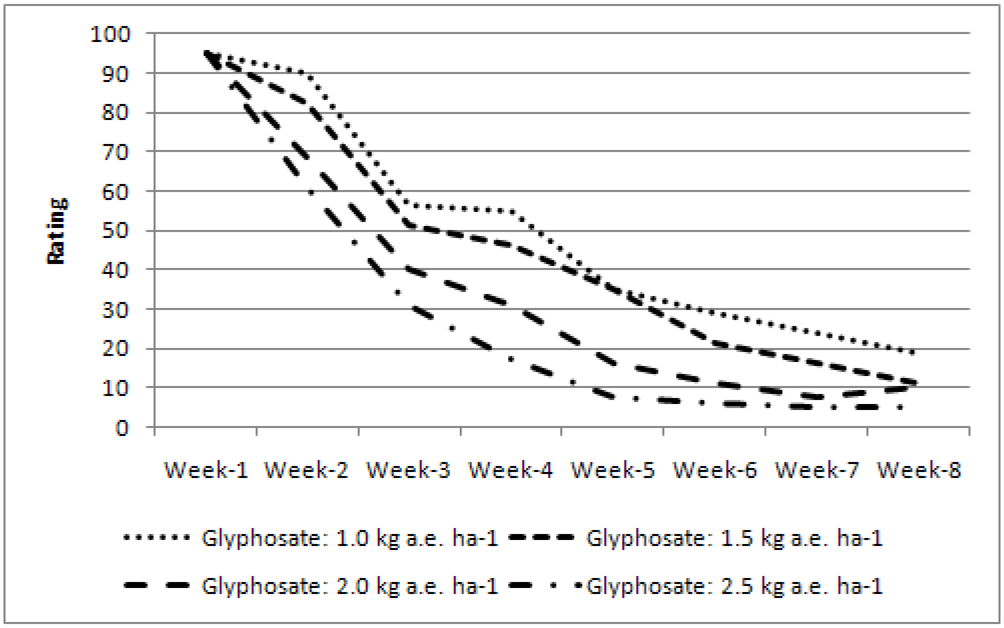

Figure D-3.4 Field herbicide rate study: visual ratings of red clover over eight weeks following glyphosate application in spring 2010.

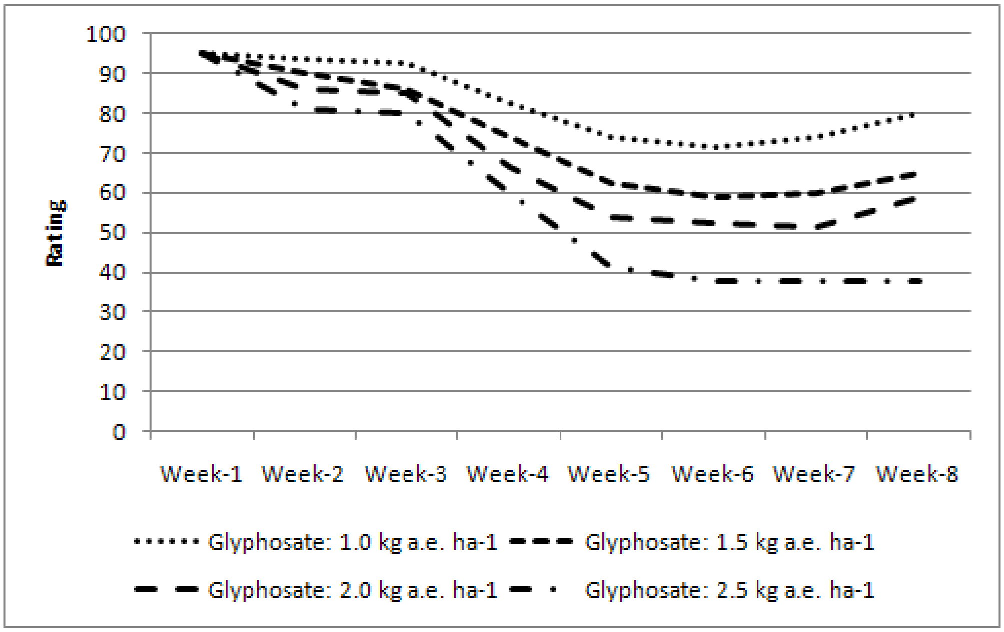

Figure D-3.5 Field herbicide rate study: visual ratings of white clover over eight weeks following glyphosate application in spring 2010.

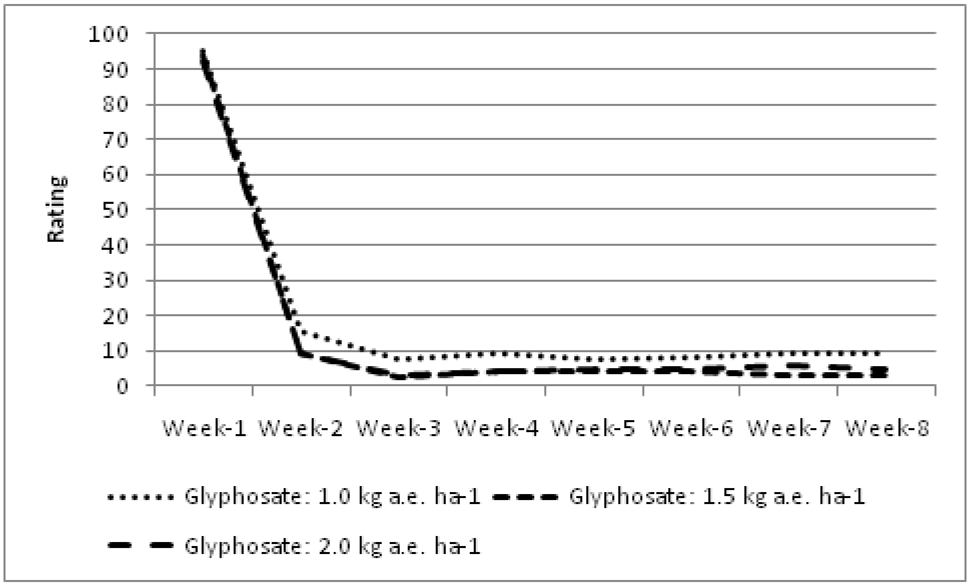

Figure D-3.6 Greenhouse herbicide rate study: visual ratings of alfalfa over eight weeks following glyphosate application in spring 2010.

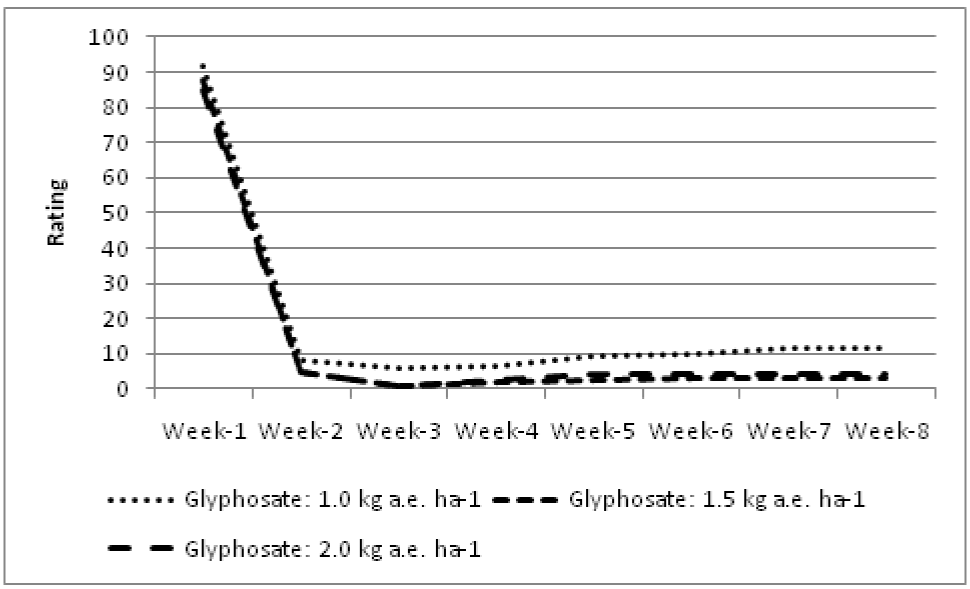

Figure D-3.7 Greenhouse herbicide rate study: visual ratings of birdsfoot trefoil over eight weeks following glyphosate application in spring 2010.

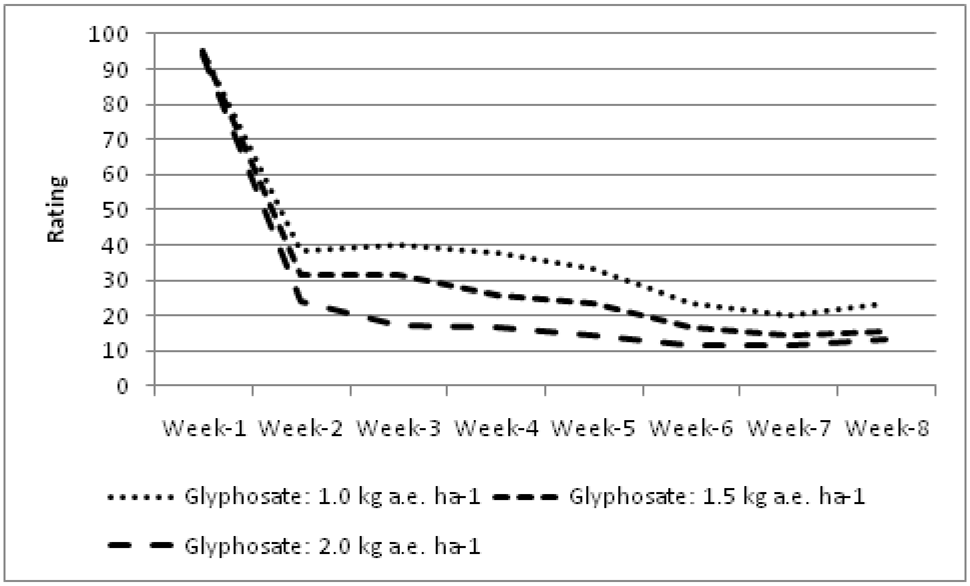

Figure D-3.8 Greenhouse herbicide rate study: visual ratings of kura clover over eight weeks following glyphosate application in spring 2010.

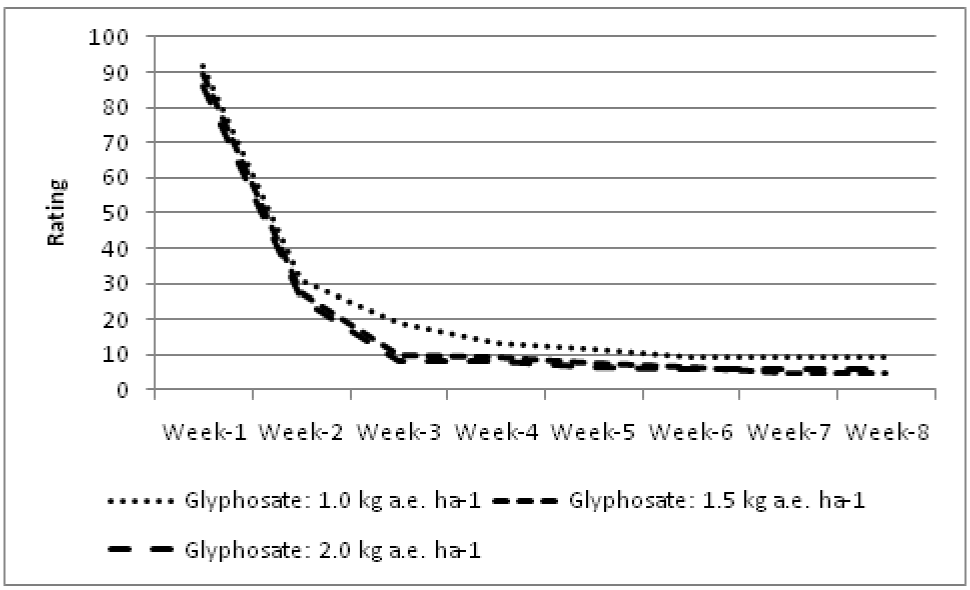

Figure D-3.9 Greenhouse herbicide rate study: visual ratings of red clover over eight weeks following glyphosate application in spring 2010.

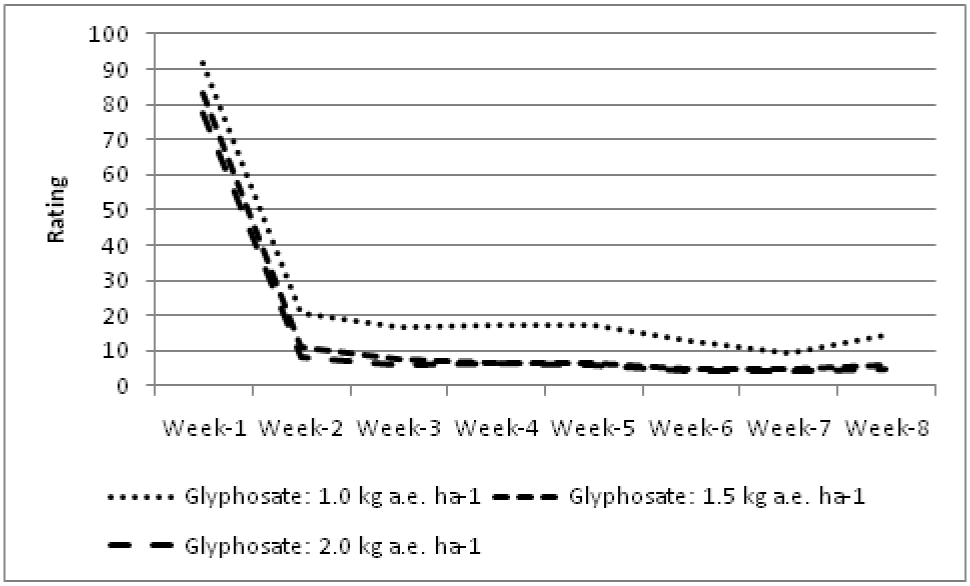

Figure D-3.10 Greenhouse herbicide rate study: visual ratings of white clover over eight weeks following glyphosate application in spring 2010.

APPENDIX D-4: RECOVERY OF POTENTIAL LEGUMINOUS LIVING MULCHES AFTER SPRING GLYPHOSATE APPLICATION-LINEAR REGRESSIONS

Table D-4.1. Linear regressions of above-ground legume biomass (percent of control) from field study eight weeks after herbicide application in spring 2010.

	Degrees of freedom	T value	P value
alfalfa	44	-0.74	0.4619
birdsfoot trefoil	44	-1.31	0.1966
red clover	44	-5.14	<.0001
white clover	44	-6.93	<.0001

Table D-4.2. Linear regressions of above-ground legume biomass (percent of control) from field study sixteen weeks after herbicide application in spring 2010.

	Degrees of freedom	T value	P value
alfalfa	44	-1.39	0.1720
birdsfoot trefoil	44	-4.59	<.0001
red clover	44	-3.89	0.0003
white clover	44	-0.02	0.9859

Table D-4.3. Linear regressions of above-ground legume biomass (percent of control) from pot study eight weeks after herbicide application in spring 2010.

	Degrees of freedom	T value	P value
alfalfa	78	-0.64	0.5224
birdsfoot trefoil	78	-2.44	0.0171
kura clover	78	-5.12	<.0001
red clover	78	-0.70	0.4851
white clover	78	-4.21	<.0001

Table D-4.4. Linear regressions of legume root/crown biomass (percent of control) from pot study eight weeks after herbicide application in spring 2010.

	Degrees of freedom	T value	P value
alfalfa	78	-1.12	0.2661
birdsfoot trefoil	78	-2.21	0.0299
kura clover	78	-1.88	0.0632
red clover	78	-1.57	0.1195
white clover	78	-3.24	0.0017