

LIVING MULCH: ITS USE IN REDUCING PHYTOPHTHORA BLIGHT  
DAMAGE TO BELL PEPPER, SUPPRESSING WEEDS, AND THE  
EFFECTIVENESS OF USING TWO-SPECIES MIXTURES VERSUS  
MONOCULTURE.

A Thesis

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## ABSTRACT

Polythene-mulched beds, used for weed control in the production of high-value horticultural crops, often have bare soil-alleyways between them, which allows soil-borne diseases to splash onto aboveground plant-parts. Living mulch species, broadcast-sown, in monoculture, in two-species mixtures, mowed and unmowed, were grown in the alleyways between polythene-mulched beds of 'Revolution' bell peppers in a *Phytophthora* blight-inoculated field in NY. Pepper yield and disease-incidence were not affected by the presence of living mulch compared to a bare soil control. Annual ryegrass, annual ryegrass-Dutch white clover mix (both sowed at 50% recommended seeding rate) and teff were effective in suppressing weeds. Mowing reduces living mulch height and with a suitable species it increases living mulch groundcover and biomass; thus helping suppress weeds. Combining two living mulch species at reduced seeding rates results in >20% more efficient land-use than monoculture-cropping and provides equally effective weed control.

## BIOGRAPHICAL SKETCH

Edward James Miles first learned an appreciation for plants in the gardens of his parents and grandmother in the city of Stoke-on-Trent, in England. A change in job-direction from architecture to horticulture was the beginning of his successful career. Edward has been involved in horticulture for more than 13 years; either through employment, as a student, or a combination of the two. His career has included time in Yorkshire, England, working for the Royal Horticultural Society, in North Wales and at the Royal Botanic Garden Edinburgh and Scottish Agricultural College. During his time in Scotland studying for his B.Sc. in Horticulture with Plantsmanship he was accepted as a summer intern at a field research station in the Chiquibul rainforest, Belize, studying tropical tree seedlings and mycorrhizal associations. While as an undergraduate student at the Welsh College of Horticulture he was employed as supervisor of a 24 acre stock-free, organic, commercial farm growing fruits, vegetables, herbs and ornamental plants.

Edward was awarded the Martin McLaren Horticultural Scholarship/ Garden Club of America Interchange Fellowship in 2007, allowing him the opportunity to study at an American university for the first year of a graduate program. Having chosen Cornell University and been accepted to the Department of Horticulture Edward arrived in July 2008. During his time at the university he was elected as co-president of the department's student organization, as chair of the graduate and professional student assembly mental wellness subcommittee, and served as the department's graduate student representative at the student assembly and on the department's seminar scheduling committee.

In January 2012 Edward began employment with Nunhems, USA Inc. as the assistant processing tomato breeder; based in Acampo, California.

**“Knowing ignorance is strength.  
Ignoring knowledge is sickness.”**

Lao Tzu. c.500BC. The Tao Te Ching. Verse 71.

(From G.F. Feng and J. English translation)

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## Chapter 1: Literature review

Intensive vegetable production incurs high costs, leading growers to seek out cultural practices to reduce costs, maximize marketable yield, and extend the growing season. Plasticulture is one such cultural practice and is widely used with high-value horticultural crops, such as bell pepper, that require repeat hand-harvesting.

Mulching beds with polyethylene, into which a crop is transplanted provides economic and practical advantages to the grower. Between these polythene-mulched beds are alleyways that require management throughout the growing-season for controlling weeds and soil compaction. These areas may harbor diseases; some of which are soil-borne and can be spread to aerial parts of crop plants through soil and water splashing during rainfall or overhead irrigation. One such disease is Phytophthora Blight caused by *Phytophthora capsici* Leon..

Polyethylene mulch can reduce soil splash in the immediate vicinity of the crop. Having mulch covering the soil of the alleyway could further reduce splashing and also reduce disease spread. Living mulch, a subcategory of cover crops, grown between polyethylene-mulched beds may reduce the incidence of aboveground symptoms of Phytophthora blight on bell pepper plants and reduce weed groundcover density without affecting pepper yield.

This paper will review literature pertaining to the current understanding of fresh market bell pepper production, *Phytophthora capsici*, the use of polythene-mulch, and the living mulch system. While this study will explore the broad understanding of these four topics it is intended for the primary focus to be on living mulch.

We begin by describing an important culinary crop that is used throughout the world: bell pepper (*Capsicum annuum* L.). With its origins of domestication in Central and Southern America, when grown in temperate regions of the world, bell pepper is a warm season annual crop, whereas in tropical regions it may be cultivated as an herbaceous perennial (Univ. of Kentucky, College of Agr. Coop. Ext. Serv., 2010; Univ. of Georgia, College of Agr. & Environ. Sci., Coop. Ext., 2009). In 2010 the total economic value of bell peppers to US agriculture was \$637 million dollars from a total harvested area of 21,326 hectares (52,700 acres) and a fresh weight of 713,910 metric tons (786,951 short tons). Bell pepper is ranked as the sixth most valuable fresh market crop in the top 24 vegetable crops grown in the US (U.S. Department of Agr. and Nat. Agr. Statistics Service, 2011).

Although the bell pepper is grown for both fresh and processing markets, most are grown for fresh market (Penn State College of Agr. Sci., Agr. Res. and Coop. Ext., 2000). The fruit are mostly hand-harvested for fresh market, and typically this takes place at mature green stage, before the fruit is ripe, because of a higher percentage of marketable fruit being guaranteed at this stage of maturity than when it is riper. Fruit are boxed in bushel cartons; between 12.7 kg (28lbs) and 13.6 kg (30lbs) (Univ. of Kentucky, College of Agr. Coop. Ext. Serv., 2010).

Bell pepper cultivars differ in horticultural characteristics such as fruit size, shape, number of lobes, flavor and disease resistance (Univ. of Kentucky, College of Agr. Coop. Ext. Serv., 2010). Most commercial cultivars grown in the US are from hybrid seed, which are expensive and therefore require specific germination conditions to ensure the most cost effective use of the seed. Typically for field production, plants are raised in greenhouses in multi-celled trays at 26.7 °C (80 °F), hardened off, then transplanted into raised, plastic-mulched beds when transplants

are between 10 cm (4 inches) and 15 cm (6 inches) tall (Lamont, Jr., 1996; Univ. of Georgia, College of Agr. & Environ. Sci., Coop. Ext., 2009).

For highest and earliest yield of bell pepper fruit it is recommended to plant transplants in a double-row because these produce more fruit by number and weight than single rows (Locascio and Stall, 1982; Hutton and Handley, 2007), although if the market demands large fruit (U.S. No. 1 and Fancy grade), single-row spacing is recommended (Khan and Leskovar, 2006). In-row spacing also effects fruit size. Twelve inch in-row spacing yielded 25-30% more fruit than from pepper plants at 23 cm (9 inches) spacing (Locascio and Stall, 1982). Thirty centimeters (12 inches) in-row spacing is often used, whereas 45 cm (18 inches) spacing will result in larger fruit size (Univ. of Kentucky, College of Agr. Coop. Ext. Serv., 2010). As population density of bell pepper plants in a field increases, the vegetative plant weight and fruit yield declines on a per-plant basis, but increases on a per-land basis. However, different population densities do not significantly differ in terms of fruit size or average weight, according to a study by Gaye et al. (1992).

Windbreaks, every four or more beds apart, are suggested for increasing yield of field-produced bell peppers, although results from their use have been variable (Univ. of Georgia, College of Agr. & Environ. Sci., Coop. Ext., 2009; Monette and Stewart, 1987). More consistent increase in marketable yield is achieved through planting into black plastic mulched beds. Pepper plants grown through plastic mulch yielded significantly more U.S. No. 1 grade fruit, had higher total yield, and accumulated a greater total amount of N in shoots, immature fruit and harvested fruit than plants grown in bare soil, with no mulch (Monette and Stewart, 1987; Locascio et al., 1985). There has been minimal adoption of reduced tillage cultivation for commercial bell pepper production due to weeds and diseases being difficult to control and therefore limiting

viability of the production system (Univ. of Georgia, College of Agr. & Environ. Sci., Coop. Ext., 2009).

Fruit set and weight is affected by temperature and humidity (Bakker, 1989a; Bakker, 1989b). High daytime humidity improves fruit set and high nighttime humidity increases average fruit weight. There is a positive correlation between increase in daily air temperature amplitude and increase in incidence of bud abortion. A high mean air temperature leads to a decrease in fruit growth period, and a low mean air temperature delays flowering. Optimal fruit-set takes place with nighttime temperatures of 14 °C -16 °C (57 °F – 61 °F) (Rylski and Spigelman, 1982). Fruit that set and developed below this temperature were deformed, and increasing nighttime temperature significantly increased blossom drop.

Bell pepper plants require deep, fertile, well-drained soil with good water-holding capacity, and a pH of 5.8 to 6.6 (Berke et al., 1999; Univ. of Kentucky, College of Agr. Coop. Ext. Serv., 2010; Penn State College of Agr. Sci., Agr. Res. and Coop. Ext. 2000). In good soil pepper plant roots can grow to between 91 cm (36 inches) and 122 cm (48 inches) deep (Univ. of Georgia, College of Agr. & Environ. Sci., Coop. Ext., 2009), but as the study by Gough (2001) showed, 40% of bell pepper plant roots are in the upper 5 cm (2 inches) of soil and 70% in the upper 10 cm (4 inches) of soil. Being as the plants are shallow-rooted and therefore intolerant of flooding or drought, peppers should not be planted in a field that is slow to drain after heavy rain, in low-lying parts of fields or close to bodies of water due to increased risk of flooding and disease incidence (Berke et al., 1999; Univ. of Kentucky, College of Agr. Coop. Ext. Serv., 2010). Drip or furrow irrigation is recommended. Do not use overhead irrigation, as wet leaves, stems and fruit are more likely to succumb to disease infection and splashing increases spread of disease around the field (Berke et al., 1999).

*Phytophthora capsici* causes the disease Phytophthora blight, also known as Phytophthora crown and root rot and Phytophthora fruit rot, and is reported in North, Central and South America, Europe and Asia (Gevens et al., 2008a). Significant economic losses can occur from the effects of Phytophthora blight. Hausbeck and Lamour (2004) estimated that ¼ of vegetables grown in Michigan alone were highly susceptible to Phytophthora blight. The cost to a single farm of a losing a single crop to this disease can amount to hundreds of thousands of dollars. Growers of Midwest bell peppers have reported losses of up to 100% of their fields due to Phytophthora blight (Walters et al., 2007).

This pathogen has a broad host range that includes crop plants and field weeds. Some vegetable crops from the Solanaceae, Cucurbitaceae, Chenopodiaceae, Brassicaceae and Leguminosae families are susceptible (Gevens et al., 2008a; Gevens et al., 2008b; Tian and Babadoost, 2004). A pathogenicity study of 36 vegetable crop species identified 22 species that became symptomatic for *P. capsici*; with cucurbits and peppers being the most susceptible hosts (Tian and Babadoost, 2004). Other crop plants reportedly susceptible include cacao, chayote, marigold, macadamia nut, papaya and Fraser fir (Gevens et al., 2008a; Quesada-Ocampo et al., 2009). This latter crop is particularly significant as it is often grown in rotation on vegetable farms in northern states of North America.

Weeds may be alternate hosts and therefore a significant mode for *P. capsici* survival between growing seasons. It can survive as a weak root pathogen or as a root colonizer on some weeds, such as common purslane, velvetleaf, American black nightshade and Carolina geranium (Ploetz and Haynes, 2000; Tian and Babadoost, 2004; French-Monar et al., 2006).

Phytophthora blight was first discovered on Chile peppers by Leon Leonian in 1918, and it was from peppers that he successfully isolated the pathogen one year after his discovery (Leonian,

1922). Despite much having been learnt about this pathosystem, Leonian's initial description of the symptoms he observed remain accurate; a small, water-soaked, dull green spot or elongated lesions that occur most frequently at the stem-end or the blossom-end of fruit, and when lesions form on stems they may girdle the stem, killing the plant tissue above that point of the plant, while other parts of the plant remain healthy.

The pathogen can infect every part of the pepper plant, causing root and crown rot, stem and leaf lesions, and fruit rot (Ristaino, 2003; Ristaino and Johnston, 1999). Current technology allows us to identify the pathogen through morphological characteristics, molecular methods and through isolation on semi-selective media by direct or dilution plating.

*Phytophthora capsici* reproduces sexually and asexually and may be polycyclic within seasons. The heterothallic pathogen produces compatible mating types, A1 and A2, which are often present in the same field in similar quantities. When in close proximity and in response to each other's released hormones, gametangia differentiation takes place. The oogonium (female gametangium) grows through the opposite mating types amphigynous antheridium (male gametangium), whereupon, following meiosis, plasmogamy and karyogamy result in the formation of a circular oospore (Lamour and Hausbeck, 2000).

Asexual reproduction occurs through the formation of an ovoid sporangium, with a pronounced papilla at its apex, borne at the tip of a branched sporangiophore. Sporangia are formed in large amounts and are easily released. On one naturally-infected spaghetti squash fruit there was an estimated 44 million deciduous sporangia (Hausbeck and Lamour, 2004). Clones do not survive temperate winters whereas the thick wall of an oospore, containing  $\beta$ -glucan and cellulose, enables it to overwinter, survive for at least 5 years in the soil and serve as a significant source

of inoculum (Lamour and Hausbeck, 2002; Lamour and Hausbeck, 2003; Ristaino, 2003; Lamour and Hausbeck, 2000; Babadoost et al., 2008; Hausbeck and Lamour, 2004).

Primary infection of a healthy pepper plant is most commonly caused by an inoculum source in the soil that causes root infection, which then spreads to the crown of the plant. This initial infection becomes the center from which the disease spreads outward (Ristaino and Johnston, 1999; Sujkowski et al., 2000; Ristaino, 2003). Primary mechanisms for infection include

- growing roots come into contact with inoculum in the soil,
- growth, or movement in the case of zoospores, of the inoculum until it is in contact with plant roots, and
- plant root-to-root contact transferring inoculum from infected to healthy plant roots, most often within a row.

Bowers et al. (1990) showed that plant-to-plant spread of the pathogen from the primary source point was within a row before across rows and between beds and Ristaino et al. (1997) identified that this occurs primarily due to root-to-root contact. Growth, and movement, towards plant roots shows that *P. capsici* inoculum chemotactically follows nutrient gradients (Hausbeck and Lamour, 2004).

Phytophthora species are oomycetes, commonly referred to as water molds, and are more closely related to algae than to true fungi (Hausbeck and Lamour, 2004). *Phytophthora capsici*'s pathogenicity and epidemiology depend greatly on free water in the growing environment, as is highlighted by three of the following secondary mechanisms for infection being associated with water:

- splash dispersal,

- surface water movement ,
- use of infested water for irrigation, and
- poor hygiene of workers and equipment in the field transferring the disease.

Oospores of *P. capsici* germinate in two ways: directly through the formation of a germ tube, or indirectly through the formation of sporangia on germ tubes (Ristaino and Johnston, 1999; Ristaino, 2003). Ninety-four percent of oospores germinate indirectly and this can occur within 5 days of favorable conditions, which are oospores being in soil, in the dark, at 24 °C (75 °F), and cyclic soil moisture changes (Hord and Ristaino, 1991). Constant soil saturation inhibits oospore germination (Ristaino and Johnston, 1999). Periodic flooding results in greater bell pepper mortality from Phytophthora blight than at constant soil-water matric potentials. At constant soil-water matric potentials of -2.5 kPa and -12.5 kPa, one of 15 and zero bell pepper plants, respectively, became infected with Phytophthora blight. One, two, or three, twenty-four hour-long flooding occurrences during a 10 day period resulted in mortality rates of 20%, 53% and 100% respectively (Bowers and Mitchell, 1990).

When immersed in free moisture each sporangium germinates indirectly through it differentiating and releasing between 20 and 40 motile, bi-flagellate zoospores through the papilla (Hausbeck and Lamour, 2004; Ristaino and Johnston, 1999; Ristaino, 2003). Zoospores are highly effective inoculum. Only 5 zoospores suspended in water and placed onto the base of expanding pepper leaves caused 100% mortality of plants grown in high humidity. Introducing 10 and 25 zoospores to pots with 1 cm (0.4 inch) of surface water, simulating flooding conditions, resulted in between 75% and 95% mortality of pepper plants respectively (Bowers and Mitchell, 1991). At relatively low inoculation levels, in the presence of ample free moisture and in contact with susceptible plant material, zoospores will encyst, germinate and grow a germ tube that either penetrates the plants cuticle layer or enters through open stomata

(Hausbeck and Lamour, 2004). Infection can take place at temperatures between 9 °C (48 °F) and 32 °C (90 °F), with most occurring at 19 °C (66 °F) or above, and within 10 minutes of inoculation (Granke and Hausbeck, 2010; Biles et al., 1995).

Water is an important vector for *P. capsici*'s propagules along and between rows of plants, in and above the soil, in a naturally-infested field (Ristaino et al., 1997; Ristaino, 2003; Hausbeck and Lamour, 2004). Sporangia are primarily moved about a field by water. They are easily dislodged from sporangiophores by a variety of mechanical means, including by water splashes and through capillary action (Granke et al., 2009; Ristaino and Johnston, 1999).

A study of furrow-irrigated fields of tomato, pepper and squash plants, irrigated every 14 days and inoculated in isolated locations with *P. capsici*, showed that viable, infectious propagules of the pathogen could be transported approximately 70 meters (230 feet) in the flow of surface water and could move 2 meters (6.6 feet) upstream from the initial point of inoculation (Café Filho and Duniway, 1995).

Splashing of water by rainfall or overhead-irrigation disperses propagules from inoculum on the surface of soil or plastic-mulch to the aboveground parts of plants (Ristaino et al., 1997; Sujkowski et al., 2000). Splash dispersal is noted as occurring later in an epidemic by Ristaino et al. (1997); however, it is nevertheless important in epidemic development.

Wind-driven rain, first hypothesized by Bowers et al. (1990) as having the greatest significant effect on disease spread, has been proven to increase the spread of *P. capsici*, and in the prevailing wind direction (Granke et al., 2009). There is a positive correlation between airborne concentrations of *P. capsici* sporangia and rainfall events, indicating dispersal is through splashing (Granke et al., 2009). Substantial dispersal can occur in as short a duration of rainfall

as less than 2 minutes (Madden, 1997). As volume of rainfall in both 24 hour and 1 hour periods increase, disease progress increases, and number of days of rainfall is highly correlated with disease progress (Bowers et al., 1990; Ntahimpera et al., 1998). Studies of raindrop size and its effect on *Phytophthora* species dispersal have shown that as initial droplet size increases, the number of spores transported and flight distance of the secondary droplets increase, due to an increase in velocity and kinetic energy. Splash dispersal of *P. capsici* occurs over short distances, of less than 15 cm (6 inches), unlike *P. cactorum* that may have propagules dispersed up to 120cm (47 inches) (Grove et al., 1985; Madden, 1997).

The first signs of infection following artificial inoculation of susceptible plant tissue appear within 24 hours, and extensive signs are noticeable within 3 days (Leonian, 1922). This delay in symptoms is a problem post-harvest. Shipments may be rejected due to asymptomatic, infected vegetables may be harvested, packaged and shipped, during which time symptoms develop (Hausbeck and Lamour, 2004). The key method to avoiding this occurring is through avoiding risk of *Phytophthora* blight-infection. A rotation of greater than 5 years is necessary between susceptible crops (Lamour and Hausbeck, 2002). In some areas of the United States, finding land that is not infested with *Phytophthora capsici* in order to maintain an effective crop rotation is difficult (Hausbeck and Lamour, 2004).

A variety of cultural control measures are available to growers of *Phytophthora* blight-susceptible crops, although as Hausbeck and Lamour (2004) point out, the adoption of these practices may be limited to growers of high-value horticultural crops than growers of processing crops because the former may be more able to afford the additional expenses to production costs. Ristaino and Johnston (1999) recommended the adoption of the following *Phytophthora* blight management strategies:

1. utilize genetic resistance in cultivars

2. monitor and reduce propagules in the soil and water sources
3. choose and apply fungicides appropriately
4. transplant on top of a small ridge, raised or crowned bed
5. reduce high soil moisture conditions
6. reduce soil splash.

When Leonian first described *P. capsici* he noted that it did not cause root rot of Chile peppers (Leonian, 1922). He described localized immunity to infection from the pathogen, which halted lesion growth from the initial point of infection. He was observing the plants resistance to root and crown rot infection which has since been utilized in the breeding of some *P. capsici*-tolerant cultivars of bell pepper. Tolerance, or resistance, to crown and root infection is not an indicator of resistance to above ground infection of stems, leaves and fruit (Hausbeck and Lamour, 2004; Dunn et al., 2010). In studies of commercially available bell pepper cultivars and their tolerance to high *P. capsici* disease pressure in naturally-infested fields, between 93% and 97% of 'Paladin' plants were asymptomatic for Phytophthora blight at the end of the season. Disease incidence in non-resistant cultivars ranged from 52% for tolerant cultivar 'Aristotle X3R' to 98% for the susceptible cultivar 'Cal Wonder' (Walters et al., 2007; Babadoost, 2009). 'Paladin', marketed as a resistant-cultivar, has desirably high tolerance to Phytophthora blight. However, its resistance is to root and crown rot symptoms. Therefore leaves and fruit remain susceptible to infection. No complete resistance is currently available in a bell pepper cultivar.

Where a history of Phytophthora blight is known in a field or area, monitoring and reduction of propagules is necessary. Oospores and mycelium of *P. capsici* may be effectively controlled in soil through fumigation using methyl bromide and chloropicrin, although alternative controls such as soil solarization or white-on-black plastic mulch allow survival of viable propagules (French-Monar et al., 2007). The use of a preceding cover crop, into which the cash crop is

planted, shows great potential for reducing Phytophthora blight incidence. Ristaino et al. (1997) transplanted a bell pepper crop into bare ground, black plastic mulch and stubble of a fall-sown winter wheat cover crop that had been herbicide-killed and mown in spring. They found final disease incidence on plants grown in the stubble was significantly less than on plants in other treatments. Disease incidence was between 2.5% and 43% for the stubble treatment, compared to between 71% and 72% disease incidence for bare ground and between 42% and 78% disease incidence for black plastic mulch.

Water contaminated with *P. capsici* is a significant source of primary inoculum and should be avoided or treated effectively if it is necessary to use it. Baiting water sources with green pears or cucumber fruit is a cheap and effective method for monitoring for the presence of *P. capsici* (Hausbeck et al., 2006). Due to Phytophthora's similarity to algae, algaecides have been studied for their potential use in controlling the pathogen in water sources. In laboratory assays, Granke and Hausbeck (2010) found algaecides containing copper sulfate, chelated copper or sodium carbonate peroxyhydrate (SCP) active ingredients completely inhibited zoospore motility within 3 minutes of treatment and caused significant zoospore mortality. Treatment times of 30 minutes or more with several copper-based and one SCP-based algaecides showed similar zoospore mortality rates as the positive control of bleach. Eight of the 11 algaecide treatments resulted in, or very close to, 100% zoospore mortality, indicating potential for their use in treating *P. capsici*-infested irrigation water.

Monitoring the growing environment must include identifying weeds that may serve as alternative hosts to the pathogen and controlling these to prevent increase and survival of the pathogen (French-Monar et al., 2006; Ploetz and Haynes, 2000; Tian and Babadoost, 2004).

Diseased plant material should never be plowed into the soil, and should be removed for incineration or burial in landfill. Epidemic onset occurs earlier and proceeds at an increased rate when diseased plant material, such as infected fruit, remains in the field, on the surface of the soil and particularly on the surface of polythene mulch (Sujkowski et al., 2000).

Fungicides are suitable control methods for Phytophthora blight management if chemicals are alternated so as to prevent build-up of resistance in the *P. capsici* population being treated. Fields which only had mefenoxam (Ridomil Gold) applied had a much greater number of resistant isolates than fields where the chemical had been used in rotation with other fungicides. Fungicide insensitivity, particularly for metalaxyl and its enantiomer, mefenoxam, has become a significant problem for growers of susceptible crops since the last decade of the twentieth century (Parra and Ristaino, 2001).

For a long time growers relied on a small range of fungicides for Phytophthora blight control, in particular fungicides within the phenylamide class, which includes mefenoxam and metalaxyl (Hausbeck and Lamour, 2004). *Phytophthora capsici* populations evolved insensitivity through a combination of the repeated use of these chemicals, the chemicals site-specific mode of action, selection pressure and polycyclic lifecycle of the pathogen. Sexual recombination can lead to progeny fully insensitive to mefenoxam due to sensitivity being inherited as a single incompletely dominant gene (Lamour and Hausbeck, 2002). Mefenoxam-resistant populations are as virulent as sensitive populations on bell pepper (Café-Filho and Ristaino, 2008). Once mefenoxam use ceases on a field with a known mefenoxam-insensitive *P. capsici* population, the insensitivity-trait has been shown to exist in future generations of the population, due to long-term survival of oospores within the soil carrying forward the trait (Lamour and Hausbeck, 2001).

Due to the translocated action of some fungicides like metalaxyl, repeated applications of these through an irrigation system can lead to significant reduction in final disease incidence on aboveground plant parts (Ristaino et al., 1997). Regular foliar-applications of copper-containing fungicides from mid-season onwards are also recommended for effective foliar blight control (Ristaino and Johnston, 1999). Both these methods of using fungicides do not provide control of root and crown-rot.

Growers should avoid planting susceptible crops in low-lying areas of a field, particularly those areas prone to flooding, as this is where *Phytophthora* blight-infection is likely to occur first. It should be noted that infection may still occur in well-drained soil when environmental conditions are favorable and an inoculum source is present (Hausbeck and Lamour, 2004). Soils humic and water content, porosity and levels of net mineralizable N are positively correlated with final disease incidence of *Phytophthora* blight on bell pepper seedlings, and soil bulk density is negatively correlated (Liu et al., 2008). Therefore soils with high organic-matter content and/or that retain moisture well are most likely to have higher disease incidence than mineral soils that drain freely. To minimize *P. capsici*-infection of roots and crown, planting on a raised, crowned bed increases water runoff away from the plants into the furrow, and cultivations to form the bed makes a more friable, porous soil texture that improves drainage in the root-zone.

Controlling soil moisture is an effective method of controlling *Phytophthora* blight disease incidence. Use of subsurface drip irrigation is considered preferable to using shallow or surface drip irrigation. Maintaining a consistent soil-water matric potential is desirable, as it is known that excessive soil moisture or cyclic changes in soil-water potential induce germination of *P. capsici* propagules (Bowers and Mitchell, 1990). Soil-water matric potential should be kept between 20 and 40 J/kg (Ristaino, 2003).

It is recommended to plant at low-density populations in order to reduce foliar blight disease incidence. By increasing air-flow around plants, the time aboveground plant parts remain covered in a film of water following rainfall is decreased (Hausbeck and Lamour, 2004).

The characteristics of ground cover and plant canopy have a significant effect on disease spread. Mulching with straw or establishing living mulch between crop plants significantly reduces splash dispersal compared to bare soil. And increasing surface roughness and leaf area index of the living mulch canopy reduces splash dispersal of *P. capsici*. Dense cover of tall sudangrass living mulch disperses *P. capsici* spores further than short grass (Ristaino and Johnston, 1999; Madden and Ellis, 1990; Madden, 1997).

Growers of high-value horticultural crops will often lay polythene-mulch at the time of bed-formation as a relatively cheap and easy-to-manage method of weed control. Other benefits of plasticulture to crop production include:

- Earlier crop production (7-21 days)
- Higher yields per acre (2-3 times higher)
- More efficient use of water resources
- Reduced leaching of fertilizers
- More efficient use of fertilizer inputs
- Reduced soil and wind erosion
- Better management of certain insect pests
- Fewer weed problems
- Reduced soil compaction and elimination of root pruning
- Opportunity to double- or triple-crop with maximum efficiency (Lamont, Jr., 1996; Lamont, Jr., 2005).

Use of polythene-mulch is reported as having contradictory effects on the incidence of P. blight. Its use may result in a greater percent of wilted bell pepper plants early in the growing season and at the end, due to incidence of crown and root rot, than plants grown through organic and living mulches (Ristaino et al., 1997; Roe et al., 1994). Using polythene-mulch reduces soil splash in the immediate vicinity of crop plants. Preventing soil splash reduces aboveground P. blight incidence, the number of lesions on leaves and fruit, and in doing so it increases the marketable yield of the crop and reduces the need for applications of foliar fungicides (Stevens et al., 1993; Univ. of Georgia, College of Agr. & Environ. Sci., Coop. Ext., 2009). Careful soil moisture management beneath polythene-mulch mitigates the problem of crown and root rot.

In temperate climates, where there is a short growing season and premium early-season prices, use of black polythene mulch results in greater total yield, early yield, yield per plant and larger fruit, compared to no mulch or organic mulches applied to the bed surface (Emmert, 1956; Teasdale and Abdul-Baki, 1997; Roe et al., 1994; Decoteau et al., 1989). If plastic-mulched beds are prepared in fall, earlier spring planting can be achieved that may lead to an even earlier first harvest (Reiners et al., 1997).

Black polyethylene, 1.25 mil (0.031 mm) thick, is the most popular sheet mulch used for weed control and may have a lifespan of 1-3 years depending on its use and weather exposure. Thicker and embossed sheeting lasts longer due to being reinforced and less prone to mechanical damage (Lamont, Jr., 1996; Grundy and Bond, 2007; Univ. of Georgia, College of Agr. & Environ. Sci., Coop. Ext., 2009). Black-colored polythene sheet mulch degrades significantly slower than white, infrared-transmitting (IRT), grey and clear polyethylene mulches (Ngouajio and Ernest, 2005). The color of plastic mulch also changes the microclimate surrounding the plant: the spectral balance, quantity of light and root zone temperature

(Decoteau et al., 1989; Decoteau et al., 1990). Black polythene absorbs most ultraviolet, visible and infrared wavelengths and reflects this back as heat, giving it the ability to reduce weed seed germination, growth of weeds, and in the cooler periods of the growing season can help moderate temperature both above and below the soil; soil temperature below this material can be 2°C (35.6°F) higher than bare soil, white or silver mulches (Lamont, Jr., 1996; Grundy and Bond, 2007; Decoteau et al., 1989), leading to earlier harvests and crop growth extending later into the season. In addition, plastic mulch may modify the growing environment surrounding a transplant through the carbon dioxide that builds up beneath plastic mulch, from aerobic respiration of plant roots and soil organisms, being released through planting holes. Grundy and Bond (2007) suggest this may lead to an enhancement of a crop plants ability to photosynthesize.

Weeds are controlled well by plastic mulch; although niches such as planting holes, holes due to mechanical or animal damage, edges of plastic sheets, and depressions on the surface of plastic that become filled with soil and decayed organic matter, can all become places in which weeds are able to grow (Emmert, 1956; Grundy and Bond, 2007).

As methyl bromide use is ruled out for soil-borne pest, disease and weed control polythene mulches, especially translucent wavelength selective, reflective or nonselective mulches, show great potential as alternative sterilization methods; this includes them being used as a control of pernicious perennial weeds such as purple nutsedge (Lamont Jr., 1996; Grundy and Bond, 2007; Patterson, 1998).

Elimination of weed competition because of plastic mulch, combined with efficient management of water and fertility supply to the crop through use of drip- and trickle-irrigation systems with appropriate emitter spacing, results in increased fruit yield, a reduction in number of culled fruit

from sunscald and blossom-end rot and maximum economic returns (Madramootoo and Rigby, 1991; Singh et al., 2009). Additionally, use of micro-irrigation methods beneath polyethylene film mulch significantly reduces nitrate leaching into groundwater, compared to no mulch or biodegradable mulch alternatives (Emmert, 1956; Romic et al., 2003).

Despite many advantages to using polythene mulches, there are some disadvantages also (Univ. of Georgia, College of Agr. & Environ. Sci., Coop. Ext., 2009; Grundy and Bond, 2007; Kozlowski, 1984; Rice et al., 2001), namely

- expensive and time-consuming laying, removal and disposal of plastic mulch,
- increased fire risk in the field due to the flammable material being used,
- some weeds remain difficult to control in the plasticulture system,
- soil beneath polythene mulch remains flooded for longer than soil without mulch following a flooding event, leading to depletion of soil oxygen and accumulation of phytotoxic compounds that may harm and ultimately kill crop plants,
- increased water, sediment and pesticide runoff from fields where polythene mulch is used.

Covering a field with between 50-75% impermeable polyethylene mulch, as is often the case in intensive, high-value, fresh-market vegetable production systems, greatly increases soil erosion and watercourse pollution from agriculture. Water runoff increases up to 4 times and more than 3 times the volume of sediment is lost compared to plots mulched with hairy vetch residue. Water runoff takes with it sediment-bound and dissolved pesticides to watercourses (Rice et al., 2001).

In 2006, 83% of acreage for bell pepper production had fungicides applied, totaling 298,010 kg (657,000 lbs). Fifty-one percent of this acreage had copper hydroxide applied, the second most used active ingredient in a pesticide, totaling 48,988 kg (108,000 lbs) (U.S. Department of Agr. and Nat. Agr. Statistics Service, 2007). Runoff from vegetable production utilizing polyethylene mulch can contain up to 35% of copper applied to crops in fungicides. A significant amount of the copper that leaves the fields is adsorbed to suspended particulates of soil. Copper is quickly released when soil particles enter fresh and saline waterways. The desorbed copper becomes soluble and amounts to levels of toxicity beyond LC<sub>50</sub> values for some aquatic organisms. Preventing movement of sediment into a watercourse can reduce toxic pesticide loads entering water by up to 90% (Dietrich and Gallagher, 2002; Rice et al., 2004; Rice et al., 2007).

One of the greatest costs to growers of any crop is management of weeds. Permeable-sheeting and plant-residue mulch materials do not control weeds or remain viable as long as plastic sheeting, therefore growers of high-value crops prefer to transplant into plastic mulch.

Conventional practice has been to maintain weed-free, bare soil between plastic-mulched beds using herbicides and occasional cultivation, which also improves water infiltration (Basher and Ross, 2001). However, an alternative method of weed control in the alleyway is sometimes used, called living mulch. Having living cereal rye growing between polythene-mulched beds of tomatoes during their production period reduced water runoff volume by more than 40%, soil erosion by more than 80% and pesticide loads by between 48% and 74% (Rice et al., 2007). Cereal rye did not reduce tomato yield when grown in this situation.

The use of living mulch is the intercropping strategy, whereby a managed 'companion crop' is planted before, with or after a summer cash crop is planted, and grows alongside this cash crop for the duration of its production cycle, primarily for weed control (Robinson and Dunham, 1954; Hartwig and Ammon, 2002; Hoffman and Regnier, 2006) . Pioneers of this system include

Robinson and Dunham (1954) for their development of the system of using a 'companion crop' with soybean and Lilly (1965) for establishing the "sleeping sod" system of planting warm-season crops into suppressed, cool-season perennial grasses.

Dr. Robert Sweet of Cornell University is credited with having first coined the term "living mulch" in 1979, in order to distinguish the management of plants within the cropping system, amongst cash crops, as being different from other uses of cover crops. The term "living mulch" is one of a variety of terms referring to specific uses for cover crops (Teasdale et al., 2007). Other terms include 'green manure' to signify use of a cover crop for the purposes of introducing fertility into a system, 'smother crop' to indicate a cover crop's use to outcompete weed species growth, and 'catch crop', meaning a fast-growing cover crop species of economic value that may be grown in niches between main crop growing periods or simultaneously with the main crop but harvested earlier, providing benefits both to the soil from having been covered and economically to the farmer, by them having two crops from one area of land. Living mulch performs the functions of a smother crop, although a smother crop is grown alone, without any cash crop.

The principles of the living mulch system were first developed in mountainous regions with high precipitation levels; often in vineyards. This occurred following the observation of natural plant succession and plant-soil interaction during the use of alternative groundcover management strategies. Soil erosion, being a problem in these areas, means herbicide use is often inappropriate, due to it resulting in negligible weed biomass being left on the soil surface as dead mulch to protect soil from further erosion. Also, herbicide products, through repeated use, have reduced efficacy. It has been observed that certain weed species show increasing levels of resistance following repeated exposure to the same herbicide and colonize treated land. The alternative weed-management strategy of hand-weeding results in more volume of dead mulch from weed biomass but the high labor costs make this prohibitive. In the early days of the

adoption of living mulches, wine-grape growers noted that weed species such as common chickweed grew quickly, effectively covering the ground, was low-growing with shallow roots, and reduced soil erosion while not reducing grape yield (Hartwig and Ammon, 2002). This led to these 'weedy' plant species being allowed to establish in the vineyard alleyways and growers began learning how to manage them for groundcover.

The practice of utilizing plants for living groundcover has since been applied to almost all field-based crop production systems, including both annual and perennial edible crops and perennial ornamental crops (Bond and Grundy, 2001; Hartwig and Ammon, 2002; Cripps and Bates, 1993; William, 1987; Hughes and Sweet, 1979). Where dormant trees or shrubs are harvested in winter, having living mulch growing in the aisles helps accessibility to the field. For some of these crops, cereals are grown between the rows for use as windbreaks (William, 1987). Fall-sown tree and shrub seeds in raised beds have a living mulch, such as cereal rye, sown simultaneously with the crop seed as the cereal rye germinates quickly, forming a protective layer of vegetation over the beds to prevent soil erosion and predation over winter (Hawkins, 2004).

Growing living mulches with cash crops is consistent with the goals of organic and sustainable agricultural practice, provides numerous benefits to the agroecosystem, and plays an important role in supporting ecosystem services ( Hoffman and Regnier, 2006; Graglia et al, 2006; Leary and DeFrank, 2000; Teasdale et al., 2007). Sustainable agricultural systems seek to sustain or improve all elements of productivity, whether that be from crops harvested and their associated economic gains, or soil health and environmental quality (Hoffman and Regnier, 2006). The living mulch system plays a role in mitigating the serious soil problems caused by continuous cropping (Hughes and Sweet, 1979).

As Paine and Harrison (1993) point out, once plant mineral nutrition was better understood, due to research carried out in the 19<sup>th</sup> century, and significant events of the 20<sup>th</sup> century took place, the study of soil amendments and tillage practices increased. Conventional tillage practice during a prolonged drought brought about the US Great Plains Dust Bowl in the 1930's, that lead to adoption of minimum tillage in the affected areas; although the rest of the country's farmers continued with routine tillage, despite the warning of its potential to cause harm to the soil. Around the mid-20<sup>th</sup> century a reaction began building in farmers, researchers and conservationists, in response to intensive agricultural mechanization and the widespread adoption of manufactured inorganic fertilizers and pesticides for the production of food. Agriculture's dependence on fossil-fuels through mechanization and manufactured crop-management products by the 1980's was to prove to be costly, both environmentally and economically. The oil crisis of the 1970's sharply increased food production costs, leading to farmer's seeking less intensive but equally as effective methods of crop management. This led to greater adoption of reduced-tillage practices in combination with cover crops. Organic farmers in particular saw living mulches, green manures and reduced- or no-tillage as a sustainable way forward for maintaining healthy soil, naturally controlling weed competition and for growing healthful food (Leary and DeFrank, 2000).

Those who have adopted and studied the use of living mulches have identified preferred characteristics used for the selection of suitable species. Williams (1987) summarizes the criteria as being that the living mulch species will

1. establish rapidly to suppress weeds and provide early trafficability and erosion control;
2. provide adequate wear tolerance and persistence;
3. tolerate drought and low fertility;
4. reduce costs associated with mowing intervals, fertilizer needs, thatch removal, or chemical mowing; and

5. enhance crop yield and quality.

Uniform, rapid, early growth, resulting in dense, competitive groundcover is key to the success of living mulch suppressing weeds (Teasdale, 1998; Nicholson and Wien, 1983; Bertin et al., 2009). Some authors also identify that a living mulch should possess allelopathic ability (Hartwig and Ammon, 2002; Bertin et al., 2009). Its morphology and physiology should be both noncompetitive to the primary crop and suppressive to weeds. Groundcover is more important than height for suppressive ability of living mulch. The shorter and less vigorous turfgrasses and white clover showed the greatest promise for use as living mulch through their combined ability to provide good weed suppression while not reduce yield of sweet corn or cabbage (Nicholson and Wien, 1983).

Although height may not be a measure of suitability for use as living mulch, the amount of aboveground biomass grown is an indication of that plants ability to suppress weeds, be an effective groundcover, result in organic matter being added to the soil, help retain nutrients, water and soil, and the amount of nutrients, particularly nitrates, that it will make available to subsequent crops. Biomass production is therefore an additional selection criterion for choosing living mulches and is dependent on soil-type and conditions, seeding rate, stand establishment and environmental conditions (Patten et al., 1990; Burgos et al., 2006).

Some plants are poor at competing with weeds, and others may be susceptible to insects and diseases and may therefore become alternative hosts to crop pests and diseases. Use of any living mulch within the tree row of an apple orchard elevates the amount of meadow vole damage to trees (Wiman et al., 2009), especially when legumes were included in the seed mix. It is therefore important to understand the biotic, as well as the abiotic, interactions any potential living mulch plant has, within the environment it is expected to be utilized, before it is fully

introduced into the cropping system (Hoffman and Regnier, 2006). Screening of potential candidate species is essential, such as the screening of 50 legumes and 50 grasses by Sweet (1982).

Whether to include legume plants in the living mulch is an important consideration. Legumes are an important addition to the living mulch cover crop system, whether as a pure stand or in a mixture combined with small grains and other grasses, especially in the organic system where they play an important role in both weed management and for provision of nitrogen (Hoffman and Regnier, 2006; Teasdale et al., 2007; Creamer and Baldwin, 2000; Gaskell and Smith, 2007).

When a combination of grass and legume species are grown together in a mixture a general trend is for the resulting mixtures biomass to be the same as or greater than either of the individual species grown in pure stands (Burgos et al., 2006). Regardless of seeding rates, grasses tend to be the greatest contributors to the resulting biomass of a mixture. Combining benefits of legume and non-legume plants adds to the effectiveness of the living mulch. Many cereals suppress weeds more than legume plants. Legumes fix atmospheric-nitrogen through a symbiotic relationship with soil-borne bacteria (Gaskell and Smith, 2007). A good balance is desired for a legume/non-legume mixture to be successful. Economic, biological and physical factors need to be considered.

Most research on the use of living mulches has concentrated on using small grain, forage (including legume) and turf-grass species. Birds foot trefoil, sheep's fescue, alfalfa and white clover were effective living mulches grown with wheat (Carof et al., 2007) and Ladino clover suppressed weeds adequately when grown as living mulch in corn (Echtenkamp and Moomaw, 1989).

Living mulches with a concurrent growth period to the cash crop may be undesirable for most crops due to them being competitive for resources. This is believed to be yet more significant if crop and living mulch have a similar root spatial range (Nicholson and Wien, 1983).

Subterranean clover shows potential for use with crops grown during hot summers as its main growth takes place over winter when rains provide much of the necessary irrigation requirements. Weed suppression from winter extends into much of the summer growing season. Cash crops can be planted into the living clover through the creation of herbicide-killed strips or strip-tilled planting zones. Once senescence takes place in Spring, as temperatures rise, the dehiscid seeds of subterranean clover remain dormant in the soil surface. Upon cooler temperatures and increase in soil moisture in fall, seeds germinate and this annual specie reestablishes (Lanini et al, 1989). Studies of its use have shown it suppresses weeds more than conventional tillage plus herbicide treatment, conventional tillage alone or rye dead mulch, and while weeds were effectively controlled yield of cabbage, field corn, sweet corn, snap beans, tomato, corn silage and grain from plots treated with this living mulch were the same or higher than yields from the other treatments (Enache and Ilnicki, 1990; Ilnicki and Enache, 1992).

Much more research into alternative species for use as living mulches is required, such as other crop plants and even weed species. Ellis et al. (2000) made a two-year study into the use of a plant specie familiar to many vegetable growers as a weed: common purslane. Their choice for using this plant was because of its attributes as a successful weed being so similar to the desired characteristics of living mulch for use with summer crops: aggressive summer growth, prostrate habit, rapid establishment, dense canopy, tolerant of a variety of growing conditions and reproduces easily both sexually and vegetatively. The study found that common purslane was an economically viable living mulch.

The living mulch system performs the following roles within the agroecosystem, as proposed by Hughes and Sweet (1979),

1. continuous groundcover especially important for establishment of tender plants,
2. erosion control,
3. reduction of leaching losses,
4. increased organic matter return,
5. less energy consumption in terms of fuel for tillage and chemicals, and
6. reduced disease, insect, and weed problems.

It has been noted that there is less amplitude in soil temperature in plots treated with living mulch, compared to no mulch or cover crop residue (Sweet, 1982; Teasdale, 1998). Compared to a dead mulch of the same species, a living mulch of Kura clover stored between 37mm (1.5 inches) and 50mm (2 inches) less soil water in spring, and retained 29mm (1.1 inches) to 36mm (1.4 inches) more water in summer, when used for no-till corn production. Drainage was not reduced by the presence of the living mulch (Ochsner et al., 2011). Growing crops in combination with living mulches may increase arbuscular mycorrhizal colonization of the primary crop plants roots, and therefore increases the cash crop's uptake of phosphorus from the soil and increase its ability to establish early and successfully (Deguchi et al., 2005). Living mulches moderate the growing environment, benefiting young and tender plants.

Water leaving a field may carry soil particles, nutrients and pesticide residues, leading to pollution of water courses, loss of resources and increased expense to the farmer (Rice et al., 2002; Rice et al., 2004; Rice et al., 2007). Overland flow of water, either due to furrow-irrigation or a heavy rainfall event is a particular problem. It has been shown that living mulch reduces surface runoff of water, and allows more water penetration (Sweet, 1982).

Vegetation either within the furrow or as filter strips at the upper and lower ends of the crop mitigate soil erosion, are a relatively low-cost solution and, depending on plant species used in the filter strips, may give economic returns, unlike some alternative solutions, such as sediment retention basins (Carter et al., 1993). Clean-tilled aisles in tree and shrub nurseries recorded sediment concentrations in surface water runoff, after rainfall events, between 1.9 to 8.8 times greater than living mulch-covered aisles (Cripps and Bates, 1993).

Where some growers may leave stover or stalks of a harvested crop, such as soybean or corn, overwinter to aid with the intention of reducing soil erosion, interseeding with grasses, such as annual ryegrass or creeping red fescue, or legumes such as crownvetch or birdsfoot trefoil, at the last cultivation of the primary crop is more effective than the dead mulch alone (Hall et al., 1984; Hively and Cox, 2001; Singer and Pedersen, 2005). A living mulch of crownvetch, into which no-till corn was drilled, reduced water runoff, soil erosion and herbicide runoff between 95% and 99% (Hartwig, 1985). Using simulated storm rainfall events on a sloping site Schwab and Albrecht (2011) noted preliminary results showing the use of Kura clover living mulch in no-till corn reduced both soil erosion and phosphorus runoff more than 50%. A living mulch of Kura clover may also reduce nitrate-N leachate between 31% and 74% when compared to a control of dead mulch (Ochsner et al., 2011). Living mulch roots will help retain soil structure, uptake leachates, improve drainage and may have positive effects on the soil ecosystem. Indeed, Sweet (1982) believed that 90% of the benefits of the living mulch both to the soil and cropping system are derived from its roots and wrote of the need for studies to include belowground biomass and interactions evaluations.

A living mulch of cereal rye growing between polythene-mulched beds reduces runoff volume by more than 40%, soil erosion by more than 80% and pesticide loads by between 48% and 74% (Rice et al., 2003; Rice et al., 2004; Rice et al., 2007). In some agricultural regions of the US,

leached pesticides have reached such significant levels in groundwater sources that the contamination level can be measured and where pollution has reached riparian habitats wildlife is threatened (Sweet, 1982; Dietrich and Gallagher, 2002). The use of living mulch to filter surface water runoff, improve drainage and increase soil organic matter content shows great potential for mitigating agriculture's impacts on the local surrounding environment.

Use of living mulches has the potential to improve the positive effects of agriculture on the environment through the increase in carbon sequestration, biodiversity and soil organic matter (Carof et al., 2007). It was proposed by the National Wildlife Federation (2011) that in order for America to meet its goal of reducing greenhouse gas emissions 17% by 2020, while still meeting the needs for food, fuel and fiber, carbon sequestration on agricultural land would play a significant role. The Farm Bill conservation title funds sustainable farming practices, including the use of cover crops. If US farmers were to grow cover crops on all acres of cultivated land suitable for them, approximately 74.9 million hectares (185 million acres), an estimated 4% of annual greenhouse gas emissions could be mitigated. Living mulches would play an important role in this advancement as they would fix carbon all year round, including during the cropping cycle.

Part of the role living mulches play in reducing agriculture's impact on the environment is through reducing dependency on fossil fuels. Integrated pest management systems, including non-chemical pest and disease control methods, are desirable as increasing numbers of consumers have concerns about food safety and the effects of pesticide use on human health and the environment, and chemical control measures alone are not always adequately effective (Bond, 1992; Robinson and Dunham, 1954). Some applications of chemical treatment for insect control may be able to be eliminated through use of living mulches (Andow et al., 1986).

Conventional weed control methods, by periodic tillage, use of polythene mulch, or herbicide application, rely heavily on fossil fuels. Living mulches may give equal or better weed control as these conventional weed control methods (DeGregorio and Ashley, 1986; Zumwinkle and Rosen, 1991; Ellis et al, 2000).

Reducing the number of trips a farmer makes across a field also reduces fuel use and costs and is a benefit of an integrated system such as one that combines reduced tillage and living mulch (Lilly, 1965; Burgos et al., 2006).

Plant disease can be reduced through use of living mulch. Apple trees grown with mown and herbicide-controlled living mulch ground cover of sod grass or crown vetch remained free of symptoms of *Phytophthora* crown or root rots for the four year duration of the study, whereas 35% of trees mulched with straw had developed symptoms. Living mulch plots maintained lower levels of soil moisture throughout the season, compared to straw mulched plots, therefore reducing disease incidence from *Phytophthora* species (Merwin et al., 1992). Use of the living mulch Rhodes grass showed potential for nematode control in eggplant production (Valenzuela and DeFrank, 1994).

While there may be certain reports of living mulch plants reducing disease damage to cash crops consideration must be given to whether they may be alternate hosts to diseases if used with different cash crops. Sweet (1982) reported that Zitter, a plant pathologist at Cornell University, found several clover species are excellent alternative hosts for some viruses; most notably, viruses of cucurbit crops. While Ellis et al. (2000) may recommend the use of common purslane as living mulch its use needs to be considered in relation to the crop with which it will be grown. Common purslane is documented as a host of *P. capsici* and therefore should not be grown with crops susceptible to this pathogen (Ploetz and Haynes, 2000).

Similar to the potential for being a host for diseases, living mulches have the potential to increase pest damage to crops. Damage to harvestable vegetable produce by herbivorous insects is higher from plots with vegetative cover than from weed-free plots (Altieri et al., 1985). Likewise, vegetation provides food and shelter for rodents, such as the pocket gopher, meadow mouse or vole. Damage by rodents varies according to crop-type, and may include chewing of roots, stems, leaves and fruits, and girdling the bark at the base of orchard tree trunks. Damage may still occur whether the living mulch is growing adjacent to the crop plants or if there is a bare-ground border maintained. There are reports of rodents traveling up to a meter from heavily grassed plots, causing damage to orchard trees. Instead of maintaining large areas of bare soil around crops in order to prevent rodent damage, keeping living mulch vegetation cut short, below 30cm (12 inches) tall is a good control measure for this problem. Most rodents seek shelter among plants taller than one foot, so as to hide from predators (deCalesta, 1982; Sullivan, 2006; Wiman et al., 2009).

However, living mulches may also reduce damage from crop pests. Populations of cucumber beetles and pests of cabbages were significantly lower on their respective crop plants when grown in living mulch-treated plots than without any surface mulch (Amirault and Caldwell, 1998; Andow et al., 1986). More predatory beetles and spiders are found in plots covered by vegetation of either weeds or living mulch, than in straw-mulch treated or bare soil plots. In general there is a trend for more herbivores to be found in weedy plots and more predatory insects to be found in living mulch-covered plots (Altieri et al., 1985). Reducing populations of herbivorous insects around crop plants also reduces incidence of insect-transmitted disease such as viruses. Whether using living mulch or weeds around zucchini plants aphid population density was reduced on the crop, indicating the benefit of diversified cropping systems to disrupt

aphid's ability to colonize cash crops by reducing the contrast between the cash crop and its surroundings (Hooks et al., 1998)

In 1954, Robinson and Dunham proposed a new method of weed control: the use of companion crop competition. They proved it to be a successful and relatively inexpensive form of weed control, compared to chemical or tillage methods. They found that sowing wheat, rye or peas immediately after sowing soybeans resulted in the best establishment of the companion crops: today referred to as living mulches.

From their own research as well as from reviewing other researchers work, Teasdale et al. (2007) conclude that living mulches suppress weeds throughout the season and at all stages of the weed's life cycle better than a mulch of cover crop residue (Table 1).

**Table 1.1:** Potential impact of typical cover crop residue or live cover crop (living mulch) on inhibition of weeds at various life cycle stages. From Teasdale et al., (2007).

Weed life cycle stage	Cover crop residue	Live cover crop
Germination	Moderate	High
Emergence/establishment	Moderate	High
Growth	Low	High
Seed production	Low	Moderate
Seed survival	None? <sup>a</sup>	Moderate? <sup>a</sup>
Perennial structure survival	None? <sup>a</sup>	Low-moderate? <sup>a,b</sup>

<sup>a</sup> More research is needed to provide definite estimates of cover crop influences on these processes.

<sup>b</sup> Perennial structures may be more effectively reduced when a living mulch is mowed, as discussed in Graglia et al. (2006).

Sweet (1982) stated that “The first plant that occupies the land is going to dominate it for the next several months or maybe for the next year, depending on the crop”. A weed emerging from the soil into an established stand of living mulch is at a disadvantage and highly unlikely to be able to compete for essential resources (Teasdale, 1998). By planting living mulch to replace weeds and through managing its growth, more success of managing the entire production system can be achieved.

Living mulches intercept and absorb light radiation, resulting in the inhibition of phytochrome-mediated germination of weed seeds. Some living mulches release allelochemicals from their shoots and roots, adding to their effectiveness in controlling weeds (Creamer et al., 1996). Effective weed control, comparative to use of herbicides, can be achieved using appropriate living mulch species in combination with suitable tillage and planting practices for the primary crop (Enache and Ilnicki, 1990; Infante and Morse, 1996).

Even pernicious perennial weeds like *Cirsium arvense*, Canadian thistle, that are typically difficult to control, particularly under organic farming conditions where herbicides are not permitted, are controlled more effectively through the presence of a grass-clover living mulch mixture, periodically mowed, than cultivation treatments (Graglia et al., 2006; Lukashyk et al., 2008).

Use of living mulches can be effective weed control and there are studies that show some crops grown in the system may yield the same or greater than conventional bare soil treatment (Infante and Morse, 1996). However, some living mulches may suppress cash crop yield unless species are chosen and managed appropriately (Nicholson and Wien, 1983; Andow et al., 1986; Teasdale, 1998; Chase and Mbuya, 2008). Wheat yield was suppressed up to 81% in 14 of 18 undersown living mulches (Carof et al., 2007). Vigorous, uncontrolled growth of living mulches,

such as perennial ryegrass and cereal rye, may be too suppressive for crop plants even when the living mulch is cultivated into strips or grown between plastic-mulched beds (Neilsen and Anderson, 1989; Reiners and Wickerhauser, 1995). Despite modifying the planting system for a cash crop of cucurbits from single-row to double-row, increasing yield 4.8 times, total yield from plots with living mulch was still only 72% of the yield of production from bare soil plots without living mulch (Amirault and Caldwell, 1998). What researchers observe is a negative correlation between living mulch dry matter and crop yield (Sweet, 1982; Nicholson and Wien, 1983; Carof et al., 2007).

Understanding the competitive relationships between the cash crop and the living mulch are essential in order to prevent yield loss in living mulch systems (Carof et al., 2007).

Crop plants will be tolerable of living mulch up to a critical threshold without suffering any or significant reduction in yield (Ellis et al., 2000). Beyond this threshold yield can be reduced in crop plants grown in living mulch compared to plants grown in weed-free plots (Degregorio and Ashley, 1986). Water and nitrogen are the main elements that a cash crop and living mulch will compete most for (Hartwig and Ammon, 2002).

Competition between living mulch and crop plants is likely to occur early in the season when the crop is still young and especially if cool-season living mulch species were established during the previous year. Establishing living mulch at the same time or after planting the cash crop may help reduce this problem (Nicholson and Wien, 1983).

Typically, in order to prevent yield reduction of the cash crop when using living mulch, some form of suppression is required. Timing of suppression, degree of suppression, and the mulches root growth characteristics are important factors to consider for successful management of the living mulch system (Wiles et al, 1989). The most referenced methods of living mulch control are

varying the seeding rate, timing and method of seeding, applying herbicides, and mechanical mowing. It may be that if herbicide use is compared to mechanical methods of suppression, both are equally successful in preventing yield reduction compared to yield when the crop is grown in bare soil (Leary and DeFrank, 2004). In another situation herbicide may be more effective control than mechanical suppression (Lindgren and Ashley, 1986). However, the best method of suppression will vary according to the choice of crop, living mulch, method of crop production and the regions climate. In regions with precipitation greater than 1,100 mm (43 inches), living mulch can be maintained all-season and controlled using mechanical means. However, in drier regions living mulches will likely have a greater impact on primary crop yield because of less availability of water and some nutrients. Sub-lethal doses of herbicides may also be required to suppress living mulch growth (Echtenkamp and Moomaw, 1989; Hartwig and Ammon, 2002).

Relatively high densities of living mulch plants are needed for strong suppression of weeds. High density planting intercepts the greatest amount of light, therefore outcompeting weeds better. Seeding rates affect the timing of when the canopy closes. Mowing living mulch may alter the relationship between seed rate and weed density (Gibson et al., 2011). High seeding rate results in highest dry weight biomass and low seeding rate results in lowest dry weight biomass of the same mixture. This correlates to the best weed control being with high seeding rate and the worst weed suppression being in plots sowed at the low seeding rate for mixtures or a monoculture (Akemo et al., 2000).

The most effective weed control is achieved through early establishment of living mulches. In the situation of using living mulches with sweet corn or soybean, adequate weed control is still possible when living mulches are sown the same time as the cash crop. An explanation for this is that due to the primary crop differing considerably from living mulch in both root system and

growth habit, so there was no or negligible competition between the two (Vrabel et al., 1980; Robinson and Dunham, 1954).

Sowing living mulches in narrow strips prior to, simultaneously, or after planting or sowing of the primary crop reduces or prevents yield reduction. Broadcast sowing living mulch seed can only take place following establishment of the cash crop for it not to reduce yield. In some instances seeding later in the season, such as after the cash crop is planted, requires weed control within the living mulch, which defeats the purpose of using a living mulch to suppress weeds (Vrabel et al., 1980).

Success of the living mulch system is dependent on the growth of the plants used being competitive with weeds at appropriate times of the latter's life cycle. However, it is possible that through repeated use of a monoculture of any living mulch, combined with a routine management strategy, that selection may occur, within that field's weed population, for those species that are able to persist under these treatments. Mohler (1991) saw weeds colonize living mulch stands of white clover into which sweet corn was sown annually for four years. Combining use of living mulch with alternative management strategies may not prevent weed colonization. When using buckwheat, seedbank densities increased for common purslane and carpetweed that escaped mowing due to their prostrate habits (Gibson et al., 2011). This limitation of using single specie living mulches leads to the consideration for employing mixed species living mulches or alternating monocultures with each sowing. It would be the intention to select species with contrasting growth habits in order that they provide a broad range of competitiveness to a wide range of weeds. Growing a mixture of species has the advantage of increasing overall biomass production and so increasing weed suppression. A combined stand of Italian ryegrass with Kura clover, where ryegrass was between 16% and 25% of the mixture, increased forage production by 15% (Contreras-Govea and Albrecht, 2005). A further important

observation from this study was that in combination with Kura clover, Italian ryegrass survived winter in Wisconsin, unlike when grown in monoculture, suggesting a possible 'nursing' effect of the Kura clover.

Finding the correct balance of species in a living mulch mixture is important. Mixtures of cereal rye and field pea with more than 50% proportion being rye gave the best weed suppression compared to pure stands of both crops and mixtures with 50% or less of rye; only 2% groundcover was weeds in rye-pea mixes compared to 73% in pea-only living mulch (Akemo et al., 2000).

Many living mulch systems rely on the use of herbicides to control growth of the mulch. Where it is appropriate to use them, herbicides should not be excluded from the living mulch system, but instead should be considered a tool by which the system achieves preservation of soil resources (Teasdale, 1998).

Management of living mulches using herbicides requires careful testing of application rates for each chemical. Pioneers of the living mulch system began with using herbicides to control growth of perennial ryegrass, wheat, oats and rye with varying degrees of success. They reported low application rates of herbicides having little to no effect, whereas higher rates resulted in mortality of the living mulches followed by high weed populations flourishing in mulches that disintegrated quickly after death. Where control of the living mulches was ineffective, interplanted cash crops suffered significant, unacceptable competition (Lilly, 1965; Hughes and Sweet, 1979; Echtenkamp and Moomaw, 1989). Through altering doses of herbicides it was soon discovered that living mulch could be managed by these new chemical products, into which crops could be successfully planted and acceptable yields achieved (Sweet, 1982; Hall et al., 1984). Also known as "chemical-mowing", sub-lethal doses of

herbicides cause stunting, chlorosis and puckering of treated plants. This technique reduces shoot and total biomass yield of the living mulch, as well as leaf area duration and biomass duration, and some herbicides reduce shoot growth more than root growth (Wiles et al, 1989; Echtenkamp and Moomaw, 1989).

Methods of herbicide application for use in the living mulch system include non-lethal doses of herbicide sprayed broadly for suppression or lethal doses applied in bands for use when a crop is to be transplanted or sowed into an established living mulch stand (Ilnicki and Vitolo, 1986; Teasdale, 1998; William, 1987). Great care needs to be taken to prevent crop injury and reduction in yield due to herbicide-damage when applying herbicides to living mulch (Lindgren and Ashley, 1986).

Sub-lethal doses of herbicides and selective herbicides have potential for use in controlling living mulch growth in intercropping systems (Lilly, 1965; Gupton, 1997; Leary and DeFrank, 2004; Wiles et al, 1989; Carof et al., 2007). However, studies to define what are the appropriate herbicide application rates for controlling growth while not causing mortality are needed for each living mulch specie and consideration must be given to which herbicide products are labeled for the cash crop in which the living mulch will be grown (Gupton, 1997). Some slower establishing living mulch, such as birdsfoot trefoil, requires chemical weed control in order to accomplish a full stand (Hall and Cherney, n.d.).

Mowing has been used as a non-chemical weed control method by growers and has also been adopted to control living mulches. The need for using mowing control of living mulch is dependent on the growth habit of both the living mulch plant and the cash crop (Chase and Mbuya, 2008). Mowing is particularly necessary for tall living mulch species in order to reduce shading of a crop plant (Teasdale, 1998).

Some living mulch plants respond better to mowing than others and it is this response that affects their ability to re-close their canopy and effectively control weeds. Sweet (1982) noted that white clover responded well to mowing, maintaining good cover, whereas alfalfa did not respond well and weeds colonized gaps in its canopy. Removal of a large percentage of the living mulch canopy by mowing leads to a significant reduction in light interception by the cover, which allows weed seeds to germinate and for prostrate weeds which are unaffected by the mowing to complete their life cycle. However, this same treatment is also effective for maintaining or reducing weed seed banks (Gibson et al, 2011).

A range of mowing technology is available, allowing choices for a variety of cropping situations, the desired height of cut and size of the clippings. Rotary mowers mow two to three times faster than other mowing equipment and side discharge of material is an option that allows for windrowing clippings around the base of adjacent crops for use as a dead mulch. Flail mowers use more power, are able to cut plants shorter, and reduce cut material into smaller-sized pieces than other machines, which is advantageous if leaving clippings in situ. Large clippings may suppress regrowth of the living mulch further, causing gaps in which weeds colonize and the smaller clippings form dead mulch in the small gaps between living mulch plants, which reduces moisture loss (William, 1987; Patten et al., 1990). Sickle-bar mowers make one cut close to the base of each living mulch plant and leave the largest-sized clippings. Modified with an implement such as a v-plow, the sickle-bar mower can be used to mechanically deliver living mulch material to the base of an adjacent cash crop. Distributing cut living mulch around the base of a cash crop provides it with some of its nitrogen requirements (Zumwinkle and Rosen, 1991).

Donald (2005) writes that mowing alone is not an adequate measure for controlling weeds. Combining plant competition between living mulch and weeds with repeated mowing increases control of even the most pernicious weeds, and this is a more effective control measure than repeated tillage and cultivation of forage crops. Six passes with a mower over a living mulch stand of grass and white clover mixture reduced aboveground *Cirsium arvense* biomass by 69% (Graglia et al., 2006). Both the repeated removal of the *Cirsium arvense* shoots by mowing and the suppression of the weed by the grass-clover mix proved 97% effective in reducing shoot re-growth (Lukashyk et al., 2008). For planting a crop into established living mulch of perennial grasses the best results were achieved through a combination of mowing to between 10 cm and 15 cm (4 inches and 6 inches) and spraying selective herbicide to reduce obstruction of planting equipment by foliage (Lilly, 1965).

Contrasting results have been observed when mowing living mulch. Mowed plots of subterranean clover had significantly greater weed biomass compared to unmowed plots when grown among squash. When used among tomatoes, mowed subclover plots reduced yield compared to unmowed plots. This latter observation contrasts with results for cabbage and sweet corn, grown as part of the same study, which showed that mowing subclover prior to planting increased yields, compared to unmowed plots. The use of mowing to control growth of living mulch and improve weed control needs to be considered for each cash crop within which the living mulch is being managed and studies should be undertaken to test for efficacy and effect of this method (Ilnicki and Enache, 1992). In addition the tests should include analysis of methods to encourage greatest success with the living mulch system.

In contrast to suppression, there may be occasions when supplemental fertilizer, irrigation, or just carefully choosing the time of year to sow may greatly improve the living mulch stand and its benefits. Turfgrass species maintain vigor and weed suppression ability as living mulch with

the addition of a small amount of fertilizer (William, 1987). Broadcast sowing a cool-season living mulch, such as Dutch white clover, in June in a southern US state is quite likely to result in slow or failed germination, poor stand establishment and subsequent unacceptably low weed suppression. Instead a cool-season living mulch should be sowed in spring or the previous fall, if the specie is hardy for the region's winter climate (Law et al., 2006; Smith and Valenzuela, 2002).

It is possible for the right balance to be struck between effective weed control by living mulch and crop yield being the same or higher than yields from conventionally managed, no-mulch systems (Enache and Ilnicki, 1990). While some plants used in the living mulch system may require managing, the most successful approach to adoption of this system depends on choosing the right combination of living mulch and primary crop. Finding the successful combination may require testing of a wide range of living mulch species in conjunction with suitable management practices. Identifying significant biological-interactions between crop and living mulch are vital; for example, what the threshold at which living mulch biomass is suppressive to the cash crop yield, and at what stage in the crop's development competition by living mulch most damaging (Carof et al., 2007).

The specie of the crop plant, cultivar vigor and the method of its establishment will affect decisions on how best to apply living mulch. Developing squash transplants were affected by the presence of subclover living mulch, whereas direct-sowed snap beans were not affected. Planting time of the cash crops and the vegetative stage of the living mulch, actively growing and senescing respectively had a significant effect (Ilnicki and Enache, 1992). More vigorous of ten eggplant cultivars grew and yielded equally well whether grown in plastic-mulched beds or in a living mulch of Rhodes grass, whereas less vigorous cultivars were suppressed by the Rhodes grass (Valenzuela and DeFrank, 1994). Use of larger transplants and timely control of

the living mulch enabled broccoli to tolerate common purslane as living mulch without a reduction in its yield (Ellis et al., 2000). Choosing a suitable combination of life cycles of cash crop and living mulch is important

As has already been described, many high-value horticultural crops, such as bell pepper, are transplanted into polythene-mulched beds that cover between 50% and 75% of a field (Rice et al., 2007). This plasticulture system is ideally suited to having living mulch covering the remaining 25% to 50% of the field, for all the benefits to growers and the environment that have been described.

As a method for controlling weeds, the living mulch system is summarized by Teasdale (1998) as “replacing an unmanageable weed population with a manageable cover crop species”. As the highlighted research in this paper has shown, the system offers many more benefits beyond weed management. However, its use is always focused around one key concept: keeping soil between crop plants covered with living plant material under the control of the farmer. Lilly’s (1965) “sleeping sod” system is a model example of the living mulch system. This dormant sod, into which row crops are slit-planted, remains almost dormant throughout the season then ‘awakens’ following harvest of the cash crop and continues its protection of the soil and the surrounding environment.

For a long time it was thought that leaving land fallow was sufficient to improve soil health characteristics. Sweet (1982) suggested that “if you put a third of your crop land in sod for two or three years out of ten, that would keep it in very nice condition”. With increasing world population yield per acre must continue to rise, agricultural land must be kept in production for extended periods of time, and where there is shortage of cultivated land, adoption of marginal land is necessary. ‘Resting’ land is no longer viable due to current demands on crop production.

A compromise is to adopt an intercropping strategy of maintaining and improving soil surrounding a growing cash crop using living mulch. Intercropping systems typically provide good weed control, exhibit less crop damage from pests and diseases, may be more efficient than monocultures at exploiting limited resources and as such can increase yield per acre (Coolman and Hoyt, 1993).

From this study of living mulch literature, parallels can be drawn to Donald's (2005) findings on the use of mowing for weed management. There is a lack of systematic research and scientific understanding of the living mulch system that is necessary to optimize its use and validate its adoption to skeptical growers. Few, if any, overall conclusions or even recommendations can be drawn from the array of studies on living mulch, other than that there are many instances that suggest positive benefits from application of the system. While disparate research groups undertake an assortment of relatively short-term experiments, focusing primarily on field-based research of the topic, questions about much of the science of the system will remain unanswered. Adoption by farmers will remain limited to those who hold to the pioneer spirit, rather than by a wider audience wishing to make use of a well-honed, practical tool, refined by replicated scientific studies.

Paine and Harrison (1993), in their review paper 'The Historical Roots of Living Mulch and Related Practices', began by highlighting that "maintaining the productivity of the soil that feeds a population is essential", and also that it is necessary for a surplus of crops to be produced for the continued "rise of civilizations". A well-managed living mulch system can maintain productivity and even exceed conventional production levels. Living mulch requires an interdisciplinary understanding for the effective and appropriate employment of this complex system. Without doubt there are many variables to be considered before choosing what living mulch to grow with what crop and how it should be managed. Interactions between the crop,

living mulch and the growing environment are crop- and site-specific and so careful research and practical experimentation must be undertaken before widely adopting the living mulch system (Coolman and Hoyt, 1993; Wiles et al., 1989).

Living mulch is not suitable for every growing situation, although it would appear it is applicable for use in plasticulture. Some of the competitiveness between living mulch and crop seen in other situations is reduced due to polythene-sheeting and micro-irrigation being in the vicinity of the cash crop and therefore limiting the need for suppression of the mulch. Conflicting results regarding the effect on crop yield of growing living mulch between polythene-mulched beds, where the same living mulch was utilized but the crops were different, suggests growers must carefully consider the combination of living mulch and crop they choose (Reiners and Wickerhauser, 1995; Rice et al., 2007). When the right combination is identified the use of living mulch can bring significant and diverse benefits to a cropping system for years (Paine and Harrison, 1993; Clark, 2007).

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## **Chapter 2: Living mulch in alleyways for Phytophthora blight and weed control**

### **Introduction**

The use of living mulch is the intercropping strategy used primarily for weed control, whereby a managed 'companion crop' is planted before, with or after a cash crop is planted, and grows alongside this cash crop for the duration of its production cycle (Robinson and Dunham, 1954; Hartwig and Ammon, 2002; Hoffman and Regnier, 2006). Pioneers of this system include Robinson and Dunham (1954) for their development of the system of using a 'companion crop' with soybean and Lilly (1965) for establishing the "sleeping sod" system of planting warm-season crops into suppressed, cool-season perennial grasses.

Dr. Robert Sweet is credited with having first coined the term "living mulch" to distinguish the management of plants within the cropping system grown among cash crops as being different from other specific uses of cover crops such as 'green manure', 'smother crop' and 'catch crop' (Hughes and Sweet, 1979; Teasdale et al., 2007). Living mulch performs the functions of a smother crop to suppress weeds but it is distinguished from smother crops since it is grown at the same time as the cash crop, and not prior. The living mulch system also plays a role in mitigating the serious soil problems caused by continuous cropping (Hughes and Sweet, 1979).

Uniform, rapid, early growth, resulting in dense, competitive groundcover is key to the success of living mulch suppressing weeds (Teasdale, 1998; Nicholson and Wien, 1983; Bertin et al., 2009). The living mulch's morphology and physiology should be both noncompetitive to the primary crop and suppressive to weeds (Nicholson and Wien, 1983; Lanini et al, 1989). Height is not a measure of plants suitability for use as living mulch (Nicholson and Wien, 1983).

Groundcover and total biomass are more reliable indicators of ability to suppress weeds. Both

are dependent on growing conditions, seeding rate and stand establishment (Patten et al., 1990; Burgos et al., 2006).

Just as there is negative correlation between living mulch dry matter and amount of weed biomass, there can be a similar reduction in crop yield due to living mulch biomass (Sweet, 1982; Nicholson and Wien, 1983; Carof et al., 2007). To prevent yield reduction of the cash crop when using living mulch, some form of suppression of the living mulch is required. Popular methods of living mulch suppression include altering seeding techniques by reducing seeding rates and broadcast sowing rather than drilling, herbicides at sub-lethal doses and mechanical mowing, applied alone or in combination (Akemo et al., 2000; Vrabel et al., 1980; Teasdale, 1998; Sweet, 1982; Hall et al., 1984; Echtenkamp and Moomaw, 1989; Lilly, 1965; Gupton, 1997; Leary and DeFrank, 2004; Wiles et al., 1989; Carof et al., 2007; Hall and Cherney, n.d.; Chase and Mbuya, 2008; Zumwinkle and Rosen, 1991; Ilnicki and Enache, 1992). Timing of suppression, degree of suppression and mulch root growth patterns are important factors to consider for successful management of the living mulch system (Wiles et al., 1989).

Mixing species, particularly a combination of grass and legume, can be preferable compared to growing a single species living mulch, especially in the organic system where legumes play an important role in providing nitrogen. Legumes fix atmospheric-nitrogen through a symbiotic relationship with soil-borne bacteria (Hoffman and Regnier, 2006; Teasdale et al., 2007; Creamer and Baldwin, 2000; Gaskell and Smith, 2007). Aboveground biomass from a mixed-species stand of a grass mixed with a legume may be similar or greater than either species grown in monoculture, indicating increased groundcover and weed suppression (Contreras-Govea and Albrecht, 2005; Burgos et al., 2006). A 'nursing' effect between the two plant species is believed to exist, which is where both are of mutual benefit to the other through them modifying the growing environment (Contreras-Govea and Albrecht, 2005).

It is important to understand the biotic, as well as the abiotic, interactions any potential living mulch plant has, within the environment it is expected to be utilized, before it is fully introduced into the cropping system (Hoffman and Regnier, 2006). For example, legumes planted in a living mulch mix for an apple orchard increased vole damage (Wiman et al., 2009). Despite a recommendation for the use of common purslane as living mulch by Ellis et al. (2000), this plant is a host of *Phytophthora capsici* and therefore is not suitable to be grown with crops susceptible to this pathogen (Ploetz and Haynes, 2000). Screening of numerous potential candidate species is essential as results may show only small numbers of species being suitable (Sweet, 1982). Research on the use of living mulches has concentrated on using small grain, forage and turf-grass species (Carof et al., 2007; Echtenkamp and Moomaw, 1989; Nicholson and Wien, 1983).

The practice of utilizing plants for living groundcover has since been applied to almost all field-based crop production systems, including both annual and perennial edible crops and perennial ornamental crops (Bond and Grundy, 2001; Hartwig and Ammon, 2002; Cripps and Bates, 1993; William, 1987; Hughes and Sweet, 1979; Hawkins, 2004). Growing living mulches with cash crops is consistent with the goals of organic and sustainable agricultural practice, provides numerous benefits to the agroecosystem, and plays an important role in supporting ecosystem services ( Hoffman and Regnier, 2006; Graglia et al, 2006; Leary and DeFrank, 2000; Teasdale et al., 2007).

Use of living mulches has the potential to improve the positive effects of agriculture on the environment through reducing dependency on fossil fuels, and increasing carbon sequestration, biodiversity and soil organic matter (Carof et al., 2007; National Wildlife Federation, 2011). Conventional weed control methods, by periodic tillage, use of polythene mulch, or herbicide

application, rely heavily on fossil fuels. Living mulch can give equal or better weed control as these conventional methods and when integrated into a reduced tillage system it may further reduce the number of trips across a field a farmer has to make (DeGregorio and Ashley, 1986; Zumwinkle and Rosen, 1991; Ellis et al, 2000; Lilly, 1965; Burgos et al., 2006). Applications of chemical treatment for pest control may be reduced or eliminated through use of living mulches, which addresses consumers concerns over pesticide use for food production on human health and the environment (Bond, 1992; Robinson and Dunham, 1954; Andow et al., 1986).

Since the first description of the concept of using living mulch by Robinson and Dunham (1954), most research and adoption of the system has been related to weed control. A weed emerging from the soil into an established stand of living mulch is at a disadvantage and highly unlikely to be able to compete for essential resources (Teasdale, 1998; Sweet, 1982). Studies have shown that living mulch suppresses weeds throughout the season and at all stages of the weed's lifecycle better than a mulch of cover crop residue (Teasdale et al., 2007). Living mulches intercept and absorb light radiation, resulting in the inhibition of phytochrome-mediated germination of weed seeds and some release allelochemicals from their shoots and roots, adding to their effectiveness in controlling weeds (Creamer et al., 1996). Effective weed control, even for pernicious perennial weeds, comparative to use of herbicides, can be achieved using the living mulch system, especially in combination with suitable tillage and planting practices for the primary crop (Enache and Ilnicki, 1990; Infante and Morse, 1996; Graglia et al., 2006; Lukashyk et al., 2008).

Living mulch moderates soil temperature and soil water content, and may increase arbuscular mycorrhizal colonization of the primary crops roots and therefore is beneficial to young and tender plants (Sweet, 1982; Teasdale, 1998; Ochsner et al., 2011; Deguchi et al., 2005; Contreras-Govea and Albrecht, 2005). Some crops grown in the living mulch system may yield

the same or greater than a conventional, bare-soil, production system (Infante and Morse, 1996).

Incidence of plant disease can be reduced through use of living mulch, due to the modification of the growing environment by the living mulch (Merwin et al., 1992; Valenzuela and DeFrank, 1994). Pest damage can also be reduced due to use of living mulch. Fewer herbivores and more predators are observed in crop fields that have living mulch grown in the system (Amirault and Caldwell, 1998; Andow et al., 1986; Altieri et al., 1985). A reduction in certain crop-pests consequently reduces insect-transmitted diseases such as viruses (Hooks et al., 1998).

However, living mulch may also be alternate hosts to diseases and can also increase crop damage from diseases and pests unless selected and managed appropriately (Sweet, 1982; Ploetz and Haynes, 2000; Altieri et al., 1985; deCalesta, 1982; Sullivan, 2006; Wiman et al., 2009).

Water leaving a field may carry soil particles, nutrients and pesticide residues, leading to pollution of water courses, loss of resources and increased expense to the farmer (Rice et al., 2002; Rice et al., 2004; Rice et al., 2007; Sweet, 1982; Dietrich and Gallagher, 2002). Overland flow of water, either due to furrow-irrigation or a heavy rainfall event is a particular problem. It has been shown that living mulch reduces surface runoff of water, and allows more water penetration (Sweet, 1982). Consequently living mulch reduces soil erosion, nutrient leaching and herbicide runoff, often by a significant amount (Carter et al., 1993; Cripps and Bates, 1993; Hall et al., 1984; Hively and Cox, 2001; Singer and Pedersen, 2005; Hartwig, 1985; Schwab and Albrecht, 2011; Ochsner et al., 2011; Rice et al., 2004; Rice et al., 2007). Living mulch roots will help retain soil structure, uptake water and leachates, improve drainage and may have further positive effects on the soil ecosystem. Indeed, Sweet (1982) believed that 90% of the benefits of the living mulch both to the soil and cropping system are derived from its roots.

The use of living mulch to reduce soil erosion and movement of nutrients and pesticides from the field to water courses has its greatest potential in the plasticulture system. In intensive, high-value, fresh-market vegetable production a field may have between 50% and 75% of its area covered with impermeable polythene mulch, leading to a four-fold increase in water runoff and three-fold increase in volume of lost sediment compared to plots mulched with cover crop residue (Rice et al., 2001). Runoff from vegetable production utilizing polyethylene mulch can contain up to 35% of copper applied to crops in fungicides, which can be toxic to aquatic organisms. Preventing movement of sediment into a watercourse can reduce toxic pesticide loads entering water by up to 90% (Dietrich and Gallagher, 2002; Rice et al., 2004; Rice et al., 2007). Permeable-sheeting and plant-residue mulch materials have been recommended as polythene mulch alternatives, but they do not control weeds or remain viable as long as plastic sheeting and therefore are not widely adopted (Rice et al., 2001; Grundy and Bond, 2007).

Combining the use of polythene mulch with micro-irrigation methods, surface and subsurface, has improved control of weeds, crop yield and also some soil erosion. Water, fertilizer and fungicide can be supplied in exact amounts and with timely precision through trickle irrigation tubing; improving efficiency of their use and minimizing the availability of these resources to weeds (Madramootoo and Rigby, 1991; Singh et al., 2009; Ristaino et al., 1997). Nitrate-leaching to groundwater is significantly reduced by this system, compared to no mulch or biodegradable mulch (Emmert, 1956; Romic et al., 2003). The ability to control fertigation with such precision reduces the amount of culled fruit due to disorders brought on by water and mineral excess or deficiency. Irrigation to the soil and roots also reduces diseases that may otherwise result from repeated wetting of aboveground plant parts and splashing of pathogen propagules by overhead-irrigation (Ristaino et al., 1997; Sujkowski et al., 2000). Overhead- and

furrow-irrigation lead to movement of soil about and away from the field, whereas no soil movement or loss occurs due to micro-irrigation (Carter et al., 1993).

In temperate climates, where there is a short growing season and premium early-season prices, use of black polythene mulch for a relatively cheap and easy-to-manage method of weed control results in greater total yield, early yield, yield per plant and larger fruit, compared to no mulch or organic mulches applied to the bed surface (Emmert, 1956; Teasdale and Abdul-Baki, 1997; Roe et al., 1994; Decoteau et al., 1989). Preparation of plastic-mulched beds in fall allows earlier spring planting that may lead to an even earlier first harvest (Reiners et al., 1997).

The many benefits of plasticulture to crop production are described by Emmert (1956) and Lamont, Jr. (1996 and 2005) and include

- Earlier crop production (7-21 days)
- Higher yields per acre (2-3 times higher)
- More efficient use of water resources
- Reduced leaching of fertilizers
- More efficient use of fertilizer inputs
- Reduced soil and wind erosion
- Better management of certain insect pests
- Fewer weed problems
- Reduced soil compaction and elimination of root pruning
- Opportunity to double- or triple-crop with maximum efficiency

Black polyethylene, 1.25 mil (0.031 mm) thick, is the most popular sheet mulch used and has a lifespan of up to 3 years depending on its use and weather exposure; thicker and embossed sheeting may last longer (Lamont, Jr., 1996; Grundy and Bond, 2007; Univ. of Georgia, College of Agr. & Environ. Sci., Coop. Ext., 2009). The color of plastic mulch changes the microclimate surrounding the plant: the spectral balance, quantity of light and root zone temperature (Decoteau et al., 1989; Decoteau et al., 1990). Black polythene absorbs most ultraviolet, visible and infrared wavelengths and reflects this back as heat, giving it the ability to reduce weed seed germination, growth of weeds, and in the cooler periods of the growing season can help moderate temperature both above and below the soil; soil temperature below this material can be 36°F (2°C) higher than bare soil, white or silver mulches (Lamont, Jr., 1996; Grundy and Bond, 2007; Decoteau et al., 1989), leading to earlier harvests and crop growth extending later into the season. In addition, plastic mulch may modify the growing environment surrounding a transplant through the carbon dioxide that builds up beneath plastic mulch, from aerobic respiration of plant roots and soil organisms, being released through planting holes. Grundy and Bond (2007) suggest this may lead to an enhancement of a crop plants ability to photosynthesize.

A high value, fresh market vegetable crop that benefits from being planted through polythene mulch is bell pepper (*Capsicum annuum* L.). Grown as a warm season annual plant in temperate regions this New World crop's economic value to US agriculture in 2010 was \$637 million dollars, making it the sixth most valuable fresh market crop (Univ. of Kentucky, College of Agr. Coop. Ext. Serv., 2010; U.S. Department of Agr. and Nat. Agr. Statistics Service, 2011). Four to six inches (10cm to 15 cm) tall bell pepper plants, propagated from seed in greenhouses then hardened-off, are transplanted to polythene mulched beds in single or double rows, at between 18 inches and 12 inches (45 cm and 30 cm) in-row spacing, depending on whether large fruit or high yields, respectively, are required (Univ. of Georgia, College of Agr. & Environ.

Sci., Coop. Ext., 2009; Locascio and Stall, 1982; Hutton and Handley, 2007; Khan and Leskovar, 2006; Univ. of Kentucky, College of Agr. Coop. Ext. Serv., 2010). Despite different plant population densities being adopted, fruit size or weight do not significantly differ (Gaye et al., 1992).

Peppers should not be planted on land that is slow to drain or is close to water-bodies due to an increased risk of flooding and disease. They are intolerant of flooding, drought and weed competition. Despite 70% of bell pepper roots being in the upper 4 inches (10 cm) of the soil profile, the plants require deep, fertile, well-drained soil with good water-holding capacity and pH 5.8 to 6.6 (Gough, 2001; Berke et al., 1999; Univ. of Kentucky, College of Agr. Coop. Ext. Serv., 2010; Penn State College of Agr. Sci., Agr. Res. and Coop. Ext. 2000).

Through its modification of the growing environment, as described above, polythene-mulched raised beds have been shown to increase total yield, number of U.S. No.1 grade fruit and marketable yield of bell pepper, and fruit accumulate greater total amount of nitrogen in shoots and fruit, compared to plants grown in bare soil (Monette and Stewart, 1987; Locascio et al., 1985). Of particular importance to this crop is that weed competition is significantly reduced due to the polythene. The combination of the process of bed-formation and it being covered with polythene improves water retention and drainage in the root-zone. Trickle-irrigation is typically used for the production of peppers and is laid, preferably beneath the soil surface, when the bed is formed. For the reasons already described this irrigation method is beneficial; especially for prevention of foliar diseases.

A significant pathogen of a broad range of annual and perennial crops, notably in the Solanaceae, Cucurbitaceae, Chenopodiaceae, Brassicaceae and Leguminosae families, that is easily spread about a field due to flooded soil or splashing is *Phytophthora capsici* Leon.

(Gevens et al., 2008a; Gevens et al., 2008b; Tian and Babadoost, 2004). This oomycete causes the disease Phytophthora blight, which is also known as Phytophthora crown and root rot and Phytophthora fruit rot, and is reported in the America's, Europe and Asia (Ristaino, 2003; Gevens et al., 2008a). Cucurbits and bell peppers are the most susceptible crops to this disease (Tian and Babadoost, 2004). Single farms growing these crops have reported economic losses amounting to hundreds of thousands of dollars and up to 100% loss of a crop due to Phytophthora blight (Hausbeck and Lamour, 2004).

First discovered on Chile peppers by Leon Leonian in 1918, the pathogen is known to infect every part of a bell pepper plant, causing root, crown and fruit rot, and stem and leaf lesions (Leonian, 1922; Ristaino, 2003; Ristaino and Johnston, 1999). Symptoms are small, water-soaked, dull green spots or elongated lesions that occur on fruit, leaves or stems. Plants and, most importantly, harvested fruit may be asymptomatic for up to 24 hours following infection (Leonian, 1922; Hausbeck and Lamour, 2004).

Primary infection of a healthy pepper plant is most commonly caused by an inoculum source in the soil that causes a root or crown infection. *Phytophthora capsici* reproduces sexually and asexually and may be polycyclic within a season. An oospore results from sexual recombination of compatible mating types, A1 and A2, and due to its thick cell wall containing  $\beta$ -glucan and cellulose it remains viable in soil overwinter in temperate climates, surviving for more than 5 years, and serves as a significant source of inoculum in soil (Ristaino and Johnston, 1999; Hausbeck and Lamour, 2004; Lamour and Hausbeck, 2000; Lamour and Hausbeck, 2002). Asexual reproduction occurs through the formation of an ovoid sporangium borne at the tip of a branched sporangiophore (Ristaino and Johnston, 1999; Ristaino, 2003). Sporangia are formed in large amounts and are easily released but do not survive temperate winters (Ristaino, 2003; Hausbeck and Lamour, 2004).

Pathogenicity and epidemiology of *P. capsici* depends greatly on the availability of free water in the growing environment. Germination of oospores relies on cyclic changes of soil-water matrix potential; a constant level is inhibitory (Hord and Ristaino, 1991; Ristaino and Johnston, 1999; Bowers and Mitchell, 1990). When immersed in free moisture each sporangium germinates indirectly releasing 20 to 40 motile, bi-flagellate zoospores through a papilla at its apex (Hausbeck and Lamour, 2004; Ristaino and Johnston, 1999; Ristaino, 2003).

Initial infection occurs by growing roots coming into contact with inoculum in the soil, or the pathogen growing, or moving, toward plant roots chemotactically, following a nutrient gradient (Bowers et al., 1990; Hausbeck and Lamour, 2004; Ristaino, 2003; Sujkowski et al., 2000). Plant-to-plant spread of the pathogen within a row also occurs, primarily due to root-to-root contact (Ristaino et al., 1997).

Water is an important vector for pathogen propagules along and between rows of plants, in and above the soil, in a naturally-infested field (Ristaino et al., 1997; Ristaino, 2003; Hausbeck and Lamour, 2004). Sporangia are easily dislodged in water by mechanical and capillary action, and zoospores are capable of swimming (Granke et al., 2009; Ristaino and Johnston, 1999).

Infectious *P. capsici* propagules can be transported up to 230 feet (70 meters) downstream and 6.6 feet (2 meters) upstream from an initial point of inoculum in furrow-irrigated fields (Café Filho and Duniway, 1995). *Phytophthora capsici*-infested irrigation water can be a significant source of primary inoculum (Hausbeck et al., 2006).

Wind-driven rain can cause significant dispersal of *P. capsici* from inoculum on the surface of soil or plastic-mulch to the aboveground parts of plants within such a short time-period of rainfall as less than 2 minutes (Bowers et al., 1990; Granke et al., 2009; Madden, 1997). Increases in

volume of water fallen within a time-period and frequency of rainfall events are correlated to an increase in disease progress (Bowers et al., 1990; Ntahimpera et al., 1998). Water droplet size is highly correlated to the number of spores transported and flight distance of the secondary droplets. Although splash dispersal of propagules by rainfall or overhead irrigation occurs over short distances of less than 6 inches (15 cm) it is important in epidemic development in this pathosystem (Grove et al., 1985; Madden, 1997).

Control measures for this pathogen include utilizing genetic resistance in cultivars, monitoring and reducing propagules in soil and water, application of fungicides and reduction of plants exposure to excessive water (Ristaino and Johnston, 1999; French-Monar et al., 2007). Utilizing at least a 5-year rotation in the cropping system is ideal, although in areas where *P. capsici* infestation is rife, finding land to include in such as rotation can be difficult (Hausbeck and Lamour, 2004).

There are a limited number of *P. capsici*-tolerant and -resistant bell pepper cultivars currently available to growers, and where this does exist it is limited to a reduction in susceptibility to crown and root rot symptoms. Genetic resistance to below ground symptoms is not an indicator of resistance to infection of stems, leaves and fruit (Hausbeck and Lamour, 2004; Dunn et al., 2010; Walters et al., 2007; Babadoost, 2009).

Being aware of the presence of *P. capsici* in the field, removing all diseased plant material from the field to landfill or for incineration, eradicating the presence of weeds that are known to be alternate hosts and sampling water sources to avoid contaminated irrigation water are effective control measures. Treating infested water with algaecides containing copper sulfate, chelated copper or sodium carbonate peroxhydrate (SCP) active ingredients have been shown as

possible alternative control treatments (French-Monar et al., 2006; Ploetz and Haynes, 2000; Tian and Babadoost, 2004; Hausbeck et al., 2006; Granke and Hausbeck, 2010).

Repeated use of the same fungicides, particularly mefenoxam and metalaxyl within the phenylamide class, has led to fungicide-insensitivity and -resistance in some *P. capsici* isolates and their progeny (Parra and Ristaino, 2001; Lamour and Hausbeck, 2001; Lamour and Hausbeck, 2002; Café-Filho and Ristaino, 2008). Despite this, the use of fungicides for the control of Phytophthora blight is still recommended, as long as chemistries are rotated. Translocated and copper-based fungicides help prevent aboveground symptoms of the disease (Ristaino et al., 1997; Ristaino and Johnston, 1999).

Planting susceptible crops away from low-lying areas of a field, on raised, crowned beds to increase water runoff away from the plants to the furrow reduces exposure of the crop to excess water and reduces risk of infection; although Phytophthora blight may affect plants in well-drained soil when environmental conditions are favorable and an inoculum source is present (Hausbeck and Lamour, 2004). Cultivations to form the bed make a more friable, porous soil texture thus improving drainage in the root-zone. If organic matter is to be applied to land where there is a risk of *P. capsici* being present, its use should be considered carefully and application kept moderate or in small quantities, particularly on soils that have poor drainage, because humic and water content and levels of net mineralizable N are positively correlated with final disease incidence on bell pepper seedlings (Liu et al., 2008). Constant soil water matrix potential should be maintained and controlled through the use of subsurface drip irrigation (Ristaino, 2003; Bowers and Mitchell, 1990). Irrigation via this method prevents irrigation wetting of aboveground plant parts, and combined with planting at lower densities that increases airflow around plants to speed their drying after rain, these can greatly reduce infection (Hausbeck and Lamour, 2004).

The effective control of a pathogen such as *P. capsici* depends on a combined management approach, using the full range of available control measures, including growing crops through polythene mulch. Use of polythene mulch moderates soil moisture levels and greatly reduces splash dispersal in the immediate vicinity of the crop plant, therefore reducing aboveground disease incidence, reducing the need for foliar fungicide applications, and increasing the marketable yield of the crop (Stevens et al., 1993; Univ. of Georgia, College of Agr. & Environ. Sci., Coop. Ext., 2009). However, there can be a greater percent of wilted bell pepper plants early in the growing season and at the end, due to incidence of crown and root rot, than plants grown through organic and living mulches (Ristaino et al., 1997; Roe et al., 1994) and propagules of *P. capsici* may still be splashed from the bare soil of the alleyway onto plants or to the surface of the mulch, from where it is then splashed to plants.

Mitigation of splash dispersal of soil from alleyways has the potential to reduce the need for fungicide applications. Use of mulches to reduce splash dispersal of pathogens has been studied and the characteristics of ground cover and plant canopy have a significant effect on disease spread. Mulching with straw or establishing living mulch between crop plants significantly reduces splash dispersal compared to bare soil. And, increasing surface roughness and leaf area index of the living mulch canopy reduces splash dispersal of *P. capsici*. Dense cover of tall grass living mulch disperses *P. capsici* spores further than short grass (Ristaino and Johnston, 1999; Madden and Ellis, 1990; Madden, 1997).

This study examined the use of living mulch between polythene mulched beds to reduce the incidence of foliar blight and fruit rot symptoms on bell pepper. The objectives were to

- evaluate a range of plant species for use as living mulch in this system,
- evaluate their effectiveness in covering the surface of soil and suppressing weeds,

- evaluate their response to mowing,
- assess crop yield to ensure growing living mulch is not costly to the cash crop, and
- evaluation of the living mulch's ability to reduce splash dispersal of *P. capsici* from alleyways.

The hypothesis was that living mulch growing between polythene mulched beds, providing good groundcover, does not reduce crop yield, provides good weed suppression, which would be improved by mowing, and reduces incidence of Phytophthora blight symptoms on aboveground tissue of pepper plants compared to bare soil or dead mulch. A further hypothesis was that plots treated with tall, dense living mulch would have higher Phytophthora blight incidence than short, or mowed, living mulch, because of increased *P. capsici* spore dispersal, as described above.

## **Methods**

Experiments took place in 2009 and 2010 at the Phytophthora Blight farm, part of Cornell University's New York State Agricultural Experiment Station (NYSAES), in Geneva, NY (42°52'51.84"N, 77°00'48.10"W). Soil type was Odessa silt loam (fine, illitic, mesic Aeric Endoaqualfs). Soil samples were taken prior to both years' experiments and analyzed by Morgan extraction at Cornell Nutrient Analysis Laboratory (Ithaca, NY). Soil organic matter and pH were within normal ranges in both years. Phosphorus and potassium were added according to soil test recommendations.

## **Experimental design**

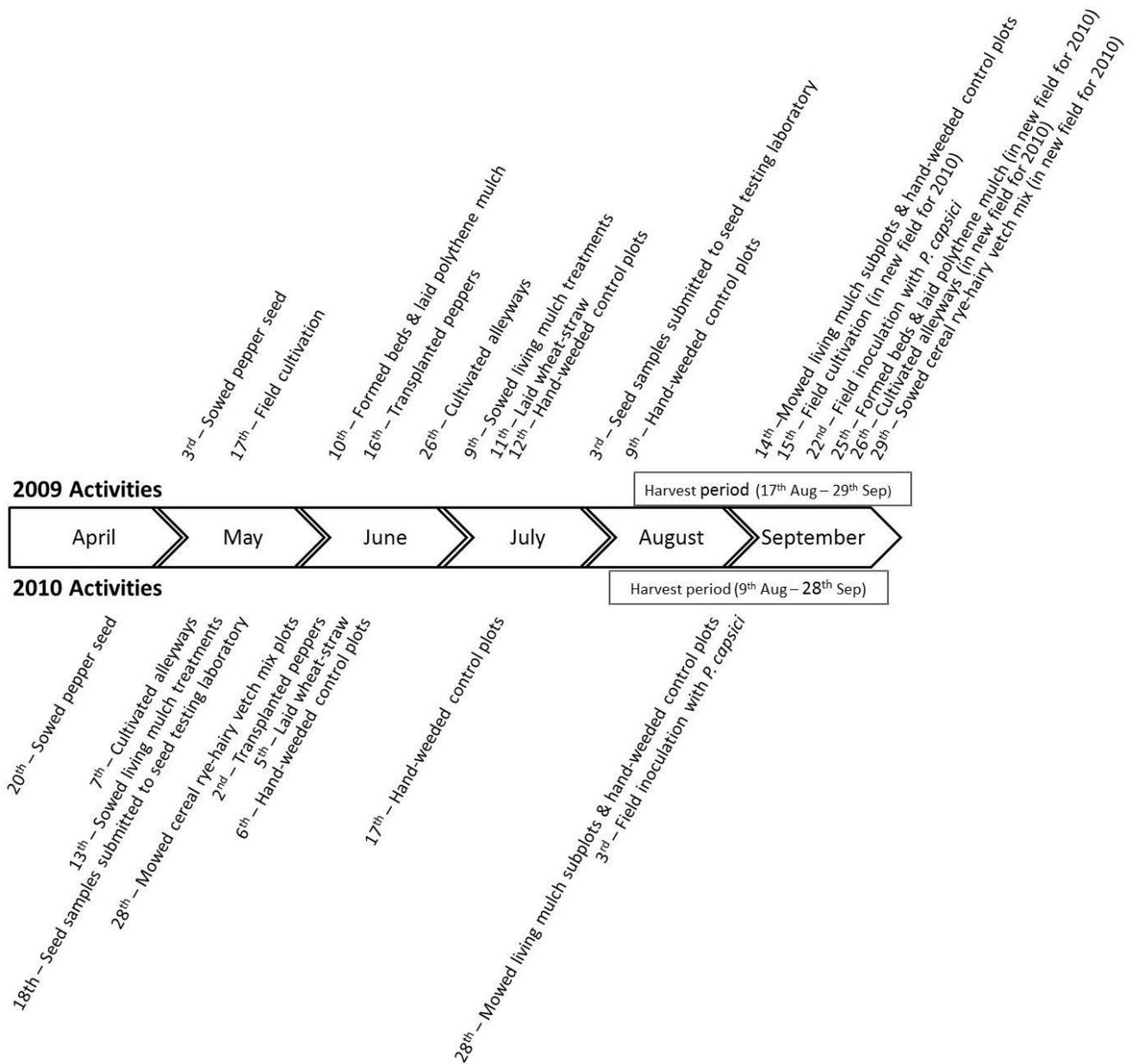
Seeds of 'Revolution' bell pepper, a large-fruited, blocky-type, with intermediate resistance to *P.* blight (Source: Harris Moran), were seeded into 72-cell plastic trays [cell size 1.75 inches by

1.75 inches (4.5 cm by 4.5 cm)] filled with Cornell Mix, a soilless potting mixture, on May 3, 2009 and April 20, 2010 (Figure 2.1) and grown within a climate-controlled greenhouse environment until late May, approximately two weeks prior to planting, when seedlings were moved to open cold-frames for hardening and holding. Watering and fertigation was by hand, as needed.

For the 2009 study, a field was prepared in May using a disc-harrow, followed by a tined-harrow with rear crumbler-cage to incorporate overwintered plant debris. For the 2010 study, the field was prepared in mid-September 2009. A summer cover crop of annual ryegrass (*Lolium multiflorum* Lam.) was herbicide-killed and cultivated using the same procedure as for the 2009 study.

Flat beds, 36 inches (92 cm) wide, mulched with embossed black plastic [1 mil (0.0254 mm thick)] were laid on 84 inch (2.13 m) centers. A single line of drip irrigation [8 mil (0.2032 mm)] T-Tape was laid along the center of each bed on the soil-surface, beneath the plastic-mulch, during the forming of each bed. Beds were made for the 2009 study on June 10 and for the 2010 study on September 25, 2009 (Figure 2.1). Irrigation was applied as needed through the season.

On June 16, 2009, a single row of 'Revolution' plants, were hand-planted 18 inches (45 cm) apart into fertigated holes in each bed. Holes were punched through the plastic by a tractor-towed, single-row, water-wheel transplanter, which provided approximately 8 fluid ounces (237 mL) starter-solution of Peters Excel All Purpose soluble fertilizer, 21-5-20 kg.ha<sup>-1</sup>, N - P<sub>2</sub>O<sub>5</sub> - K<sub>2</sub>O, at a rate of 10 lbs/A of N, into each planting hole. Each plot contained sixteen plants and subplots had eight.



**Figure 2.1:** Timeline of activities for 2009 and 2010, which took place at the Phytophthora Blight farm in Geneva, NY.

On June 2 2010, plants were hand-planted through the plastic-mulch at staggered double-row spacing: rows 18 inches (45 cm) apart and plants 12 inches (30 cm) apart within row. Plots

contained thirty-six plants, and subplots contained eighteen. Upon completion of planting, all beds were irrigated. No starter-solution was applied.

In 2009, fertigation took place approximately every two weeks for the first ten weeks of the trial, using a venturi-type injector system from a stock solution of 10-10-10 kg.ha<sup>-1</sup>, N - P<sub>2</sub>O<sub>5</sub> - K<sub>2</sub>O, soluble fertilizer, applied to the field at a rate of 10 lbs N.ac. In 2010 weekly fertigation took place for six weeks: five applications of 10-10-10, at a rate of 10 lbs.ac N, followed by one application of 20-10-20 N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O .ha<sup>-1</sup>, at a rate of 10lb/A N.

In both years, experimental design was randomized block, split-plot design with five replications, with a single factor of mowing (two levels: mowed or unmowed) for living mulch treatments. To account for variability in soil conditions across the field, each bed and the adjacent alleyways, both sides, were defined as a block. Randomly assigned along each block were evenly-sized plots: Plots were 26 feet (7.9 m) long in 2009 and 20 feet (6.1 m) in 2010.

On June 26, 2009, alleyways between beds were rototilled, and a stale-seedbed was created. On July 9, 2009, seed for living mulches was broadcast-sown by hand and raked into the soil. During the first three weeks after sowing, overhead irrigation of seedbeds, using oscillating sprinklers, was required twice. Wheat-straw was laid soon after living mulches were seeded.

In 2009, soil on both sides of a plot was treated with one of three different broadcast-sown living mulches, annual ryegrass, creeping red fescue (*Festuca rubra* L. ssp. *arenaria* (Osbeck) F. Aresch.), or Dutch white clover (*Trifolium repens* L.). Two additional treatments were included. Wheat-straw mulch laid approximately 3 inches (7.6 cm) deep was applied by hand. Finally a

bare soil treatment served as a control, which was maintained by hand weeding. See Table 2.1 for the list of treatments.

For the 2010 trial, additional living mulches were included. Treatments included annual ryegrass, Dutch white clover, birdsfoot trefoil (*Lotus corniculatus* L.), Teff (*Eragrostis tef* (Zucc.) Trotter), or three LM mixes, cereal rye (*Secale cereale* L.) and hairy vetch (*Vicia villosa* Roth) mix, annual ryegrass and Dutch white clover mix, buckwheat (*Fagopyrum esculentum* Moench.) and Dutch white clover mix, or straw and bare-soil treatments; nine treatments in total. See Table 2.1 for a complete list of treatments.

Since beds were made in the fall of 2009, seeds for the cereal rye-hairy vetch mix were broadcast-sown on September 29, 2009, after alleyways had been rototilled and prepared for sowing. Seed was raked into the soil-surface and plots were covered with Reemay® row cover. Once seedlings had grown to approximately 2 inches (5 cm) tall, October 19, the row cover was removed during the day and replaced in the evening, until the end of October, when the Reemay® was removed completely.

All other living mulches were broadcast-sown on May 13, 2010, at their respective seeding rates, into rototilled and raked soil, then raked in and rolled. No irrigation was needed following sowing. Annual ryegrass-Dutch white clover mix and buckwheat-Dutch white clover mix plots had seed mixed in containers, and small seeds of teff were mixed with coarse sand. Straw plots were laid as in 2009, soon after pepper planting.

The cereal rye-hairy vetch mix plots grew until most inflorescences were newly open, before the entire plot was mown as close to the ground as possible using a two-wheel tractor with front-

mounted, 45 inches (114 cm) wide, sickle bar mower, on May 28, 2010. Cut plant material was spread evenly across the respective plots by hand.

**Table 2.1:** Alleyway treatments used in 2009 and 2010 experiments at the Phytophthora Blight farm in Geneva, NY.

<b>Living Mulch Treatments</b>	<b>Broadcast seeding rate (kgs.ha<sup>-1</sup>)</b>	
	<b>2009 Experiment</b>	<b>2010 Experiment</b>
Annual Ryegrass (AR)	34	34
Birdsfoot Trefoil (BT)	-	20
Creeping Red Fescue (RF)	6	-
Dutch White Clover (WC)	16	16
Teff (TF)	-	6
Annual Ryegrass with Dutch White Clover Mix (AC)*	-	17 & 8
Buckwheat with Dutch White Clover Mix (BC) *	-	48 & 8
Cereal Rye with Hairy Vetch Mix (RV) **	-	56 & 157
<b>Non-living mulch treatments</b>		
Control - Bare soil (SO)	n/a	n/a
Wheat-straw (ST)	n/a	n/a

Letters in parentheses are used as the treatment acronym in results [e.g. (AR)]

'-' indicates treatment not used

'n/a' indicates data not applicable

\* Mixtures sown at 50% of the species recommended seeding rate

\*\* Mixture sown at 200% of the species recommended seeding rate

All living mulch plots were split evenly in two and subplots assigned the factor of mowing were cut once, to within approximately 0.6 inch (1.5 cm) of the soil-surface using a sickle bar mower; this included mowing cereal rye-hairy vetch mix subplots again. Mowing of all living mulch treatments took place when the majority of the living mulch treatments had most inflorescences fully-opened and prior to seed formation: September 14, 2009 and August 28, 2010. Two passes of the mower were required to ensure most living mulch shoots were mown. Cut plant material was redistributed evenly across the respective subplot by hand.

Control plots of bare-soil, were hand-weeded three times (Figure 2.1), as required, during both growing seasons, following the initial stale seedbed preparation and pepper planting.

### **Field inoculation with *Phytophthora* Blight**

Inoculation of the 2009 experiment used *P. capsici* isolate 0664-1 (A1 mating type, sensitive to mefenoxam) cultured on V8® juice-vermiculite media. Nineteen gallons (72 liters) of inoculum material was prepared using the protocol described by Sujkowski et al (2000). An autoclaved mixture of V8® juice and vermiculite was inoculated with plugs of isolate 0664-1, taken from approximately 10-day-old cultures; one petri-dish culture was used for each 1 liter flask of inoculum. The inoculated media was incubated at 77°F (25 °C) for 3 ½ weeks in a room with natural daylight.

A band of inoculum, approximately 4 inches (10 cm) wide was laid, by-hand, in the alleyway alongside the edge of both sides of the plastic mulch, on September 22, 2009. Approximately 34 fluid ounces (1 liter) of inoculum material was used per single-side of a plot and was placed on the soil surface, beneath or between any dead or living mulch, in order to emulate *P. capsici* being present in the soil. Soil was moist at time of inoculum being applied and further precipitation fell shortly after, so no overhead irrigation was required.

On August 28, 2010, approximately six-hundred fruit of zucchini and cucumber, both *P. capsici*-susceptible crops, were inoculated with *P. capsici* isolate 0664-1 zoospores in liquid-suspension in a separate area of the field to the experiment, using distilled water and sprayed through a handheld pressure sprayer. Production of sporangia took place in the Smart Lab, at NYSAES, whereupon they were harvested in water and incubated to release zoospores. An estimate of approximately 776,000 zoospores per milliliter of solution was made using a hemacytometer. This concentration was diluted at a 1:10 ratio before being sprayed on the fruits. Surface of the

fruit was kept moist with water twice daily for a week. By September 3, mycelium was visible on the underside of most of the inoculated fruit indicating infection. These infected fruits were then distributed evenly among plots; placed within 6 inches (15 cm) of the edge of the plastic-mulch, beneath dead or cut mulch or amongst living mulch, as close to the soil as possible. For a total of six hours following inoculum being introduced to the field, overhead irrigation was applied, at a rate of approximately 0.25 inch (0.64 cm) per hour.

### **Sampling**

Seed samples of living mulch plant species, were taken from the proprietary bags of seed, labeled and submitted to the New York State Seed Testing Laboratory, Geneva, NY, for seed viability analysis on August 3, 2009 and May 18, 2010; insufficient seed of cereal rye and hairy vetch was available for testing. Sampling and analysis was undertaken according to the Association of Official Seed Analysts (AOSA) procedures.

Pepper fruit were harvested by hand, per subplot, every two weeks from August 17 to September 29, 2009, and weekly from August 9 to September 28, 2010. Harvested fruit was graded according to the United States Standards for Grades of Sweet Peppers (USDA, 2005): U.S. No.2, U.S. No.1 and U.S. Fancy. All grades were weighed and numbers of fruit were counted. Culled fruit were also recorded by number and weight, along with the reason for being culled. Phytophthora blight diseased fruits were counted and weighed in both years. All harvest data was extrapolated to provide weight (tons) and numbers of fruit per hectare.

Also in 2010, visible incidence of Phytophthora blight on pepper plants was recorded. Following inoculation and until the termination of the experiment, fourteen plants per subplot, excluding two at each end as border-plants, were assessed twice-a-week for the visible signs of Phytophthora blight. Observations of stem or leaf lesions, fruit-rot, wilting or death were

recorded in binary form for each plant. Numbers of affected plants per subplot were recorded and a percentage number of diseased plants and the disease incidence were calculated. From this data the area under disease progress curve (AUDPC), described by Shaner et al. (1977), was calculated using the formula:

$$\text{AUDPC} = \sum_{i=1}^n [(Y_{i+1} + Y_i)/2] [X_{i+1} - X_i]$$

in which  $Y_i$  = disease incidence at the  $i$ th observation,  $X_i$  = time (days) at the  $i$ th observation since inoculation took place, and  $n$  = total number of observations.

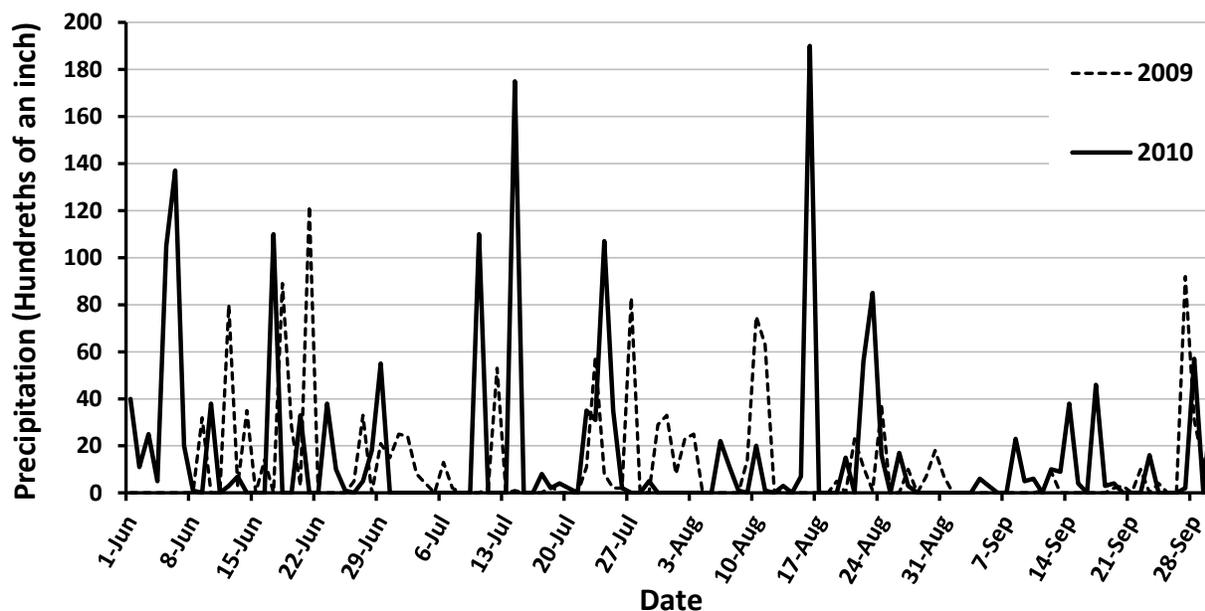
At the end of the growing season each living pepper plant had its height measured to the uppermost leaf tip and approximately twenty of the most recently matured leaves of healthy pepper plants in each subplot were excised. After being rinsed in distilled water and dried for 48 hours at 149 °F (65 °C), leaves were ground, packaged and sent to Cornell Plant Nutrient Analysis Laboratory, Ithaca, NY, for analysis of percent N, and quantity of K, Ca, Mg, P, Fe, Mn, Zn, B and Cu per subplot sample.

Subplots were measured for living mulch plant height, straw thickness and field penetration resistance readings were taken at both 6 inch (15cm) and 18 inch (45cm) depth in 2010. Percent groundcover of mulch and weeds, and above-ground living mulch biomass dry-weight were recorded in both years.

Living mulch height was measured from the soil surface to the uppermost tip of a plant, whether leaf or inflorescence, and repeated six times per subplot. Field penetration resistance readings were taken six times per subplot, using a hand-held soil compaction tester (penetrometer) according to the protocol described in the Cornell soil health assessment training manual (Gugino et al., 2007). Percent groundcover was by visual estimation, using Bayley's (2001)

diagram of “Distribution of ground cover to assist in determining percentage cover” as a guide. A 12 inch (30 cm) square quadrat of aboveground plant biomass was cut from both sides of a living mulch subplot and combined. Plant material was cut at the height of mowing-cut, separated into living mulch and weeds, placed in labeled paper-bags, dried for 48 hours at 149 °F (65 °C) then had dry-weight recorded. These assessments were undertaken at the termination of the experiments and biomass cuts were also taken from both subplots per plot, prior to mowing.

Subplots affected by prolonged periods of flooding after precipitation (more than two days) were noted from visual observations in both years. Flooding occurred on several occasions in both years, with 2010 being greatly affected. Precipitation events of daily accumulation exceeding 1 inch (2.5 cm) took place once in 2009 and six times in 2010 from the beginning of June through to the end of September (Cornell University, CALS, NYSAES, 2011) (Figure 2.2).



**Figure 2.2:** Precipitation levels for the 2009 and 2010 field trials, using data from the Vegetable Research Farm weather station, New York State Agricultural Experiment Station, Geneva, NY.

## **Data analysis**

During statistical analysis of data, when mowing did not have a significant effect on comparison of living mulch treatments, data for subplots was averaged to provide data per plot.

The repeated incidence of flooding in 2010 resulted in death of many young pepper plants: notably in straw plots. Only plants in one straw replicate survived, therefore 2010 straw data was excluded from statistical analysis that required assessment of pepper plants; this included harvest, disease incidence, pepper plant height and plant nutrient content analysis. All other treatments in 2010 had a minimum of three replicates; most had four. Subplots were excluded if less than seven plants per subplot were alive at the point of field inoculation. Flooding was included as a covariate in the initial model and remained if it had a significant affect, or removed if it had no significant effect.

Significance of differences among treatments, of the interaction between living mulch and mowing, and of the effects of mowing and the random variable of flooding, were determined using least-squares regression analysis. Significant differences ( $P \leq 0.05$ ) between treatments or in levels of main effect variables were determined using least significant difference (LSD) in JMP®, version 9.0.0 (SAS Institute, Inc., 2010). When residuals of data were not normally-distributed it was transformed using natural log or square root.

## **Results**

### **Seed viability**

Annual ryegrass had consistently high germination rate, most viable seed, and germinated over a short time period (Table 2.2). Birdsfoot trefoil had the lowest germination rate (71%) due to a

high percentage of hard seed (11%); “hard seed” being the un-germinated seed that remains at the end of testing due to the seed testa being harder than usual, resulting in no, or much slower, imbibition, which prevents germination. Creeping red fescue was the slowest to germinate (29 days) and also had low germination rate. Buckwheat, Dutch white clover and teff had similarly high germination rates, ranging from 82% for Dutch white clover to 90% for buckwheat.

**Table 2.2:** Seed viability analysis of living mulch plant species.

Living Mulch Treatments	2009				2010			
	Days to germination	Germination (%)	Hard seed (%)	Viable seed (%)	Days to germination	Germination (%)	Hard seed (%)	Viable seed (%)
Annual ryegrass	8	98	0	98	9	98	0	98
Birdsfoot trefoil	-	-	-	-	15	71	11	82
Buckwheat	-	-	-	-	9	90	0	90
Cereal rye	-	-	-	-	--	--	--	--
Creeping red fescue	29	73	0	73	-	-	-	-
Dutch white clover	7	86	2	88	9	82	3	85
Hairy vetch	-	-	-	-	--	--	--	--
Teff	-	-	-	-	15	86	0	86

Seed samples tested by the NYS Seed Testing Laboratory, Geneva, NY, according to Association of Official Seed Analysts (AOSA) procedures.

'-' indicates specie was not used.

'--' indicates samples were not submitted due to insufficient seed being available.

## Disease Incidence

In 2009 straw treatment had the greatest percentage of P. blight-diseased fruit of all treatments, although not significantly different, by weight (8%) and by number (11%) (Table 2.3). Creeping red fescue-treated plots yielded the least amount of Phytophthora blight-infected fruit.

Flooding significantly affected the number of infected pepper fruit in 2010. Non-flooded plots had significantly more infected fruit than flooded plots; an average of 30% infected fruit, by count, compared to 9% from flooded plots ( $P = 0.0014$ ) (Table 2.3).

In 2010 control plots yielded the greatest percent of harvested fruit with *Phytophthora* blight: 41% by weight and 32% by number of fruit (Table 2.3). There was no statistically significant difference of percent of diseased fruit among treatments. Buckwheat-Dutch white clover-treated plots had the lowest percent of infected pepper fruit by weight and teff-treated plots had the lowest percent of infected fruit by number.

Analysis of area under the disease progress curve (AUDPC) from 2010 data, indicated no significant difference ( $P = 0.3274$ ) between the least squared means of treatments (Figure 2.3). Differences of disease on above-ground plant parts diminished in severity between treatments in the following order: control > annual ryegrass-Dutch white clover mix > Dutch white clover > birdsfoot trefoil > annual ryegrass > cereal rye-hairy vetch mix > buckwheat-Dutch white clover mix > teff.

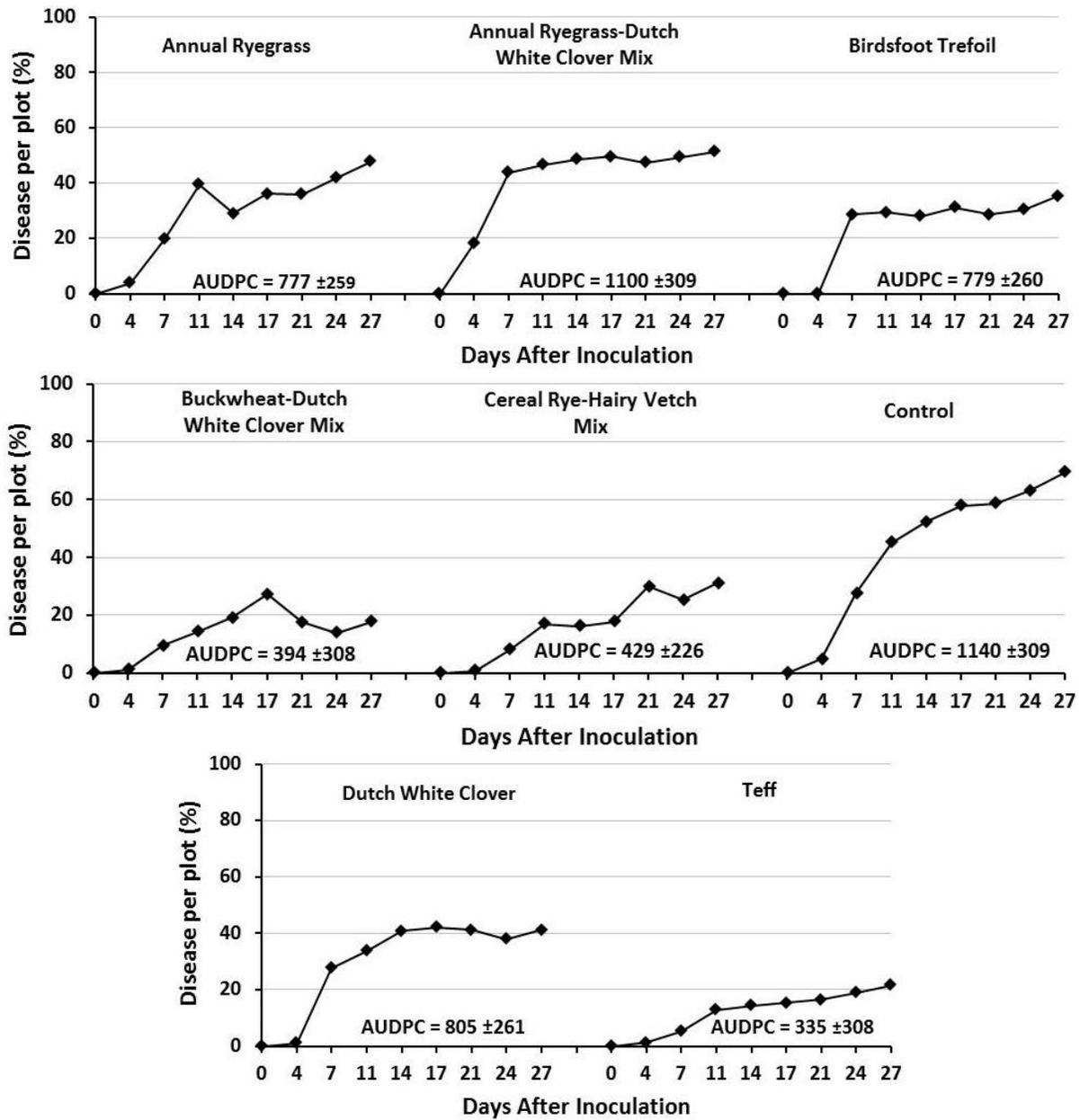
**Table 2.3:** The percent of total harvested fruit, post field-inoculation, with Phytophthora blight according to the effect of treatment and flooding. Least squared means followed by different letters within each column indicate statistically significant differences ( $P < 0.05$ ) between effects. Least squared means analysis was carried out separately for 2009 and 2010, and separately for the effect of flooding and treatments in 2010.

Effect Test	Total harvested fruit per treatment with P. Blight, by weight (%)		Total harvested fruit per treatment with P. Blight, by number (%)	
	2009	2010	2009	2010
Treatment	P = 0.0020	P = 0.5611	P = 0.0063	P = 0.2222
Flooding	-	-*	-	P = 0.0014
<b>LSMeans Differences</b>				
Unflooded:Flooded	-	-*	-	30 a 9 b
S.E. for Flooding	-	-*	-	3.9
Annual ryegrass	6 a	23	7 a	21
Annual ryegrass-Dutch white clover mix	-	29	-	24
Birdsfoot trefoil	-	29	-	29
Buckwheat-Dutch white clover mix	-	10	-	8
Cereal rye-Hairy vetch mix	-	11	-	10
Control	3 b	41	4 ab	32
Creeping red fescue	0.3 b	-	1 b	-
Dutch white clover	6 ab	17	7 ab	22
Straw	8 a	--	11 a	--
Teff	-	11	-	7
S.E. for Treatment	2.2	12	2.5	7.7

'-' indicates data was unavailable due to the treatment not being included.

'--' indicates data was unavailable due to pepper plants having died in this treatment's plots.

'-\*' indicates flooding was not included as a covariate due to it not having a significant effect in this analysis.



**Figure 2.3:** Phytophthora blight disease progress in 2010, shown as the percentage of experimental units ('Revolution' bell pepper plants) per treatment with visible symptoms. Treatments least squared means for AUDPC (Area under the disease progress curve) were not significantly different ( $P = 0.3724$ ); these LS Means are shown followed by the standard error for that respective treatment.

**Table 2.4:** ‘Revolution’ bell pepper plant height on the last day of trial assessment for the 2009 and 2010 growing seasons according to the effect of treatment and flooding. Least squared means followed by different letters within each column indicate statistically significant differences ( $P < 0.05$ ) between effects. Least squared means analysis was carried out separately for 2009 and 2010, and separately for the effect of treatment and flooding.

Effect Test	Pepper plant height (cm)	
	2009	2010
Treatment	P = 0.7974	P = 0.1239
Flooding	P = 0.0008	P = 0.0003
<b>LSMeans Differences</b>		
Unflooded:Flooded	42 a	32 b
S.E. for Flooding	1.5	3.2
Annual ryegrass	37	34
Annual ryegrass-Dutch white clover mix	-	37
Birdsfoot trefoil	-	26
Buckwheat-Dutch white clover mix	-	44
Cereal rye-Hairy vetch mix	-	30
Control	38	23
Creeping red fescue	35	-
Dutch white clover	40	33
Straw	36	--
Teff	-	34
S.E. for Treatment	2.4	5.0

'-' indicates data was unavailable due to treatments not being included.

'--' indicates data was unavailable due to pepper plants having died in this treatment's plots.

### Pepper Plant Height

Pepper plant heights were not significantly different between treatments in either year (Table 2.4). Pepper plants were significantly shorter in plots affected by flooding in both years.

In 2009, Dutch white clover-treated plots had the tallest median plant height, 16 inches (40 cm), and creeping red fescue-treated plots had the shortest plants, 14 inches (35 cm). The tallest pepper plants in 2010 were in buckwheat-Dutch white clover mix-treated plots; median plant height was 17 inches (44 cm), with one plot having an average plant height of 28 inches (71 cm)

(data not shown). Pepper plants in 2010 control plots were the shortest, at just 9 inches (23 cm) tall.

## **Nutrients**

2009:

'Revolution' pepper plants growing in all treatments had N, P, K, Mg, Ca, Fe, Cu, B and Zn leaf nutrient content within acceptable ranges (Mills and Jones, 1996) (Table 2.5). Pepper plants from all treatments were below the recommended level for Mn.

There was a significant interaction between treatment and mowing for both Mg and Zn in pepper leaves (Table 2.5). Pepper plants grown in mowed Dutch white clover subplots contained the most Mg, and those grown in unmowed creeping red fescue subplots contained the least Mg. Pepper plants grown in mowed Dutch white clover subplots contained significantly less Zn than plants grown in unmowed Dutch white clover subplots; the latter subplots contained the greatest amount of Zn of living mulch treatments in 2009.

Fe and Cu content of pepper plants grown in control plots was significantly greater than in plants grown in annual ryegrass- and straw-treated plots (Table 2.5).

There was a significant effect of flooding on both Cu and Zn content of pepper leaves (Table 2.5). Plants grown in unflooded plots contained more Cu and Zn than those plants exposed to flooding.

**Table 2.5:** Leaf nutrient content for 'Revolution' bell pepper plants grown in 2009. Macronutrient (N, P, K, Ca and Mg) content is expressed as a percentage; micronutrient (Fe, Cu, B, Mn and Zn) content is expressed as parts per million. Least squared means followed by different letters within in each column indicates statistically significant differences ( $P < 0.05$ ) between effects or treatments. Least squared means analysis was carried out separately for 2009 and 2010 and separately for effects and treatments within both years.

**2009**

Effect Test	N (%)	P (%)	K (%)	Mg (%)	Ca (%)	Fe (ppm)	Cu (ppm)	B (ppm)	Mn (ppm)	Zn (ppm)
Treatment	P = 0.1638	P = 0.6916	P = 0.8091	P = 0.3309	P = 0.1771	P = 0.0054	P = 0.0003	P = 0.0479	P = 0.9039	P = 0.3898
Treatment*Mowing	-	-	-	P = 0.0413	-	-	-	-	-	P = 0.0218
Mowing	-	-	-	P = 0.6133	-	-	-	-	-	P = 0.5784
Flooding	-	-	-	-	-	-	P < .0001	-	-	P = 0.0359
<b>LSMeans Differences</b>										
Unflooded:Flooded	-	-	-	-	-	-	11.6 a 6.5 b	-	-	65.8 a 53.0 b
S.E. for Flooding	-	-	-	-	-	-	0.6	-	-	3.8
Annual ryegrass	3.33	0.20	2.03	0.61 ab 0.53 b	3.86	169 b	6.4 b	44.4 a	45.1	54.5 b 55.3 b
Control	3.77	0.20	2.02	--	3.83	453 a	13.0 a	44.1 a	42.0	--
Creeping red fescue	3.70	0.21	1.97	0.56 b 0.61 ab	3.55	435 ab	9.8 ab	41.8 ab	44.8	54.4 b 64.0 ab
Dutch white clover	3.48	0.19	1.89	0.60 ab 0.66 a	3.74	219 ab	9.8 ab	44.9 a	43.2	72.0 a 56.2 b
Straw	3.48	0.20	1.91	--	3.16	194 b	6.1 b	37.0 b	38.5	--
S.E. for Treatment or Treatment*Mowing	0.1	0.02	0.4	0.1	0.3	105.4	1.0	2.2	7.2	5.2

'-' indicates covariate was not included due to it not having a significant effect.

'--' indicates data was unavailable due to there being a significant interaction between living mulch treatment and mowing.

**Table 2.6:** Leaf nutrient content for 'Revolution' bell pepper plants grown in 2010. Macronutrient (N, P, K, Ca and Mg) content is expressed as a percentage; micronutrient (Fe, Cu, B, Mn and Zn) content is expressed as parts per million. Least squared means followed by different letters within in each column indicates statistically significant differences ( $P < 0.05$ ) between effects or treatments. Least squared means analysis was carried out separately for 2009 and 2010 and separately for effects and treatments within both years.

<b>2010</b>										
<b>Effect Test</b>	<b>N (%)</b>	<b>P (%)</b>	<b>K (%)</b>	<b>Mg (%)</b>	<b>Ca (%)</b>	<b>Fe (ppm)</b>	<b>Cu (ppm)</b>	<b>B (ppm)</b>	<b>Mn (ppm)</b>	<b>Zn (ppm)</b>
Treatment	P = 0.8337	P = 0.6029	P = 0.9829	P = 0.0480	P = 0.0336	P = 0.0887	P = 0.5194	P = 0.1957	P = 0.0913	P = 0.6517
Treatment*Mowing	-	-	-	-	P = 0.0425	-	-	-	-	-
Mowing	P = 0.0227	-	P = 0.0013	P = 0.0452	P = 0.0050	-	-	P = 0.0113	P = 0.0131	-
Flooding	P = 0.0028	-	-	P = 0.0199	-	P = 0.0497	P = 0.0070	-	P < .0001	P = 0.0188
<b>LSMeans Differences</b>										
Unmowed:Mowed	2.55 b 2.76 a	-	4.17 a 3.71 b	0.63 a 0.59 b	2.70 a 2.36 ab	-	-	67.4 a 60.2 b	37.7 a 32.5 b	-
S.E. for Mowing	0.1	-	0.2	0.02	0.1	-	-	2.5	1.5	-
Unflooded:Flooded	2.95 a 2.37 b	-	-	0.57 b 0.65 a	-	52.8 b 63.4 a	18.7 a 15.0 b	-	27.4 b 42.8 a	81.4 b 93.9 a
S.E. for Flooding	0.1	-	-	0.03	-	4.6	1.3	-	1.6	4.1
Annual ryegrass	2.69	0.59	3.92	0.55 c	2.35 ab 2.54 ab	51.8	16.9	66.7	34.0	92.1
Annual ryegrass-Dutch white clover mix	2.46	0.55	3.79	0.55 bc	2.99 ab 2.55 ab	46.8	16.4	61.5	31.7	82.1
Birdsfoot trefoil	2.67	0.51	4.09	0.70 a	3.53 a 2.54 ab	66.2	15.9	64.6	41.1	95.9
Buckwheat-Dutch white clover mix	2.77	0.72	3.90	0.58 bc	2.13 ab 2.07 ab	51.1	19.9	58.3	31.2	88.8
Cereal rye-hairy vetch mix	2.57	0.53	4.01	0.66 ab	2.54 ab 2.48 ab	67.6	16.2	70.8	39.9	89.6
Control	--	0.49	--	--	--	75.7	14.5	--	--	80.7
Dutch white clover	2.85	0.63	4.12	0.56 bc	1.89 b 1.83 b	54.9	17.7	55.8	30.5	81.7
Teff	2.58	0.54	3.73	0.67 ab	3.47 a 2.48 ab	51	17.4	68.6	37.4	90.6
S.E. for Treatment or Treatment*Mowing	0.2	0.1	0.4	0.04	0.3	7.1	1.9	4.7	3.0	10.9

'-' indicates covariate was not included due to it not having a significant effect.

'--' indicates data was unavailable due to there being a significant interaction between living mulch treatment and mowing.

2010:

In 2010 plant leaf nutrients, except Mn and Fe, were at adequate levels (Mills and Jones, 1996) for all treatments. For Mn and Fe, all treatments had levels below the recommended minimum (Table 2.6).

Mowing had a significant effect on N, K, Mg, Ca, B and Mn content (Table 2.6). Mowing living mulches reduced these nutrients in pepper plants, with the exception of N; mowing increased N content of leaves. Pepper plants grown in flooded plots had significantly more Mg, Fe, Mn and Zn, and significantly less N and Cu.

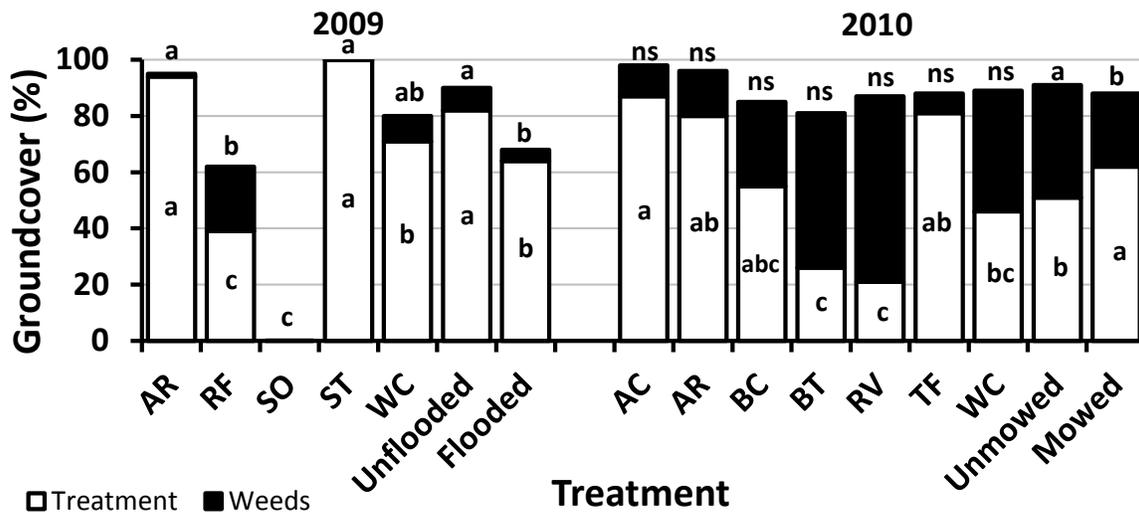
Pepper plants from living mulch-treated plots had higher P, Cu and Zn content than those grown in control plots, whereas plants from control plots had higher Fe content (Table 2.6).

### **Groundcover**

Mowing significantly reduced overall area of ground covered by plant material (living mulch plus weeds) in 2010 ( $P = 0.0263$ ), but increased the percent groundcover that was living mulch ( $P = 0.0127$ ) (Figure 2.4). In both years flooded plots had significantly less groundcover than plots that were not flooded ( $P < 0.05$ ). The exception to this was in 2010, when the area of ground covered by weeds increased due to flooding ( $P = 0.0183$ ) (Appendix Table A.1.1).

In 2009 straw and annual ryegrass treatments had similarly high percent of total groundcover (Figure 2.4). Of the living mulch treatments, annual ryegrass plots had the lowest percentage of weed coverage, 1%, whereas creeping red fescue plots had the greatest percent of weed coverage, 23%, and significantly less living mulch ground cover than annual ryegrass- and Dutch white clover-treated plots: 39%.

In 2010 mowing decreased the area of ground covered with weeds in treatments (Figure 2.4), with the exception of teff (Appendix Table A.1.1). In this treatment, the percent of ground covered by weeds rose from 6% in unmowed subplots, to 14%, following mowing of teff. Weed infestation remained highest (>20%) in mowed subplots of cereal rye-hairy vetch mix and birdsfoot trefoil.



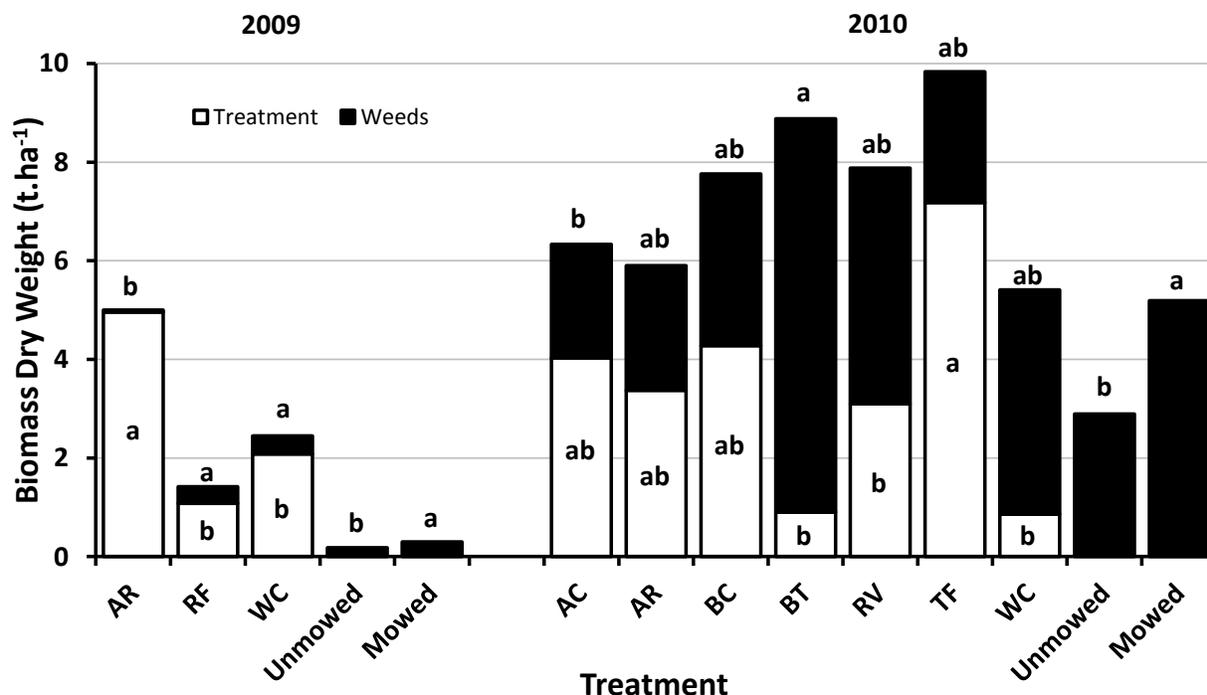
**Figure 2.4:** The percent area of ground covered in alleyway plots in 2009 and 2010. Treatments are as follows: AC = Annual ryegrass-Dutch white clover mix, AR = Annual ryegrass, BC = Buckwheat-Dutch white clover mix, BT = Birdsfoot trefoil, RF = Creeping red fescue, RV = Cereal rye-hairy vetch mix, SO = Bare soil (Control), ST = Wheat-straw, TF = Teff, WC = Dutch white clover. Treatments (or effects) with different letters above the bar or within the white area of the bar have statistically significant differences ( $P < 0.05$ ) between their least squared means for the total area of ground covered in that treatment's plot or the area of ground covered by the applied treatment, respectively. Least squared means analysis was carried out separately for 2009 and 2010 and separately for treatments and effects within both years.

Although there was no significant difference between treatments for total area of ground covered by plant material in 2010 ( $P = 0.0621$ ), there were significantly different amounts of ground covered by living mulch ( $P < .0001$ ) (Appendix Table A.1.1). Both birdsfoot trefoil and cereal rye-hairy vetch mix treatments had significantly less area of ground covered by their respective living mulch species than annual ryegrass-, annual ryegrass-Dutch white clover mix- and teff-treated plots (Figure 2.4). Annual ryegrass-Dutch white clover mix-treated plots had the most area of ground covered by living mulch: 87%. Cereal rye-hairy vetch mix had the smallest percent of ground covered by living mulch, 21%, and highest percent weed coverage of all treatments: 71% weed coverage for unmowed and 62% for mowed subplots.

### **Biomass**

In both years treatments grew significantly different amounts of above ground living mulch biomass during the growing season (Figure 2.5 and Appendix Table A.1.2). In 2009 annual ryegrass-treated plots produced  $4.96 \text{ t}\cdot\text{ha}^{-1}$  on average; significantly greater than both Dutch white clover- ( $2.08 \text{ t}\cdot\text{ha}^{-1}$ ) and creeping red fescue-treated ( $1.08 \text{ t}\cdot\text{ha}^{-1}$ ) plots. In 2010 teff grew  $7.18 \text{ t}\cdot\text{ha}^{-1}$  of living mulch biomass; significantly more than birdsfoot trefoil, cereal rye-hairy vetch mix and Dutch white clover treatments, and  $2.9 \text{ t}\cdot\text{ha}^{-1}$  more biomass than buckwheat-Dutch white clover, which produced the second greatest amount of biomass.

Weed pressure within treatment plots was greater in 2010 than 2009 (Figure 2.5 and Appendix Table A.1.2). Both annual ryegrass and Dutch white clover treatments had a great increase in weed biomass and a corresponding reduction in living mulch biomass in 2010 compared to data for 2009. Birdsfoot trefoil-treated plots yielded the most weed biomass:  $7.98 \text{ t}\cdot\text{ha}^{-1}$  of dry weed biomass. Mowing significantly increased weed biomass in treatment plots in both years.



**Figure 2.5:** Dry weight of above ground biomass grown by living mulch treatments during 2009 and 2010. Treatments are as follows: AC = Annual ryegrass-Dutch white clover mix, AR = Annual ryegrass, BC = Buckwheat-Dutch white clover mix, BT = Birdsfoot trefoil, RF = Creeping red fescue, RV = Cereal rye-hairy vetch mix, TF = Teff, WC = Dutch white clover. Treatments (or effects) with different letters above the bar or within the white area of the bar have statistically significant differences ( $P < 0.05$ ) between their least squared means for the total weight of aboveground biomass or weight of living mulch biomass, respectively. Least squared means analysis was carried out separately for 2009 and 2010 and separately for treatments and effects within both years.

### Living mulch plant height

In both years living mulch height at the end of the study period (Table 2.7) varied significantly between treatments, between mowed and unmowed subplots, and there was significant effect of the interaction between treatment and mowing. In 2010 living mulch treatments affected by

**Table 2.7:** Living mulch treatments' heights in 2009 and 2010 and the effect of mowing and flooding. Least squared means followed by different letters indicates statistically significant differences ( $P < 0.05$ ) between effects or treatments. Least squared means analysis was carried out separately for 2009 and 2010 and separately for effects and treatments within both years.

Effect Test	Living mulch height (cm)			
	2009		2010	
Treatment	P < .0001		P < .0001	
Mowing	P < .0001		P < .0001	
Treatment*Mowing	P < .0001		P < .0001	
Flooding	-*		P = 0.0098	
<b>LSMeans Differences</b>				
Unmowed:Mowed	24.4 a	13.2 b	64 a	17 b
S.E. for Mowing	1.3		1.9	
Unflooded:Flooded	-*		44 a	37 b
S.E. for Flooding	-*		2.0	
Annual ryegrass	41.7 a	19.3 b	89 a	18 bcd
Annual ryegrass-Dutch white clover mix	-		93 a	25 bc
Birdsfoot trefoil	-		19 bc	7 de
Buckwheat-Dutch white clover mix	-		94 a	16 bcde
Cereal rye-Hairy vetch mix	-		26 bc	6 e
Creeping red fescue	14.0 bc	8.2 c	-	
Dutch white clover	17.4 bc	12.3 bc	20 bcd	13 cde
Teff	-		106 a	33 b
S.E. for Treatment*Mowing	2.0		5.1	

'-' indicates data was unavailable due to the treatment not being included.

'-\*' indicates flooding was not included as a covariate due to it not having a significant effect in this analysis.

flooding were shorter than those growing in unflooded plots. Mowed plots were significantly shorter than unmowed plots.

Unmowed annual ryegrass was significantly taller than all other treatments at the end of the 2009 growing season, and it also re-grew more than other treatments. In 2009, unmowed

annual ryegrass did not grow to half the height reached in 2010, but it re-grew to a similar height in 2009 following mowing.

In 2010 the tallest subplots were unmowed teff: 42 inches (106 cm). Unmowed buckwheat-Dutch white clover mix, 37 inches (94 cm), unmowed annual ryegrass-Dutch white clover mix, 37 inches (93 cm), unmowed annual ryegrass, 35 inches (89 cm), and unmowed teff were significantly taller than all other treatments that year. Re-growth after mowing was greater in teff and annual ryegrass-Dutch white clover mix subplots than in other treatments.

### **Soil compaction**

Data was only recorded for the 2010 season. At 6 inches (15 cm) depth, all living mulch treatments had penetrometer measurements between 200 and 300 p.s.i. (Table 2.8). There was a significant interaction between treatment and mowing ( $P = 0.0212$ ). Unmowed subplots of cereal rye-hairy vetch mix required significantly more pressure to penetrate the soil to 6 inches depth than unmowed subplots of annual ryegrass, birdsfoot trefoil and buckwheat-Dutch white clover mix treatments. At 18 inches (45 cm) depth all treatments had readings of 300 p.s.i. or greater (data not shown).

**Table 2.8:** Soil resistance at 6 inches (15 cm) below the soil surface according to living mulch treatments in 2010 and the effects of mowing and flooding. Measurements were taken using a soil penetrometer and are reported in pounds per square inch (psi). Least squared means followed by different letters indicates statistically significant differences ( $P < 0.05$ ) between effects or treatments.

<b>Effect Test</b>	<b>Soil resistance at 6 inches (psi)</b>	
Treatment	P = 0.1037	
Treatment*Mowing	P = 0.0212	
Mowing	P = 0.0015	
Flooding	P < 0.0001	
<b>LSMeans Differences</b>		
Unmowed:Mowed	232 b	246 a
S.E. for Mowing	11.0	
Unflooded:Flooded	259 a	219 b
S.E. for Flooding	11.3	
	Unmowed	Mowed
Annual ryegrass	221 b	243 ab
Annual ryegrass-Dutch white clover mix	254 ab	223 ab
Birdsfoot trefoil	218 b	237 ab
Buckwheat-Dutch white clover mix	211 b	248 ab
Cereal rye-hairy vetch mix	273 a	254 ab
Dutch white clover	236 ab	243 ab
Teff	240 ab	243 ab
S.E. for Treatment*Mowing	14.4	

## Yield

Total season yield data was compared for this analysis. No significant difference between treatments was observed for total, culled, marketable, or any grade of marketable pepper fruit yield in either 2009 or 2010, whether compared by weight ( $t \cdot ha^{-1}$ ) (Figure 2.6 and Appendix Table A.1.3) or by number of fruit per hectare (Figure 2.7 and Appendix Table A.1.4).

2009:

Dutch white clover-treated plots yielded the highest mean weight and number of marketable bell pepper fruit (No. 2, No.1 and Fancy grades combined), 8.4 t.ha<sup>-1</sup> (Figure 2.6, Appendix Table A.1.3) and 48,053 fruit.ha<sup>-1</sup> (Figure 2.7, Appendix Table A.1.4) respectively, which was 0.2 t.ha<sup>-1</sup> and 4,661 fruit.ha<sup>-1</sup> more than the control treatment. All other treatments yielded less marketable fruit than the control treatment. Dutch white clover-treated plots yielded the greatest amount of Fancy grade fruit of all treatments: 8.1 t.ha<sup>-1</sup> and 45,277 fruit.ha<sup>-1</sup>.

Straw-treated plots yielded the least weight and number of marketable fruit, 4.9 t.ha<sup>-1</sup> (Figure 2.6 and Appendix Table A.1.3) and 29,201 fruit.ha<sup>-1</sup> (Figure 2.7 and Appendix Table A.1.4) respectively, and the highest amount of culled pepper fruit, 1.10 t.ha<sup>-1</sup> and 10,251 fruit.ha<sup>-1</sup> respectively. Creeping red fescue-treated plots had the lowest mean weight of culled fruit, 0.75 t.ha<sup>-1</sup>, and, along with annual ryegrass-treated plots, had the least number of culled fruit, 7,859 fruit.ha<sup>-1</sup>.

Flooding significantly decreased the amount of fancy grade and marketable fruit harvested from treatment plots in 2009 (Appendix Tables A.1.3 and A.1.4).

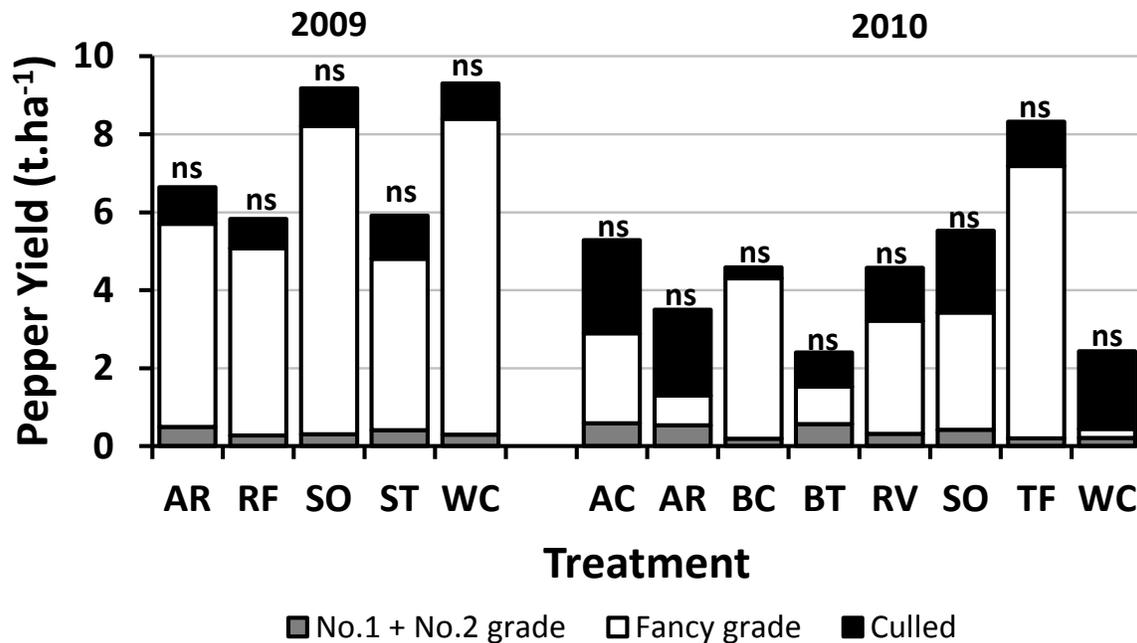
2010:

In 2010 there was a reduction in yield of marketable bell pepper fruit for the treatments used in both years (annual ryegrass, control and Dutch white clover) and an increase in culled fruit in 2010 (Figures 2.6 and 2.7, and Appendix Tables A.1.3 and A.1.4). 'Revolution' pepper plants yielded different quantities of fruit in different treatments, however, this can only be described as a trend and not statistically significant at  $\alpha = 0.05$ .

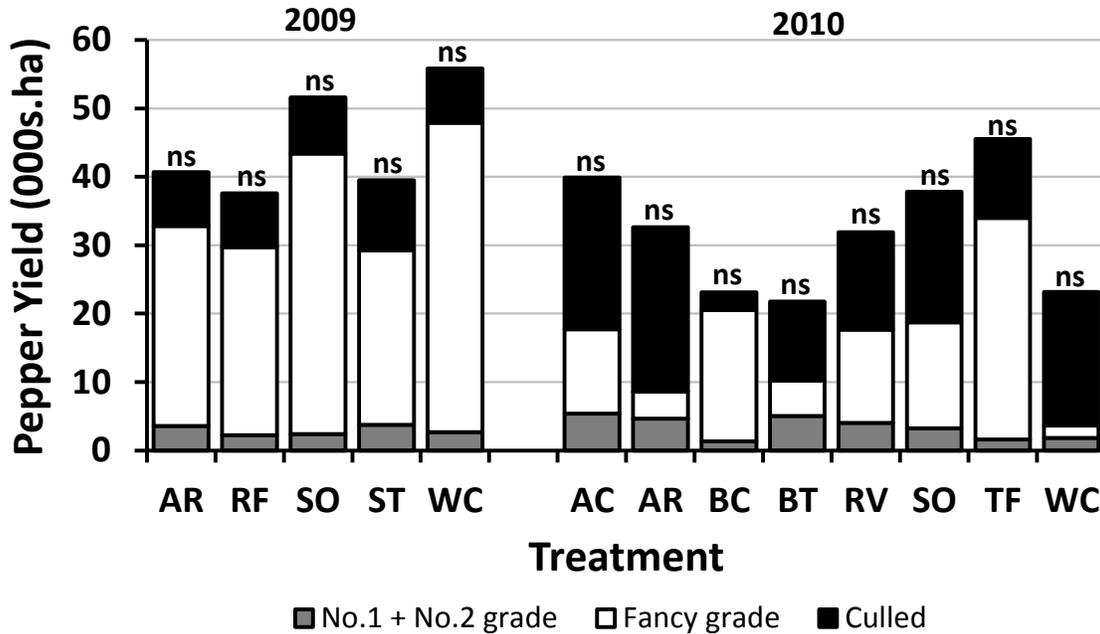
Teff-treated plots had the highest yield of Fancy-grade fruit, 6.99 t.ha<sup>-1</sup> (Figure 2.6) and 32,383 fruit.ha<sup>-1</sup> (Figure 2.7) and marketable fruit (Appendix Tables A.1.3 and A.1.4) of all treatments. Dutch white clover-treated plots yielded least Fancy grade fruit. Both buckwheat-Dutch white clover mix- and teff-treated plots yielded more Fancy-grade fruit and less culled fruit than control plots.

Annual ryegrass-Dutch white clover mix-treated plots yielded the greatest weight of culled fruit, 2.39 t.ha<sup>-1</sup> (Figure 2.6), and annual ryegrass yielded the greatest number of culled fruit, 24,034 fruit.ha<sup>-1</sup> (Figure 2.7). These two treatments were the only ones to yield more culled fruit than the control treatment. Buckwheat-Dutch white clover mix-treated plots yielded the least amount of culled fruit: 0.28 t.ha<sup>-1</sup> and 2,635 fruit.ha<sup>-1</sup>.

Flooding significantly decreased the amount of both marketable and culled fruit harvested from plots in 2010 (Appendix Tables A.1.3 and A.1.4).



**Figure 2.6:** Tons per hectare of ‘Revolution’ bell pepper fruit harvested in 2009 and 2010 according to treatments applied in the respective years. Yield for each treatment is split into grades. Treatments are as follows: AC = Annual ryegrass-Dutch white clover mix, AR = Annual ryegrass, BC = Buckwheat-Dutch white clover mix, BT = Birdsfoot trefoil, RF = Creeping red fescue, RV = Cereal rye-hairy vetch mix, SO = Bare soil (Control), ST = Wheat-straw, TF = Teff, WC = Dutch white clover. There were no statistically significant differences ( $P < 0.05$ ) between least squared means of treatments for total yield or grade of fruit. Least squared means analysis was carried out separately for 2009 and 2010.



**Figure 2.7:** The number of ‘Revolution’ bell pepper fruit harvested in 2009 and 2010 according to treatments applied in the respective years. Yield for each treatment is split into grades. Treatments are as follows: AC = Annual ryegrass-Dutch white clover mix, AR = Annual ryegrass, BC = Buckwheat-Dutch white clover mix, BT = Birdsfoot trefoil, RF = Creeping red fescue, RV = Cereal rye-hairy vetch mix, SO = Bare soil (Control), ST = Wheat-straw, TF = Teff, WC = Dutch white clover. There were no statistically significant differences ( $P < 0.05$ ) between least squared means of treatments for total yield or grade of fruit. Least squared means analysis was carried out separately for 2009 and 2010.

## Discussion

For evaluation of the effectiveness of a living mulch, characteristics to evaluate include competitiveness with the cash crop, effectiveness in suppressing weeds, adequate strategies for suppression, and any modification to growing environment.

One of the many advantages of growing a crop in polythene mulched beds is that competition between the crop plant and weeds for essential resources is greatly reduced (Emmert, 1956; Teasdale and Abdul-Baki, 1997; Roe et al., 1994; Decoteau et al., 1989). It would be fair to assume that the use of polythene mulch would also reduce competition between crop plants and living mulch, due to the latter, like weeds, being limited to growth and resources available in the furrows between mulched beds. The exception may be if the plant growing in the furrow had either a rhizomatous or stoloniferous growth habit that would enable it to greatly expand its growing area laterally, beneath the polythene mulch, or above and into planting holes of the mulch. Studies have however proven this assumption incorrect. Vigorously growing living mulch of both cereal rye and perennial ryegrass, maintained either in strips away from the cash crop or in furrows between polythene mulched beds suppressed crop yield (Nielsen and Anderson, 1989; Reiners and Wickerhauser, 1995). Competition between living mulch and crop plants is likely to occur early in the season when the crop is still young and especially if cool-season living mulch species were established during the previous year. Establishing living mulch at the same time or after planting the cash crop may help reduce this problem (Nicholson and Wien, 1983).

Growing living mulch in alleyways between beds of peppers did not affect bell pepper yield, based on the range of plants used in this study (Figures 2.6 and 2.7, and Appendix Tables A1.3 and A.1.4). When the cash crop is fertigated through trickle-tape placed near the crop and beneath polythene-mulch, limited competition for nutrients and water from the living mulches is expected to take place. However, it has been shown that even with the use of polythene mulch, competition between crop and living mulch can occur, leading to suppression of yield (Reiners and Wickerhauser, 1995). In 2009 living mulch was excluded by the polythene mulch for approximately 18 inches (45 cm) either side of the pepper plants and in 2010 approximately 9

inches (23 cm). In both situations, in the presence of living mulches no significant difference in yield was recorded, compared to the control having no living mulch.

Quality of harvested fruit varied between treatments in 2010, with teff- and buckwheat-Dutch white clover mix-treated plots yielding more Fancy-grade peppers than all other treatments; teff-treated plots yielded most of all. Of note is that buckwheat-Dutch white clover mix-treated plots yielded less total weight than plots treated with annual ryegrass-Dutch white clover mix, cereal rye-hairy vetch mix, the control, and yielded less numbers of fruit than these same treatments and plots treated with annual ryegrass, but due to the combination of buckwheat and Dutch white clover reducing incidence of the most frequent causes for fruit to be discarded, *P. blight* fruit rot and sunscald, a greater amount of marketable fruit remained.

In both years, pepper plants in control plots yielded more fruit, both total and marketable quantities, than plants grown in annual ryegrass-treated plots; although not statistically significant. Plots containing annual ryegrass in 2010 had more culled fruit than the control treatment, and had high *P. blight* disease incidence. Annual ryegrass' tall, dense foliage may cause a reduction in air flow and a corresponding increase in humidity and temperature around pepper plants. Reduced air flow slows drying of plant surfaces following a rain event. This allows pathogens, such as *P. capsici*, that cause foliar diseases and benefit from being in a film of water, an extended period of time to infect plant tissue (Hausbeck and Lamour, 2004). Being as buckwheat-Dutch white clover mix and teff both were taller living mulch treatments than annual ryegrass-treatments (Table 2.7) we may expect them to have similar or higher *P. blight* disease incidence. Instead, these treatments had the lowest percent of total number of harvested fruit infected with *P. blight* (Table 2.3) and the smallest AUDPC (Figure 2.3). Density of foliage was not measured, but it may be that despite their height, both buckwheat and teff had a less dense canopy than annual ryegrass, which therefore would allow better airflow and

drying of aboveground pepper plant tissue. Density of canopy of living mulch may be affected by plant population density, plant growth habit and amount of groundcover. There was a trend for buckwheat-Dutch white clover mix-treated plots and teff-treated plots to have less total area of groundcover than plots treated with annual ryegrass (Appendix Table A.1.1). Although not a significant difference, less groundcover is an indication of lower plant population density that would allow better airflow around pepper plants.

No statistically significant differences in harvested fruit from treated plots were recorded in either year. However, in 2010 less fruit was harvested from plots treated with birdsfoot trefoil, cereal rye-hairy vetch mix and Dutch white clover than from control plots. The output of pepper fruit from Dutch white clover-treated plots in 2010 was in contrast to the treatment's performance in 2009, which was equal to or better than the control. Pepper plants growing in several replicates of plots treated with Dutch white clover were affected by the repeated flooding events of 2010 due to their location in the field being more often flooded than others. Dutch white clover would perform better than the results of 2010 suggest if fewer flooding events occurred in a season; perhaps the same could also be said for all treatments used in 2010. Further repetitions of this study in future would be necessary to evaluate this assumption.

Despite some living mulch plants in this study growing up to 42 inches (106 cm) tall, such as unmowed teff, the average height of bell pepper plants was not affected. Similarly, nutrient content of bell pepper plants was consistently within recommended boundaries despite significantly different percent area of groundcover between mowed and unmowed plots in 2010, between treatments in 2009, and significantly different amounts of biomass between treatments and mowed and unmowed plots in both years. Supplying required crop nutrients directly to the root-zone through trickle-tape is efficient, economical, ensures sufficient supply is provided throughout the crops growing cycle, increases marketable yield and reduces nitrate leaching to

groundwater (Emmert, 1956; Romic et al., 2003; Madramootoo and Rigby, 1991; Singh et al., 2009).

As stated, all macro- and micronutrients in pepper plants were within acceptable boundaries in both years. Mowing living mulch increased N-content of pepper leaves. If we consider that overall biomass produced during the growing season was significantly greater from mowed subplots than from non-mowed subplots, N-demand of living mulches was greater from mowed subplots and we would consequently anticipate seeing less N within leaves of pepper plants from these subplots. However, despite an increased demand for N in mowed subplots due to plant regrowth, pepper N-content was higher from mowed subplots. The presence of a living mulch increases soil biological activity, including nitrification (Masciandaro et al., 1997). The action of mowing plant material, both living mulch and weeds, within the alleyway is likely to have increased the rate of decomposition of this organic material and release of nitrogen. Once nitrification has taken place this element is likely to have moved with soil water and been taken up by neighboring pepper plants. A study by Thornton and Millard (1997) showed that defoliation of grass species, whether on one occasion or repeatedly, resulted in a reduction in total plant N uptake, which would therefore leave more N available in the soil and could be an explanation for the observation in our study.

A sickle-bar mower, as used in this study, leaves relatively large pieces of plant residue on the soil surface. Rotary and flail-mowers cut plant material into much smaller pieces and if employed in this system could perhaps increase release of N into soil water to be available as a supplemental source of this element to the crop and to support the living mulch itself. As this system is refined in future, the method and timing of mowing could be tailored to the N-requirement of the crop plant.

The result of there being significantly less potassium, magnesium, calcium, boron and manganese in pepper leaves from mowed subplots is less clear to make assumptions about the reason for this result. It is possible that mowing increased demand for these elements in the living mulch and weeds that regrew. The increased growth of the mowed plants in the alleyways may have resulted in more of these elements being taken up by these plants than the pepper plants, when compared to the non-mowed subplots.

It is hypothesized that results from this study are different to those that would be recorded from a similar study with an absence of polyethylene mulch and trickle-tape. If fertility for the cash crop was incorporated into the soil pre-planting, competition between roots of the cash crop, the living mulch and any weeds would be more likely to occur. Also, root-zone nutrients would be more likely to be lost through leaching, due to irrigation or precipitation, leading to less availability to plants in the system, and greater competition between cash crop and living mulch for the limited resources that would remain. Several studies have identified suppression of crop yield due to living mulch (Sweet, 1982; Nicholson and Wien, 1983; Nielsen and Anderson, 1989; Andow et al., 1986; Echtenkamp and Moomaw, 1989; Degregorio and Ashley, 1986; Altieri et al., 1985).

For successful weed suppression living mulches must germinate and emerge quickly after sowing, have fast growth and cover the ground well, either by an upright or spreading growth habit or a combination of the two (Williams, 1987; Bertin et al., 2009; Nicholson and Wien, 1983). Living mulch height alone is not a good indicator of weed suppression ability (Nicholson and Wien, 1983). The amount of biomass living mulch produces is directly related to the degree of weed suppression it provides (Burgos et al., 2006).

Although no quantitative field data on germination, and emergence, was collected, observations indicated that laboratory-based seed viability testing is not a good indicator of field performance. In both years, laboratory test showed Dutch white clover seed to be quick to germinate; even a day faster than annual ryegrass in 2009. Field observations in this study, and others, were that Dutch white clover is slow to emerge, and when it does, emergence is non-synchronous (Law et al., 2006). In contrast, laboratory tests showed teff to be slower to germinate and having just 1% more viable seed than Dutch white clover. However, field observations were that teff was faster to emerge than Dutch white clover and quickly forms a uniform seedling-carpet over the treated area that enables it to begin competing with weed seedlings sooner than Dutch white clover.

Both creeping red fescue and birdsfoot trefoil were slow to germinate, had low germination rates, are low growing species and as a result covered the ground poorly and had more weeds present in their plots than other treatments. In contrast, teff was the most successful living mulch for suppressing weeds. Beyond the speed of its emergence and stand establishment, teff's success at suppressing weeds was associated with its height and aboveground biomass; significantly higher than birdsfoot trefoil, cereal rye-hairy vetch mix or Dutch white clover. Mowing increased teff's biomass production, as was seen by it re-growing more than all other treatments.

Both teff and annual ryegrass (including annual ryegrass grown in combination with Dutch white clover) stood apart from other living mulch species for weed suppression ability through their combined speed of emergence, synchronous stand establishment, percent of ground covered with living mulch and the amount of aboveground living mulch biomass. Both plant species excelled at competing with weeds; illustrated by the low weight of weed biomass and by the small area of ground covered with weeds in these treatment's plots.

In 2009 the dead mulch of straw was the most effective treatment for suppressing weeds, as is shown by the very small area of ground covered with weeds (Figure 2.4). However, the use of dead mulch has its problems. Non-living mulches made from light particles, for example organic material such as straw, are susceptible to wind blowing, equipment and pedestrian traffic can disturb the mulch, even incorporate the material into the soil, and additionally, during flooding, they are lifted and moved with the flow of water (Grundy and Bond, 2007). In all situations, bare soil is exposed. Such types of mulch are more effective if laid over a permeable membrane; although this adds to the cost of its use. To lay an adequate depth of dead mulch for successful weed suppression, more than 2 inches, is an expensive task and likely to be something most farmers would avoid undertaking.

An important observation from this study was that the straw itself, due to remnant, viable wheat seed being present, became a source of weeds; the growth of volunteer wheat plants is undesirable when using dead mulch.

Winter-hardy cereal rye and hairy vetch were among the first cover crops used in mulch systems for weed suppression and are frequently used today (Hoffman and Regnier, 2006). In North America these two crops, often grown in a mixture, are typically Fall-sown and killed in late spring or early summer. Their growth takes place in milder weather during winter and spring. Early sowing in fall increases the amount of biomass living mulch produces, improves weed suppression for the following growing season and increases the amount of nitrogen scavenged from the soil before it would otherwise be leached (Clark, 2007).

The fall-sown cereal rye-hairy vetch mix used in this study was ineffective in suppressing weeds. Good groundcover was achieved by the time the plots were mown. Mowing killed cereal

rye and most hairy vetch plants. Insufficient plant biomass resulted in poor groundcover by the residue and minimal weed suppression.

Mohler and Teasdale (1993) used the same seeding rate for both cereal rye ( $56\text{kg}\cdot\text{ha}^{-1}$ ) and hairy vetch ( $157\text{ kg}\cdot\text{ha}^{-1}$ ) as was used in this study. They found that the 'natural' rate of residue was insufficient to control most weed species. Through supplementing with cut cereal rye and hairy vetch plant material from additional plots in order to vary mulch rates, they found that it took 2.0 or 4.0 times as much 'natural' residue rate for weed emergence to be adequately suppressed. Weeds penetrated hairy vetch earlier in the season than cereal rye, however the relative response to residue rates were similar. An observation they made was that hairy vetch decayed quicker than cereal rye. This exposed bare soil earlier and potentially increased soil nitrate availability; therefore improving the growing environment for weed emergence and colonization to take place. Mowing cereal rye and hairy vetch causes them to release allelochemicals that help prevent weed seed germination (Hoffman and Regnier, 2006; Mohler and Teasdale, 1993). However, in the absence of adequate quantities of plant material this method of weed suppression has limited effect. It is not economically feasible for a farmer to grow between 2 to 4 times the acreage of this plant mix that is required to be mulched, and incur the expense of killing, cutting, transporting and laying the cut material for weed suppression during the following summers growing season.

Mowing took place on September 14<sup>th</sup> 2009 and August 28<sup>th</sup> 2010 in this study (Figure 2.1). A single mowing of living mulch had no significant effect on pepper yield, plant height or plant nutrient content, or on incidence of Phytophthora blight. For all living mulches, mowing increased total biomass production for the season, and suppressed almost all living mulch plant height. While the treatment of mowing subplots was applied around 2 ½ to 3 months after sowing the living mulch, applying this treatment earlier in the season may have resulted in more

effective suppression of weeds by more of the living mulch species. Mowing early would be hoped to substantially decrease the early weed population, while allowing, or encouraging, further expansion of groundcover by the living mulch. Thereafter, mowing may be required only a small number of further occasions to maintain accessibility for harvesting; for low growing species no further mowing may be necessary.

An observation in this study, although not quantified through recorded data, was that tall living mulch with numerous upright stems or leaf blades, when cut using the sickle-bar mower, form a mat of dead mulch above the soil surface and remaining living plant parts. The mat of plant residue was effective at suppressing both weeds and the growth of some of the remaining living mulch; the latter being undesirable due to it reducing continuity of the living mulch system. Shorter living mulch species, such as Dutch white clover and birdsfoot trefoil, increased groundcover in response to being mowed and showed no signs of growth suppression. Sweet (1982) found that white clover responded well to mowing. In his study, he found that when mowed, white clover maintained a dense cover and provided satisfactory weed control.

Some cover crops are killed by mowing and are therefore inappropriate for use as living mulch. The exception to this is if the living mulch is grown in combination with a plant able to withstand the treatment and would continue as living groundcover. Buckwheat, cereal rye, and most hairy vetch plants were killed by mowing. Some hairy vetch plants did regrow from nodes beneath the height of the cut. However, the groundcover provided by the living hairy vetch was inadequate and the spreading habit of the plant sometimes led to it growing over the plastic mulch and among the pepper plants.

This study utilized a front, mid-mounted, sickle bar mower. There are two other main types of mower appropriate for use in the living mulch system: rotary and flail. Mower-type affects the

resulting size of cut plant material and how the plant residue is distributed in the plot; sickle bar leaves plant material in large pieces, whereas rotary and flail mowers cut plant material numerous times, resulting in much smaller pieces. Size of cut plant material determines its rate of decomposition as well as how much light is excluded from reaching the surface of the soil. When plant material is cut small, decomposition rate is faster than larger pieces of plant material (Williams, 1987; Patten et al., 1990). Plant residue cut by a sickle bar mower tends to lay in a continuous direction; a mid-mounted sickle bar splits the residue into two equal swaths of material, lying at approximately  $\pm 145^\circ$  from the direction of travel, with no residue covering a central strip. Rotary mowers either deposit residue over the surface of the ground covered by the width of the mowing deck, or, if a side-chute is opened, may disperse residue across a wide area to the side of the direction of travel. Flail mowers tend to deposit cut material within the area of the cutting deck; almost in the exact same location the plant material grew.

Use of the sickle bar mower between plastic-mulched beds had difficulties. The reciprocating blades, if positioned too close to the bed's edge, caught the polythene and cut through it; rendering the polythene prone to further tearing at that point and the exposed soil beneath to colonization of weeds. Mowing close enough to the bed edge, to effectively cut plant material closest to it, is impossible with this piece of equipment, due to the lateral shaking of the cutter-blades. It is our belief that either a flail or a rotary mower (with chute closed so it is used as a 'mulch-mower') would be more appropriate than a sickle bar mower for use with the living mulch system where there are such exacting boundaries for mowing. Having fixed mowing decks and blade positions would reduce damage to polythene while allowing mowing close to the bed edge, keep cut plant material within the alleyway and, due to the small-sized pieces of plant residue, living mulch groundcover could be maintained. More research is required on the efficacy of mowing living mulches, the best timing, associated effects of mowing living mulches, if multiple mowing is suitable, and the type of technology to use for the operation.

A suitable alternative to repeated mowing could be the effective combination of two or more living mulch species; one of which establishes quickly and out-competes fast germinating weed species, and is perhaps then killed by mowing, which would then allow for the slower, lower-growing understory living mulch to successfully colonize the soil surface, preventing the need for further mowing. This was the anticipated effect of combining buckwheat with Dutch white clover in the 2010 study. In some replicates of the treatment this combination of species and mowing resulted in good groundcover of Dutch white clover by the end of the growing season. In plots where buckwheat grew vigorously, growth of Dutch white clover was reduced and subsequent groundcover was affected. Altering seeding rates or timing of first mowing may lead to a better balance between buckwheat and Dutch white clover and worthy research for the future. Consideration should be given to using a mix of living mulch species for soil-fertility benefits of combining a legume with a grass-specie, to enable an increased range of environmental tolerances and for increased weed suppression (Teasdale et al., 2007; Burgos et al., 2006).

Results from this study show that growing living mulch between polythene mulched beds of bell peppers makes no statistically significant difference to *Phytophthora* blight disease incidence on aboveground parts of the crop plant, compared to a control of bare soil. The living mulch aboveground biomass, percent groundcover, the species chosen, and the treatment of mowing, had no significant effect on disease incidence.

Straw and annual ryegrass had highest disease incidence in 2009; although overall disease incidence was low in this year. Creeping red fescue had lowest disease incidence in this year. Teff, buckwheat-Dutch white clover mix and cereal rye-hairy vetch mix all recorded lower AUDPC scores than other treatments, and teff and buckwheat-Dutch white clover mix had the

lowest percent of plants in plots affected by the disease at the end of the 2010 trial; around 20% (Figure 2.3). The control and annual ryegrass-Dutch white clover mixture treatment had the highest AUDPC scores, and control plots had the highest percent of plants affected by the disease at the end of that year's trial; approximately 70%.

In 2009 field inoculation took place late in the season, just one week before the end of the trial, which led to only one disease assessment taking place, which was based on harvested fruit only, and not taking into account symptoms on all aboveground plant parts. Spread of the disease by soil splash was hampered by there being unfavorable environmental conditions; infrequent and low volume of rainfall, and high daytime temperatures with cold nights. In 2010 several periods of heavy rainfall events during the growing season led to repeated occurrences of flooded plots before living mulches were established. Also, heavy weed pressure quickly developed in many plots, despite stale seedbed establishment prior to sowing. Without uniform stands of the living mulch species to compare between each other and against bare soil and straw, the treatments true effect on disease incidence is unclear. It is recommended that disease data should be given due consideration for the fact that the results are incomparable between years and that only two years data is reported here; especially considering repeated flooding of 2010's field led to lost data.

Flat-topped beds were used in this study that, due to an uneven surface, often collected pockets of soil, water and decaying plant material in shallow depressions. It is possible that spread of *P. capsici* was increased because of this and led to a reduction in effect of the alleyway treatment. Sources of inoculum on the surface of polythene significantly increase the onset and rate of an epidemic of *P. capsici* (Bowers et al., 1990; Grove et al., 1985; Ristaino et al., 1997; Sujkowski et al., 2000). An even soil surface beneath polythene mulch when laying polythene mulch is essential for water and organic material to be shed from its surface. A crowned bed should be

formed for the most efficient shedding of materials from the surface of polythene mulch (Ristaino and Johnston, 1999).

Straw had high disease incidence, least amount of marketable fruit and most culled fruit in 2009 and straw-treated plots in 2010 retained water after flooding much longer than other treatments, resulting in pepper plant mortality due either to root death from pathogens or toxicity from anaerobic soil conditions. Merwin et al. (1992) reported that apple trees treated with either mowed or herbicide controlled living mulch groundcover remained free of symptoms of *Phytophthora* crown or root rots (PCRR), whereas 35% of trees mulched with straw developed symptoms over a 4-year period. Straw-mulched plots had prolonged periods of soil saturation which led to the increased incidence of PCRR. It is recommended to avoid using straw mulch in fields with poor drainage.

An observation from both years, although not quantitatively recorded, was that despite observing clear evidence of rodent activity within the living mulch in the form of droppings, cleared pathways through living mulch and chewed plant material, particularly in unmowed plots, no fruit were culled due to rodent damage. Contrary to those studies that suggest an increase in damage to crops from rodents, observations during this study indicate that although there may be rodents present they do not cause damage to intensively managed annual vegetable crops (deCalesta, 1982; Sullivan, 2006; Wiman et al., 2009). The treatment of mowing is suggested as effective in reducing rodent activity in the field (deCalesta, 1982).

In 2009 there were more beetles on fruit and feeding damage to fruit, believed to be from the beetles, in living mulch plots than from control or straw-treated plots; data was not recorded. Some studies have indicated that the presence of vegetative cover in a cropping system can increase damage to harvestable vegetable produce by herbivorous insects (Altieri et al., 1985).

However, the presence of living mulch may reduce damage from these crop pests and encourage increased populations of predatory insects (Amirault and Caldwell, 1998; Andow et al., 1986; Altieri et al., 1985).

Soil penetrometer testing in 2010 indicated there was sub-surface compaction across the field and soil at 6 inches (15 cm) depth was moderately compacted (Table 2.8). Despite there being significant difference between surface penetrometer readings of mowed and unmowed plots, flooded and unflooded plots, and due to an interaction between treatment and mowing, all recorded measurements were in a close range. A possible reason for statistical differences could be due to traffic, both pedestrian and machinery, passing across the unflooded, mowed subplots with higher frequency, than the flooded, taller, unmowed subplots; therefore an increase in traffic may have led to increased soil compaction.

Analysis of living mulch effects on soil health was not carried out in this study. It may be hypothesized that physical properties of the soil may not change within the period of use for the living mulch but it may be possible for chemical and biological elements to alter due to effects of the living mulch root mass and exudates, and its effectiveness in moderating soil temperature and moisture. A study by Masciandaro et al. (1997) showed that the use of living mulch has positive effects on soil physical and metabolic properties. In the presence of living mulch biological activity of soil organisms and plant roots was seen to increase, causing more surface-cracking of soil and stimulating enzyme activities that led to increased water-soluble carbon and NO<sub>3</sub>/NH<sub>4</sub> nitrification. These responses to the living mulch treatment were seen as indications of improvements in soil physical and biochemical properties and microbial activities.

Insufficient significant differences were found between the living mulches used; thus preventing conclusions on what living mulch should be used in alleyways between polythene-mulched

beds. The choice of living mulch specie, cash crop, method of application and management are greatly important elements for the potential for the systems success.

More screening of suitable plants for use as living mulches is vital. Much of living mulch research has concentrated on using small grain, forage (including legume) and turf-grass species. Research into alternative species is required. Ellis et al. (2000) undertook a two-year living mulch study into the adoption of a plant familiar to many vegetable growers as a weed: common purslane. Their choice for using this plant was because of its attributes as a successful weed being parallel to the desired characteristics of living mulch for use with summer crops: aggressive summer growth, prostrate habit, rapid establishment, dense canopy, tolerant of a variety of growing conditions and reproduces easily both sexually and vegetatively. The study found that establishment of common purslane for living mulch in broccoli was “more economically feasible than black plastic mulch to manage weeds” and had similar costs to chemical weed control. A possible strategy for future study of living mulch could be through the identification of a region’s most successful, annual or ephemeral weed specie/s, followed by their selection and domestication.

As was discovered with this research project and others, establishment of living mulch in spring and early summer can be difficult due to limiting environmental conditions and competitive weed growth (Law et al., 2006). Also at this time, the grower may require repeated access to the alleyways during the early stages of the cash crops establishment that would impede growth of the young living mulch. Whenever possible, establishing living mulch in fall is recommended (Lanini et al., 1989; Smith and Valenzuela, 2002). This would be particularly appropriate if polythene-mulched beds were to be laid at this time in order to capitalize on early harvesting (Reiners et al., 1997). Having living mulch established by winter would help prevent soil erosion and leaching, and may increase the opportunity of earlier spring transplanting, whilst also

increasing effectiveness of weed control. Living mulch-treated land retains less water in winter and more water in summer than a killed cover-crop of the same specie, while also not impeding drainage; thus permitting earlier access to fields and less water-stress for the crop plant during summer (Ochsner et al., 2011).

Cash crop yield from a living mulch-treated plot can be equal or greater than yield from plots kept weed-free. It is unclear as to the mechanisms by which growing teff or buckwheat-Dutch white clover mix in alleyways between bell peppers may result in increased marketable yield, compared to control plots. A reduction in Phytophthora blight damage to fruit from plots treated with these living mulches suggests a possible reason. Taller plants have a larger foliage volume per unit of soil surface area and so cover the soil more thoroughly and reduce soil splash better than plants with erect leaves. Lateral-spreading leaves and species with a high plant population per area provide the most effective groundcover and therefore most reduction in splash dispersal of diseases (Ram et al., 1960; Ntahimpera et al., 1998). Teff has tall leaves that have a weeping habit, therefore covering more surface area, and shed water well. Buckwheat and Dutch white clover combine the benefits of tall plants with spreading leaves and good groundcover.

Weeds within a cropping system can be effectively controlled by living mulch and this system should be seen as a viable alternative to the use of cultivation or herbicides. Robinson and Dunham (1954) noted that use of companion crops between rows of soybean was equally successful for weed control as was repeated cultivation. Mowing living mulch increases its effectiveness at controlling weeds. Mowing living mulch once during the growing season increases the overall amount of biomass produced and reduces the area of land covered with weeds. Future studies should compare different mowing strategies, including differences in timing of mowing, height of cut and type of mowing equipment used.

No economic analysis was carried out for this study, and no literature was found that referred to the cost of including living mulch in the production of any crop. If marketable yield from living mulch plots were to be consistently higher than from bare soil plots, this increase in economic return would be an important, and potentially attractive, variable in the production cost calculation of adopting the living mulch system for a grower.

There is great potential for the living mulch system to be applied to the production of high value horticulture crops in plasticulture and for it to be a viable alternative to maintaining bare soil. More research needs to be undertaken to study the cultivation of living mulch and its economic effects on crop production.

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## Chapter 3: Living mulch mixtures and mowing

### Introduction

The intercropping strategy of growing living mulch among or alongside a cash crop has been researched, refined and applied to the production of many crops since the publication of the Robinson and Dunham's 1954 paper 'Companion crops for weed control in soybeans'. Lilly (1965) described the system of planting warm season cash crops into a living cover crop of cool season perennial grasses, called the "sleeping sod" system. Whereas cover crops are typically grown in niches between cropping cycles for a variety of purposes, including as green manure, catch crop or as a smother crop, and killed prior to the subsequent cash crop, the "living mulch", as it was named by Sweet (Hughes and Sweet, 1979), involves planting a managed cover crop before, with or after a summer cash crop is planted. The living mulch then grows for the duration of the cash crop's production cycle; primarily, although not exclusively, for the purpose of weed control.

Growing living mulches with cash crops is consistent with the goals of organic and sustainable agricultural practice, provides numerous benefits to the agroecosystem, and plays an important role in supporting ecosystem services ( Hoffman and Regnier, 2006; Graglia et al, 2006; Leary and DeFrank, 2000; Teasdale et al., 2007). Use of living mulches has the potential to improve the positive effects of agriculture on the environment through the increase in carbon sequestration, biodiversity and soil organic matter (Carof et al., 2007). It was proposed by the National Wildlife Federation (2011) that in order for America to meet its goal of reducing greenhouse gas emissions 17% by 2020, while still meeting the needs for food, fuel and fiber, carbon sequestration on agricultural land would play a significant role. The Farm Bill conservation title funds sustainable farming practices, including the use of cover crops. If US farmers were to grow cover crops on all acres of cultivated land suitable for them, approximately

74.9 million hectares (185 million acres), an estimated 4% of annual greenhouse gas emissions could be mitigated. Living mulches would play an important role in this advancement as they would contribute to the carbon pool all year round, including during the cropping cycle.

From its earliest stages of development as an alternative groundcover management tool in vineyards of mountainous regions both for weed and soil erosion control, the living mulch system has been proven to play a role in mitigating the serious soil problems caused by continuous cropping (Hartwig and Ammon, 2002; Hughes and Sweet, 1979). Water leaving a field may carry soil particles, nutrients and pesticide residues, leading to pollution of water courses, loss of resources and increased expense to the farmer (Rice et al., 2002; Rice et al., 2004; Rice et al., 2007). In some agricultural regions of the US, leached pesticides have reached such significant levels in groundwater sources that the contamination level can be measured and where pollution has reached riparian habitats wildlife is threatened (Sweet, 1982; Dietrich and Gallagher, 2002). Clean-tilled aisles in tree and shrub nurseries recorded sediment concentrations in surface water runoff, after rainfall events, between 1.9 to 8.8 times greater than living mulch-covered aisles (Cripps and Bates, 1993). Preventing movement of sediment into a watercourse can reduce toxic pesticide loads entering water by up to 90% (Dietrich and Gallagher, 2002; Rice et al., 2004; Rice et al., 2007). Living mulch reduces surface runoff of water, and allows more water penetration (Sweet, 1982). Cereal rye growing between polythene-mulched beds reduces runoff volume by more than 40%, soil erosion by more than 80% and pesticide loads by between 48% and 74% (Rice et al., 2004; Rice et al., 2007). Crownvetch, into which no-till corn was drilled, reduced water runoff, soil erosion and herbicide runoff between 95% and 99% (Hartwig, 1985). On a sloping site the use of Kura clover in no-till corn reduced both soil erosion and phosphorus runoff more than 50% (Schwab and Albrecht, 2011). Kura clover may also reduce nitrate-N leachate between 31% and 74% when compared to a control of dead mulch (Ochsner et al., 2011).

Living mulch can be integrated into almost all field-based cropping systems, including annual and perennial edible crops, and perennial ornamental crops (Bond and Grundy, 2001; Hartwig and Ammon, 2002; Cripps and Bates, 1993; William, 1987; Hughes and Sweet, 1979), and will perform many, if not all, of the following roles within the agroecosystem, as proposed by Hughes and Sweet (1979)

1. continuous groundcover that is especially important for establishment of tender plants,
2. erosion control,
3. reduction of leaching losses,
4. increased organic matter return,
5. less energy consumption in terms of fuel for tillage and chemicals, and
6. reduced disease, insect, and weed problems.

Success of the living mulch system is dependent on the growth of the plants used being competitive with weeds at appropriate times of the latter's life cycle. However, it is possible that through repeated use of a monoculture of any living mulch, combined with a routine and limited management strategy, that selection may occur within that field's weed population, for those species that are able to persist under these treatments. Mohler (1991) saw weeds colonize living mulch stands of white clover into which sweetcorn was sown annually for four years. Combining use of living mulch with alternative weed management strategies may not always prevent weed colonization. When using buckwheat, seedbank densities increased for common purslane and carpetweed that escaped mowing due to their prostrate growth habits (Gibson et al., 2011). This limitation of using single specie living mulches leads to the consideration for employing mixed species living mulch or alternating monoculture species with each sowing. It would be the intention to select species for use as living mulch that have contrasting growth

habits to each other in order that they provide a broad range of competitiveness to a wide range of weed species.

Most research on the use of living mulches has concentrated on using single species of small grain, forage and turf-grass species rather than growing a species mixture. When a combination of grass and legume species are grown together in a mixture a general advantageous trend is for the resulting overall biomass to be the same as or greater than either of the individual species grown in pure stands and so increasing weed suppression (Burgos et al., 2006). The amount of aboveground biomass grown is an indication of the living mulches ability to suppress weeds, be an effective groundcover, result in organic matter being added to the soil, help retain nutrients, water and soil, and the amount of nutrients, particularly nitrates that it will make available to subsequent crops. Biomass production is therefore a significant selection criterion for choosing living mulch specie or mixture, and is dependent on soil-type and conditions, seeding rate, stand establishment and environmental conditions (Patten et al., 1990; Burgos et al., 2006). Using a forage crop study as an example of the effect of using a species mixture compared to monoculture we see that a combined stand of Italian ryegrass with Kura clover, where ryegrass was between 16% and 25% of the mixture, increased forage production by 15% (Contreras-Govea and Albrecht, 2005). A further important observation from this study was that in combination with Kura clover, Italian ryegrass survived winter in Wisconsin, unlike when grown in monoculture, suggesting a possible 'nursing' effect of the Kura clover.

Relatively high densities of living mulch plants are needed for strong suppression of weeds. High density planting intercepts the greatest amount of light, therefore outcompeting weeds better. Seeding rates affect the timing of when the canopy closes. Mowing living mulch may alter the relationship between seed rate and weed density (Gibson et al., 2011). High seeding rate results in highest dry weight biomass and low seeding rate results in lowest dry weight

biomass of the same mixture. This correlates to the best weed control being at a high seeding rate and the worst weed suppression being in plots sown at the low seeding rate for mixtures or a monoculture (Akemo et al., 2000).

Regardless of seeding rates, grasses tend to be the greatest contributors to the resulting biomass of a mixture. Combining benefits of legume and non-legume plants adds to the effectiveness of the living mulch. Many cereals suppress weeds more than legume plants. Whether to include legume plants in the living mulch is an important consideration. Legumes are an important addition to the living mulch cover crop system, whether as a pure stand or in a mixture combined with small grains and other grasses, especially in the organic system where they play an important role in both weed management and for provision of nitrogen (Hoffman and Regnier, 2006; Teasdale et al., 2007; Creamer and Baldwin, 2000; Gaskell and Smith, 2007). Legumes fix atmospheric-nitrogen through a symbiotic relationship with soil-borne bacteria (Gaskell and Smith, 2007). A good balance is desired for a legume/non-legume mixture to be successful. Economic, biological and physical factors need to be considered.

Finding the correct balance of species in a living mulch mixture is important. Mixtures of cereal rye and field pea with more than 50% proportion being rye gave the best weed suppression compared to pure stands of both crops and mixtures with 50% or less of rye; only 2% groundcover was weeds in rye-pea mixes compared to 73% in pea-only living mulch (Akemo et al., 2000).

Use of living mulches can be effective weed control and there are studies that show some crops grown in the system may yield the same or greater than conventional bare soil treatment (Infante and Morse, 1996). However, some living mulches may suppress cash crop yield unless species are chosen and managed appropriately (Nicholson and Wien, 1983; Andow et al., 1986;

Teasdale, 1998; Chase and Mbuya, 2008). Wheat yield was suppressed up to 81% in 14 of 18 undersown living mulches (Carof et al., 2007). Vigorous, uncontrolled growth of living mulches, such as perennial ryegrass and cereal rye, can be too suppressive for crop plants even when the living mulch is cultivated into strips or grown between plastic-mulched beds (Neilsen and Anderson, 1989; Reiners and Wickerhauser, 1995).

Often studies combine the use of living mulch with some form of suppression of their growth. The method, timing and degree of suppression and the root characteristics of both the crop and living mulch are important factors to consider for successful management of the system (Wiles et al, 1989; Vrabel et al., 1980; Robinson and Dunham, 1954). Suppression techniques most frequently employed include use of sub-lethal doses of herbicides (“chemical mowing”), mechanized mowing and cultivation.

Mowing is a non-chemical weed control method adopted by growers and is successful in controlling living mulch growth. Mowing alone is not an adequate measure for weed control (Donald, 2005). However, when it is combined with living mulch and the competition between these plants and weeds, the system can be effective at controlling even pernicious perennial weeds (Graglia et al., 2006; Lukashyk et al., 2008). The need for using mowing suppression of living mulch is dependent on the growth habit of both the living mulch plant and the cash crop (Chase and Mbuya, 2008). Mowing is particularly necessary for tall living mulch species in order to reduce shading of a crop plant (Teasdale, 1998).

Some living mulch plants respond better to mowing than others and it is this response that affects their ability to re-close their canopy and effectively control weeds. White clover responded well to mowing, maintaining good cover, whereas alfalfa did not respond well and weeds colonized gaps in its canopy (Sweet, 1982). Mowed plots of subterranean clover had

significantly greater weed biomass compared to unmowed plots, when grown among squash (Ilnicki and Enache, 1992). Removal of a large percentage of the living mulch canopy by mowing leads to a significant reduction in light interception by the cover, which allows weed seeds to germinate and for prostrate weeds which are unaffected by the mowing to complete their life cycle. However, this same treatment is also effective for maintaining or reducing weed seed banks (Gibson et al, 2011).

While it was once suggested keeping land “in sod for two or three years out of ten, that would keep it in very nice condition” (Sweet, 1982), due to current global population pressures on farmers to increase production per acre, agricultural land must now be kept in production for extended periods of time, and where there is shortage of cultivated land, adoption of marginal land is necessary. A compromise is to adopt an intercropping strategy of maintaining and improving soil surrounding a growing cash crop using living mulch. Intercropping systems typically provide good weed control, exhibit less crop damage from pests and diseases, may be more efficient than monocultures at exploiting limited resources and as such can increase yield per acre (Coolman and Hoyt, 1993).

This study tested the hypothesis that growing a living mulch of mixed species increases biomass production and thus improves weed suppression, compared to monocultures of the same species. Dutch white clover was grown in combination with annual ryegrass, and in combination with buckwheat, and results were compared to plots of the three individual species. We aimed to identify what effects on weed suppression and biomass production of the overall living mulch there were from combining them at different seeding ratios compared to when they were grown separately. Additionally, subplots of the mixed living mulch and individual species were mowed once during the growing season to observe the effect of mowing on suppression of living mulch and weed growth.

The crop production scenario chosen for this study was utilizing living mulch in alleyways between black polythene-mulched beds. This is because, as is often the case in intensive, high-value, fresh-market vegetable production systems, growers cover between 50-75% the area of a field with impermeable polyethylene mulch and maintain weed-free, bare soil in the alleyways via herbicide use and occasional cultivation. Studies have shown that this production system significantly increases soil erosion and watercourse pollution from agriculture, and therefore further research is needed on technologies to mitigate this issue (Rice et al., 2001; Basher and Ross, 2001). While the scenario used in this study is specific, the findings will be pertinent to the use of this system in a variety of other field-based cropping situations where living mulch use is appropriate.

## **Methods**

Experiments took place in 2010 at the Phytophthora Blight farm, part of Cornell University's New York State Agricultural Experiment Station (NYSAES), in Geneva, NY (42°52'51.84"N, 77°00'48.10"W). Soil type was Odessa silt loam (fine, illitic, mesic Aeric Endoaqualfs). Soil samples were taken in April 2010 and analyzed by Morgan extraction at Cornell Nutrient Analysis Laboratory (Ithaca, NY). Soil organic matter (4%) and pH (6.9) were adequate, as were other nutrients, so no fertilizer or lime was added.

## **Experimental design**

On September 22, 2009 a summer cover crop of annual ryegrass (*Lolium multiflorum* Lam.) was herbicide-killed, the soil was cultivated using a disc-harrow and tined-harrow with rear crumbler-cage, and beds were immediately prepared. Flat beds, 36 inches (92 cm) wide, were mulched

with embossed black plastic (1 mil [0.0254 mm] thick) on 7 foot (2.13 m) centers. The experimental design was a randomized block, split-plot design with five replications, with a single factor of mowing (two levels: mown or not mown) applied to subplots. Each living mulch treatment was randomly assigned a plot that was 10 feet (3 m) long and 4 feet (1.2 m) wide.

Seed mixtures of annual ryegrass with Dutch white clover and buckwheat with Dutch white clover were weighed-out in appropriate quantities for the seeding rates and size of plots, and put into containers. Seeding rates for the mixtures were chosen to reflect percentages of average recommended broadcast seeding rate: See Table 3.1 for a list of treatments. For comparison, all three living mulches were sown at their usual sowing rates; in effect these were check treatments.

All living-mulches were broadcast-sown by hand on May 13, 2010, into rototilled and raked soil; then seeds were raked in and plots were rolled. No irrigation was applied to any seeded plots.

All plots were split evenly in two. Subplots assigned the factor of mowing were cut once to within approximately 0.6 inch (1.5 cm) of the soil-surface using a two-wheel tractor with front-mounted, 45 inch (114 cm) wide, sickle bar mower. Mowing took place when the buckwheat and annual ryegrass treatments had begun flowering and prior to seed formation: August 28, 2010. Two passes of the mower were required to ensure most living mulch shoots were mown. Cut plant material was redistributed evenly across the respective subplot by hand.

**Table 3.1:** Broadcast seeding rates used for living mulch treatments in field experiments carried out at the New York State Agricultural Experiment Station, Geneva, NY.

Living Mulch Treatments	Broadcast seeding rate (kgs.ha <sup>-1</sup> )
Annual Ryegrass (AR)	34
Buckwheat (BW)	96
Dutch White Clover (WC)	16
Annual Ryegrass with Dutch White Clover Mix (AC25:75) *	8.5 & 12
Annual Ryegrass with Dutch White Clover Mix (AC50:50) *	17 & 8
Buckwheat with Dutch White Clover Mix (BC25:75) *	24 & 12
Buckwheat with Dutch White Clover Mix (BC50:50) **	48 & 8

Letters in parentheses are used as the treatment acronym in results [e.g. (AR)].

\* Mixtures sown at 25% and 75% of AR or BW and WC recommended seeding rate, respectively.

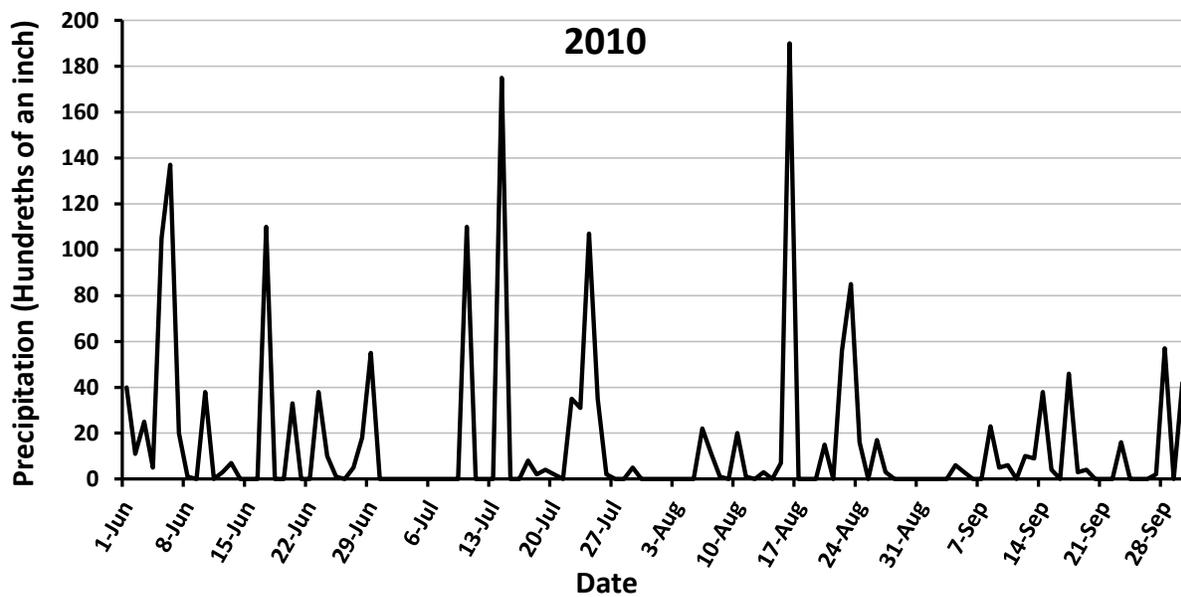
\*\* Mixtures sown at 50% of the species recommended seeding rate

## Sampling

Seed samples for all plant species were taken from the proprietary bags of seed, labeled and submitted to the New York State Seed Testing Laboratory, Geneva, NY, for germination analysis on May 18, 2010. Subplots were measured for percent groundcover of mulch and weeds, above-ground living mulch biomass dry-weight, and field penetration resistance readings were taken at both 6 inch (15cm) and 18 inch (45cm) depth. Percent groundcover was by visual estimation, using Bayley's (2001) diagram of "Distribution of ground cover to assist in determining percentage cover" as a guide. A 12 inch (30 cm) square quadrat of above-ground plant biomass was taken from each living mulch subplot. Plant material was cut at the soil surface, separated into living mulch and weeds, placed in labeled paper-bags, dried for 48 hours at 149 °F (65 °C) then had dry-weight recorded. Biomass samples were extrapolated to provide tons per-hectare (t.ha<sup>-1</sup>) values. Field penetration resistance readings were taken six times per subplot, using a hand-held soil compaction tester (penetrometer) according to the

protocol described in the Cornell soil health assessment training manual (Gugino et al., 2007). These assessments were undertaken at the termination of the experiments and biomass cuts were also taken from both subplots per plot, prior to mowing.

Subplots affected by prolonged periods of flooding after precipitation (more than two days) were recorded. Flooding occurred on several occasions in 2010. Six precipitation events of daily accumulation exceeding 1 inch (2.5 cm) took place from the sowing of the living mulches to the end of the experiments; the greatest being 1.9 inches (4.8 cm) (Cornell University, CALS, NYSAES, 2011) (Figure 3.1).



**Figure 3.1:** Precipitation levels for 2010 field trial, using data from the Vegetable Research Farm weather station, New York State Agricultural Experiment Station, Geneva, NY.

## Data analysis

Land equivalent ratio (LER) was calculated using aboveground biomass yield data for both the studies and for both the mixed seeding rates in order to identify how intercropping the living mulch species compared to growing the individual species alone. The calculation was based on Mead and Willey (1980) for an intercrop using two species:

$$\text{LER} = \text{LER}_{\text{LM1}} + \text{LER}_{\text{LM2}} = \frac{B_{i\text{LM1}}}{B_{m\text{LM1}}} + \frac{B_{i\text{LM2}}}{B_{m\text{LM2}}}$$

$\text{LER}_{\text{LM1}}$  is the land equivalent ratio for living mulch annual ryegrass or buckwheat, and  $\text{LER}_{\text{LM2}}$  is LER of Dutch white clover.  $B_i$  is the biomass yield of the living mulch specie when grown in the intercrop mix and  $B_m$  is the biomass yield of the same living mulch specie grown in monoculture. The calculation is undertaken for each replication of both mixes before being statistically analyzed for differences between LER of mixes and the effect of mowing on LER. In order to understand the influence of the choice of denominator for either living mulch in the mixture, calculations of LER were carried out using the maximum monoculture biomass yield recorded during the study, the average monoculture biomass yield across all five replicates, and the monoculture biomass yield for each replicate.

Additionally, yield proportion of both annual ryegrass and buckwheat aboveground biomass was calculated using  $\text{LER}_{\text{LM1}} / (\text{LER}_{\text{LM1}} + \text{LER}_{\text{LM2}})$ , as defined by Mead and Willey (1980). The yield proportion was also calculated three times for both mixes using the three calculations of the LER.

Normal log-transformation of data was required for LER analysis of annual ryegrass with Dutch white clover using monoculture biomass yield for each replicate and for all LER analysis of buckwheat with Dutch white clover for normalization of residuals.

Significance of differences between treatments, of the interaction between living mulch and mowing, and of main effects, were determined using least-squares regression analysis. Once the model including main effects and the interaction was analyzed non-significant variables were removed. Significant differences ( $P \leq 0.05$ ) between treatments or in levels of main effect variables were determined using least significant difference (LSD) in JMP®, version 9.0.0 (SAS Institute, Inc., 2010). Whenever necessary, data was transformed using natural log or square root.

## Results

### Seed viability

All living mulch species germinated in 9 days (Table 3.2). Annual ryegrass had the highest percent germination and total percent of viable seed: both 98%. Dutch white clover scored the lowest percent for the same two categories: 82% germination and 85% viable seed.

**Table 3.2:** Seed viability analysis of living mulch plant species.

<b>Living Mulch Treatments</b>	<b>Days to germination</b>	<b>Germination (%)</b>	<b>Hard seed (%)</b>	<b>Viable seed (%)</b>
Annual ryegrass	9	98	0	98
Buckwheat	9	90	0	90
Dutch white clover	9	82	3	85

**Table 3.3:** Cost of living mulch seed at different seeding rates and in mixtures, as of 2011. Low cost is the dollars per pound rate, based upon purchasing 50lb seed bags, multiplied by the seeding rate. High cost is the dollars per pound rate, based upon 1lb seed bags, multiplied by the seeding rate. Source of seed prices is Johnny’s Selected Seeds online-catalog ([www.johnnyseeds.com](http://www.johnnyseeds.com)).

Living Mulch Treatments (% of seeding rate)	Broadcast seeding rate (kgs.ha <sup>-1</sup> )	Cost (\$.ac)	
		Low	High
Annual ryegrass (100)	34	41.10	142.50
Annual ryegrass-Dutch white clover mix (50:50)	17 & 8	55.27	148.60
Annual ryegrass-Dutch white clover mix (25:75)	8.5 & 12	62.36	151.66
Dutch white clover (100)	16	69.44	154.70
Buckwheat (100)	96	115.20	792.00
Buckwheat-Dutch white clover mix (50:50)	48 & 8	92.32	473.35
Buckwheat-Dutch white clover mix (25:75)	24 & 12	80.88	314.03
Dutch White Clover (100)	16	69.44	154.70

### Seed cost

Seed prices increased from annual ryegrass to buckwheat, to Dutch white clover. As seeding rate of annual ryegrass decreased and the rate of Dutch white clover increased, the cost of the treatment’s seed increased also (Table 3.3). The opposite trend was observed in the buckwheat-Dutch white clover study; as the seeding rate of Dutch white clover increased, the cost of seed decreased.

The cheapest treatment’s seed used in these two studies was annual ryegrass grown in monoculture, followed by the mix of annual ryegrass and Dutch white clover at 50% of both their seeding rates. The most expensive treatment’s seed was buckwheat grown in monoculture, followed by the mix of buckwheat and Dutch white clover at 50% of both their seeding rates.

In both studies the greatest cost variation was between the full broadcast seeding rate for annual ryegrass or buckwheat and the mix of these species with Dutch white clover at 50% of both their recommended seeding rates. The cost variation was an increase in the annual ryegrass-Dutch white clover study, and a decrease in the buckwheat-Dutch white clover study.

### **Biomass from living mulch-treated plots**

Annual ryegrass and Dutch white clover study:

There was a statistically significant difference between biomass dry weights of living mulch treatments ( $P < .0001$ ), with the monoculture of Dutch white clover growing less above ground biomass than treatments that included annual ryegrass (Figure 3.2). Dutch white clover produced  $0.8 \text{ t}\cdot\text{ha}^{-1}$  biomass. Annual ryegrass-Dutch white clover 50-50 mix produced the greatest weight of living mulch biomass;  $7.1 \text{ t}\cdot\text{ha}^{-1}$ .

Mowing significantly increased the weight of living mulch biomass that plots produced ( $P < .0001$ ); an increase of  $1.7 \text{ t}\cdot\text{ha}^{-1}$ .

There was  $3.6 \text{ t}\cdot\text{ha}^{-1}$  more weed biomass produced in Dutch white clover-treated plots than in annual ryegrass-Dutch white clover 50-50 mix-treated plots; this is significantly more weed biomass ( $P = 0.0003$ ). Eighty-four percent of the total above ground dry biomass collected from Dutch white clover plots was weeds; significantly more than all other treatments ( $P < .0001$ ).

Flooding had a significant effect on amount of weed biomass produced ( $P = 0.0498$ ). Unflooded plots produced  $5.4 \text{ t}\cdot\text{ha}^{-1}$  weeds; significantly more than flooded plots, which produced  $3.7 \text{ t}\cdot\text{ha}^{-1}$ .

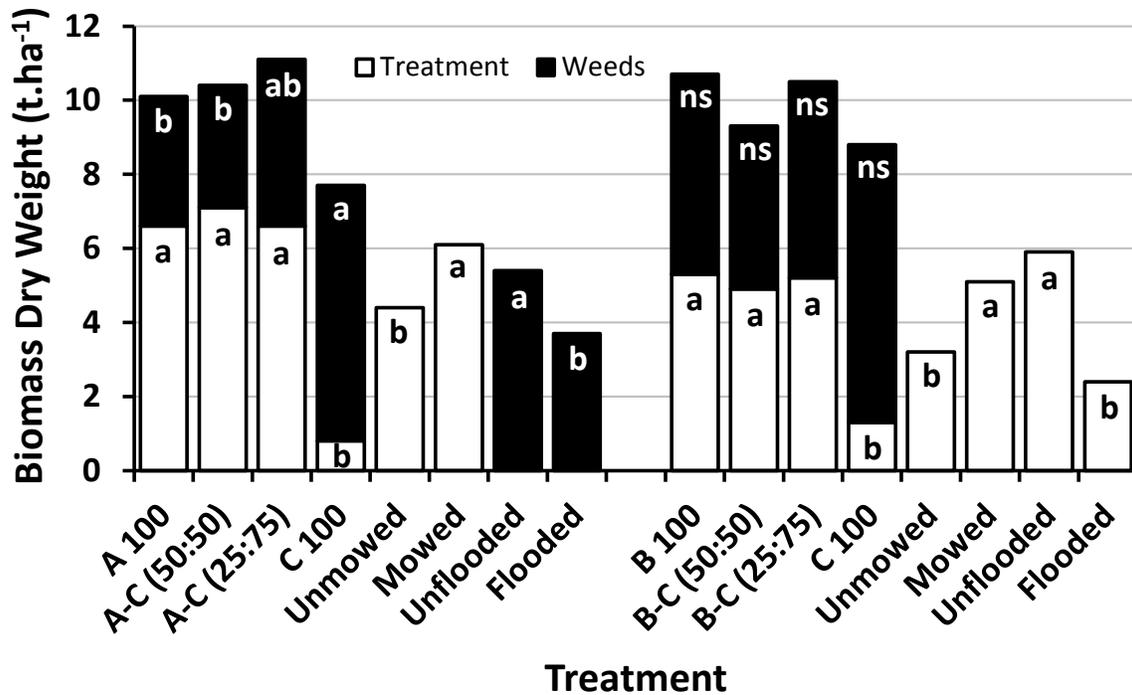
Buckwheat and Dutch white clover study:

There was significantly less living mulch biomass harvested from the Dutch white clover-treated plots (C 100) than all other treatments ( $P = 0.0006$ ) (Figure 3.2). Dutch white clover-treated plots produced  $1.3 \text{ t}\cdot\text{ha}^{-1}$ . Buckwheat-treated plots (B100) produced the most living mulch biomass:  $5.3 \text{ t}\cdot\text{ha}^{-1}$ , and between the two mixes, more living mulch biomass was produced from 25:75 mix plots ( $5.2 \text{ t}\cdot\text{ha}^{-1}$ ) than 50:50 plots ( $4.9 \text{ t}\cdot\text{ha}^{-1}$ ).

The amount of weed biomass was not significantly different between treatments ( $P = 0.2224$ ). Dutch white clover-treated plots had the greatest amount of weeds,  $7.5 \text{ t}\cdot\text{ha}^{-1}$ , and the buckwheat-Dutch white clover 50-50 mix had the least amount of weeds,  $4.4 \text{ t}\cdot\text{ha}^{-1}$ .

Mowing significantly increased living mulch biomass production ( $P = 0.0004$ ) in all treatments. Unmowed plots produced least living mulch biomass:  $3.2 \text{ t}\cdot\text{ha}^{-1}$ . Mowed plots produced  $5.1 \text{ t}\cdot\text{ha}^{-1}$ .

Flooding significantly reduced living mulch biomass production ( $P = 0.0024$ ). Unflooded plots produced  $5.9 \text{ t}\cdot\text{ha}^{-1}$  of living mulch biomass, compared to  $2.4 \text{ t}\cdot\text{ha}^{-1}$  of living mulch biomass from flooded plots.



**Figure 3.2:** Above ground biomass grown by living mulch treatments from two studies in 2010, and the effects of mowing and flooding. Living mulch treatments included monocultures of annual ryegrass (A 100) or buckwheat (B100) and Dutch white clover (C 100), and in both studies there were two mixes of either annual ryegrass and Dutch white clover [A-C (50:50) and A-C (25:75)], or buckwheat and Dutch white clover [B-C (50:50) and B-C (25:75)]. Mixes were a combination of the two living mulch species and at modified seeding rates; percentages of monoculture seeding rate are indicated by the associated numbers in the mix-title. Treatments with different letters within the white or black area of the bar have statistically significant differences ( $P < 0.05$ ) between their respective least squared means for living mulch and weed biomass respectively. Least squared means analyses were carried out separately for treatment, mowing and flooding for each study.

### **Biomass of individual living mulch species**

Annual ryegrass and Dutch white clover study:

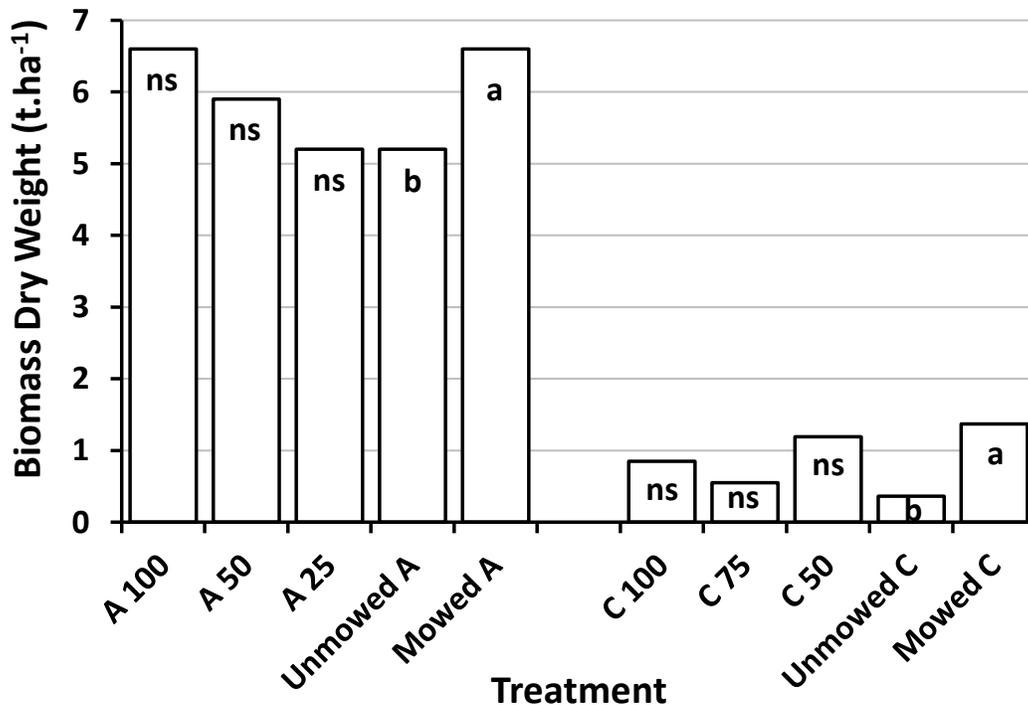
There was no significant difference between least square means for the three different treatments including annual ryegrass ( $P = 0.3017$ ) (Appendix 2, Table A.2.1) or for the three treatments including Dutch white clover ( $P = 0.1861$ ) (Figure 3.3 and Appendix 2, Table A.2.2).

The percentage of total biomass collected from a plot containing annual ryegrass differed significantly ( $P = 0.0442$ ) between treatments. Monoculture plots had highest percent of annual ryegrass (71%); significantly more than percent of annual ryegrass from annual ryegrass-Dutch white clover 25-75 mix-treated plots that had 51%.

Annual ryegrass 100 produced the most annual ryegrass biomass per plot,  $6.6 \text{ t}\cdot\text{ha}^{-1}$ , and the annual ryegrass-Dutch white clover 25:75 mix produced the least amount of annual ryegrass biomass,  $5.2 \text{ t}\cdot\text{ha}^{-1}$ . Mowing significantly increased annual ryegrass biomass in all treatments using this living mulch specie ( $P = 0.0408$ ); from  $5.2 \text{ t}\cdot\text{ha}^{-1}$  to  $6.6 \text{ t}\cdot\text{ha}^{-1}$ .

Biomass from the annual ryegrass-Dutch white clover 50:50 mix-treated plots had the greatest weight of Dutch white clover,  $1.19 \text{ t}\cdot\text{ha}^{-1}$ , of all treatments including this living mulch specie (Figure 3.3 and Appendix 2, Table A.2.2). Mowing significantly increased the amount of Dutch white clover in a treated plot ( $P < .0001$ ); increasing the weight from  $0.36 \text{ t}\cdot\text{ha}^{-1}$  to  $1.37 \text{ t}\cdot\text{ha}^{-1}$ .

Mowed and flooded plots had a significantly higher percent of Dutch white clover biomass harvested from them;  $P = 0.0005$  and  $P = 0.0056$  respectively. Mowing increased percent of Dutch white clover from 5% to 18%, and flooding increased the percent from 9% to 14%.

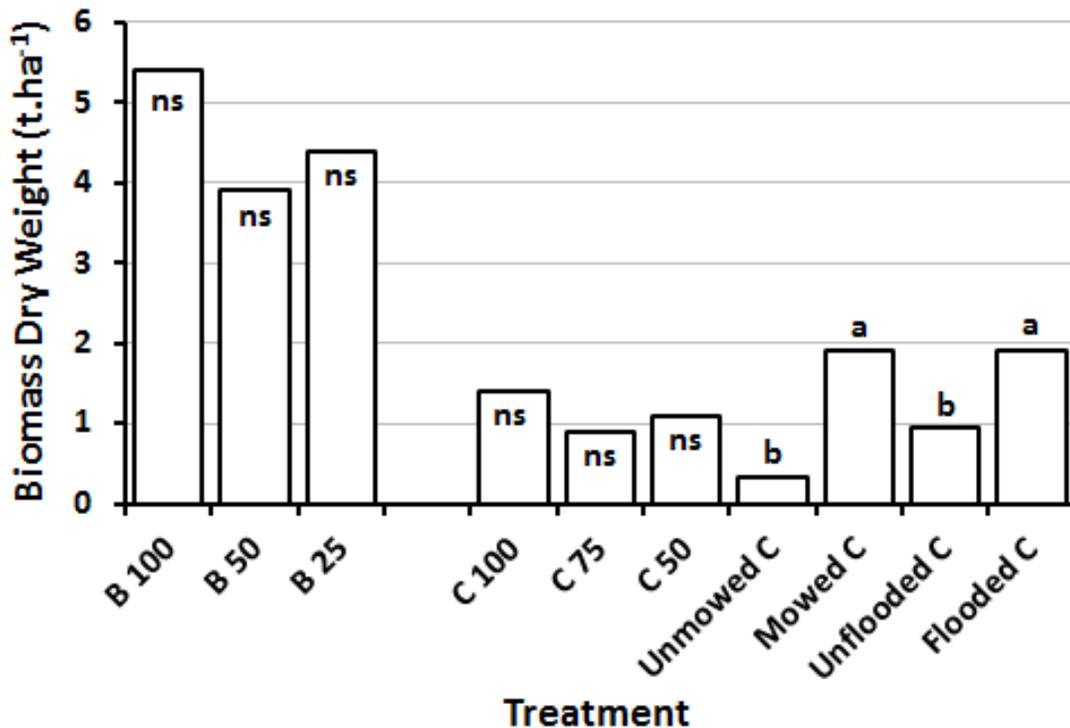


**Figure 3.3:** Above ground biomass for the living mulches, annual ryegrass (A) and Dutch white clover (C), that were grown in monoculture (A 100 and C 100) and in mixes [A-C (50:50) and A-C (25:75)], and the effect of mowing on the biomass produced in 2010. Numbers in the treatment titles are the percentage of the living mulches recommended seeding rate used. Treatments with different letters within the white bars have statistically significant differences ( $P < 0.05$ ) between their respective least squared means for living mulch biomass. Annual ryegrass, Dutch white clover and main effects were analyzed separately for differences between least squared means.

Buckwheat and Dutch white clover study:

Buckwheat biomass was not significantly different between treatments ( $P = 0.1781$ ) (Figure 3.4 and Appendix 2, Table A.2.3). Dry weight ranged from 3.9 t.ha<sup>-1</sup> for buckwheat-Dutch white clover 50:50 mix, to 5.4 t.ha<sup>-1</sup> for buckwheat 100. There was no significant difference between

percent of total biomass collected from plots that was buckwheat ( $P = 0.2255$ ). However, mowing ( $P = 0.0281$ ) and flooding ( $P = 0.0006$ ) both significantly affected the percent of buckwheat in the total biomass from plots. Mowing increased the percentage of buckwheat from 31% to 39%. Flooding decreased the percentage of buckwheat from 53% to 16%.



**Figure 3.4:** Above ground biomass for buckwheat (B) and Dutch white clover (C) living mulch that were grown in monoculture (B 100 and C 100) and in mixes [B-C (50:50) and B-C (25:75)]. Also, the effect of mowing and flooding on the amount of Dutch white clover biomass are illustrated. Numbers in the treatment titles are the percentage of the living mulches recommended seeding rate used. Treatments with different letters above the white bars have statistically significant differences ( $P < 0.05$ ) between their respective least squared means for weight of living mulch biomass. Buckwheat, Dutch white clover and main effects were analyzed separately for differences between least squared means.

There was also no significant difference in weight of Dutch white clover biomass for the three different treatments containing this living mulch. Dutch white clover biomass ranged from 0.9 t.ha<sup>-1</sup>, at 75% seeding rate in a mix with buckwheat (C 75), to 1.4 t.ha<sup>-1</sup>, when seeded at 100% of the recommended rate (C 100) in monoculture (Figure 3.4 and Appendix 2, Table A.2.4).

Mowing and flooding significantly increased Dutch white clover biomass production ( $P < .0001$  and  $P = 0.0059$  respectively), and the percentage of Dutch white clover in the total biomass for plots ( $P < .0001$  and  $P = 0.0010$  respectively). Biomass increased from 0.3 t.ha<sup>-1</sup>, produced in unmowed plots, to 1.9 t.ha<sup>-1</sup> in mowed plots. Flooding increased biomass from 0.9 t.ha<sup>-1</sup> to 1.3 t.ha<sup>-1</sup>. The Dutch white clover percent of total biomass from plots increased from 6% to 25% due to mowing and from 10% to 20% due to flooding.

### **Land Equivalent Ratio (LER)**

Sowing mixtures of living mulch at different seeding rates did not result in significantly different land equivalent ratios (Table 3.4 and Table 3.5).

Living mulch mixtures, when calculated using either the monoculture biomass yield per replicate or the average monoculture yield, had LER's greater than one; indicating that more land is required when growing monocultures of the two crops in order to produce the equivalent aboveground biomass harvested from a unit area of the mixed-species treatments. LER results using the average monoculture yield in the calculation were higher for the 50:50 mix than the 25:75 mix in both studies. The same trend is observed when the calculation uses the maximum monoculture yield for both studies and each replicates monoculture yield in the annual ryegrass-Dutch white clover study (Table 3.4). When using the monoculture yield in each replicate of the

buckwheat-Dutch white clover study, the 25:75 mixture has a larger LER than the 50:50 mix (Table 3.5).

The effect of mowing on the LER of mixed-species living mulch plots differed between the two studies. For the annual ryegrass-Dutch white clover mixture study mowing plots significantly reduced the LER ( $P \leq 0.0212$ ) (Table 3.4); even to the extent of lowering the LER value from  $>1$  to  $<1$ . For plots of buckwheat and Dutch white clover mixtures, mowing increased LER (Table 3.5). This was statistically significant when calculation of LER for mowed and unmowed plots used either the maximum monoculture yield of the study or the average monoculture yield. Indeed, the latter calculation resulted in the respective LER rise from 0.79 for unmowed plots to 1.79 for mowed plots.

**Table 3.4:** Land Equivalent Ratios (LER) for two living mulch mixes involving annual ryegrass and Dutch white clover sown at different ratios, and the effect of mowing. LER's are calculated three different ways based upon use of three different monoculture yield figures. The three different calculations and effect of mowing were analyzed separately for least squared means differences.

	Calculations per mix using monoculture yield:					
	maximum		average		per replicate	
	<u>25:75</u>	<u>50:50</u>	<u>25:75</u>	<u>50:50</u>	<u>25:75</u>	<u>50:50</u>
<b>Annual ryegrass</b>	0.46	0.53	0.83	0.95	1.26	1.52
<b>Dutch white clover</b>	0.38	0.37	0.78	0.74	1.83	1.61
<b>Total LER</b>	<b>0.85</b>	<b>0.90</b>	<b>1.60</b>	<b>1.69</b>	<b>3.09</b>	<b>3.13</b>
S.E.	0.15		0.30		1.10	
P-value	P = 0.8139		P = 0.8366		P = 0.7381	
	<u>Unmowed</u>	<u>Mowed</u>	<u>Unmowed</u>	<u>Mowed</u>	<u>Unmowed</u>	<u>Mowed</u>
<b>Mowing effect on LER</b>	1.31	0.43	2.49	0.80	4.82	1.41
S.E.	0.15		0.30		1.10	
P-value for effect	P = 0.0008		P = 0.0010		P = 0.0212	

**Table 3.5:** Land Equivalent Ratios (LER) for two living mulch mixes involving buckwheat and Dutch white clover sown at different ratios, and the effect of mowing. LER's are calculated three different ways based upon use of three different monoculture yield figures. The three different calculations and effect of mowing were analyzed separately for least squared means differences.

	Calculations per mix made using monoculture yield:					
	maximum		average		per replicate	
	<u>25:75</u>	<u>50:50</u>	<u>25:75</u>	<u>50:50</u>	<u>25:75</u>	<u>50:50</u>
<b>Buckwheat</b>	0.13	0.18	0.49	0.65	1.48	0.86
<b>Dutch white clover</b>	0.21	0.21	0.71	0.74	1.95	0.81
<b>Total LER</b>	<b>0.33</b>	<b>0.39</b>	<b>1.20</b>	<b>1.39</b>	<b>3.43</b>	<b>1.67</b>
S.E.	0.05		0.21		1.25	
P-value	P = 0.4140		P = 0.3911		P = 0.6928	
	<u>Unmowed</u>	<u>Mowed</u>	<u>Unmowed</u>	<u>Mowed</u>	<u>Unmowed</u>	<u>Mowed</u>
<b>Mowing effect on LER</b>	0.21	0.51	0.79	1.79	2.38	2.72
S.E.	0.05		0.21		1.25	
P-value for effect	P = 0.0008		P = 0.0013		P = 0.1676	

### Yield Proportion

The proportion of aboveground annual ryegrass biomass harvested from annual ryegrass-Dutch white clover mixed-species plots increased with the increase in sowing rate (Table 3.6).

Depending on how calculations were made the proportion of annual ryegrass ranged between 41% and 55% for the 25:75 mix plots to between 48% and 59% for the 50:50 mix plots. Mowing this mixture increased the proportion of aboveground annual ryegrass biomass.

The proportion of buckwheat biomass also increased with an increase in sowing rate (Table 3.7). The proportion of buckwheat ranged from between 38% and 43% for the 25:75 treatment to between 45% and 52% for the 50:50 treatment. In contrast to annual ryegrass, mowing either made no change or decreased the proportion of aboveground buckwheat biomass in

buckwheat-Dutch white clover-treated plots. Unmowed plots had between 47% and 58% buckwheat in harvested biomass, whereas mowed plots yielded between 28% and 47% buckwheat biomass.

The choice of numerical datum or statistic to use for the monoculture comparison in the calculation of LER affects the total LER and yield proportion results and subsequently the effective LER curve. Changing from using the maximum biomass yield recorded for a monoculture-treated plot to using the data for monoculture-treated plots in each replicate increases LER (Table 3.6 and Table 3.7). This same change in use of data has different effects on yield proportion results, depending upon the living mulch species in question. The proportion of annual ryegrass biomass decreased from 59% to 41% (Table 3.6), and the proportion of buckwheat biomass increased from 38% to 52% (Table 3.7). The effect of mowing showed contrasting changes depending on the living mulch being treated. For annual ryegrass the change in calculation increased the difference between mowed and unmowed plots (Table 3.6), whereas for buckwheat, the calculation change decreased the difference (Table 3.7).

### **“Effective” LER**

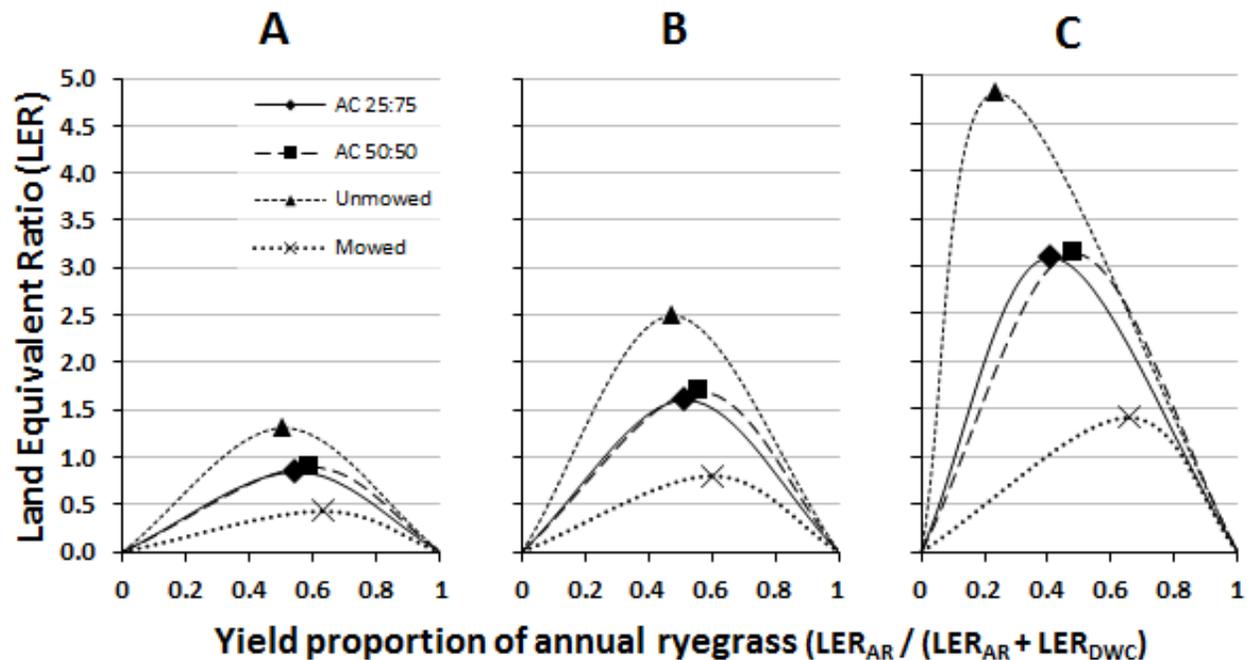
The effective LER curves indicate no difference in biological efficiency between the two living mulch mixtures that include annual ryegrass, if the desired proportion of annual ryegrass aboveground biomass is between 41% and 59%. Greater biological efficiency is achieved with an annual ryegrass-Dutch white clover mixture if it is not mowed, assuming it is acceptable to have a proportion of annual ryegrass between 24% and 51% (Figure 3.5 and Table 3.6). In contrast, if the proportion of buckwheat biomass is acceptable to be between 28% and 47%, mowing increased biological efficiency of a buckwheat-Dutch white clover mixture (Figure 3.6 and Table 3.7). The 50:50 buckwheat-Dutch white clover mix was only very slightly more biologically efficient than the 25:75 mixture if using either the maximum monoculture yield or

**Table 3.6:** The proportion of aboveground biomass that is annual ryegrass for two living mulch mixes involving annual ryegrass and Dutch white clover sown at different ratios, and the effect of mowing. Yield proportions are calculated three different ways based upon use of three different monoculture yield figures. The three different calculations and effect of mowing were analyzed separately for least squared means differences.

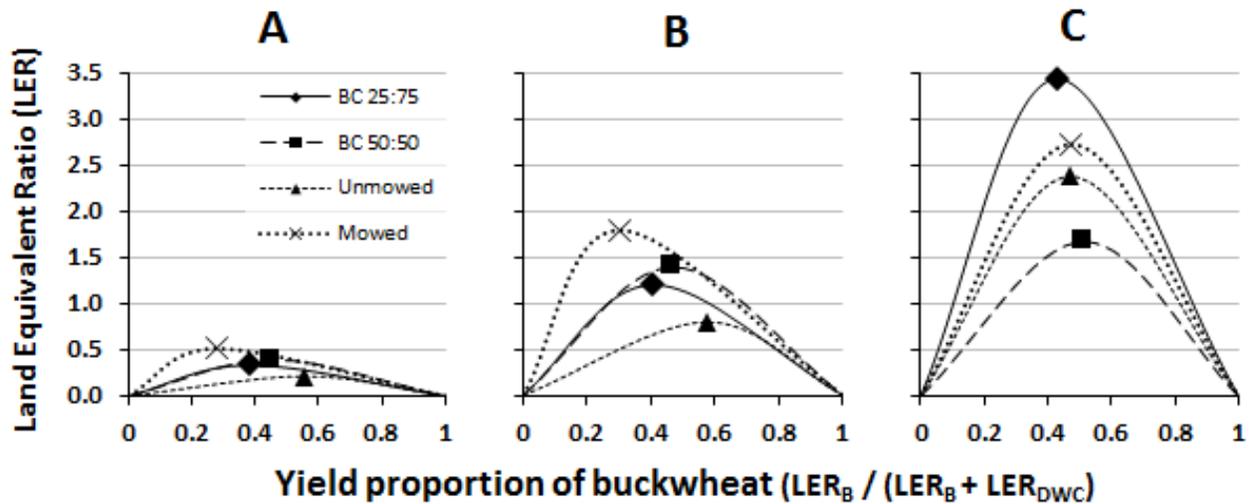
	Calculations per mix made using monoculture yield:					
	maximum		average		per replicate	
	<u>25:75</u>	<u>50:50</u>	<u>25:75</u>	<u>50:50</u>	<u>25:75</u>	<u>50:50</u>
<b>Yield proportion of annual ryegrass</b>	<b>0.55</b>	<b>0.59</b>	<b>0.52</b>	<b>0.56</b>	<b>0.41</b>	<b>0.48</b>
S.E.	0.07		0.07		0.06	
P-value	P = 0.6622		P = 0.6690		P = 0.3693	
<b>Mowing effect on yield proportion of annual ryegrass</b>	<u>Unmowed</u>	<u>Mowed</u>	<u>Unmowed</u>	<u>Mowed</u>	<u>Unmowed</u>	<u>Mowed</u>
	0.51	0.63	0.48	0.60	0.24	0.66
S.E.	0.07		0.07		0.06	
P-value for effect	P = 0.2330		P = 0.2375		P = <.0001	

**Table 3.7:** The proportion of aboveground biomass that is buckwheat for two living mulch mixes involving buckwheat and Dutch white clover sown at different ratios, and the effect of mowing. Yield proportions are calculated three different ways based upon use of three different monoculture yield figures. The three different calculations and effect of mowing were analyzed separately for least squared means differences.

	Calculations per mix made using monoculture yield:					
	maximum		average		per replicate	
	<u>25:75</u>	<u>50:50</u>	<u>25:75</u>	<u>50:50</u>	<u>25:75</u>	<u>50:50</u>
<b>Yield proportion of buckwheat</b>	<b>0.38</b>	<b>0.45</b>	<b>0.41</b>	<b>0.47</b>	<b>0.43</b>	<b>0.52</b>
S.E.	0.12		0.12		0.13	
P-value	P = 0.6646		P = 0.7158		P = 0.6377	
<b>Mowing effect on yield proportion of buckwheat</b>	<u>Unmowed</u>	<u>Mowed</u>	<u>Unmowed</u>	<u>Mowed</u>	<u>Unmowed</u>	<u>Mowed</u>
	0.56	0.28	0.58	0.30	0.47	0.47
S.E.	0.12		0.12		0.13	
P-value for effect	P = 0.1042		P = 0.1153		P = 0.9876	



**Figure 3.5:** “Effective LER” curves for two different living mulch mixtures of annual ryegrass with Dutch white clover, broadcast-sown at a percentage of the recommended sowing rate. AC 25:75 is 25% of the recommended annual ryegrass sowing rate with 75% rate of Dutch white clover, and AC 50:50 is 50% of both species recommended sowing rate. Charts show the standardized monoculture biomass yields are based on (A) the maximum yield per specie recorded during the study, (B) the average yield across the five replicates, and (C) the respective monoculture yields in each replicate. No significant differences between the two mixtures were noted for yield proportion of annual ryegrass ( $LER_A / [LER_A + LER_C]$ ) or LER at  $\alpha = 0.05$ . The effect of mowing caused significant differences between mowed and unmowed subplots for the yield proportion of annual ryegrass ( $P < 0.0001$ ) when the standardized monoculture biomass yield was based on yields from each replicate. Mowing caused significant differences in LER when using any standardized monoculture biomass yield ( $P \leq 0.0212$ ).



**Figure 3.6:** “Effective LER” curves for two different living mulch mixtures of buckwheat with Dutch white clover, broadcast-sown at a percentage of the recommended sowing rate. BC 25:75 is 25% of the recommended buckwheat sowing rate with 75% rate of Dutch white clover, and BC 50:50 is 50% of both species recommended sowing rate. Charts show the standardized monoculture biomass yields are based on **(A)** the maximum yield per specie recorded during the study, **(B)** the average yield across the five replicates, and **(C)** the respective monoculture yields in each replicate. No significant differences between the two mixtures were noted for yield proportion of buckwheat ( $LER_B / [LER_B + LER_C]$ ) or LER at  $\alpha = 0.05$ . The effect of mowing caused significant differences between mowed and unmowed subplots for the LER when the standardized monoculture biomass yield was based on yields from either the maximum yield per specie ( $P = 0.0008$ ) or the average yield across the five replicates ( $P = 0.0013$ ).

average monoculture yield data in calculations. However, if calculations are made using each replicates monoculture yield, the effective LER curve for the 25:75 mix is almost twice as large as the 50:50mix, indicating a much greater biological efficiency (Figure 3.6).

### **Groundcover in living mulch-treated plots**

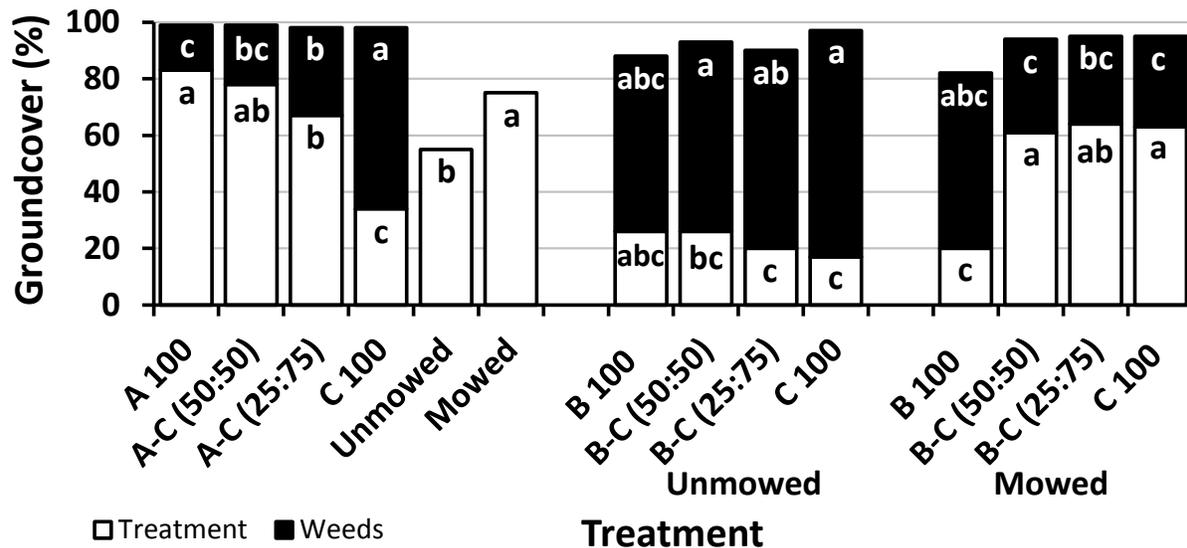
Annual ryegrass and Dutch white clover study:

Total area of ground covered in plots containing annual ryegrass and/or Dutch white clover was between 98% and 99% ( $P = 0.7748$ ) (Figure 3.7). Annual ryegrass grown in monoculture resulted in the greatest area of land that was covered with living mulch (83%); significantly more living mulch covering the ground than both annual ryegrass-Dutch white clover 25:75 mix treatment and Dutch white clover grown in monoculture ( $P < .0001$ ). The area of ground covered with weeds was the inverse of the percent area of ground covered by living mulch. Therefore annual ryegrass grown in monoculture had significantly less area of its plots covered in weeds than both annual ryegrass-Dutch white clover 25:75 mix treatment and Dutch white clover grown in monoculture ( $P < .0001$ ). Mowing significantly increased the area of land covered with living mulch and reduced the area of land covered with weeds ( $P < .0001$ ). Area of ground covered by living mulch increased from 55% to 75% due to mowing.

Buckwheat and Dutch white clover study:

The total area of ground covered was not significantly different between treatments ( $P = 0.1153$ ). There was a significant effect of the interaction between treatment and mowing on the area of ground covered by living mulch ( $P = 0.0003$ ) (Figure 3.7). Mowing the two mix-treatments and Dutch white clover treatment significantly increased the area of ground covered by living mulch. Least squared means analysis of living mulch groundcover showed that mowed plots of the two mixes and Dutch white clover covered significantly more ground than unmowed plots of buckwheat-Dutch white clover 25:75 mix and Dutch white clover and mowed plots of

buckwheat; between 35% and 47% more groundcover. Mowed buckwheat-Dutch white clover 25:75 mix-treatment covered the most ground with living mulch (64%) and unmowed Dutch white clover covered the least area (17%).



**Figure 3.7:** Area of ground covered by living mulch treatments and the effect of mowing from two studies in 2010. Living mulch treatments included monocultures of annual ryegrass (A 100) or buckwheat (B100) and Dutch white clover (C 100), and in both studies there were two mixes of either annual ryegrass and Dutch white clover [A-C (50:50) and A-C (25:75)], or buckwheat and Dutch white clover [B-C (50:50) and B-C (25:75)]. Mixes were a combination of the two living mulch species and at modified seeding rates; percentages of monoculture seeding rate are indicated by the associated numbers in the mix-title. Treatments with different letters in the white or black area of the bars have statistically significant differences ( $P < 0.05$ ) between their least squared means for the percentage of ground covered by living mulch or weeds respectively. Least squared means analyses for living mulch, weeds and for the effect of mowing were carried out separately for each study.

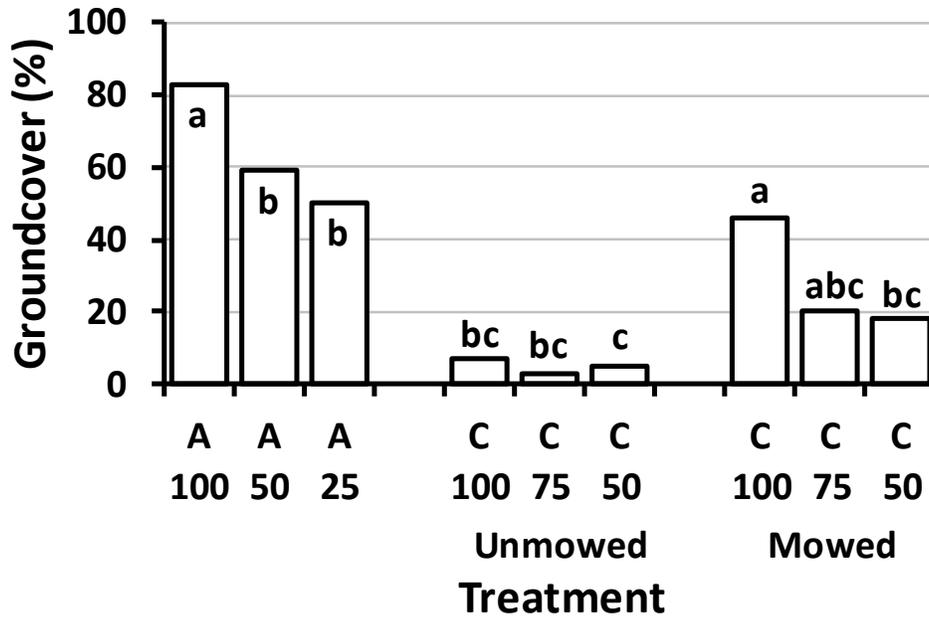
The area of ground covered by weeds was also affected by the interaction between treatment and mowing ( $P = 0.0103$ ). Mowed plots of the two mixes and Dutch white clover had significantly less weeds covering the ground than unmowed plots of buckwheat-Dutch white clover 50:50 mix and Dutch white clover. Mowed buckwheat-Dutch white clover 25:75 mix-treatment had the least area of ground covered by weeds: 31%. Unmowed Dutch white clover had the greatest amount of weed-cover: 80%. Mowing had no effect on groundcover of weeds in buckwheat treatment; both unmowed and mowed plots had 62% of ground covered by weeds.

### **Groundcover of individual living mulch species**

Annual ryegrass and Dutch white clover study:

Ground coverage by annual ryegrass was significantly greater in monoculture treated plots than from either mix-treatment ( $P = 0.0001$ ) (Figure 3.8). Eighty-three percent of ground was covered with this living mulch in annual ryegrass monoculture plots. The area of ground covered with annual ryegrass decreased as seeding rate of this living mulch decreased in mixes with Dutch white clover.

Area of ground covered with Dutch white clover was significantly affected by the interaction between treatment and mowing ( $P = 0.0097$ ). Mowing Dutch white clover increased the percentage area of ground covered by this living mulch specie. The mowed plots of Dutch white clover grown in monoculture covered the greatest area of ground with Dutch white clover (46%). This was significantly greater than all unmowed treatments and the mowed annual ryegrass-Dutch white clover 50:50 mix-treatment. Unmowed plots covered between 3% and 7% of ground with Dutch white clover.



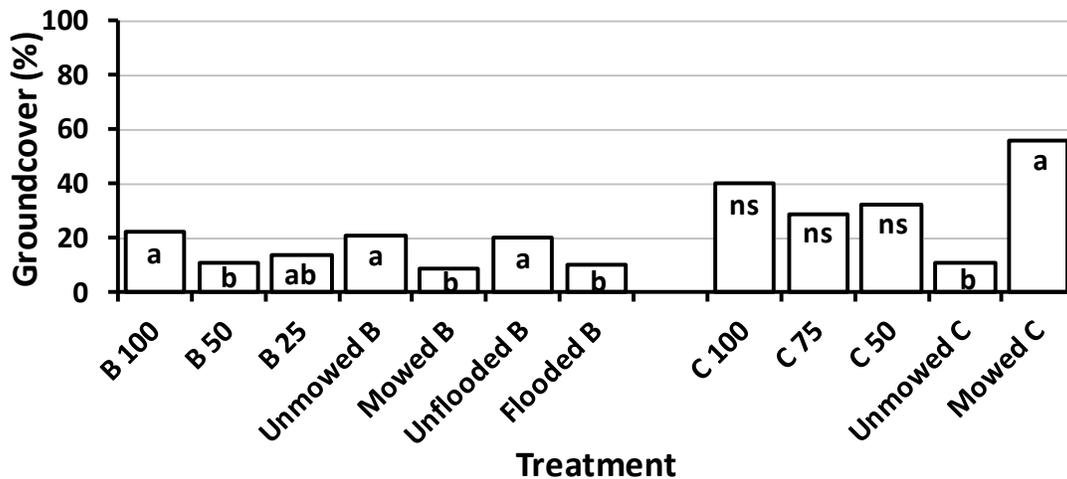
**Figure 3.8:** Area of ground covered by annual ryegrass (A) or Dutch white clover (C) living mulches grown either in monoculture at 100% seeding rate or in two mixes [A-C (50:50) and A-C (25:75)]; the constituent proportions of living mulches for the mixes are shown separately. Numbers in the treatment titles are the percentage of the living mulches recommended seeding rate used. For Dutch white clover the interaction between treatment and mowing was significant ( $P = 0.0097$ ). Treatments with different letters within or above white bars have statistically significant differences ( $P < 0.05$ ) between their respective least squared means for the area of ground covered by that living mulch specie. Least squared means analysis was carried out separately for annual ryegrass and Dutch white clover.

Buckwheat and Dutch white clover study:

Area of ground covered by buckwheat differed significantly between the monoculture treatment and the buckwheat-Dutch white clover 50:50 mix-treated plots ( $P = 0.0325$ ) (Figure 3.9). A monoculture of buckwheat covered twice as much ground as buckwheat in the 50:50 mix with Dutch white clover. The monoculture of buckwheat covered 22% of the soil surface. There was

no significant difference between area of ground covered by Dutch white clover at different seeding rates.

Mowing significantly affected the area of ground covered by both buckwheat ( $P = 0.0081$ ) and Dutch white clover ( $P < .0001$ ) in contrasting ways. Mowing decreased the area of ground covered by buckwheat by 12% and increased the area of ground covered by Dutch white clover by 45% (Figure 3.9). Flooding was a significant effect on buckwheat groundcover ( $P = 0.0255$ ); reducing it by 10%.



**Figure 3.9:** Area of ground covered by buckwheat (B) or Dutch white clover (C) living mulches grown either in monoculture at 100% seeding rate or in two mixes [B-C (50:50) and B-C (25:75)]; the constituent proportions of living mulches for the mixes are shown separately. Numbers in the treatment titles are the percentage of the living mulches recommended seeding rate used. Treatments with different letters within white bars have statistically significant differences ( $P < 0.05$ ) between their respective least squared means for the area of ground covered by that living mulch specie. Least squared means analyses for buckwheat, Dutch white clover and for mowing and flooding effects were carried out separately.

## **Living mulch plant height**

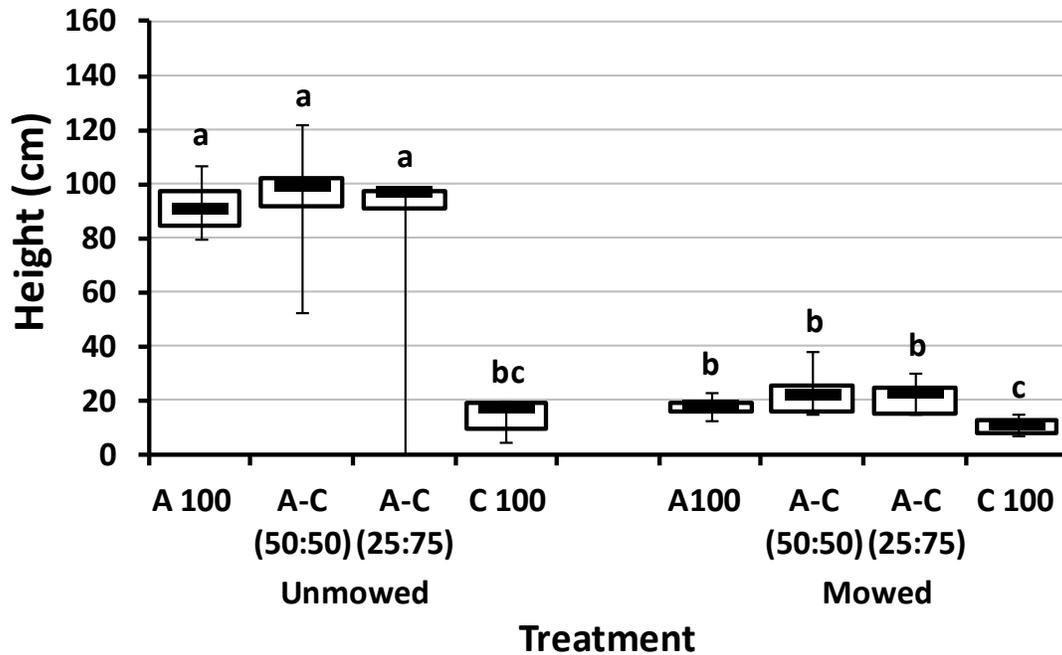
Annual ryegrass and Dutch white clover study:

The interaction between treatment and mowing had a significant effect on living mulch plant height in the annual ryegrass and Dutch white clover study ( $P < .0001$ ). Unmowed treatments containing annual ryegrass [A 100, A-C (50:50) and A-C (25:75)] were all significantly taller than mowed treatments and unmowed Dutch white clover (Figure 3.10). Mowed treatments containing annual ryegrass were significantly taller than mowed Dutch white clover. Unmowed annual ryegrass-Dutch white clover 25:75 mix treatment had the tallest living mulch plants [38 inches (96 cm)], whereas annual ryegrass-Dutch white clover 50:50 mix treatment was the tallest of all treatments after mowing [9 inches (23 cm)]. Mowing Dutch white clover did not significantly reduce this living mulch's height; Dutch white clover height was reduced from 6 inches (15 cm) to 4 inches (11 cm) due to mowing.

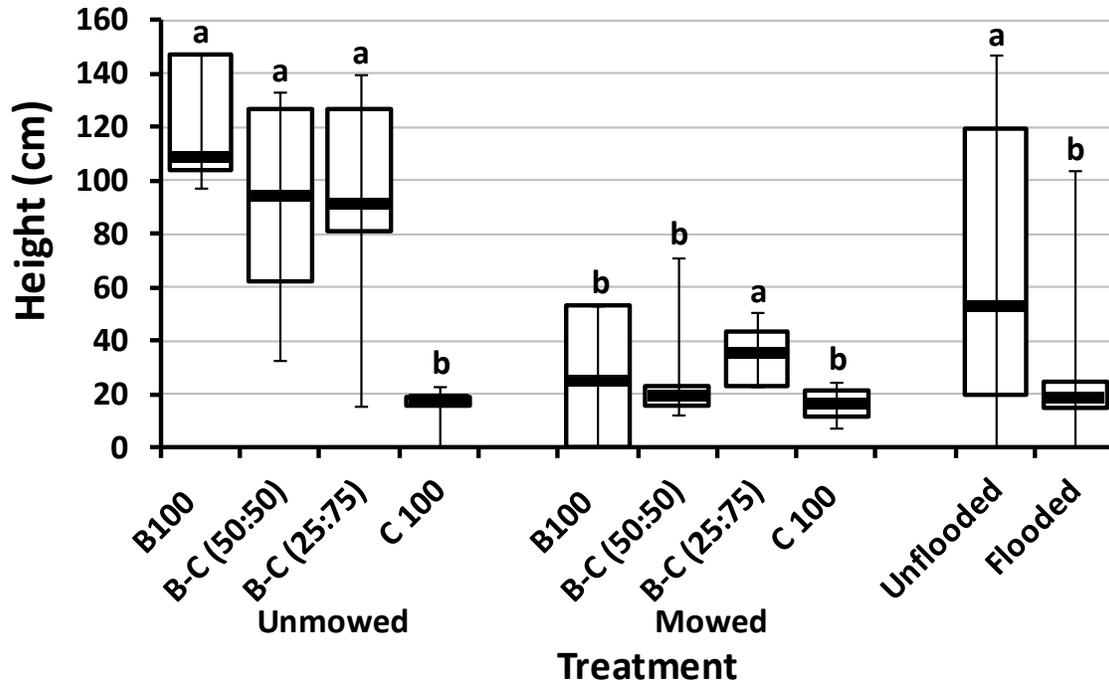
Buckwheat and Dutch white clover study:

Unmowed Dutch white clover was significantly shorter than all unmowed treatments containing buckwheat and the mowed buckwheat-Dutch white clover 25-75 mix treatment ( $P < .0001$ ) (Figure 3.11). This latter mix-treatment was also significantly taller than all other mowed treatments; growing to [13 inches (32 cm)]. Mowing had no effect on plots containing only Dutch white clover; there was less than a 0.8 inch (2 cm) difference in height due to mowing.

Unmowed buckwheat (B 100) had the tallest living mulch plants; on average they were 45 inches (114 cm) tall (Figure 3.11). Flooding significantly reduced the height of living mulches ( $P < .0001$ ); reducing the least squared means for living mulch height in unflooded plots from 26 inches (65 cm) down to 15 inches (39 cm).



**Figure 3.10:** Box-and-whisker plot showing height of living mulch-treated plots of annual ryegrass (A) and Dutch white clover (C) grown in monocultures (A 100 and C 100) and in two mixes [A-C (50:50) and A-C (25-75)]. Numbers in the treatment titles are the percentage of the living mulches recommended seeding rate used. Living mulch height was significantly influenced by the interaction between treatment and mowing ( $P < .0001$ ). Treatments with different letters above the boxplot have statistically significant differences ( $P < 0.05$ ) between their respective least squared means for living mulch heights.



**Figure 3.11:** Box-and-whisker plot showing height of living mulch-treated plots of buckwheat (B) and Dutch white clover (C) grown in monocultures (B 100 and C 100) and in two mixes [B-C (50:50) and B-C (25:75)]. Numbers in the treatment titles are the percentage of the living mulches recommended seeding rate used. Living mulch height was significantly influenced by the interaction between treatment and mowing ( $P < .0001$ ), and by flooding ( $P = 0.0001$ ). Treatments with different letters above the boxplot have statistically significant differences ( $P < 0.05$ ) between their respective least squared means for living mulch heights. Least squared means analyses for treatment and the flooding effect were carried out separately.

**Table 3.8:** Soil resistance, as a measure of soil compaction, within two studies of living mulches grown in 2010. In separate studies annual ryegrass and buckwheat were grown in monoculture and in two mixes with Dutch white clover; this latter plant specie was also grown in monoculture in both studies. Mixes were a combination of the two living mulch species and at modified seeding rates; percentages of monoculture seeding rate are indicated by the associated numbers in the mix-title. Soil resistance was measured at 6 inches (15 cm) below the soil surface using a soil penetrometer and is reported in pounds per square inch (p.s.i.). Least squared means followed by different letters indicates statistically significant differences ( $P < 0.05$ ) between effects or treatments. Least squared means analyses were carried out separately for treatment, mowing and flooding for each study.

<b>Effect Test</b>	<b>Annual ryegrass</b>		<b>Buckwheat</b>	
Treatment	P = 0.5828		P = 0.6952	
Mowing	P = 0.0355		P = 0.0126	
Flooding	P < 0.0001		P = 0.0027	
<b>LSMeans Differences</b>	<b>Soil resistance (psi)</b>			
Unmowed:Mowed	224.5 b	239.8 a	214.8 b	236.3 a
S.E. for Mowing	7.8		9.1	
Unflooded:Flooded	254.0 a	210.3 b	244.6 a	206.5 b
S.E. for Flooding	8.6		10.2	
Annual ryegrass/Buckwheat	231.7		227.2	
Annual ryegrass/Buckwheat-Dutch white clover 50:50 mix	233.2		218.6	
Annual ryegrass/Buckwheat-Dutch white clover 25:75 mix	239.2		226.2	
Dutch white clover	224.3		230.2	
S.E. for Treatment	9.5		10.9	

### Soil compaction testing

At 6 inch (15 cm) depth no significant difference in penetrometer readings from treatment plots was observed in either study ( $P > 0.05$ ) (Table 3.8). All soil resistance measurements were within the 200 to 300 p.s.i. range. All readings taken at the 18 inches (45 cm) depth were greater than 300 p.s.i. or greater (data not shown).

In both studies mowing significantly increased soil resistance;  $P = 0.0355$  for the annual ryegrass-Dutch white clover study and  $P = 0.0126$  for the buckwheat-Dutch white clover study.

Flooded plots in both studies had significantly less soil resistance than unflooded plots;  $P < .0001$  for the annual ryegrass-Dutch white clover study and  $P = 0.0027$  for the buckwheat-Dutch white clover study.

## **Discussion**

The two studies delivered contrasting results about what seeding rate worked best, leading us to the conclusion that the most effective seeding ratio for a mixed-species living mulch is dependent on the plant species being utilized. For annual ryegrass with Dutch white clover the 50:50 mix was the best combination based on biomass yield, groundcover, weed suppression, and these benefits to the system equaled and, in many cases, exceeded those achieved by annual ryegrass alone. This latter treatment was most similar to AC50:50 for effectiveness. In the buckwheat with Dutch white clover study the 25:75 mix appeared to be the best treatment, although there was little difference between this mix and either the 50:50 mix or buckwheat alone for their comparative benefits to the system. It is possible that the reason for the differences between the two studies, for which treatment provided the most desirable outcomes, is due to the continued growth of both living mulches in the annual ryegrass-Dutch white clover study and the early termination of buckwheat, due to both natural senescence and because of the mowing treatment.

Living mulch treatments in this study were mowed at a time when most of the buckwheat plants were at early flowering stage, which coincided with the earliest flowering of annual ryegrass. Buckwheat flowers quickly in summer; with this being advanced in response to environmental stress (Clark, 2007). By this developmental stage both flowering species were almost at their maximum height of around 3 ft (1 m) tall. The height the plants reached before being mowed had both benefits and limitations in the scenario being trialled.

The purpose of withholding the mowing treatment until this time was to maximize biomass production while minimizing seed production and senescence of the species; unfortunately mowing killed buckwheat plants, resulting in senescence of the residual plant material. While biomass production is believed to have been maximized, resulting in the best possible weed suppression before mowing, most of plots that included buckwheat had begun lodging while the annual ryegrass leaves spread beyond the cultivated area, which in both situations has the potential for becoming problematic for the growth of a neighboring cash crop. The scenario used in this study was growing living mulch in wide alleyways, 42 inches (105 cm) wide, between plastic-mulched beds 36 inches (90 cm) wide. There was substantial encroachment by living mulch over the surface of the bed. Additionally, use of living mulch in this way severely hampers pedestrian access for any maintenance or harvesting of the crop prior to the time mowing took place, and for unmowed subplots the problem would continue throughout the season. It is therefore our belief that mowing, or any other kind of method of living mulch suppression, should be carried out earlier in the development of tall-growing plant species, when the plants are shorter, in order to maintain field accessibility and minimize living mulch suppression of the cash crop; the latter being a reported problem of using the living mulch system, even between plastic-mulched beds (Amirault and Caldwell, 1998; Nicholson and Wien, 1983; Andow et al., 1986; Teasdale, 1998; Chase and Mbuya, 2008; Neilsen and Anderson, 1989; Reiners and Wickerhauser, 1995).

As a measure of how well living mulch treatments suppressed weeds, biomass and groundcover of both the living mulch and weeds were recorded. Significant differences between treatments were observed. The overall difference was that annual ryegrass with or without Dutch white clover suppressed weeds better than buckwheat with or without Dutch white clover, as is shown by less weed biomass (Figure 3.1) and ground covered by weeds (Figure 3.7) in the former. The reasons for better weed suppression by treatments including annual ryegrass, compared to those including buckwheat, are perhaps due to high seed viability that results in early establishment of an evenly distributed population of vigorously growing plants that form excellent groundcover and grow at a faster rate than the weed population that is present; therefore suppressing weeds early in the season. The pioneer specie that occupies land will dominate for some time after before succession takes place (Sweet, 1982). Additionally, when annual ryegrass is combined with Dutch white clover there is a positive effect of this combination on the average living mulch height, whereas for buckwheat adding Dutch white clover reduces the average height of plots. Height alone is no indicator of a plant's weed suppression ability (Nicholson and Wien, 1983). However, plant height combined with a dense canopy, which gives good groundcover and is often the result of a high seeding rate, results in effective light interception by the living mulch (Akemo et al., 2000). This reduces light availability for existing weeds and for phytochrome-mediated germination of weed seeds (Gibson et al., 2011). Annual ryegrass, from observations, maintains a dense canopy for the majority of its overall height, whereas buckwheat provides good groundcover and dense canopy for much less of its overall height. As the buckwheat stand grows beyond the first couple of nodes formation, and particularly once the plant reaches maturity and begins developing inflorescences, internode length extends to such an extent that light passes through the canopy. Combine this observation with the plants preponderance for lodging and it is clear why buckwheat does not suppress weeds for an extended period of time such as the full growing season of a summer

cash crop. Buckwheat is typically grown over a short period and killed early in its lifecycle; before flowering (Clark, 2007). It has a short life cycle, resulting in it flowering and developing seed in a relatively short period of time; this is expedited when the plants are exposed to environmental stresses.

In both studies, a 50:50 percent broadcast seeding rate mixture yielded the least dry weight of weeds (3.3 t.ha<sup>-1</sup> in A-C 50:50, and 4.4 t.ha<sup>-1</sup> in B-C 50:50), and 100% Dutch white clover produced the greatest dry weight of weeds: 7.5 t.ha<sup>-1</sup> in the buckwheat-Dutch white clover study, and 6.9 t.ha<sup>-1</sup> in the annual ryegrass-Dutch white clover study. This indicates the effectiveness of using Dutch white clover in a mixture with a more vigorously growing specie for weed control, compared to using this specie alone. Dutch white clover produced significantly less biomass than other treatments in both the annual ryegrass-Dutch white clover study ( $P < .0001$ ) and the buckwheat-Dutch white clover study ( $P = 0.0006$ ). Dutch white clover sown at 100% produced only 1.3 t.ha<sup>-1</sup> of living mulch biomass in the buckwheat-Dutch white clover study and 0.8 t.ha<sup>-1</sup> in the annual ryegrass-Dutch white clover study. When compared to the 5 to 6 t.ha<sup>-1</sup> of annual ryegrass and around 4 to 5 t.ha<sup>-1</sup> of buckwheat, Dutch white clover biomass production is poor and an indication of why it inadequately suppressed weeds. This plant had the lowest seed viability results, was slow to germinate in the field, despite laboratory results reporting it germinated as quickly as other samples, and it took a long while to establish and form much groundcover. This protracted establishment period for Dutch white clover allowed weeds to colonize monoculture plots (C100) quicker, and with little competition they flourished until the mowing treatment was applied to respective subplots. Dutch white clover showed significant increase in biomass and groundcover due to mowing, which led to a corresponding decrease in weed biomass and groundcover in plots that included this specie; the most notable change being in groundcover for mixed-species and C100 subplots in the buckwheat-Dutch white clover study (Figure 3.7). Dutch white clover is a cool-season perennial and as such may

have not performed as well as it could otherwise have. Seeds were broadcast sown on May 13, 2010. Had sowing taken place the previous fall or earlier in the year, when temperatures were more moderate and soil moisture was more consistent the rate of establishment for Dutch white clover and its ability to suppress weeds are likely to have been improved.

This study found that mowing significantly increased production of the aboveground biomass of all living mulch treatments ( $P \leq 0.0004$ ), increased the area of ground covered with living mulch, decreased the area of ground covered with weeds for two of the three living mulch species used, significantly reduced the height of taller-growing species, and significantly increased soil resistance. An increase in aboveground living mulch biomass due to mowing is desirable as biomass is an indicator of living mulch weed suppression ability (Patten et al., 1990; Burgos et al., 2006). Additionally, following the termination of the cash crop, the remaining aboveground biomass, including living mulch will, at some time, be incorporated into the soil. Therefore an increase in biomass will result in an increase in organic matter addition to the soil, which is beneficial to the farm's cropping system (Gugino et al., 2007).

Improving groundcover leads to improved weed suppression. Greater surface area of the ground was colonized with living mulch of both annual ryegrass and Dutch white clover than weeds by the end of the trial period due to the mowing treatment. Mowing reduced the area of ground covered with weeds. In mowed subplots living mulch growth suppressed most additional growth of existing weeds and also growth of newly emerged weeds; thus living mulch effectively suppressed weed colonization of the soil surface. The method of mowing may also have affected results and is something worthy of future consideration for similar studies. A sickle-bar mower, as used in this study, leaves relatively large pieces of plant residue on the soil surface. Rotary and flail-mowers cut plant material into much smaller pieces and if employed in this system could perhaps result in changes to groundcover and biomass production. Large

clippings may suppress regrowth of the living mulch further, causing gaps in which weeds colonize, whereas smaller clippings form dead mulch in the small gaps between living mulch plants, which further assists in weed suppression and reduces moisture loss; therefore aiding the living mulch (William, 1987; Patten et al., 1990)

Total weed biomass for the season did not differ between mowed and unmowed subplots following mowing; these subplots did not differ in weed biomass prior to mowing. This result seemed counter to our expectations and not reflective of the weed suppression that did take place in this study and in others (Graglia et al., 2006; Lukashyk et al., 2008; Lilly, 1965). It is likely that weeds in unmowed plots continued to grow throughout the season. If mowing had suppressed further weed growth then unmowed subplots would very likely have had significantly greater weed biomass recorded than mowed subplots. This was not observed in the results of this study. As groundcover results suggest a reduction in weed coverage (Figure 3.7), albeit not total removal, and an increase in living mulch ground coverage, it is likely that weeds that survived mowing continued to grow; therefore adding to total weed biomass yield for the season. In part also, weeds in unmowed subplots may have had a slowing of growth rate, perhaps even senescence, after mowing-time, leading to very little additional biomass being produced.

Of important note is that the living mulch response to mowing appears to depend on the species being treated. Work by Thornton and Millard (1997) showed that grass species, *Lolium perenne* and *Festuca rubra*, regrew most leaves after a single defoliation, compared to repeated defoliation. In this study, treatments were mowed once, using a sickle-bar mower, to approximately 0.6 inches (1.5 cm) above the soil surface. Annual ryegrass and Dutch white clover both responded well to being mowed. For mixtures of annual ryegrass and Dutch white clover mowing decreased the land equivalent ratio (LER) and bio-efficiency, and increased the

proportion of annual ryegrass biomass. In contrast, mixtures of buckwheat and Dutch white clover showed an increase in LER and bio-efficiency resulting from mowing and a reduction in proportion of buckwheat biomass. Most buckwheat plants died and almost no shoots emerged from above- or belowground nodes following mowing. New buckwheat seedlings emerged in the time period following the application of the mowing treatment in both subplots due to seeds having developed and senesced just prior to, or following, treatment. New seedlings emerged in small numbers and unevenly within subplots, and grew very little, making them of little use for weed suppression. It is unclear whether buckwheat would have survived and regrown following mowing if treatment had been applied earlier in the plants lifecycle or at a higher cutting-height.

The success of living mulch depends on it remaining alive. Living mulch that is killed or senesces before the majority of the cropping cycle is complete has limited use within a cropping system. Once the mulch is killed there is significantly less competition for a broad range of resources for weed plants to overcome. Plant residue is not as successful at suppressing weeds as a managed mulch of living plants (Teasdale et al., 2007). A living mulch crop should not require supplemental irrigation or fertility for its success, beyond what resources are naturally occurring and species should be selected with this in mind.

As Mead and Willey (1980) highlight in their discussion on the topic of standardization of sole crop yields “the method of standardization should vary according to the form and objective of the experiment”. For the purposes of this study the term ‘sole crop’ is referred to by its synonym, monoculture. While three different calculations were made for LER, using standardized monoculture yields for (A) the maximum biomass yield recorded during the study, (B) the average monoculture biomass yield and (C) the monoculture biomass yield per replicate (Table 3.4 and Table 3.5), all for the respective plant species, we chose to draw conclusions from the results of using standardized monoculture yields (A) maximum yield and (B) average yield. The

reason for this is that both these statistics are useful to compare the biomass yield of species in both mixes to. It is important to know how the mixes performed compared to the average yield of growing both plant species in monoculture, as well as comparing the mixes to those same species at their maximum potential. It would seem appropriate to place more emphasis on conclusions drawn from using the standardizing factor of the average monoculture biomass yield, while still giving due consideration for mixes performance when compared to the maximum monoculture yield potential. For the purpose of considering living mulch, a crop with no tangible economic return, a farmer is unlikely to have the desire to compare using a mixed-species living mulch to maximum biomass potential of growing the same species in monoculture. However, depending on weed pressure, cost of seed and use for the companion crop, use of maximum yield potential is nevertheless a worthy standardizing factor to consider using in LER calculations. The reason to discard using monoculture yield recorded for each replicate is that it greatly inflates LER and has much greater error than using other standardizing factors.

A total LER greater than 1 indicates the mixed-species crop has a yield advantage over growing the same crops in monoculture. A total LER of 1 indicates parity between the yields of monocultures and the mixed crop from the same area of land. Total LER of less than 1 indicates yield loss from the area of land growing the mixed crop compared to the same area of land if it was divided into two and had the crops grown in monoculture. In both studies the LER for a 50:50 mix was higher than the 25:75 mix, and compared to the average monoculture yield both mixes in both studies had LER's greater than 1. Annual ryegrass with Dutch white clover made better land use than buckwheat with Dutch white clover. Between 60% and 69% more land would have been required to produce the same amount of aboveground biomass from the average yielding monoculture stands of annual ryegrass and Dutch white clover than if the two were combined in either 25:75 or 50:50 mixes, respectively. Twenty percent to 39% more land

would have been required to produce the equivalent biomass from the average yielding monoculture stands of buckwheat with Dutch white clover than if they were grown together in the same respective mix-ratios. Such figures show significant improvement to biomass production per land area unit when living mulch plants are grown in mixes, compared to when they are grown in monoculture stands.

Comparing the living mulch mixes to the maximum biomass yields we see, as should be expected, a reduction in efficiency of land use. Buckwheat with Dutch white clover mixes, 25:75 and 50:50, were just 33% and 39% of the maximum recorded yields, respectively, meaning that to equal the maximum yields 67% and 61% more land of both these respective mixes would have to be grown. However, annual ryegrass with Dutch white clover mixes, 25:75 and 50:50, produced total LER's equivalent to 85% and 90% of the maximum recorded yields, which would seem quite respectable ratios. Just 15% and 10% more land would be required for either of these respective mixes to produce the equivalent aboveground biomass recorded in the highest yielding monoculture plots.

This study did not include sampling of root biomass, length, architecture and any biotic interactions, however this would be worthy of future research. Sweet (1982) believed that 90% of the benefit of living mulch is to be found in its roots. It would be worthwhile to study living mulch roots and how intercropping and mowing effects their growth. Studies have published conflicting opinions on how excision of aboveground plant tissue alters root biomass, architecture and exudates, and carbon partitioning to roots and tissue nutrient content may vary by plant species, resulting in specific changes to soil fauna, plant population and ecosystem dynamics (Bardgett et al., 1998).

This experiment made use of three species with quite contrasting growth habits and involved the pairing of tall, upright-growing plants with prostrate, spreading Dutch white clover. Growing combinations of plant species that have contrasting growth habits is hypothesized to be of benefit to the living mulch system in contrast to using species with similar growth habits. After all, this is a tenet of the intercropping system; resources are more efficiently utilized when two or more species with contrasting growth habits are grown together (Coolman and Hoyt, 1993; Malézieux et al., 2009; Osman et al., 2011; Willey and Osiru, 1972; Willey, 1990). Cover cropping systems will combine a cereal crop with a legume; for example the combinations of oats with peas and cereal rye with hairy vetch (Clark, 2007). Studies reporting use of mixed plant species focus on either forage production (Contreras-Govea and Albrecht, 2005) or cover crops for use alone (i.e. not among a cash crop) (Akemo et al., 2000). Finding the correct combination and ratio of species in a living mulch mixture is important. Mixtures of cereal rye and field pea with more than 50% proportion being rye gave the best weed suppression compared to pure stands of both crops and mixtures with 50% or less of rye. Only 2% of groundcover was weeds in rye-pea mixes compared to 73% in monoculture plots of pea cover crop (Akemo et al., 2000).

Our experiment has shown that combining plant species for use as living mulch can provide similar, even equivalent, weed suppression as the most vigorous specie grown in monoculture. Of particular note, the combination of annual ryegrass with Dutch white clover sown at 50% of their respective recommended seeding rates shows great promise as a mixed-species living mulch. While it was the second cheapest treatment used, due mainly to the cheap cost of annual ryegrass seed, it showed great merit for weed suppression ability, response to mowing and almost equivalent use of land for production of aboveground biomass. With a combined stand of two species there can be increased biomass production (Burgos et al., 2006;

Contreras-Govea and Albrecht, 2005), as was seen in this study with the combination of annual ryegrass with Dutch white clover.

While there may not be conclusive results indicating the need to alter living mulch application from growing in monoculture to including multiple species, the fact that comparative results were observed between growing annual ryegrass alone and results from growing this specie in combination with Dutch white clover at 50% of their recommended rate suggests a suitable alternative. Additionally a grower should consider the benefit of adding in a legume to the living mulch that would increase the amount of nitrogen and organic matter being added to the soil, and increase species diversity in the cropping system (Hoffman and Regnier, 2006; Teasdale et al., 2007; Creamer and Baldwin, 2000; Gaskell and Smith, 2007). What is being proposed has parallels to the original living mulch work of Robinson and Dunham (1954) of using companion crops for weed control, except rather than it being companion crops being grown with soybeans this system is utilizing a vigorous living mulch specie as a companion to a less vigorous specie: Dutch white clover. Companion living mulch plants provide one another a 'nursing' effect, which is of overall benefit to the growing system (Contreras-Govea and Albrecht, 2005).

The living mulch system is an alternative to other weed control practices, but it has many more additional benefits that use of a dead mulch or conventional practices cannot offer to the agroecosystem (Carof et al., 2007; Clark, 2007; Deguchi et al., 2005; Hall et al., 1984; Hively and Cox, 2001; Hughes and Sweet, 1979; Ochsner et al., 2011; Paine and Harrison, 1993; Rice et al., 2002; Rice et al., 2004; Rice et al., 2007; Singer and Pedersen, 2005; Sweet, 1982; Teasdale, 1998). Maximizing the benefits of adopting such an alternative system for non-cropped land, but within reasonable economic and practical limitations, is paramount. Combining the biological benefits of two, or more, plants for use as living mulch is possible, practical and beneficial and can be cheaper than growing a monoculture.

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## APPENDIX 1

**Table A.1.1:** Percent groundcover in alleyway plots according to the effects of treatment, mowing, the interaction between living mulch treatment and mowing, and flooding. Percent groundcover is categorized into total area that was covered, the area of ground covered by a mulch-treatment, and the area of ground covered by weeds. Least squared means in the same column followed by different letters indicates statistically significant differences ( $P < 0.05$ ) between effects or treatments. Least squared means analysis was carried out separately for 2009 and 2010, and separately for the effects of treatment, mowing, treatment\*mowing and flooding.

Effect Test	Total area of groundcover per treatment plot (%)		Area of groundcover per plot that is treatment (%)		Area of groundcover per plot that is weeds (%)					
	2009	2010	2009	2010	2009	2010				
Treatment	P < .0001	P = 0.0621	P < .0001	P < .0001	P < .0001	P < .0001				
Mowing	--	P = 0.0263	--	P = 0.0127	--	P < .0001				
Treatment*Mowing	--	--	--	--	--	P = 0.0094				
Flooding	P = 0.0135	--	P = 0.0463	--	P = 0.0171	P = 0.0183				
<b>LSMeans Differences</b>										
Unmowed:Mowed	--	91 a	88 b	--	51 b	62 a	--	41 a	24 b	
S.E. for Mowing	--	1.7	--	--	3.8	--	--	2.5	--	
Unflooded:Flooded	72 a	59 b	--	82 a	64 b	--	8 a	4 b	27 b	38 a
S.E. for Flooding	11.6	--	--	8.7	--	--	3.4	--	2.8	
Annual ryegrass	95 a	96	94 a	80 ab	1 b	21 bcd	7 d			
Annual ryegrass-Dutch white clover mix	-	98	-	87 a	-	22 bcd	5 d			
Birdsfoot trefoil	-	81	-	26 c	-	61 a	45 abc			
Buckwheat-Dutch white clover mix	-	85	-	55 abc	-	47 ab	15 cd			
Cereal rye-Hairy vetch mix	-	87	-	21 c	-	71 a	62 a			
Control	0.1 c	-*	-	-*	0.1 c	-*				
Creeping red fescue	62 b	-	39 c	-	23 a	-				
Dutch white clover	80 ab	89	71 b	46 bc	9 a	60 a	19 bcd			
Straw	100 a	-*	100 a	-*	0.1 c	-*				
Teff	-	88	-	81 ab	-	6 d	14 cd			
S.E. for Treatment or Treatment*Mowing	5.5	3.8	3.5	8.2	3.2	6.4				

^ indicates data was unavailable due to the treatment not being included.

^ indicates the effect was not included as a covariate due to it not having a significant effect in this analysis.

^\* indicates data was unavailable due to covariate of mowing having a significant effect on living mulch treatments.

**Table A.1.2:** Dry weight of above ground biomass that grew in treatment plots during 2009 and 2010. Biomass is categorized into living mulch treatment and weed biomass, per year, and is presented in both tons per hectare (extrapolated from quadrat sampling data) and the respective percent of the total biomass harvested from that treatment's plot. Least squared means in the same column followed by different letters indicates statistically significant differences ( $P < 0.05$ ) between effects or treatments. Least squared means analysis was carried out separately for 2009 and 2010, and separately for the effects of treatment, mowing and flooding.

Effect Test	Dry weight of treatment (t.ha <sup>-1</sup> )		Total plot dry weight that is treatment (%)		Dry weight of weeds (t.ha <sup>-1</sup> )		Total plot dry weight that is weeds (%)				
	2009	2010	2009	2010	2009	2010	2009	2010			
Treatment	P < .0001	P = 0.0006	P = 0.0274	P = 0.0020	P = 0.0012	P = 0.0039	P = 0.0008	P < .0001			
Mowing	--	--	--	--	P = 0.0330	P = 0.0100	P = 0.0329	--			
Flooding	--	--	--	--	P = 0.0210	--	P = 0.0142	--			
<b>LSMeans Differences</b>											
Unmowed:Mowed	--	--	--	--	0.18 b	0.30 a	2.89 b	5.19 a	7 b	13 a	--
S.E. for Mowing	--	--	--	--	0.1	0.7	2.1	--	--	--	
Unflooded:Flooded	--	--	--	--	0.29 a	0.16 b	--	15 a	5 b	--	
S.E. for Flooding	--	--	--	--	0.1	--	2.5	--	--		
Annual ryegrass	4.96 a	3.37 ab	98 a	72 a	0.04 b	2.53 ab	2 b	28 cd			
Annual ryegrass-Dutch white clover mix	-	4.03 ab	-	73 ab	-	2.30 b	-	23 cd			
Birdsfoot trefoil	-	0.90 b	-	25 bc	-	7.98 a	-	73 ab			
Buckwheat-Dutch white clover mix	-	4.28 ab	-	60 abc	-	3.48 ab	-	49 bc			
Cereal rye-Hairy vetch mix	-	3.10 b	-	56 abc	-	4.78 ab	-	47 bc			
Control	-	-	-	-	_*	_*	_*	100 a			
Creeping red fescue	1.08 b	-	75 b	-	0.34 a	-	20 a	-			
Dutch white clover	2.08 b	0.86 b	85 ab	24 bc	0.37 a	4.55 ab	15 a	74 ab			
Straw	-	-	-	-	_*	_*	_*	11 d			
Teff	-	7.18 a	-	82 a	-	2.65 ab	-	18 cd			
S.E. for Treatment	0.4	0.8	5.3	10.0	0.1	1.3	3.0	7.0			

'-' indicates data was unavailable due to the treatment not being included.

'--' indicates the effect was not included as a covariate due to it not having a significant effect in this analysis.

'-\*' indicates data was unavailable due to covariate of mowing having a significant effect on living mulch treatments.

**Table A.1.3:** Harvested fruit of 'Revolution' bell pepper, in tons per hectare, split into grading categories and harvest per year, according to the effect of treatment and flooding. Least squared means within the same column followed by different letters indicate statistically significant differences ( $P < 0.05$ ) between effects. Least squared means analysis was carried out separately for 2009 and 2010, and separately for the effect of treatment and flooding.

Effect Test	Grades 1 & 2 (t.ha)		Fancy grade (t.ha)		Total marketable (t.ha)		Culled (t.ha)		Total (t.ha)							
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010						
Treatment	P = 0.3484	P = 8.350	P = 0.2568	P = 0.7905	P = 0.2787	P = 0.7653	P = 0.8454	P = 0.4518	P = 0.3358	P = 0.6895						
Flooding	--	--	P = 0.0017	--	P = 0.0015	P = 0.0065	--	P 0.0003	P = 0.0013	P = 0.0011						
<b>LSMeans Differences</b>																
Unflooded:Flooded	--	--	8.6 a	3.5 b	--	9.0 a	3.8 b	4.95 a	0.33 b	--	2.76 a	0.33 b	10.1 a	4.4 b	7.81 a	0.65 b
S.E. for Flooding	--	--	1.0	--	1.0	1.6	--	0.4	1.0	1.7						
Annual ryegrass	0.50	0.54	5.2	0.76	5.6	1.19	0.94	2.2	6.4	3.51						
Annual ryegrass-Dutch white clover mix	-	0.59	-	2.31	-	1.92	-	2.39	-	4.42						
Birdsfoot trefoil	-	0.57	-	0.96	-	0.13	-	0.88	-	0.92						
Buckwheat-Dutch white clover mix	-	0.19	-	4.12	-	3.08	-	0.28	-	3.4						
Cereal rye-Hairy vetch mix	-	0.32	-	2.90	-	2.76	-	1.36	-	4.11						
Control	0.31	0.42	7.9	3.01	8.2	4.23	0.97	2.1	9.1	6.39						
Creeping red fescue	0.28	-	4.8	-	5.0	-	0.75	-	5.7	-						
Dutch white clover	0.30	0.21	8.1	0.23	8.4	1.87	0.91	2	9.2	3.95						
Straw	0.41	-	4.4	-	4.9	-	1.1	-	5.8	-						
Teff	-	0.20	-	6.99	-	5.95	-	1.13	-	7.13						
S.E. for Treatment	0.1	0.3	1.5	2.6	1.5	2.3	0.3	0.7	1.6	2.6						

'-' indicates data was unavailable due to the treatment not being included.

'--' indicates the effect was not included as a covariate due to it not having a significant effect in this analysis.

**Table A.1.4:** Harvested fruit of 'Revolution' bell pepper, in number of fruit per hectare, split into grading categories and harvest per year, according to the effect of treatment and flooding. Least squared means within the same column followed by different letters indicate statistically significant differences ( $P < 0.05$ ) between effects. Least squared means analysis was carried out separately for 2009 and 2010, and separately for the effect of treatment and flooding.

Effect Test	Grades 1 & 2 (#.ha)		Fancy grade (#.ha)		Total marketable (#.ha)		Culled (#.ha)		Total (#.ha)							
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010						
Treatment	P = 0.3477	P = 0.4494	P = 0.2460	P = 0.7947	P = 0.2663	P = 0.6926	P = 0.7721	P = 0.3665	P = 0.4049	P = 0.7870						
Flooding	--	--	P = 0.0014	--	P = 0.0013	P = 0.0073	--	P = 0.0002	P = 0.0012	P = 0.0002						
<b>LSMeans Differences</b>																
Unflooded:Flooded	--	--	46,472 a	20,946 b	--	49,472 a	23,792 b	25,934 a	2,771 b	--	26,863 a	4,352 b	58,850 a	29,959 b	52,972 a	7,492 b
S.E. for Flooding	--	--	4,661	--	4,625	7,118	--	3,849	5,192	8,190						
Annual ryegrass	3,588	4,654	29,247	3,942	32,789	7,979	7,859	24,034	39,685	32,370						
Annual ryegrass-Dutch white clover mix	-	5,395	-	12,324	-	12,707	-	22,197	-	35,710						
Birdsfoot trefoil	-	5,058	-	5,170	-	3,315	-	11,547	-	15,052						
Buckwheat-Dutch white clover mix	-	1,349	-	19,164	-	14,333	-	2,635	-	17,665						
Cereal rye-Hairy vetch mix	-	4,047	-	13,597	-	15,327	-	14,259	-	29,605						
Control	2,392	3,237	41,016	15,489	43,392	22,523	8,201	19,101	51,272	40,984						
Creeping red fescue	2,221	4,047	27,518	-	29,724	-	7,859	-	37,261	-						
Dutch white clover	2,656	1,821	45,277	1,788	48,053	10,817	7,917	19,552	55,315	30,411						
Straw	3,759	-	25,488	-	29,201	-	10,251	-	38,489	-						
Teff	-	1,619	-	32,383	-	27,822	-	11,537	-	40,056						
S.E. for Treatment	692	2,254	7,083	11,823	7,028	10,770	2,007	6,343	7,889	13,341						

'-' indicates data was unavailable due to the treatment not being included.

'--' indicates the effect was not included as a covariate due to it not having a significant effect in this analysis.

## APPENDIX 2

**Table A.2.1:** Annual ryegrass aboveground biomass, by weight and percent of total cut plant material, and percent of total groundcover for monoculture and two mixed-species plots. Annual ryegrass was combined with Dutch white clover at reduced seeding rates. Least squared means in the same column followed by different letters indicates statistically significant differences ( $P < 0.05$ ) between effects or treatments. Least squared means analysis was carried out separately for the effects of treatment and mowing.

Effect Test	Total dry weight of treatment ( $t \cdot ha^{-1}$ )		Total dry weight per plot that is treatment (%)		Total area of groundcover per plot that is treatment (%)	
Treatment	P = 0.3017		P = 0.0442		P = 0.0001	
Mowing	P = 0.0408		-		-	
<b>LSMeans Differences</b>						
Unmowed:Mowed	5.2 b	6.6 a	-	-	-	-
S.E. for Mowing	0.29		-		-	
A 100	6.6		71 a		83 a	
A 50	5.9		61 ab		59 b	
A 25	5.2		51 b		50 b	
S.E. for Treatment	0.5		5.0		4.1	

'-' indicates the effect was not included as a covariate due to it not having a significant effect in this analysis.

**Table A.2.2:** Dutch white clover aboveground biomass, by weight and percent of total cut plant material, and percent of total groundcover for monoculture and two mixed-species plots. Dutch white clover was combined with annual ryegrass at reduced seeding rates. Least squared means in the same column followed by different letters indicates statistically significant differences ( $P < 0.05$ ) between effects or treatments. Least squared means analysis was carried out separately for the effects of treatment and mowing.

<b>Effect Test</b>	<b>Total dry weight of treatment (t.ha<sup>-1</sup>)</b>		<b>Total dry weight per plot that is treatment (%)</b>		<b>Total area of groundcover per plot that is treatment (%)</b>	
Treatment	P = 0.1861		P = 0.7874		P = 0.0328	
Mowing	P < .0001		P = 0.0005		P < .0001	
Treatment*Mowing	-		-		P = 0.0097	
Flooding	-		P = 0.0056		P = 0.0098	
<b>LSMeans Differences</b>						
Unmowed:Mowed	0.36 b	1.37 a	5 b	18 a	6 b	28 a
S.E. for Mowing	0.2		2.9		3.9	
Unflooded:Flooded	-		9 b	14 a	-	
S.E. for Flooding	-		2.9		-	
C 100	0.85		13		7 bc	46 a
C 75	0.55		10		3 bc	20 abc
C 50	1.19		12		5 c	18 b
S.E. for Treatment or Treatment*Mowing	0.2		3.5		5.4	

'-' indicates the effect was not included as a covariate due to it not having a significant effect in this analysis.

**Table A.2.3:** Buckwheat aboveground biomass, by weight and percent of total cut plant material, and percent of total groundcover for monoculture and two mixed-species plots. Buckwheat was combined with Dutch white clover at reduced seeding rates. Least squared means in the same column followed by different letters indicates statistically significant differences ( $P < 0.05$ ) between effects or treatments. Least squared means analysis was carried out separately for the effects of treatment and mowing.

<b>Effect Test</b>	<b>Total dry weight of treatment (t.ha<sup>-1</sup>)</b>		<b>Total dry weight per plot that is treatment (%)</b>		<b>Total area of groundcover per plot that is treatment (%)</b>	
Treatment	P = 0.1781		P = 0.2255		P = 0.0325	
Mowing	-		P = 0.0281		P = 0.0081	
Flooding	-		P = 0.0006		P = 0.0255	
<b>LSMeans Differences</b>						
Unmowed:Mowed	-	-	30.5 b	39.1 a	21 a	9 b
S.E. for Mowing	-		1.4		2.0	
Unflooded:Flooded	-		53.4 a	16.3 b	20 a	10 b
S.E. for Flooding	-		4.6		2.5	
B 100	5.4		44		22 a	
B 50	3.9		30		11 b	
B 25	4.4		31		14 ab	
S.E. for Treatment	1.5		4.9		4.1	

'-' indicates the effect was not included as a covariate due to it not having a significant effect in this analysis.

**Table A.2.4:** Dutch white clover aboveground biomass, by weight and percent of total cut plant material, and percent of total groundcover for monoculture and two mixed-species plots. Dutch white clover was combined with buckwheat at reduced seeding rates. Least squared means in the same column followed by different letters indicates statistically significant differences ( $P < 0.05$ ) between effects or treatments. Least squared means analysis was carried out separately for the effects of treatment and mowing.

<b>Effect Test</b>	<b>Total dry weight of treatment (t.ha<sup>-1</sup>)</b>		<b>Total dry weight per plot that is treatment (%)</b>		<b>Total area of groundcover per plot that is treatment (%)</b>	
Treatment	P = 0.3090		P = 0.3280		P = 0.1177	
Mowing	P < .0001		P < .0001		P < .0001	
Flooding	P = 0.0059		P = 0.0010		-	
<b>LSMeans Differences</b>						
Unmowed:Mowed	0.33 b	1.92 a	6 b	25 a	11 b	56 a
S.E. for Mowing	0.1		2.7		5.0	
Unflooded:Flooded	0.94 b	1.30 a	10 b	20 a	-	-
S.E. for Flooding	0.1		2.7		-	
C 100	1.4		21		40	
C 75	0.9		11		29	
C 50	1.1		14		32	
S.E. for Treatment	0.1		3.3		5.2	

<sup>1</sup> indicates the effect was not included as a covariate due to it not having a significant effect in this analysis.