

**EVALUATING REDUCED TILLAGE, COVER CROPS, AND LIVING MULCHES FOR  
WEED MANAGEMENT IN COTTON**

A Thesis

by

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## **ABSTRACT**

Texas is the largest organic cotton producer in the US, but tillage is the primary weed control. Little research has been done on reducing tillage and cover crops in organic cotton. A living mulch is a cover crop planted with the main crop. There has been research on living mulches in corn and soybeans, but little in cotton. Studies in 2020 and 2021 had objectives (1) developing a organic cotton production system comparing two tillage regimes (minimum tillage-MT, conventional tillage-CT) and four cover crop treatments (Austrian winter pea, Bob oat, purpletop turnip, and a mix); and (2) assessing living mulch species for weed suppression and impact on cotton yield. MT plots had low lint yield in 2020. CT: Winter pea, turnip, and oat in 2020 and turnip, oat, and mix in 2021 performed best. Top living mulch species: cowpea (Mississippi Silver), soybean, and peanut (GA-09B).

## **DEDICATION**

I would like to dedicate this thesis to the people who have supported me in this project.

To my lab mates, student workers, interns, and undergraduate research assistants who gave me their time to assist with my project and who provided laughter, friendship, and guidance throughout the process. These friendships and memories have changed the trajectory of my life for the better and I will forever hold them in my heart.

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## **CONTRIBUTORS AND FUNDING SOURCES**

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# CHAPTER I: INTRODUCTION AND REVIEW OF LITERATURE

## 1.1 Introduction

Cotton (*Gossypium* spp.; tribe *Gossypieae*, family Malvaceae) has been cultivated since about 3000 B.C. in the Indus River valley in modern-day Pakistan, and is the world's most important and influential fiber crop. Cotton's most important product is its fiber, in addition to a number of important byproducts such as cottonseed oil and protein meal (Smith & Cothren, 1999). Cottonseed is used extensively in dairy production as a feed source, and organic cottonseed is an important feed source in organic dairy production (Cotton Incorporated, 2022). Wild ancestors of cotton are rare; however, feral populations are present in different areas showing significant impact by human influence. Cotton has had a unique evolutionary history and shows a great taxonomic diversity for its morphological traits. The genus *Gossypium* has four species: *G. arboreum*, *G. herbaceum*, *G. barbadense*, and *G. hirsutum*. The main cotton variety used in modern cotton production is *G. hirsutum* (Smith & Cothren, 1999).

Cotton has been grown in Texas since 1745 when Spanish missionaries first planted it and thousands of pounds of cotton were produced each year (Gerhardt Britton et al., 1976). Since then, cotton has contributed significantly to the economic and cultural development of Texas and has been influenced by human inventions such as barbed wire, the cotton gin, and the development of the railroad (Gerhardt Britton et al., 1976). Currently, Texas produces about 25% of the cotton produced in the U.S. and it is the most important cash crop in the state (McKee Company, Inc., 2018). Cotton in Texas generated \$2.2 billion in revenue and as much as \$24 billion in cotton products in 2016 (Hawkes, 2017). In 2019, 7 million acres of cotton were planted in Texas, and 5.2 million acres were harvested (USDA, 2020). Cotton is incredibly

important in Texas both culturally and economically and has been a driving force for the state's development since it was first introduced.

Organic cotton fetches higher prices than conventionally grown cotton due to the challenges related to its production without pesticides and chemical fertilizers that result in low yield. Organic cotton production has risen in the last 30 years, increasing in the U.S. by 26% from 2018/2019 to 2019/2020 (Textile Exchange, 2021). Organic fiber sale also rose by 5% from 2019 to 2020, reaching \$2.1 billion, most of these sales being from organic cotton (OTA, 2021). Texas produces about 74% of the U.S.'s organic cotton (OTA, 2021). In 2019, there were 366 hectares of upland cotton planted in Texas that were transitioning from conventional to organic production (OTA, 2021). Organic cottonseed prices ranged from 425 to 600 dollars  $\text{ton}^{-1}$  in 2020, as compared to 165 to 210 dollars  $\text{ton}^{-1}$  for conventional cotton, giving organic producers the opportunity to make more money from each ton of cotton than its conventional counterparts (USDA-AMS, 2021). In a challenging farming economy, farmers are more interested in changing production practices to maximize profits, and many have an interest in converting all or part of their practice to organic production (Stephenson et al., 2017).

Organic crop production is prone to more weed infestation than the conventional production system (Knezevic et al., 2017). Chemical use restrictions in organic production result in cultural and mechanical methods being the main weed control options available. (Isik et al., 2009). In the absence of chemical herbicides, either of the non-chemical methods alone is rarely effective for extended periods of time, thus requires "many little hammers" for weed control (Liebman & Gallandt, 1997). Moreover, any single weed control strategy is not adequate to control diverse species of weeds, both grass and broadleaf types, primarily due to their varying life cycles, survival strategies, and dispersal mechanisms (Knezevic et al., 2017). Currently,

mechanical, thermal, non-synthetic herbicides, and biological strategies including live mulches, cover crops, naturally occurring diseases, insect predators, etc. are the primary strategies used to control weeds in organic systems.

Cover crops are typically high biomass species that are planted after harvest of the cash crop to cover the soil and terminated before planting the next crop (Hatfield & Stewart, 1994; Hatfield et al., 2011). Cover crops provide many benefits to sustainable conservation agricultural systems. For example, cover crops have been shown to reduce soil erosion and provide habitat and sustenance for soil microbes, among many other benefits (Hatfield et al., 2011). Another important benefit from cover crops is weed suppression early in the growing season, which is known to be a critical period to protect crops from the competition of weeds for resources in order to preserve crop yields (Knezevic et al., 2002). The benefit of weed suppression by cover crops is more critical in organic agriculture specifically. It not only reduces weed growth in the winter, but the residue after its termination in the spring can discourage weed growth as well (Isik et al., 2009). Forage radish as a cover crop is known to suppress weeds by competing with fall weeds, thus lowering spring weed growth (Lawley et al., 2012). Grain crops and grasses such as oat and rye as cover crops are known to have high biomass production, which can reduce nitrate concentrations in subsurface drainage water (Kaspar et al., 2012). Cover crops reduce weed germination and emergence by creating a physical barrier of biomass (Peachey et al., 2004). Therefore, cover crop biomass production is more important to weed suppression than species diversity or functional group diversity, making the selection of cover crop species a critical step for effective weed control (MacLaren et al., 2019). Lately, cover crops have been drawing significant attention from agronomists and growers for weed suppression and numerous other benefits.

Living mulches are annual or perennial species that are planted along with the main crop with the purpose of competing with weeds while limiting competition with the main crop to preserve yields (Leary & DeFrank, 2000). Planting living mulches is an old concept and practice that can have applications in modern-day challenges, such as lowering dependency on herbicides for weed control (Paine & Harrison, 1993). Planting living mulches into established cash crops is an emerging strategy for suppressing weeds by inhibiting weed seed germination and subsequent growth through multiple mechanisms such as shading, competition for aboveground and belowground resources, and allelopathy. Living mulches can also lower soil erosion, increase soil organic matter, and enhance biodiversity (Hartwig & Ammon, 2017). Living mulches with naturally short life cycles may be suppressed by the canopy of the cash crop before they reach reproductive maturity (Bhaskar et al., 2021). The ideal living mulch species could reduce herbicide use, improve soil health, and improve biodiversity in the agroecosystem to improve cotton production systems in Texas and the southern US more broadly.

## **1.2 Review of Literature**

Cotton is a perennial species that is extremely sensitive to weed pressure in its early growth stages. Cotton needs at least 8 weeks of weed free growth after emergence to achieve high yields. For example, good yields require at least 95% weed control, while excellent yields need at least 99% weed control (Cotton Incorporated, 2020). Weeds can have a significant detrimental impact on cotton yield. For example, 8 tall morning glory (*Ipomea purpurea*) plants 7.31 m<sup>-1</sup> of cotton row has been shown to lower cotton yields by up to 40% (Buchanan & Burns, 1971), while 8 common cocklebur (*Xanthium pensylvanicum*) plants and 8 redroot pigweed

(*Amaranthus retroflexus*) plants in a 7.3 m stretch of cotton row reduced cotton yield up to 40% and 70% through the growing season, respectively (Buchanan & Burns, 1971).

A 2021 survey of over 100 cotton growers and processors in Texas found that their largest reported challenge is weed control (Rasul, 2021). Consumers perceived organic farming as more environmentally friendly as it uses fewer pesticides that have the potential to be detrimental to the environment and soil microbiome. Organic farming has been noted to hold promise for restoring biodiversity in agricultural landscapes (Fuller et al., 2005). However, organic crop production faces numerous challenges, especially for disease-pest management and nutrient supply to the crop (Davies & Welsh, 2006). Weed control is the largest challenge to organic growers across agriculture, as weeds are documented to be more numerous and diverse in organic crop production than in conventional production (Ryberg & Milberg, 2012). Many organic growers are not satisfied with the available weed control options because of their low effectiveness. Concern about managing weeds is also a significant limiting factor for farmers considering transitioning to organic production (Bond & Grundy, 2001). Most methods used in organic farming research are often not ready to be scaled up to field production levels and are not easy for farmers to implement (Kuepper, 2001).

Mechanical weed control controls weeds with cultivation or other physical actions that remove, kill, damage, or create difficult growing conditions for weeds. Cultivation and destructive tillage is known to negatively impact soil quality and health (Karlen et al., 2013). Some of the most detrimental impacts of tillage are erosion, crusting on the soil surface, and lowered stability in soil aggregates (Baumhardt et al., 2015). Intensive tillage negatively impacts soil microbes as well in several ways including lowering enzyme activity and breaking up fungal hyphae networks, among others (Holland & Coleman, 1987; Lützow et al., 2006). Reduced

tillage benefits soil health by increasing organic matter present in the soil and benefitting other physical, chemical, and biological characteristics (Sharma et al., 2013; Nunes et al., 2018).

Tillage has been shown as an effective weed management tool in organic agricultural production for managing perennial weeds, in-season weed management, and soil preparation (Bond & Grundy, 2001). Under organic systems, reduced tillage can have over twice as many weeds as conventional tillage for both annual and perennial weeds (Armengot et al., 2014). Moldboard plowing is used extensively in organic production as the main weed control method, especially because the absence of herbicides typically creates more weed infestations (Armengot et al., 2012).

Thermal control, also referred to as flaming, kills weed growth by heating up the plant's tissues with a flame produced using a propane fuel source and a torch (Bond & Grundy, 2001; Datta & Knezevic, 2013). The propane torch can create temperatures as high as 1900 degrees C, raising the temperature of the plant tissues (Ascard, 1998) and breaking down proteins in the cell membranes (Lague et al., 2001; Carrubba & Millitello, 2013). Flaming may be an excellent opportunity for organic farmers for a new weed control option that is typically used to control weeds before crops emerge because of its non-selectivity (Stepanovic & Knezevic, 2013). Flaming may be more useful in reduced tillage organic systems as it leaves the soil undisturbed (Stepanovic & Knezevic, 2013).

The number of nonsynthetic herbicides that are certified for organic agriculture is limited. Strict regulations advocated by the United States Department of Agriculture (USDA), the Federal Trade Commission through the National Organic Program (NOP) regulation, and the International Working Group on Global Organic Textile Standard (GOTS IWG) involved in the production and processing of organic cotton often limit the use of several promising weed

control tools that directly or indirectly use even a negligible quantity of synthetic chemicals. Among numerous natural products, organic vinegar (30%) is an effective organic herbicide. Vinegar that contains acetic acid as the active molecule serves as a contact herbicide and burns the plant tissues when applied under sunlight (Webber III & Shrefler, 2007). To avoid crop injury, vinegar must be applied before crop emergence or using a sprayer with attachments that protect the crop from exposure. With the recent pace of transitioning cotton cultivation into organic farming, vinegar is becoming an integral part of production practices.

Living mulches are annual or perennial species that are planted between the rows of the main crop and can provide the benefit of soil erosion control, biological nitrogen fixation, and weed control through direct competition with weeds (Hartwig & Ammon, 2002; Leary and DeFrank, 2000). The practice of growing living mulches and intercropping is an ancient approach that can have applications in an integrated weed management system with the goal of lowering herbicide inputs and corresponding costs (Paine & Harrison, 1993; Ilnicki & Enache, 1992), reducing nitrate leaching (Andrews et al., 2020) and improving soil moisture conditions (Hartwig & Ammon, 2002; Leary & DeFrank, 2000). When intercrops are harvested, living mulches can continue providing weed control (Liebman & Dyck, 1993), thus can serve as an alternative to destructive tillage (Elkins et al., 1983). Living mulches compete with weeds in several ways, including allelopathy, shading out competing plants, and competing for nutrients and water in root systems (Mediene et al., 2011; Petit et al., 2018; Teasdale, 1996).

The main facet of the competitive effect of living mulch on weeds is the reduction of weed biomass production, which can directly translate into higher crop yield (Ilnicki & Enache, 1992). However, several past studies have shown that cover crops and living mulches must be terminated to avoid reducing yield loss in the main crop (Nicholson & Wien, 1983). While living



mulches suppress weeds, they may also lower crop growth and yield (Teasdale, 1996). In an unirrigated experiment, alfalfa (*Medicago sativa* L.) as living mulch caused 96% yield reduction in corn (Eberlein et al., 1992), whereas a second year legume living mulch reduced winter wheat (*Triticum aestivum* L.) yield by 70% (White & Scott, 1991). Despite a few such reports, living mulches can suppress weed growth and foster crop yield through careful species selection and management that focuses on biological characteristic differences between living mulches, crops, and weeds (De Haan et al., 1994; Liebman et al., 2001; Verret et al., 2017).

Depending on the living mulch species, the establishment and early season growth of weeds can be prevented or substantially decreased by providing early-season ground cover (Hatfield et al., 1998; Nicholson & Wien, 1983). Living mulches that are shorter in stature generally perform well as they can establish quickly, compete with weeds, and keep competition for light resources with the main crop to a minimum (De Haan et al., 1994; Echtenkamp & Moomaw, 1989; Leoni et al., 2020). Research efforts on living mulch started intensively in the 1970s; however, there has been limited adoption in the United States since then (Hughes & Sweet, 1979). Interest in living mulch is rising again because of possible benefits for lowering herbicide inputs, benefitting soil health, and lowering weed biomass (Bartel et al., 2020; Moore et al., 2019; Robačar et al., 2016). However, hurdles such as the lack of best management practices development inhibits the widespread adoption of living mulch practices (Sheaffer & Moncada, 2012).

Selection of living mulch species must consider the characteristics and biology of both the main crop and living mulch species that decides the competitive compatibility between the two counterparts. In an experiment involving corn as the main crop and kura clover (*Trifolium ambiguum* M. Bieb) as the living mulch, corn hybrids sensitive to drought resulted in lower

yields with a living mulch versus a terminated mulch (Ziyomo et al., 2013). Response of the main crop to the presence of a living mulch differed by variety within a species in other experiments, as shown with living mulch medic species (*Medicago* spp.) in dwarf and conventional height barley (Moynihan et al., 1996), and living mulch Italian ryegrass (*Lolium multiflorum*) in forage soybean (Uchino et al., 2016). Crops that are taller and drought-resistant typically perform better with living mulches than shorter, drought-sensitive crops (Bhaskar et al., 2021).

An important consideration for living mulch agronomic decisions is the planting date. A gap between crop and living mulch planting dates helps the main crop to avoid competition during germination and early growth (Liebman et al., 2001). In broccoli, rye planted at the same time as transplanted broccoli stifled weed growth but led to a reduced broccoli yield (Brainard & Bellinder, 2004). However, rye planted 10 and 20 days after broccoli transplanting had no effect on weed growth or broccoli yield. Planting living mulches later than the crop also had lower yield losses in corn and soybeans (Brooker et al., 2020; Uchino et al., 2009; Vrabel et al., 1980; Wivutvongvana, 1973), indicating the critical importance of planting date of crop and living mulch.

### **1.3 Hypotheses**

Based on the above background, the specific hypotheses of this research project were:

- i) Integration of cover crops will be sufficient to meet weed control goals in minimum-till organic cotton production
- ii) An appropriate selection of living mulch species will provide effective weed suppression in cotton without affecting the crop yield

## 1.4 Objectives

The specific objectives of this project are to,

- 1) develop a sustainable organic cotton production system comparing two systems of tillage and four cover crop treatments; and
- 2) assess different living mulch species for weed suppression and their impact on cotton yield.

For the first objective, the tillage regimes being investigated are CT that uses tillage for field preparation and in-season cultivations, and MT with a strip-till for cotton planting and one in-season cultivation on an as-needed basis during the early season. Cover crop species investigated include Austrian winter pea, Bob oat, Purple-top turnip, and a mix of the three species. For the second objective, 22 different living mulch species, namely alfalfa, berseem clover, buckwheat, cowpea (Mississippi Silver), flax, Japanese millet, four mix treatments, peanut GA-09B, peanut Schubert, slender creeping red fescue, soybean, tall fescue, and zucchini are to be assessed for their impacts on weed biomass production and cotton yield.

## **CHAPTER II: EFFECT OF TILLAGE PRACTICES AND COVER CROPS ON WEED SUPPRESSION AND AGRONOMIC PERFORMANCE OF ORGANIC COTTON**

### **2.1 Introduction**

Cotton is an important cash crop in the southern United States (U.S.), especially in Texas, where it generates about \$2.2 billion in annual revenue (Hawkes, 2017). Amongst many, weeds have been a major impediment to cotton yield in the region and beyond, especially with the evolution and spread of herbicide-resistant weeds. Lately, the injudicious application of herbicides is threatening the environment and biodiversity. Growing consumer preference towards organic cotton has catalyzed the growth of the organic cotton sector in Texas and other cotton-producing areas in the U.S. (Delate et al., 2020; Funk & Kennedy, 2016; Hustvedt & Bernard, 2008). For instance, organic cotton production and organic fiber sales increased by 5% between 2019 and 2020 (OTA, 2021). In 2019/20, the U.S. organic cotton was grown in approximately 11,728 ha, yielding 23,720 bales (5,164 MT) and sharing 0.2% of total U.S. cotton production and 2.8% of global organic cotton production (TE 2021; USDA 2020). Texas leads the U.S. in organic cotton production with a 74% share (OTA, 2021).

Despite the growth in the organic cotton market, the production of organic cotton still remains low in the U.S. (USDA 2013). Among the several constraints, weed control remains the single most important challenge with organic cotton production, due to the limited non-chemical options available for effective weed control. Organic weed control is even more challenging under conservation or reduced tillage systems (Teasdale et al., 1991).

In southern U.S. cotton, common grass weeds are barnyardgrass (*Echinochloa crus-galli* L.), red sprangletop (*Dinebra panicea* Retz.), and johnsongrass (*Sorghum halepanse* L.) (Werner

et al., 2020). The broadleaf species that are often problematic in cotton fields are pigweeds (*Amaranthus* spp.), ragweed parthenium (*Parthenium hysterophorus* L.), and lamb's quarters (*Chenopodium album* L.) (Werner et al., 2020). Weed control in cotton is critical, especially in the early growth phases as it is a perennial and does not compete well with weeds (Cotton Incorporated, 2020). The critical period of weed control, which includes the time between seedling emergence and the point at which weed pressure will not impact the crop yield beyond an acceptable threshold (Altieri, 1998; Hall et al., 1992; Knezevic et al., 2002), is about 8 weeks in cotton (Cotton Incorporated, 2020).

Organic cotton producers in Texas have reported that weed control is the greatest challenge in their crop management plan because of the higher diversity and pressure of weeds in organic production (Rasul, 2021). Moreover, the fact that perennial weeds are more likely to thrive in organic production than in conventional agriculture makes weed management more complicated under organic farming (Bond & Grundy, 2001). In order to effectively control weeds in organic cotton, multiple approaches must be deployed (Knezevic et al., 2017). The organic cotton producers heavily rely on tillage and many “little hammers” that help control weeds in an integrated approach (Liebman & Gallant, 1997). Common methods for weed control in organic cotton include organic non-synthetic herbicides, cover crops and tillage, and the more recent, creative approaches such as flaming (Ferguson, 2004).

Organic herbicides such as citric acid (vinegar), clove oil, and thyme/clove oil are used for a variety of reasons in organic agriculture, including weed control and crop defoliation (Ferguson, 2004). Several studies report organic vinegar as an effective weed control tool (Webber III & Shrefler, 2007). At a higher concentration, vinegar is more effective at killing weeds especially when they are at later growth stages (Radhakrishnan et al., 2002). Lewis et al.

(2020) and Li et al. (2019) reported that caprylic and capric acids-based organic herbicides can reduce growth of several weed species including corn spurry (*Sperula arvensis* L.) and horseweed (*Conyza canadensis* L.). However, vinegar and other organic herbicides are nonselective and can cause damage to the crop tissues upon contact (Evans et al., 2017) and vinegar specifically is more effective in reducing weed growth when weeds are small (Radhakrishnan et al., 2002).

Lately, cover crops have attracted significant attention as a weed control tool. Cover crops are planted after cash crop harvest and terminated before planting of the next crop in the spring as an agricultural conservation practice that can also suppress weeds and improve soil health (Hatfield & Stewart, 1994). Cover crops offer a number of benefits such as nitrogen fixation (with leguminous cover crop), soil erosion reduction, weed suppression, etc. (Ingels et al., 1994). They can provide weed control in the early growing season, especially during the critical weed-free period (Knezevic et al., 2002), which is important for organic cotton. Rye or hairy vetch residues reduced weed emergence and density, compared to a bare fallow field in the subsequent sweet corn crop (Teasdale et al., 1991). Likewise, a rye cover crop before sweet corn reduced weed densities by 78% (Hayden et al., 2012).

Tillage, which mechanically disturbs the soil to interrupt weed germination and growth, is still the most widely used method of weed control (Karlen et al., 2013). Reports show that tillage stimulated higher weed emergence and exhausted weed seedbank rapidly (Roberts & Dawkins, 1967). In the absence of chemical herbicides, tillage serves as the primary tool for weed control in organic systems. Compared to reduced tillage, conventional tillage reduced both annual and perennial weeds by 50% in an organic system (Armengot et al., 2014). However, destructive tillage is known to have negative impacts on soil health and quality due to increased

erosion and reduced stability with soil aggregates (Baumhardt et al., 2015; Karlen et al., 2013). Heavy tillage can reduce soil microbial communities by cutting off hyphae networks, resulting in reduced microbial enzyme activity in the soil (Holland & Coleman, 1987; Lützow et al., 2006).

Reducing tillage can improve soil health parameters such as soil microbial health, crop yields, increased microbial enzyme activity, and carbon sequestration (Freibauer, 2004; Nunes et al., 2018; Sharma et al., 2013). However, reducing tillage brings significant challenges in organic production, with higher weed density being the primary (Teasdale et al., 1991). In other experiments, cultivated plots had higher weed emergence and exhausted the weed seedbank more quickly (Roberts & Dawkins, 1967). In reduced or no-tillage systems, it has been shown that annual grass weed seed populations increase faster than in CT systems, while the effect on broadleaf species is variable (Froud-Williams et al., 1981). However, with rising concerns of soil health and environmental issues, significant efforts are being directed towards the promotion of no- or minimum- tillage practices. Reports show that reduced tillage under conservation agriculture (residue retention practices) can still provide weed suppression (Price et al., 2006).

Thermal weeding or flaming has been a novel method of weed control that usually includes a propane torch attached to a tractor that is driven through the field and targets weeds between the crop rows (Bond & Grundy, 2001; Datta & Knezevic, 2013). The propane torch heats up plant tissues and breaks down the cell membrane proteins as the temperature reaches nearly 2000 °C (Ascard, 1998; Lague et al., 2001; Carrubba & Millitello, 2013). Because flaming is so damaging to plant tissues, it is usually applied before crops emerge to avoid potential crop damage (Stepanovic & Knezevic, 2013).

Despite the growing importance of organic cotton in the US, the production practices, especially weed control options, are not well developed to achieve a sustainable and profitable organic

cotton operation. To this effect, the specific objective of this study was to develop a sustainable weed management strategy in organic cotton production involving tillage regimes and cover crops.

## **2.2 Materials and Methods**

### *Study location and experimental setup*

This experiment was conducted over two years, beginning fall 2019 through the summer of 2021, at the Texas A&M University Research Farm in Burleson County, Texas. The climate was subtropical humid, with most rainfall occurring in the winter and spring months. The soil in the experimental field was a Belk clay (fine, mixed, active, thermic Entic Hapludert). The mean annual temperature for this location is 20.6 °C, and the average annual precipitation is 1018 mm.

The experiment was organized in a randomized split-plot design with three replications. The main-plot factor was tillage with two levels: CT and MT. The sub-plot factor was cover crops with four levels: oat (*Avena sativa*), Austrian winter pea (*Pisum sativum* ssp. *arvense* L.), purple-top turnip (*Brassica campestris*), and a mix of the three. The goal of the MT treatment was to minimize tillage as much as possible, with cotton planted following strip-tillage and early-season inter-row cultivation included on an as-needed basis. A weed-free check was maintained under each tillage regime for comparison.

Depending on equipment availability, each plot (900 m<sup>2</sup>) was made of 8 rows of 15 m length with 75-cm row spacings in 2020, and 6 rows of 15 m length with 100 cm spacings in 2021. Cotton seeding rates were uniform across the plantings. All treatment combinations had a total of 24 plots in each replication, with a one-meter alley between plots. The cotton crop was managed organically, without any synthetic fertilizers or pesticides.



### *Field management, year 1*

The CT plots were cultivated using a PC10 series John Deere cultivator on October 22, 2019, to prepare the seedbed for cover crop planting. The cover crops were planted on November 4, 2019, using a Great Plains Native Grass SERIES II grain drill. Cover crop planting rates were as follows: Austrian winter pea at 56 kg ha<sup>-1</sup> (2.5 cm planting depth), purple-top turnip at 5.5 kg ha<sup>-1</sup> (1 cm depth), oat at 78 kg ha<sup>-1</sup> (4 cm depth), and the mix proportion was 10:1:14 for winter pea: purple-top turnip: oat (2.5 cm depth). The cover crops were grown as a rainfed crop, and were terminated on March 26, 2020, using a John Deere roller crimper. Fertilization.

The CT plots were cultivated entirely, while MT plots were strip-tilled with cotton planted into the tilled area. Due to staffing shortages related to the COVID-19 pandemic, cotton was planted on June 2, 2020, later than initially planned at a seeding rate of 96,000 plants hectare<sup>-1</sup> (approx. 50 kg ha<sup>-1</sup>). The entire cotton crop was irrigated on August 18, 2020. On October 22, 2020, the field was defoliated using 30% vinegar at a rate of 280 liters hectare<sup>-1</sup> ahead of cotton harvest. The remaining biomass in the field was shredded on November 2, 2020 and was burned as a form of weed seed control on November 5, 2020, a day with low wind gusts (<10 km hour<sup>-1</sup>). No insect or disease management was necessary.

### *Fertilization*

Organic chicken litter was applied on May 20, 2020, as a fertilizer at a rate of 4500 kg ha<sup>-1</sup>. Austrian winter pea and mix cover crop plots received half the dose of chicken litter, or 2250 kg ha<sup>-1</sup> because of the cover crop's ability to fix nitrogen in the soil. A soil test conducted at the

Texas A&M Soil Testing Lab confirmed that one tonne of chicken litter provided 24.3 kg of nitrogen, 17 kg of potassium, 48 kg of calcium, 3 kg of magnesium, and 2 kg sodium.

### *Weed management*

Weed-free check plots were maintained weed-free through periodic weed control activities. In the CT plots with cover crops, this was accomplished by one inter-row cultivation on July 15, 2020, whereas in the CT weed-free plots, the weed growth was removed by hand hoeing on August 10, 2020. The MT plots were not tilled, and the soil was left completely undisturbed through the season after the strip tillage event at cotton planting. In the MT weed-free check plots, weed control was attempted with a handheld propane torch on July 3, 2020 and a manual application of 30% vinegar at a rate of 470 liters hectare<sup>-1</sup> on July 8, 2020. Further, on July 15, 2020, a push lawnmower with a 51 cm swath was used for weed control between cotton rows in these check plots. None of these methods provided acceptable weed control in MT weed-free plots because of extreme weed pressure in these plots. Thus, these plots were not truly “weed-free,” but were used as “alternative weed control” test plots.

### *Data collection, year 1*

Winter cover crop biomass was collected on March 25 and 26, 2020 by harvesting 3 random 0.75 m<sup>2</sup> quadrats from each plot, weighing after 72 hours in a dryer at 60°C, and separating by major weed species and cover crop. The weeds that emerged inside three randomly placed, permanent 0.75 m<sup>2</sup> quadrats in each plot were counted three times in 2020: June 22, July 2, and August 25. After counting, these weed seedlings were removed from the quadrat to allow for more germination. On July 30 and October 30, 2020, weed biomass was collected by

harvesting weeds from three random quadrats (size: 0.75 m<sup>2</sup>) in each plot. The biomass was then dried for 72 hours in a drier at 60°C, separated by major weed species, and weighed.

Cotton height was measured on June 15, July 22, and August 5, 2020, by measuring 10 representative cotton plants in each plot. Cotton biomass was collected on August 20, 2020, by harvesting 5 representative plants from each plot and drying them for 72 hours in a drier at 60°C. Some MT plots did not have enough cotton plants present to allow a biomass harvest, so those plots were excluded from data collection. Cotton was hand-harvested on October 29, 2020 from three, 3 m sections of row in each plot, dried for 72 hours in a drier at 60°C, and ginned using a tabletop gin to separate lint and seed. Some plots did not have adequate stand counts, so in those plots the entire plot was harvested and processed similarly.

#### *Field management, year 2*

After burning the remaining biomass in the field, CT plots were tilled on November 12, 2020, to prepare for cover crop planting on November 19, 2020 using the same agronomic practices as in 2019. The Texas A&M University Farm received two snowstorms in the winter of 2020-2021, the first on January 10, 2021, and the second on February 14, 2021. In both cases, cover crops were covered with snow and so were insulated from freezing temperatures. The lowest air temperature recorded was 0.3°C on January 10, and -12.6°C on February 14. The lowest soil temperatures recorded were 7.2°C on January 10, and 3.6°C on February 14, which was measured using a soil temperature probe at 5 cm depth. Visual damage ratings did not show significant damage to any of the cover crops.

On March 18, 2021, the cover crops were terminated using a roller crimper in CT as well as MT plots. On April 16, 2021, the CT plots were tilled before planting cotton on April 19, 2021

at a rate of 50 kg ha<sup>-1</sup> (approximately 96,000 seeds ha<sup>-1</sup>). The CT plots were cultivated on May 19, 2021 to provide early-season weed control in established cotton. From May 19, 2021 to June 10, 2021 a total of 111 mm of rainfall was recorded from a weather station installed in the field. The extremely wet conditions for a prolonged period didn't allow for any weed control activity in the plots, leading to extreme weed pressure and complete loss of the cotton crop. As a result, the entire field was terminated and tilled uniformly on June 15, 2021. Cotton was replanted on June 25, 2021, at the same seeding rate used before. After cotton harvest on November 15, cotton was shredded on November 22, 2021, and the cover crops were planted on November 24, 2021, using a grain drill. The cover crops were grown and terminated following the same agronomic practices used previously.

#### *Weed control, year 2*

For weed control in MT 'alternative weed control' test plots, 30% vinegar was applied at 280 liters hectare<sup>-1</sup> on February 19, 2021. The CT weed-free check plots were cultivated the same day to control winter weeds and again hand-weeded on August 12, 2021 to maintain the plots weed-free. Both the CT and MT plots with cover crops received an in-season cultivation on July 19, 2021. This inter-row cultivation was necessary in MT plots to obtain acceptable levels of early-season weed control, as evident from the previous field season. The CT plots were cultivated again on July 26, 2021 to provide additional weed control, whereas this cultivation was excluded in MT.

#### *Data collection, year 2*

Cotton height was measured on July 5, August 3, and September 7 2021, by measuring the height of 10 representative plants in each plot. Weed emergence was counted on June 1, July 1, and August 2 in three permanent 0.75 m<sup>2</sup> quadrats in each plot. After counting, weed seedlings were removed from the quadrat to allow for subsequent emergence and enumeration. Weed biomass was collected on August 3, by harvesting three 0.75 m<sup>2</sup> quadrats from each plot, drying the biomass in a drier at 60°C for 72 hours, and separating by species and weighing. Cotton biomass was collected on September 7, by harvesting 5 random plants from each plot, drying them for 72 hours at 60°C, and weighing them. Cotton was hand-harvested on November 15 by harvesting all seed cotton in three representative 3 or 5-m linear rows in each plot depending on the maturity progression. Weed biomass was collected on November 22 by harvesting biomass from three representative 0.75 m<sup>2</sup> quadrats, which were then dried for 72 hours in a drier at 60°C, separated by species, and weighed.

### *Data analysis*

Data analysis was conducted in R software version 4.1.0 (R Core Team, 2022). Data normality was checked using the *qqnorm* function in the base R package and no transformation was required. Replications were considered random effects and ANOVA was used to check for treatment effects and significant differences using the ANOVA function and the linear model (*lm*) function in the base R package. Tukey's HSD and Fisher's protected LSD ( $\alpha < 0.05$ ) were used for mean separation and comparison of individual treatment means. Regression analysis for weedy biomass and cotton lint yield was performed using the *cor* function in the base R package with *method = "pearson"*. The explanatory variable was weed biomass, while the response variable was cotton lint yield.

## 2.3 Results and Discussion

### *Cover Crop Biomass*

In 2020, tillage regime did not have a significant impact on cover crop biomass ( $P = 0.92$ ), but biomass production differed across cover crop species ( $P < 0.05$ ) (Figure 8). Overall, CT ( $1826 \text{ kg ha}^{-1}$ ) produced more cover crop biomass than MT ( $1822 \text{ kg ha}^{-1}$ ) (Figure 8). In 2020, oat ( $3233 \text{ kg ha}^{-1}$ ) (Figure 9) and winter mix ( $2423 \text{ kg ha}^{-1}$ ) (Figure 12) under CT, and oat ( $2374 \text{ kg ha}^{-1}$ ), winter mix ( $2335 \text{ kg ha}^{-1}$ ), and winter pea ( $2130 \text{ kg ha}^{-1}$ ) (Figure 10) under MT produced similar biomass (Figure 8). These similarities across tillage regimes can be attributed to the lack of difference in the treatment of the soil at this point in the project. Oat in CT produced the most biomass numerically ( $3233 \text{ kg ha}^{-1}$ ), whereas the purple-top turnip under MT ( $452 \text{ kg ha}^{-1}$ ) (Figure 11) as well as CT ( $66 \text{ kg ha}^{-1}$ ) produced the least aboveground biomass in 2020 (Figure 8), but it is not clear why there were large differences between tillage regimes in purple-top turnip in 2020 as there was no difference in the treatment of the soil at this point in the project.

In 2021, cover crop biomass was significantly influenced by tillage regime ( $P = 0.00021$ ) as well cover crop species ( $P = 0.027$ ). On average, CT plots ( $2346 \text{ kg ha}^{-1}$ ) produced more biomass than MT plots ( $856 \text{ kg ha}^{-1}$ ). Oat in CT produced the most biomass ( $3579 \text{ kg ha}^{-1}$ ) (Figure 9) and was similar to CT-winter mix plots ( $2677 \text{ kg ha}^{-1}$ ) (Figure 12). The presence of oat in the winter mix plots possibly drove the greater biomass production in those plots. A recent study by MacLaren et al. (2019) points to biomass production being more important than cover crop diversity in aiding crop production by suppressing weed growth. MacLaren et al. (2019) demonstrated this by investigating several cover crop mixes differing in species, functional groups, and species functional type included in mixes in South Africa.

Results of this study show that tillage treatments did not influence cover crop biomass production immediately in 2020, but they significantly impacted cover crop growth in 2021. In contradiction to this study, Schomberg et al. (2006) in southeastern U.S. and Daniel et al. (1999) in Virginia did not find any effects of tillage on cover crop growth. However, Dozier et al. (2017) and Villamil et al. (2006) in Illinois found variable growth and impact of cover crops in diverse cropping systems, including different tillage regimes.

Greater biomass production by oat in our experiment is supported by Unay et al. (2005), who reported high biomass by common vetch+ oat than other winter cover crops in Turkey. Schomberg et al. (2006) reported more biomass production from black oat, another species of oat, than oilseed radish, hairy vetch, and Austrian winter pea in the southeastern U.S. Price et al. (2006) and Reeves et al. (2005) reported that biomass production of black oat is comparable to rye and wheat in conservation-tillage cotton and soybean in the southeastern U.S. Our experiment confirms the high biomass potential of oat.

### *Weed emergence*

Tillage had a significant impact on the emergence of mid-season broadleaf weeds in 2020 and 2021, while cover crops showed a significant impact only on the mid-season grass weed emergence ( $P < 0.05$ ) (Table 1). Early and late-season weed emergence were not significantly influenced by either tillage or cover crop treatments, except for early-season grass weed emergence in 2020. The findings show that overall weed emergence was similar across the field except for a few of the interactions and treatments that significantly impacted weed emergence. Previous studies found that weed emergence differs by growing season, tillage regime, and weed species (Chauhan & Opena, 2012). DeVore et al. (2012) and Norsworthy et al. (2011) in

Arkansas reported reduced Palmer amaranth emergence in cotton fields under deep tillage and cover crop treatments.

### *Cotton plant height*

Cotton height is affected by tillage, and cotton height was significantly influenced by both tillage and cover crop species in 2020 and 2021 ( $P < 0.05$ ); cotton was taller in year 2 than in year 1 ( $P < 0.05$ ) (Figures 2 and 3). The height increase in 2021 can be attributed to all plots receiving at least one cultivation during the 2021 growing season that lowered weed biomass across the field and significantly reduced competition with cotton. In 2020, all CT plots produced significantly taller plants (80 cm) than in the MT plots (42 cm) ( $P < 0.001$ ) (Figures 2 and 3). The CT weed-free (no cover crop) (59 cm) and winter pea (53 cm) treatments produced the tallest cotton plants, whereas the MT winter pea treatment produced the shortest cotton plants (25 cm) (Figure 2).

In 2021, plants under MT treatments (73 cm) were shorter than all CT treatments (88 cm), though not statistically different in all cases (Figure 2). Cotton plant height was significantly influenced by cover crops ( $P < 0.001$ ). CT plots with purple-top turnip (96 cm) had the tallest cotton, statistically similar to the weed-free (92 cm) (Figure 2). Cotton height can be significantly influenced by weed pressure throughout the season but may not lead to a direct correlation with cotton yield (Charles et al., 2019).

Figure 1 shows the progression of cotton height for each treatment through the growing season. The average percent increase in cotton height from midseason to late season for all MT plots in 2020 was 11%, while in 2021 that number was 49%, a larger increase than CT plots with a 37% change from midseason to late season in 2021 (Table 2). In 2020, the treatments with the



highest percent increase were the CT winter mix (39%), CT oat (33%), and CT weed free (31%). MT purple top turnip (1%), MT Austrian winter pea (5%), and the MT alternative weed control check (7%), all had the lowest growth rates from midseason to late season. The treatment with the largest increase in midseason to late season growth rate from 2020 to 2021 was the MT Austrian winter pea treatment, which increased from 5% to 55% (Table 2). These findings show the impact that a single tillage event has on cotton growth. When there was no tillage for weed control in 2020, MT plots cotton height increased by only 11%, when in 2021 when MT plots were subjected to a single tillage event, cotton height increased by 49%. A single tillage event allowed for continuing cotton growth throughout the season in 2020, while cotton with no tillage in 2020 were almost completely stunted in growth.

Boquet et al. (2004) in Louisiana reported significant influences of tillage and cover crops on cotton plant height, which was reflected in cotton lint yield. They showed that no-till management provided a better growth environment for cotton plants. Similar positive influences were reported by Nyakatawa et al. (2000) in Alabama where winter cover crop under no-till management improved the cotton growth. Our study corroborated these findings.

### *Cotton biomass*

There was a significant impact of tillage regime ( $P < 0.05$ ) and cover crop species ( $P < 0.001$ ) on cotton biomass in 2020 (Figures 4 and 13). The weed-free check under CT produced the highest cotton biomass (43 g). Austrian winter pea (30 g) and the weedy check (27 g) produced similar biomass, as did the purple-top turnip (20 g), oat (19 g), and the cover crop mix (17 g), as shown in Figure 2. In 2021, MT plots (25 g) continued to produce lower cotton biomass than CT (39 g), and there was a significant difference between tillage regime ( $P < 0.001$ )

(Figure 4) as well as among the cover crop treatments ( $P < 0.01$ ). The CT weed-free check resulted in the highest cotton biomass (38 g), while CT purple top turnip (38 g) and oat (38 g) treatments were similar. The MT treatments had the lowest cotton biomass: winter mix (16 g), weed-free plots with alternative weed control (35 g), and Austrian winter pea (19 g) (Figure 4).

The difference in number of cultivations during the 2020 growing season likely contributed to significant differences in several metrics between MT and CT. Regarding midseason cotton biomass in 2020, the cotton stand counts were significantly lower in MT plots, in turn contributing to lower cotton biomass. Lower cotton plant stand and biomass in MT plots in 2020 can be attributed to persistent weed pressure throughout the season, especially after the termination of cover crops. Inter-row cultivation in CT plots was very effective in reducing weed infestations. In organic cotton production, these challenges are often avoided by integrating a fallow year in order to reduce weed seedbank and the dominance of perennial weeds (Hintzsche & Wittman, 1992), which was not followed in this study.

Price et al. (2012) reported better cotton growth under reduced tillage and high-residue conservation agricultural system in Alabama. Similar results were reported by Boquet et al. (2004) in Louisiana and Nyakatawa et al. (2000) in Alabama. Cover crops and reduced tillage can improve soil quality and thereby stimulate crop growth across the cropping systems (Schomberg et al., 2006). The optimum combination of cover crops and reduced or strip tillage can increase cotton growth and lint yield significantly (Boquet et al., 2004; Vasilakoglou et al., 2006).

*Cotton end-of-season maturity*

Near the conclusion of the 2021 growing season, maturity ratings were taken on a scale of 1-5, with 1 being cotton actively flowering with no open bolls, and 5 being no cotton flowers present with >50% open bolls when cotton was considered ready for harvest after a defoliation. Crop maturity varied significantly between replications ( $P = 0.0063$ ) to the extent that some plots were flowering, while other plots had open cotton bolls that would be ready for harvest after a defoliation (Figure 7). There was no significant difference between cover crop treatments ( $P = 0.61$ ), tillage regime ( $P = 0.21$ ), or interaction of cover crop and tillage regime ( $P = 0.42$ ) (Figure 9). Moss and Downey (1971) reported that reduced water availability can delay crop maturity, which explains the difference in cotton maturity across the plots in our study, although no specific pattern was observed. Contradictory results were reported in West Tennessee, where delayed maturity in cotton was observed under no-till and heavy cover crop mulches (Hoskinson et al., 1982). Similar results were reported by Mutchler and Greer (1985) in north Mississippi and Brown et al. (1985) in Alabama, which oppose our findings.

### *Cotton yield*

There was a significant negative relationship between weed biomass production and cotton yield in 2020 as evident in the linear regression (Figure 6). The MT treatments allowed more weed biomass production, which highly contributed to lower cotton yields (Figure 5). The weed-free check under CT produced the highest cotton lint yield ( $1035 \text{ kg ha}^{-1}$ ), while the yields with purple-top turnip ( $804 \text{ kg ha}^{-1}$ ), Austrian winter pea ( $779 \text{ kg ha}^{-1}$ ), and oat ( $730 \text{ kg ha}^{-1}$ ) were statistically similar. In some MT plots, there was no harvestable cotton yield because of intense weed pressure (Figure 6). In 2021, the difference between tillage regimes was not as clear as 2020, likely due to the single cultivation applied to the MT plots. There was still a

negative relationship between weed biomass production and cotton lint yield (Figure 5). The regression calculated using all weed biomass and cotton yield data from 2020 and 2021 projects that with 0 weed biomass, maximum cotton lint yield would be 996.945 kg ha<sup>-1</sup>, and to produce 0 kg ha<sup>-1</sup> of cotton lint, 97.15 g m<sup>-1</sup> must be present. For every additional g m<sup>-1</sup> of weed biomass present, 10.262 kg ha<sup>-1</sup> cotton lint yield is lost.

The similarity in cotton yield between MT 2021 (635 kg ha<sup>-1</sup>) and CT 2020 (754 kg ha<sup>-1</sup>) show that one early-season cultivation is enough to prevent a catastrophic cotton yield loss in organic cotton (Figure 6). The difference between one and two cultivations is not large enough to justify a second cultivation (Figure 6), if the first cultivation is properly timed. This explains the lack of difference between CT 2020 (754 kg ha<sup>-1</sup>) and CT 2021 (672 kg ha<sup>-1</sup>) cotton lint yield (Figure 5). Reduced tillage systems are known to allow greater weed seed production and species diversity than more intensive tillage regimes, which can lead to lower crop yields (Santin-Montanya et al., 2016). The pattern observed in our study is similar to that of Berner et al. (2008), who found reduced yields in wheat with reduced tillage in the first few years of transitioning. In a similar study in peanut, it was found that CT produced the highest crop yield followed by MT and lastly, no-tillage systems with the lowest crop yield (Grichar & Boswell, 1987), which corroborates our results.

## **Conclusion**

Limiting tillage led to lower cotton yields, shorter cotton, and higher weed biomass production than any treatment with tillage, even treatments with only one tillage event during the growing season. Treatments where tillage was limited to a strip till event before cotton planting led to an almost complete loss of cotton yield in 2020, even with cover crop use. Integrating a

single tillage event during the growing season prevented crop failure and preserved cotton yield while lowering weed biomass production in 2021. Cover crops contributed to weed control in relation to the amount of cover crop biomass that they produced. Cover crops that produced more biomass generally led to better weed control outcomes. Oats produced the most cover crop biomass when grown in a monoculture and contributed heavily to biomass production in cover crop mix plots. In general, limiting tillage in organic production drastically limited the production potential of all cover crop treatments. Cover crop use did not replace cultivation as a weed control method in any case. Future research should investigate the ideal number of cultivation events during a growing season and additional planting rates for cover crops.

## 2.4 Figures and Tables

**Table 1.** *P* values for the effect of tillage, cover crop, and their interaction on weed seedling emergence in organic cotton in 2020 and 2021.

	2020 <sup>a</sup>								
	Early-season			Mid-season			Late-season		
	BL	G	Total	BL	G	Total	BL	G	Total
Tillage	0.242	0.900	0.478	0.009*	0.089	0.005**	0.881	0.189	0.187
Cover Crop	0.513	0.015*	0.108	0.668	0.092	0.290	0.272	0.667	0.614
Tillage x Cover Crop	0.569	0.104	0.258	0.254	0.095	0.030*	0.658	0.574	0.870
	2021 <sup>b</sup>								
	Early-season			Mid-season			Late-season		
	BL	G	Total	BL	G	Total	BL	G	Total
Tillage	0.04*	0.315	0.903	0.279	0.282	0.651	0.257	0.387	0.482
Cover Crop	0.661	0.868	0.728	0.214	0.003* *	0.031*	0.424	0.756	0.429
Tillage x Cover Crop	0.215	0.489	0.869	0.476	0.038*	0.914	0.351	0.889	0.789

\*  $P < 0.05$ , \*\*  $P < 0.005$ . BL: Broadleaf; G: Grass.

Abbreviations: BL, Broadleaf; G, Grass.

Dominant grasses included red sprangletop and browntop panicum, while dominant broadleaves included Palmer amaranth and prostrate spurge.

<sup>a</sup> Date of observations in 2020: Early-season, June 22; Mid-season, July 2; Late-season, August 25.

<sup>b</sup> Date of observations in 2021: Early-season, June 1; Mid-season, July 1; Late-season, August 2.

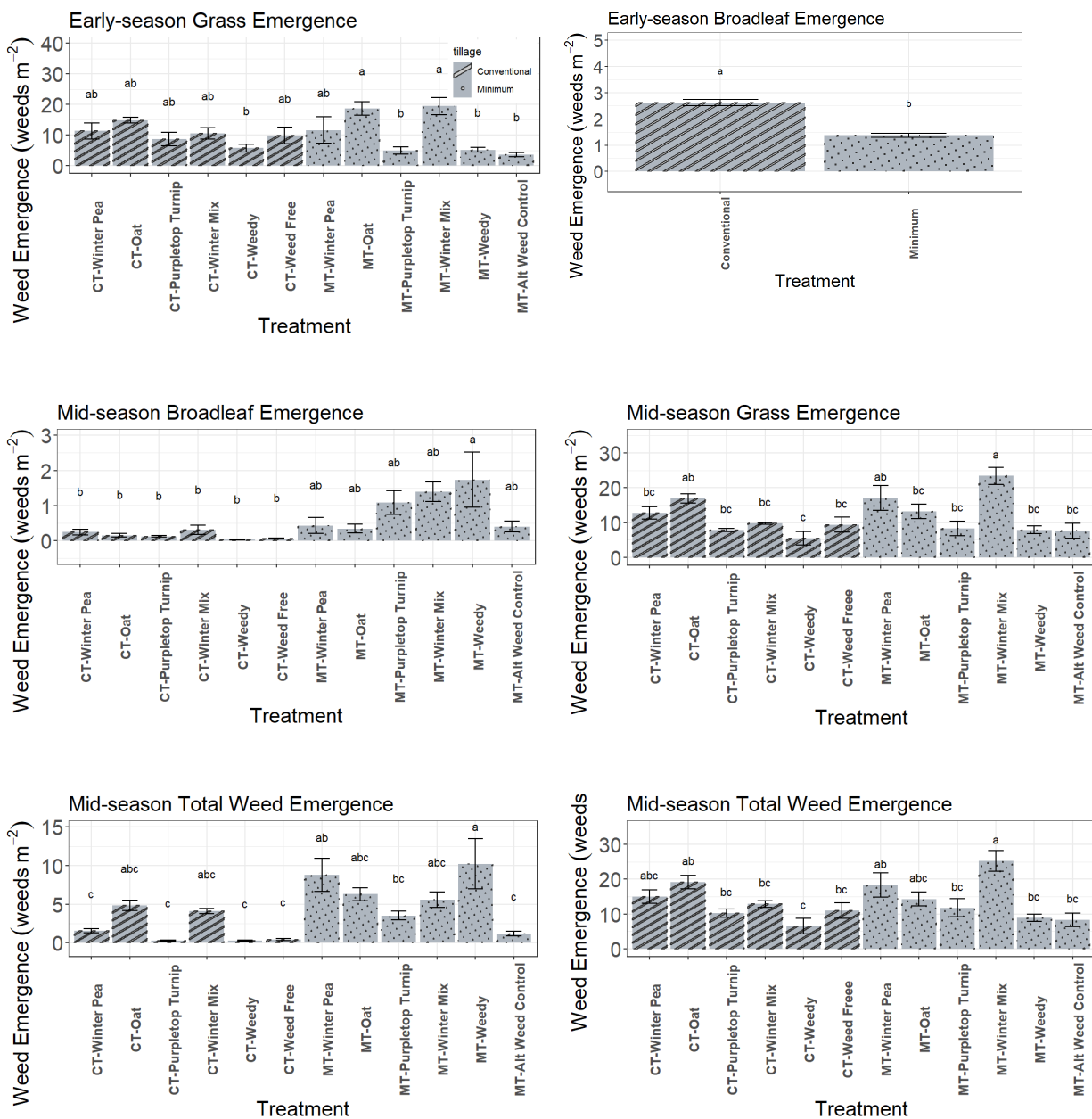
**Table 2.** Changes to mid-to late-season organic cotton plant height as influenced by tillage and cover crops in 2020 and 2021.

<b>Tillage regime</b>	<b>Cover crops</b>	<b>Increase (%) mid – late season 2020</b>	<b>Increase (%) mid – late season 2021</b>
Conventional	Austrian Winter Pea	27.9 c	45.2 d
	Oat	32.6 b	23.5 h
	Purpletop Turnip	20.9 d	36.8 f
	Winter Mix	38.8 a	41.5 e
	Weedy check	18.5 e	35.3 g
	Weed free check	30.8 b	40.4 e
	<i>Average</i>	<i>28.2</i>	<i>37.1</i>
Minimum	Austrian Winter Pea	5.0 h	54.7 a
	Oat	26.3 c	52.4 b
	Purple top Turnip	1.2 i	45.1 c
	Winter Mix	11.8 f	45.9 c
	Weedy check	12.4 f	53.8 a
	Alternative Weed Control	6.8 g	42.1 e
	<i>Average</i>	<i>10.6</i>	<i>49.0</i>

\*Alternative weed control with minimum-till plots refers to different experimental non-tillage weed control tactics implemented (including vinegar application, flaming, and mowing).

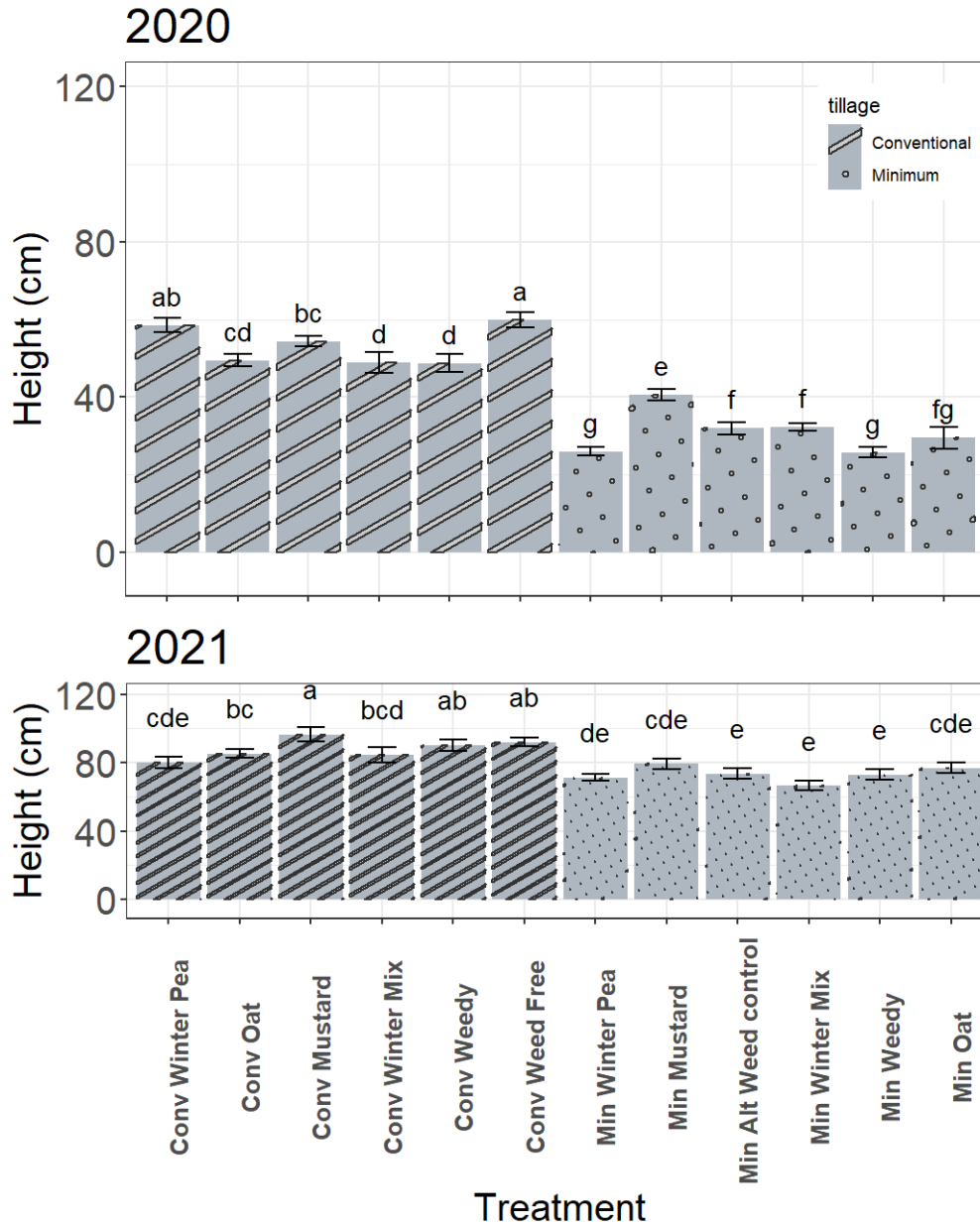
2020

2021



**Figure 1.** Weed seedling emergence in organic cotton as influenced by tillage and tillage \* cover crop interactions. Abbreviations: CT, conventional tillage; MT, minimum tillage. Alternative weed control with minimum-till plots refers to different experimental non-tillage weed control tactics implemented (including vinegar application, flaming, and mowing).

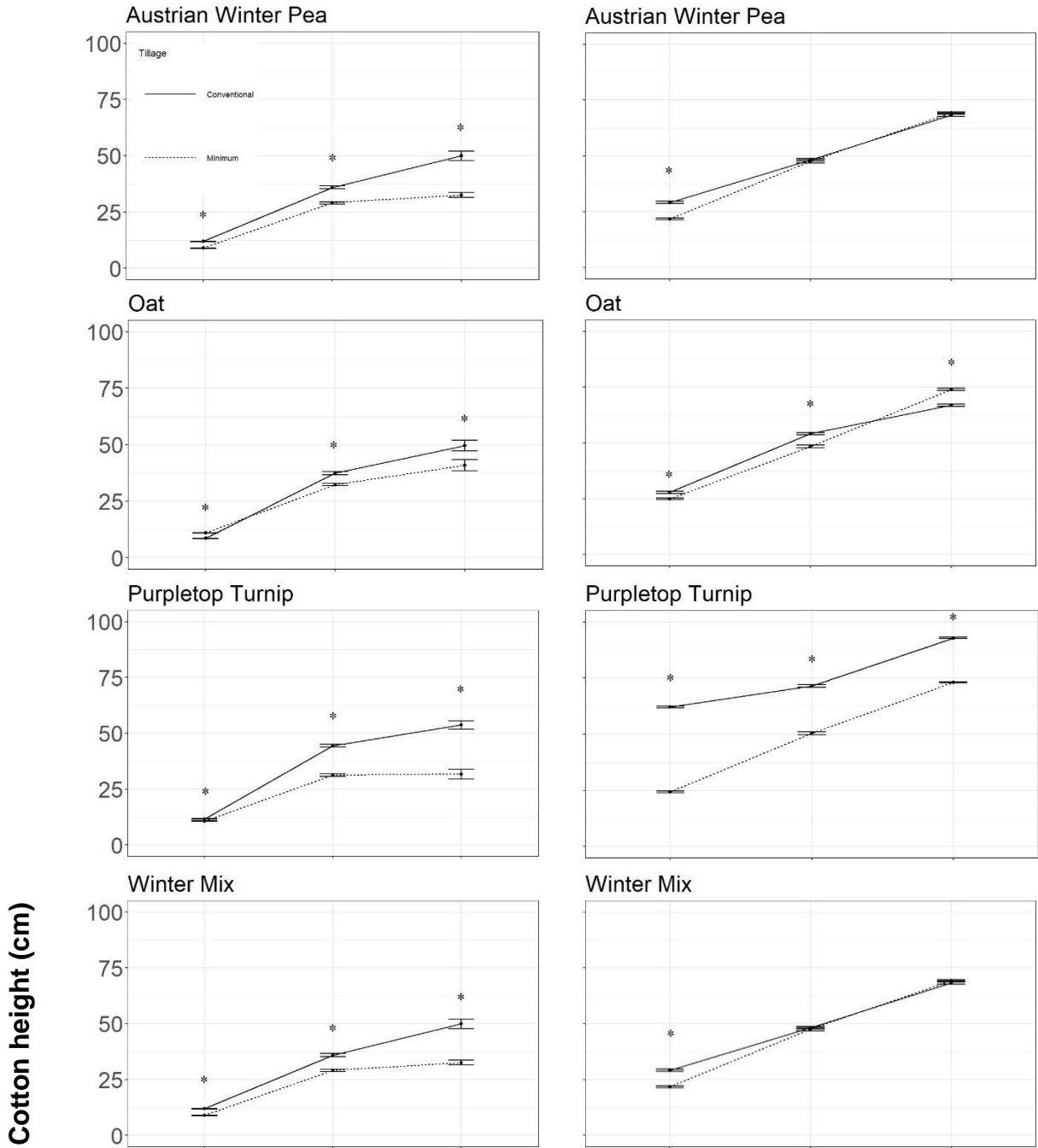


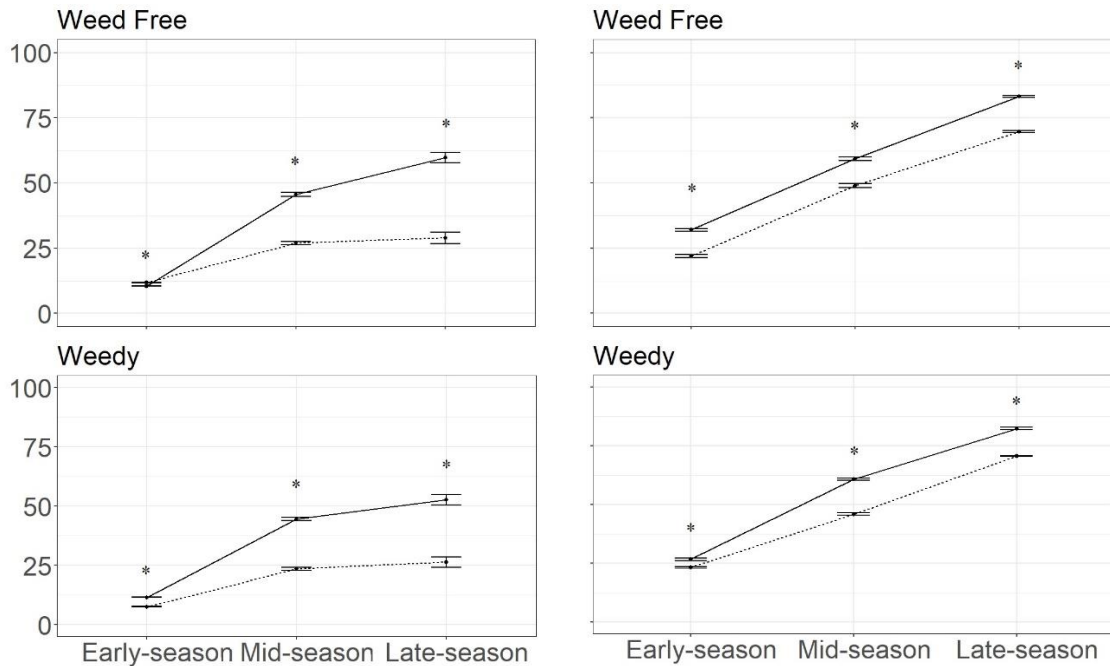


**Figure 2.** Height of cotton plants (cm) as influenced by different cover crop and tillage treatments observed during the flowering stage on August 5, 2020, and during flowering on August 3, 2021. Different letters above the standard error bars indicate significant differences based on the Fisher’s protected Least Significant Difference (LSD) test ( $\alpha=0.05$ ). Abbreviations: CT, conventional tillage; MT, minimum tillage. Alternative weed control with minimum-till plots refers to different experimental non-tillage weed control tactics implemented (including vinegar application, flaming, and mowing).

2020

2021



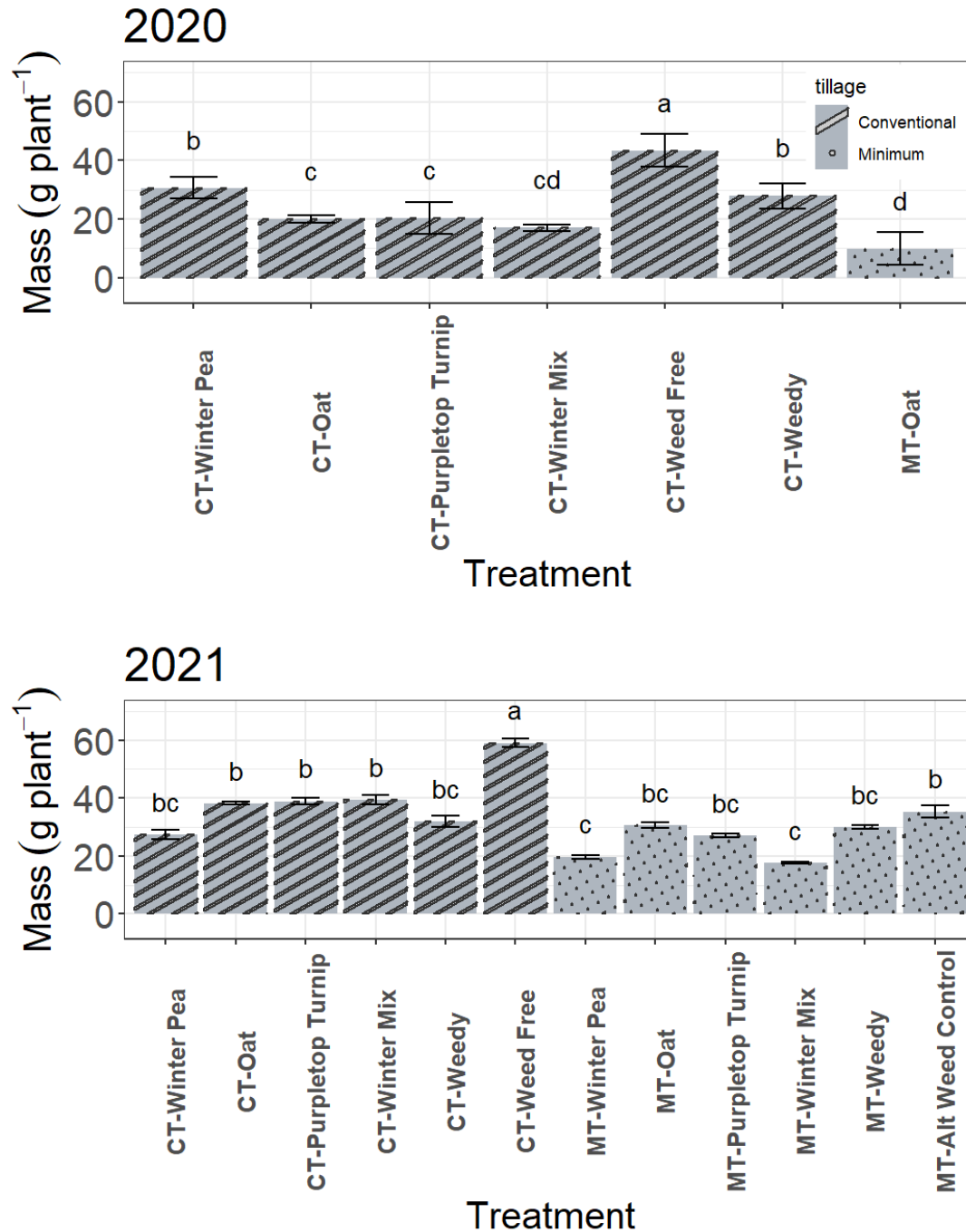


**Figure 3.** Progression of cotton height in 2020 and 2021 from early to mid and late season, separated by treatment and year. The Weed Free minimum tillage treatment in 2020 was an Alternative Weed Control treatment and these alternative weed control measures were unable to keep the plots truly weed free. In 2021 the Weed Free minimum tillage treatment was kept weed free with the conventional tillage treatment.

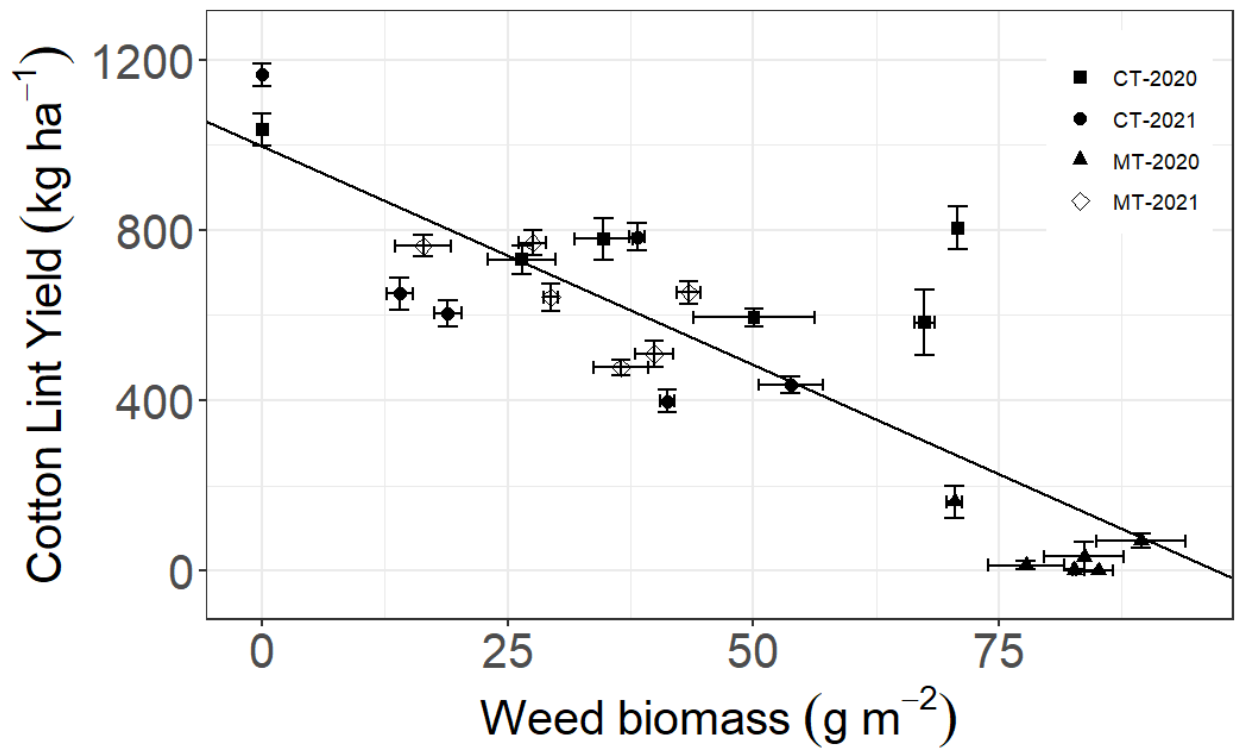
2020 Early-season: June 15 (14 days after cotton planting, DAP), Mid-season: July 22 (51 DAP), Late-season: August 5 (65 DAP).

2021 Early-season: July 5 (10 DAP), Mid-season: August 3 (39 DAP), Late-season: September 9 (76 DAP).

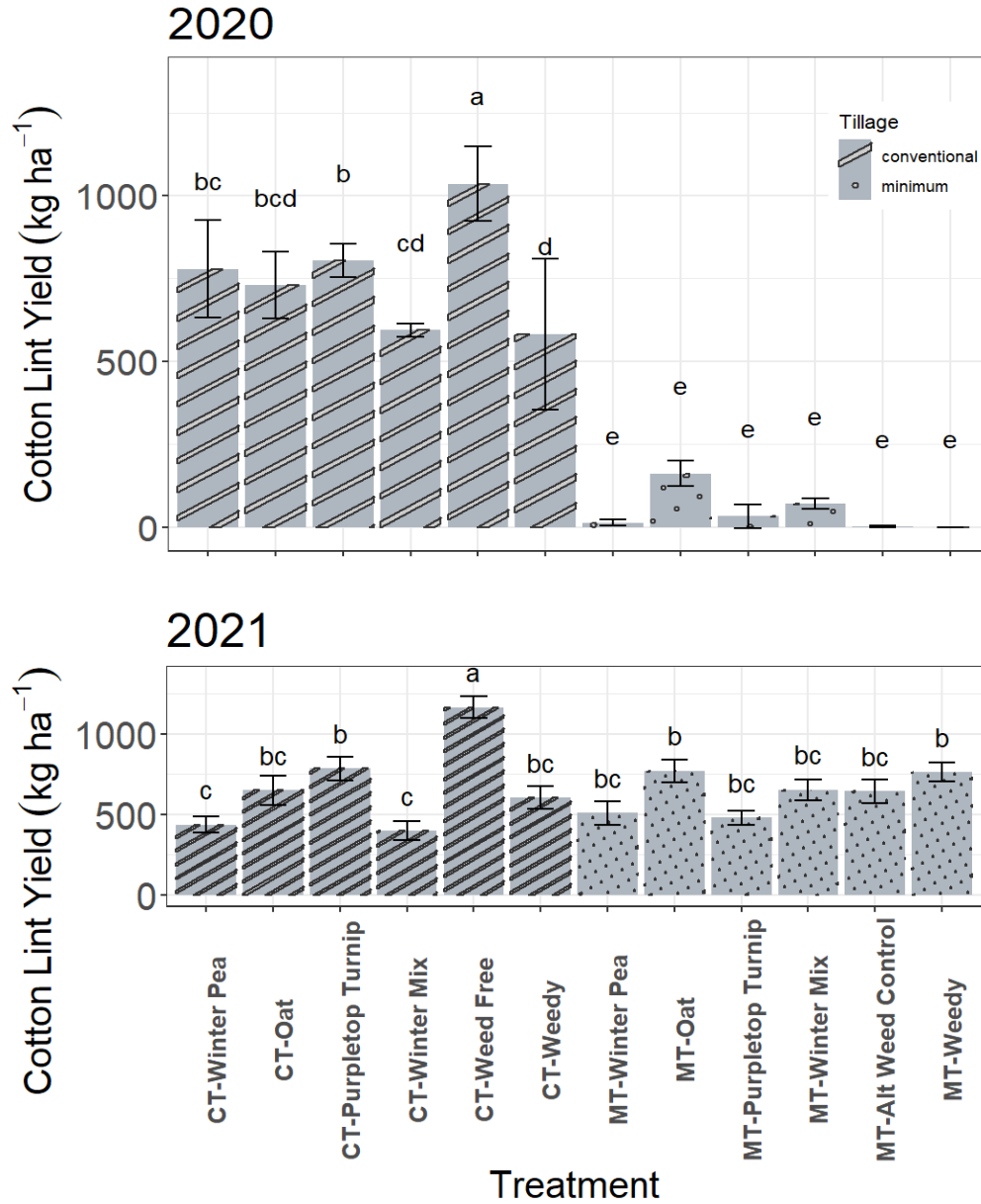
\*Indicates a significant difference between tillage treatments for a given observation timing, determined using a 2-sample Student's t-test.



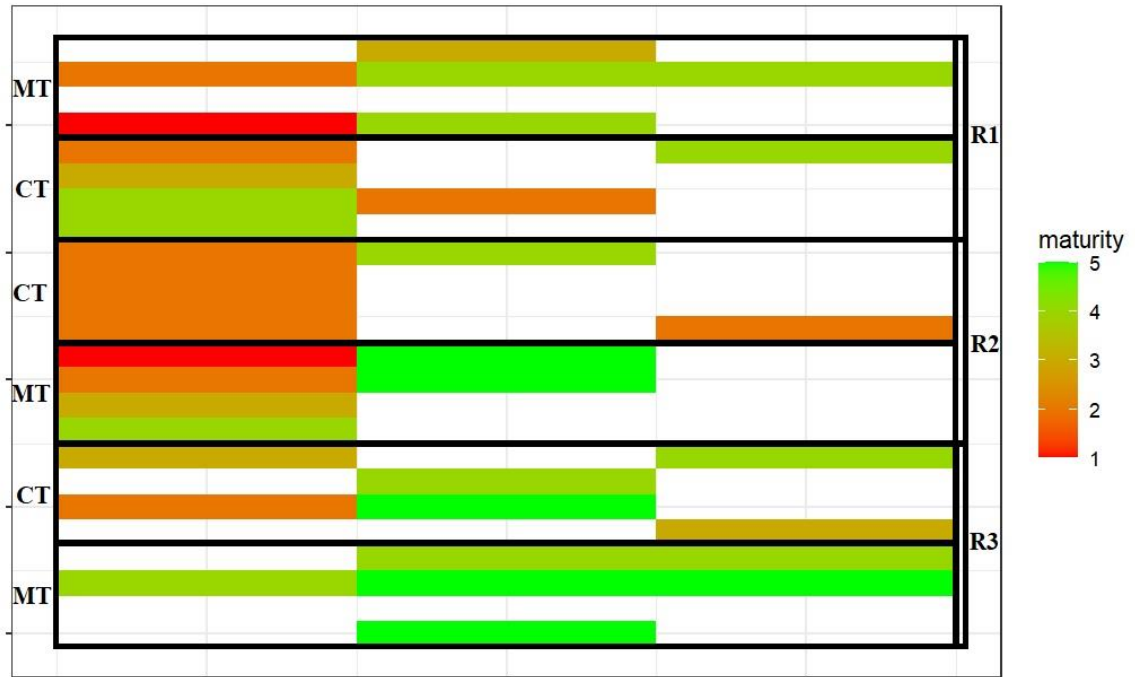
**Figure 4.** Biomass production of organic cotton plants as influenced by different cover crop and tillage treatments, observed during cotton flowering in 2020 and 2021. Different letters above the standard error bars indicate significant differences based on the Fisher's protected Least Significant Difference (LSD) test ( $\alpha=0.05$ ). Abbreviations: CT, conventional tillage; MT, minimum tillage. Alternative weed control with minimum-till plots refers to different experimental non-tillage weed control tactics implemented (including vinegar application, flaming, and mowing).



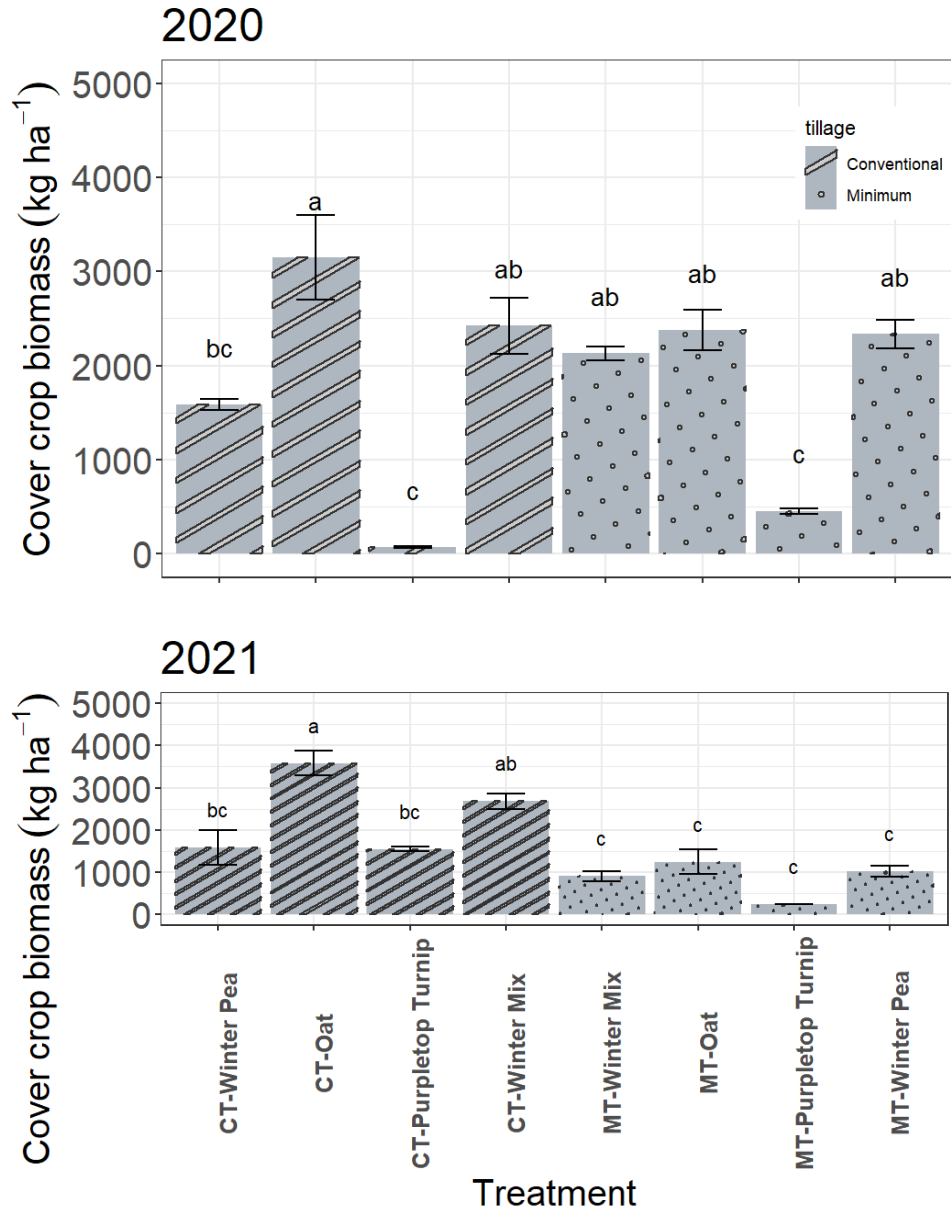
**Figure 5.** Organic cotton lint yield (kg ha<sup>-1</sup>) relative to weed biomass production (g m<sup>-1</sup>) at the time of cotton harvest for 2020 and 2021 growing seasons. The composite linear regression equation was ( $y = -10.262x + 996.945$ ) and adjusted  $r^2 = 0.71$ . Abbreviations: CT, conventional tillage; MT, minimum tillage.



**Figure 6.** Organic cotton lint yield (kg ha<sup>-1</sup>) as influenced by different cover crop and tillage treatments in 2020 and 2021. Different letters above the standard error bars indicate significant differences based on the Fisher's protected Least Significant Difference (LSD) test ( $\alpha=0.05$ ). Abbreviations: CT, conventional tillage; MT, minimum tillage. Alternative weed control with minimum-till plots refers to different experimental non-tillage weed control tactics implemented (including vinegar application, flaming, and mowing).



**Figure 7.** Maturity differences of organic cotton plots across the experimental site observed on October 28, 2021. A maturity rating of 5 was assigned to plots ready to be defoliated and harvested, and a rating of 1 was assigned to plots with cotton plants actively flowering. Abbreviations: CT, conventional tillage; MT, minimum tillage.



**Figure 8.** Cover crop biomass (kg ha<sup>-1</sup>) as influenced by different cover crop and tillage treatments observed before termination in the springs of 2020 and 2021. Different letters above the standard error bars indicate significant differences based on the Fisher's protected Least Significant Difference (LSD) test ( $\alpha=0.05$ ). Abbreviations: CT, conventional tillage; MT, minimum tillage. Alternative weed control with minimum-till plots refers to different experimental non-tillage weed control tactics implemented (including vinegar application, flaming, and mowing).





**Figure 9.** An oat cover crop in peak vegetative growth, one month prior to termination (Photo taken in February 2020).



**Figure 10.** An Austrian winter pea cover crop at peak vegetative growth, one month prior to termination (Photo taken in February 2020).



**Figure 11.** A purpletop turnip cover crop at peak vegetative growth, one month prior to termination (Photo taken in January 2022).



**Figure 12.** A cover crop mix plot (mix of oats, Austrian winter pea, and purpletop turnip) at peak vegetative growth, one month prior to termination (Photo taken in February 2020).



**Figure 13.** Cotton establishment compared between an MT plot (left) and CT plot (right) (Photo taken on July 2, 2020).

## **CHAPTER III: EVALUATION OF LIVING MULCH SPECIES FOR WEED SUPPRESSION IN COTTON**

### **3.1 Introduction**

A living mulch is an annual or perennial plant species purposely grown between the rows of a cash crop during the growing season to offer a multitude of benefits (Hartwig & Ammon, 2002; Leary & DeFrank, 2000; Paine & Harrison, 1993). Advantages of living mulches over killed cover crops may include improvements in weed suppression, erosion and leaching control, soil health, and resource-use efficiency (Bhaskar et al., 2021; Hartwig & Ammon, 2002; Leary & DeFrank, 2000). Living mulches, as opposed to intercrops, are not meant to be harvested, and because of this, have the potential to outperform the intercrops (Liebman & Dyck, 1993). Living mulches can have powerful positive effects on the agroecosystem. Leguminous living mulches can increase biological nitrogen fixation in symbiosis with rhizobacteria and reduce the leaching of nutrients such as nitrates (Power, 1987; Andrews et al., 2020). Several living mulch species are reported to exude useful nitrogen to the crop after termination (Grubinger & Minotti, 1990).

Living mulches, if selected wisely, can help suppress weeds and support cash crop growth for an extended period (Nyakatawa et al., 2000). They can lower herbicide inputs in an integrated weed management plan and improve soil moisture retention (Hartwig & Ammon, 2002; Leary & DeFrank, 2000; Paine & Harrison, 1993). Living mulches can significantly reduce weed growth through competition (Liebman et al., 2001), and thereby reduce the need for destructive tillage (Elkins et al., 1983). Living mulches suppress weeds through high biomass production (Bhaskar et al., 2021). They may also negatively impact weed growth through

allelopathy, competition for light and shading, and root competition for water and nutrients in the inter-row spaces (Mediene et al., 2011; Petit et al., 2018; Teasdale, 1996).

In a study conducted in sweet corn, white and ladino clover living mulch species have improved sweet corn yields by 75% compared to the no living mulch plots (Vrabel et al., 1981). However, the ability of the living mulch to suppress weeds may also be tied to lowering crop yields and overall growth (Teasdale, 1996). A leguminous living mulch in winter wheat decreased crop yields by about 70% (White & Scott, 1991), while under rainfed conditions, an alfalfa living mulch in corn caused yield losses up to 96% (Eberlein et al., 1992). Thus, terminating living mulches may be necessary in some crops in order to avoid significant yield losses (Nicholson & Wien, 1983). A perennial living mulch may not begin vigorous growth (Flynn et al., 2013), whereas annual species are ideally quick to establish (De Haan et al., 1994). Perennial living mulches, however, may be generally difficult to terminate with herbicides especially when the living mulch ages (Cardina & Hartwig, 1980). Annual living mulches give the grower more control over planting time and can be terminated before seed production to prevent volunteer plants in the next growing season (Teasdale, 1996).

Selecting suitable living mulches with specific biological differences from crops and weeds allows for successful management (De Haan et al., 1994; Liebman et al., 2001; Verret et al., 2017). Early-season ground cover establishment is important for suppressing early-season weed growth (Nicholson & Wien, 1983; Hatfield et al., 1998). Keeping the living mulches short allows for competition with weeds instead of the main crop (De Haan et al., 1994; Echtenkamp & Moomaw, 1989; Leoni et al., 2020). Grasses, for example, may be easier to manage than tall-growing broadleaves (Elkins et al., 1983). Legumes can fix nitrogen for crops, but the actual ability of the crop to utilize that nitrogen can vary (Germeier, 2000; Hartwig & Ammon, 2002;

Liebman & Davis, 2000; Sanders et al., 2017; Triplett, 1962). Living mulch mixes composed of multiple species may be preferable over single species since functional diversity may improve weed suppression. However, living mulch mixes may be more challenging to manage agronomically than a single species (Liebman & Dyck, 1993; Echtenkamp & Moomaw, 1989; Hartwig & Hoffman, 1975).

Amongst several factors, response to water stress is an important criterion for selecting living mulch species. A drought-sensitive crop and drought-tolerant living mulch, for example, has shown to decrease crop yields (Ziyomo et al., 2013). Corn hybrids with lower drought-tolerance had lower yields with a persisting living mulch kura clover (*Trifolium ambiguum* M. Bieb) than with a terminated living mulch (Ziyomo et al., 2013). Difference in crop varieties, for example dwarf versus conventional barley, influences the interaction of barley with a medic living mulch species (Moynihan et al., 1996; Uchino et al., 2016).

Living mulch planting date is a critical consideration, especially when dealing with crops sensitive to competition. Delaying the living mulch planting may minimize their competitive interactions with the crop, but it allows the weeds more time to grow (Liebman et al., 2001). In corn and soybeans, delaying the living mulch planting resulted in lower yield losses than planting on the same day (Brooker et al., 2020; Uchino et al., 2009; Vrabel et al., 1980; Wivutvongvana, 1973). A rye living mulch planted 10 and 20 days after transplanting broccoli neither impacted weeds nor broccoli yields, while planting at the same time significantly lowered both (Brainard & Bellinder, 2004).

Limited research has been done investigating living mulches in cotton production. This project provides a baseline assessing multiple species as living mulches in cotton in order to narrow the scope of viable living mulch species to be investigated further. The specific objective



of this project was to assess several candidate living mulch species and mixes for their usefulness in weed suppression and impact on cotton yield.

## **3.2 Materials and Methods**

### **3.2.1 Field Experiment**

#### *Study location and experimental setup*

This experiment was conducted at the Texas A&M University Research Farm in Burleson County, Texas, in the summer of 2021. The climate was subtropical humid, with most rainfall occurring in the winter and spring. The soil in the field was a Belk clay (fine, mixed, active, thermic Entic Hapludert). The mean annual temperature for this location is 20.6 °C and the average annual precipitation is 1018 mm.

This experiment was conducted in a completely randomized design with three replications. The plot factor was the living mulch species (twenty-two levels, listed in Table 4), as well as a weed-free check and a weedy check. Each plot was 24.4 meters long and included three rows of cotton with an inter-row spacing of 76 cm. Living mulch species were planted between cotton rows in the three inter-row spaces. The planting rates for each living mulch species are listed in Table 4. All seed were sourced from Justin Seed Co., except Peanut Schubert and Peanut GA-09B, which were sourced from the Texas A&M Peanut Breeding program (Courtesy: Dr. John Cason, Stephenville, TX).

Living mulch species were selected for a variety of characteristics: cold-season plants such as slender creeping red fescue were selected because they were expected to grow quickly at the start of the season, then reduce the rate of growth or self-terminate as the summer temperatures rise. Other species such as cowpea were chosen for their high biomass production

potential, while the mixes were based on summer cover crop mixes that had previously proven to be effective in biomass production in other studies.

### *Field management*

This experiment was conducted as a conventionally managed study. On April 20, 2021, there was a tank mix application of glyphosate (Cornerstone) and glufosinate (Liberty) at the rates of 2340 mL ha<sup>-1</sup> and 2120 mL ha<sup>-1</sup> (commercial product), respectively as a burn-down application before planting cotton. The field was tilled to uniformity on April 26, 2021, and LibertyLink DP 2020 B3XF cotton was planted into a clean field on April 28, 2021. An additional tank mix application of glyphosate and glufosinate at the same rates as above was made one week before living mulch planting on June 8, 2021. Living mulches were planted 5 weeks after cotton planting on June 14, 2021 at the rates listed in Table 3 using an adjustable Great Plains Native Grass SERIES II grain drill. Twin row treatments received the same overall seeding rate as the corresponding single row treatments of the same species. No insect or disease management was necessary. Cotton was terminated on October 8, 2021, with an application of paraquat as a defoliant at a rate of 2340 mL ha<sup>-1</sup> (commercial product). An additional application of paraquat was made at the same rate on October 20, 2021, to control high weed pressure in some plots. Cotton was harvested by hand on October 28, 2021, by harvesting all cotton from three randomly selected 10-foot sections of row in each plot.

### *Data collection*

Living mulch species, weed growth, and cotton performance were observed for the following characteristics throughout the summer. Initial ground cover ratings were scored on

June 27 and July 7, 2021, in five 1 m<sup>2</sup> quadrats in each plot (15 total in each species) to assess the emergence and survival of the living mulches. This was done by assessing the ground cover on a 0-10 scale, with 0 being no ground cover or emergence and 10 being 100% of the ground covered by living mulch. Weed cover was not factored into ground cover assessments.

Light measurements were taken using a ceptometer (ACCUPAR LP-80) to measure the plant available radiation (PAR) above the canopy, 15 cm below the top of the cotton plants above the living mulch, and at the ground level below the living mulch. These measurements were replicated three times in each plot. This was done every two weeks starting on July 2, 2021, two weeks after living mulch planting. This was replicated again on July 16, July 30, August 13, and August 27, for a total of five observations.

Weed biomass as well as living mulch biomass were collected on July 20 and September 6, 2021. This was done by collecting all biomass from one quadrat of 0.75 m<sup>2</sup> in each plot, separated by living mulch species and weeds, drying at 60°C for 72 hours, and then weighing. Cotton was harvested on October 28, 2021, by hand. Three 10-foot sections of each plot were hand-harvested. The seed cotton was dried at 60°C for 72 hours, ginned using an Eagle tabletop gin, and the lint and seed yields were calculated.

### **3.2.2 Crop-living Mulch Competition in the greenhouse**

The purpose of this experiment was to explore competition dynamics between cotton and living mulch species in more detail, in a controlled environment without influence from weeds and other environmental factors. The experiment was designed to minimize the differences in soil nutrient availability, water content, and other typical differences across an agricultural field

that are normally present in a field experiment. This experiment augments the living mulch field experiment by more deeply investigating the competition between cotton and living mulches.

*Experimental setup*

This experiment was conducted at the Texas A&M University Southern Crop Improvement Greenhouse Facility in College Station, Texas, between January and April 2021 and repeated in the spring of 2022, in a replacement-series design (Hart et al., 1992). Cotton and individual living mulch species (16 species) were planted in pots in five ratios: 0:4, 3:1, 2:2, 1:3, and 4:0, as shown in Figure 14. The living mulch species included in the study were: alfalfa, berseem clover, buckwheat, cantaloupe, cowpea (var. Ace, Mississippi silver), cucumber, Japanese millet, peanut (var. GA-09B, Schubert), rye, red fescue (var. slender creeping), soybean, white clover, and zucchini. The cotton cultivar used was LibertyLink DP 2020 B3XF. The experiment had four replications, thus totaling 20 pots for each living mulch species-cotton combination.

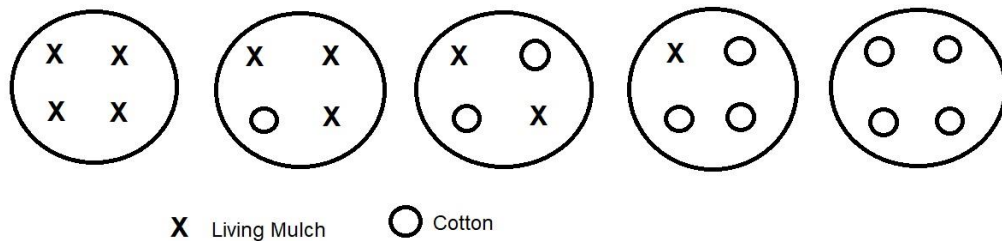


Figure 14. Arrangement of living mulch and cotton plants in the pots at five ratios in the replacement series experiment.

The experiment was conducted over a 10-week period using 7.6-liter pots (20 cm diameter x 25 cm height), filled with potting mix (Pro-Mix LP15 multipurpose). Plants were irrigated daily as needed and fertilized uniformly across all pots three times with Miracle-Gro

all-purpose plant food (24-8-16) at four, six, and eight weeks after the initiation of the study. Seed was sourced from the same source as the living mulch field experiment, Justin Seed Co. Seeds were planted directly into the potting soil and thinned to 1 plant in each hill 1 week after plant emergence. Pots were irrigated using automated irrigation and maintained soil water levels appropriate for plant growth.

#### *Data collection*

Plant height was recorded at four and ten weeks after planting by measuring the height of individual plants in each pot. Height was measured from the soil surface to the top of the plant. Aboveground biomass for each species was collected at harvest (10 weeks after planting) by removing the entire plant from the base at the soil level, followed by drying at 60°C for 72 hrs and weighing.

#### *Data analysis*

Data analysis was conducted in R software version 4.1.0. Data normality was checked using the qqnorm function in the base R package and no transformation was required. ANOVA was used to check for treatment effects and significant differences using the ANOVA function in the base R package and the linear model (lm) function in the base R package. Tukey's HSD and Fisher's protected LSD ( $\alpha = 0.05$ ) were used for mean separation and comparison of individual treatment means in the agricolae package.

Relative biomass yield was calculated following Bagavathiannan et al. (2011) and Hart et al. (1992). The relative biomass yield of each living mulch species and cotton under each planting ratio was calculated using the following equations:

Relative biomass yield (RY):

$$RY(C) = P \left( \frac{C_{mix}}{C_{mono}} \right) \quad [1]$$

$$RY(L) = (1 - P) \left( \frac{L_{mix}}{L_{mono}} \right) \quad [2]$$

where  $RY(C)$  and  $RY(L)$  are the relative biomass yields of the cotton and living mulch plants,  $P$  is the proportion of the species in mixture,  $C_{mix}$  and  $L_{mix}$  are the biomass totals of cotton and living mulch in mixture, and  $C_{mono}$  and  $L_{mono}$  are the biomass totals of cotton and living mulch in monoculture (Bagavathiannan et al., 2011; Hart et al., 1992).  $RY$  and  $RYT$  were determined for the planting proportions of 3:1, 2:2, and 1:3.

Relative biomass yield Total (RYT):

$$RYT = RY(C) + RY(L) \quad [3]$$

### 3.3 Results and Discussion

#### 3.3.1 Field Experiment

##### *Establishment and ground cover*

Among the 22 different living mulch treatments, nine species namely twin-row alfalfa, twin-row berseem clover, single-row flax, twin-row flax, twin-row peanut GA-09B, twin-row peanut Schubert, twin-row slender creeping red fescue, twin-row tall fescue, and zucchini, failed to establish in the study, and therefore were excluded from data analysis (Figure 15). There were significant differences in ground cover ratings among the different species ( $P < 0.001$ ). The five living mulch treatments with the highest ground cover ratings recorded on June 27, 2021 (on a scale of 0-10) were Mix 2 (10), twin-row Japanese millet (9.33), twin-row buckwheat (9), Mix 3 (9), and cowpea (8) (Figure 24) ( $P < 0.001$ ). The twin-row soybean (2.67) and peanut GA-09B (2.33) were the poorest performers in the 2021 experiment. Groundcover is an important metric for living mulch success because it influences weed suppression. Weed cover can be limited to 7% in instances when living mulches establish quickly and aggressively (Bhaskar et al., 2018). Living mulches with better ground cover typically suppress weeds more effectively (Ateh & Doll, 1996).

There was no significant difference in ground cover between species planted in twin rows and single rows ( $P > 0.05$ ), except soybean that produced greater ground coverage in single rows (5.33) compared to twin rows (2.5). It should be noted that these ratings are on a scale of 0 – 10, and so a rating of 5 corresponds to 50% groundcover. Peanut twin-row treatments did not establish successfully while their single row counterparts showed satisfactory establishment and ground cover (Figure 26). This could be a new avenue to investigate in the future.

The nine species that did not establish in College Station conditions were unable to survive the intense heat at the time of planting. In the spring of 2021, heavy rains delayed the planned living mulch planting at three weeks after cotton planting to June 14, 2021, which could be attributed to unsatisfactory performance of a few living mulch species in the study. Buhler et al. (2001) reported that sava medic [*Medicago scutellata* (L.) Mill.] living mulch did not perform satisfactorily when terminated 30 d after planting in Iowa. Although annual living mulches give the grower more control over planting and termination times, their establishment and performance are sensitive to these timings (Bhaskar et al., 2021; Gibson et al., 2011).

Groundcover is an important metric for living mulch and cover crop success because it influences weed suppression and crop yield. Weed cover can be limited to 7% in instances when living mulches establish quickly and aggressively (Bhaskar et al., 2018). Living mulches with better ground cover typically suppress weeds more effectively; however, there may be potential risks of crop-living mulch competition that can reduce crop yield (Ateh & Doll, 1996; Mohammadi, 2012). To facilitate widespread use of suitable living mulch across cropping systems, breeding efforts are required (Moore et al., 2019) for developing living mulch candidates based on the proposed living mulch ideotypes (Buhler et al., 1998; Flynn et al., 2013)

### *Living mulch and weed biomass*

There were significant influences of living mulch species on weed biomass production ( $P < 0.001$ ) (Figure 16). The weedy check produced the most weed biomass ( $35 \text{ g m}^{-2}$ ) ( $P < 0.05$ ) during mid-season. Single-row soybean ( $33 \text{ g m}^{-1}$ ) and twin-row soybean ( $24 \text{ g m}^{-1}$ ) produced the next highest weed biomass, followed by twin-row cowpea ( $16 \text{ g m}^{-1}$ ), twin-row buckwheat ( $14 \text{ g m}^{-1}$ ), Mix 3 ( $13 \text{ g m}^{-1}$ ), Mix 4 ( $12 \text{ g m}^{-1}$ ), buckwheat ( $11 \text{ g m}^{-1}$ ) (Figure 25), and cowpea-



Mississippi Silver ( $8 \text{ g m}^{-1}$ ) (Figure 16). The five treatments that produced the least weed biomass at the mid-season observations were Mix 1 ( $5 \text{ g m}^{-1}$ ), Mix 2 ( $4 \text{ g m}^{-1}$ ), peanut Schubert ( $3 \text{ g m}^{-1}$ ), peanut GA-09B ( $1 \text{ g m}^{-1}$ ), and twin-row Japanese millet ( $0.3 \text{ g m}^{-1}$ ) (Figure 16).

Mix 3 produced the highest living mulch biomass ( $1294 \text{ kg ha}^{-1}$ ) ( $P < 0.05$ ) (Figure 22). The mix 2 ( $853 \text{ kg ha}^{-1}$ ) and mix 3 ( $1294 \text{ kg ha}^{-1}$ ) were very competitive with weeds, as they produced the most living mulch biomass and relatively low weed biomass. The mix 2 also produced the second greatest living mulch biomass ( $853 \text{ kg ha}^{-1}$ ), while being below average in weed biomass production. Some of the lowest overall biomass production was observed in peanut Schubert, Mix 1, peanut GA-09B, and Japanese millet plots (Figure 22).

Results show that there are significant impacts of living mulch species selection on weed biomass production, and potentially on weed seed production, as reported by Wilson et al. (1995). Lowering weed seed production is vital for the long-term success of agricultural systems so that weed populations are manageable. Single and twin row soybean treatments produced statistically equal weed biomass to the weedy check. The reasons for the apparent lack of weed suppression in soybean plots is unclear, but may be attributed to low initial establishment and groundcover in soybean (5.33) and twin row soybean (2.67) treatments. The competition between living mulch and weeds can reduce living mulch biomass, but the effect can vary among the living mulch species (Enache and Ilnicki, 1990).

### *Cotton yield*

Living mulches significantly influenced the cotton lint yield ( $P < 0.001$ ) (Figure 17). Cowpea Mississippi Silver ( $3459 \text{ kg ha}^{-1}$ ) and soybean ( $3341 \text{ kg ha}^{-1}$ ) performed similarly to the weed-free check ( $3186 \text{ kg ha}^{-1}$ ). Cotton lint yield in legume plots were similar to that of the

weed-free check plots (Figure 17). This data suggests that leguminous living mulches may negate the effect of competition on cotton's lint yield, possibly due to their nitrogen-fixing ability (Germeier, 2000). As cotton growth and yield are highly sensitive to weed competition (Papamichail et al., 2002), results of this study suggest that leguminous living mulches have significant potential in cotton. The mix 3, the most competitive mix of living mulches, produced low cotton lint yields at the end of the season (1588 kg ha<sup>-1</sup>).

There was no significant relationship between cotton lint yield and living mulch biomass (Figure 18) or weed biomass (Figure 19). Mix 3 produced the greatest living mulch biomass (1293 kg ha<sup>-1</sup>) among the treatments. The top four treatments in terms of cotton yield were cowpea Mississippi Silver, soybean, peanut GA-09B, and Mix 1. Soybean produced the highest amount of weed biomass (2034 kg ha<sup>-1</sup>), but also resulted in the second highest cotton lint yield (Figure 19). It is unclear why these plots produced both high cotton yields and high weed biomass but may be connected to the biological nitrogen fixation capabilities of soybean, but these relationships were not specifically investigated in this study and would require further research. Our results corroborate with the study by Bhaskar et al. (2018), who found that in dry years living mulch biomass negatively influenced cotton yield, but in growing seasons with ample soil moisture, living mulch biomass and cotton yield had a positive relationship.

### *Light interception*

Light interception increased significantly from early to mid-season and had minimal changes from mid to late season (Figures 20 and 23). All treatments allowed more than 95% light interception at the midseason of cotton growth, from above the cotton canopy to the ground level (Figure 20) and by the end of the season, all treatments had nearly 100% light interception

from above the canopy to the ground level (Figure 20). Twin-row Japanese millet (99%) and Mix 3 (98.5%) (Figure 27) had the highest light interception (Figure 21). These treatments differed from studies such as Eberlein et al. (1992), who found that alfalfa living mulch in a corn production system had a negative trend of light interception through the growing season, but in that case the living mulches were subjected to herbicidal suppression.

Figure 23 shows the difference in light interception for each plot on July 2, 2021. The treatments with the highest light interception at the ground level were the weedy check (95.7%), Mix 3 (95.6%), Mix 1 (95.3%), Mix 2 (94.4%), and twin-row Japanese millet (93.8%) (Figure 23). Treatments with the lowest light interception included the weed-free check (83.3%), soybean (86.6%), and twin-row buckwheat (91%) (Figure 23) ( $P < 0.05$ ). Figure 20 shows treatment differences for light interception, even as early as 1 month after living mulch planting, when cotton began to close the canopy. High light interception by living mulch can in turn suppress seed production in weeds (Gibson et al., 2017).

The Mix 3 produced high living mulch biomass, high light interception, and relatively low weed biomass (Figures 21 and 22), but the yields were lower than average in general (Figure 17). Soybean produced high yields (Figure 17) but had low light interception (Figure 21) and high weed biomass (Figure 22). Differences in species and mix characteristics can explain these differences as Mix 3 was designed to be competitive with weeds, but could have also had a significantly negative effect on cotton growth, as cotton is very sensitive to competition. Soybean's lack of weed suppression may result in high weed seed production (Gibson et al., 2017), but higher yields are desired by farmers. Trade-offs such as these are important to consider when choosing and evaluating living mulches.

### 3.3.2 Crop-living Mulch Competition

#### *Relative biomass yield*

This study allowed for the isolation of competition between candidate living mulch species and cotton. These insights are valuable because of the lack of extracurricular factors such as variable weather, variable weed pressure, and the natural inconsistencies in agricultural fields. Controlling the irrigation, light, nutrient availability, and growing medium allows for the isolation of competition between cotton plants and candidate living mulch species. Investigating competition between candidate living mulch species and cotton is essential to evaluate those candidate species because of cotton's sensitivity to competition. This study dovetails with the field study by understanding the competitive effect of candidate living mulch species without interference of those factors present naturally in a field study mentioned previously. Isolating these effects allows researchers to understand each candidate species' effect on cotton and using those observations to inform decisions about living mulch species selection and weighing the usefulness of more or less competitive living mulch species. This study is limited, however, by its design to limit external influences. Competition and interspecies dynamics in an agricultural setting is not so simple as a greenhouse experiment. Outcomes in this experiment may not correlate directly to outcomes in an agricultural setting because of the influence of weather, competition with weeds, and inconsistencies in agricultural fields. This study is another tool to evaluate living mulch species and should not be used alone to select living mulch species for agricultural performance ability.

The aboveground cotton biomass was significantly influenced by living mulch species and the living mulch-cotton competition levels (Figure 28). Berseem clover, buckwheat, peanut GA-09B, peanut Schubert, slender creeping red fescue, white clover, and zucchini showed

significant differences among the competition ratios ( $P < 0.05$ ) (Figure 28). In the presence of alfalfa, berseem clover, cowpea Mississippi Silver, Japanese millet, peanut GA-09B, peanut Schubert (Figure 30), and white clover (Figure 31), cotton consistently outperformed the projected relative biomass yield, especially when cotton represented 25-50% of the mix. However, treatments such as buckwheat, cantaloupe, cucumber, rye, slender creeping red fescue, soybean, and zucchini did not allow satisfactory cotton growth.

Cowpea Mississippi Silver also had a net positive effect on cotton irrespective of proportion, while cowpea had a slight yield reduction ( $P < 0.05$ ) (Figure 28). Alfalfa and cotton performed well together at any proportion. Rye, soybean (Figure 29), peanut GA-09B, and cowpea Ace did not seem to impact cotton's growth significantly. Cantaloupe, buckwheat, Japanese millet, and zucchini had a negative effect on cotton growth. Similar negative impacts on crop growth were reported by Eberlein et al. (1992).

## **Conclusion**

Overall, twin row cowpea Mississippi Silver, soybean, and peanut GA-09B produced the best results with regards to highest cotton yields and lowest weed biomass production. These two together are strong indicators of long-term viability because of economic viability with yield and reducing or maintaining weed pressure with weed biomass and weed seed production contributing to the soil seedbank. Measurements such as light interception, weed biomass, and living mulch biomass production can be indicators of overall living mulch performance, but were not strong enough on their own to predict the performance and viability of living mulch species. Further research should investigate additional planting dates, including those dates closer to

cotton planting. Additional seeding rates and physical location in relation to the crop may also prove useful in living mulch management.

### 3.4 Tables and Figures

Table 3. Seeding rates (kg ha<sup>-1</sup>) used for different living mulch species in the 2021 field study.

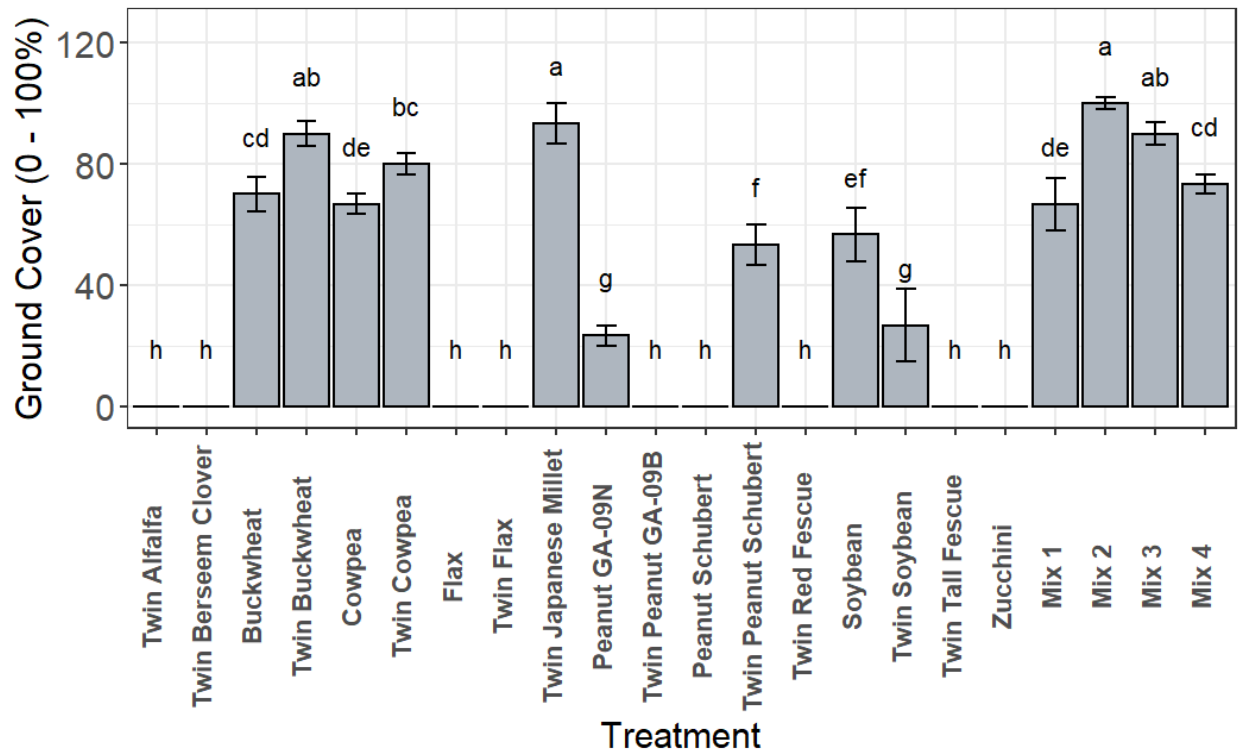
Living mulch species	Variety	Seeding rate (kg ha <sup>-1</sup> )
Alfalfa <sup>+</sup> ( <i>Medicago sativa</i> L.)	Ranger	13
Berseem clover <sup>+</sup> ( <i>Trifolium alexandrinum</i> )	BigBee	30
Buckwheat* ( <i>Fagopyrum esculentum</i> )	Common	53
Cowpea* ( <i>Vigna unguiculata</i> )	Mississippi Silver	60
Flax* ( <i>Linum usitatissimum</i> )	Culbert	20
Japanese Millet <sup>+</sup> ( <i>Echinochloa esculenta</i> )		34
Peanut* ( <i>Arachis hypogaea</i> )	GA-09B	65
Peanut* ( <i>Arachis hypogaea</i> )	Schubert	65
Slender Creeping Red Fescue+ ( <i>Festuca rubra</i> L. ssp. <i>littoralis</i> (G. Mey.) Auquier)	Boreal	45
Soybean* ( <i>Glycine max</i> )	DPL 415	50
Tall Fescue <sup>+</sup> ( <i>Festuca arundinacea</i> )	Dakota	50
Zucchini ( <i>Cucurbita pepo</i> )	Green Machine	1 plant 6 row feet <sup>-1</sup>
Mix 1 <sup>+</sup> (Broadleaf mix) Cowpea ( <i>Vigna unguiculata</i> ) (38 kg ha <sup>-1</sup> ); Sunn hemp ( <i>Crotalaria juncea</i> L.) (30 kg ha <sup>-1</sup> ); Sesame ( <i>Sesamum indicum</i> L.) (20 kg	Cowpea: ACE; Sunn hemp: Swastik (SUIN-053); Sesame: Paloma; Buckwheat: Common	108

ha <sup>-1</sup> ); Buckwheat ( <i>Fagopyrum esculentum</i> ) (20 kg ha <sup>-1</sup> )		
Mix 2 <sup>+</sup> (Grass-broadleaf mix) Sorghum-sudangrass ( <i>Sorghum x drummondii</i> ) (13 kg ha <sup>-1</sup> ); Pearl millet ( <i>Pennisetum glaucum</i> (L.) R. Br.) (15 kg ha <sup>-1</sup> ); Sesame ( <i>Sesamum indicum</i> L.) (20 kg ha <sup>-1</sup> ); Buckwheat ( <i>Fagopyrum esculentum</i> ) (25 kg ha <sup>-1</sup> )	Sorghum-sudan: Cadan WMR; Pearl millet: HGM 686; Sesame: Paloma; Buckwheat: Common	73
Mix 3 <sup>+</sup> (Grass-legume mix) Sorghum-sudangrass ( <i>Sorghum x drummondii</i> ) (13 kg ha <sup>-1</sup> ); Pearl millet ( <i>Pennisetum glaucum</i> L. R. Br.) (14 kg ha <sup>-1</sup> ); Cowpea ( <i>Vigna unguiculata</i> ) (38 kg ha <sup>-1</sup> ); Sunnhemp ( <i>Crotalaria juncea</i> L.) (30 kg ha <sup>-1</sup> )	Sorghum-sudan: Cadan WMR; Pearl millet: HGM 686; Cowpea: ACE; Sunnhemp: Swastik (SUIN-053)	95
Mix 4 <sup>+</sup> (Grass mix) Sorghum-sudangrass ( <i>Sorghum x drummondii</i> ) (13 kg ha <sup>-1</sup> ); Pearl millet ( <i>Pennisetum glaucum</i> (L.) R. Br) (14 kg ha <sup>-1</sup> ); German millet ( <i>Setaria italica</i> L.) (15 kg ha <sup>-1</sup> ); Proso millet ( <i>Panicum miliaceum</i> L.) (15 kg ha <sup>-1</sup> )	Sorghum-sudan: Cadan WMR; Pearl millet: HGM 686; German millet: Golden; Proso millet: Dawn	57

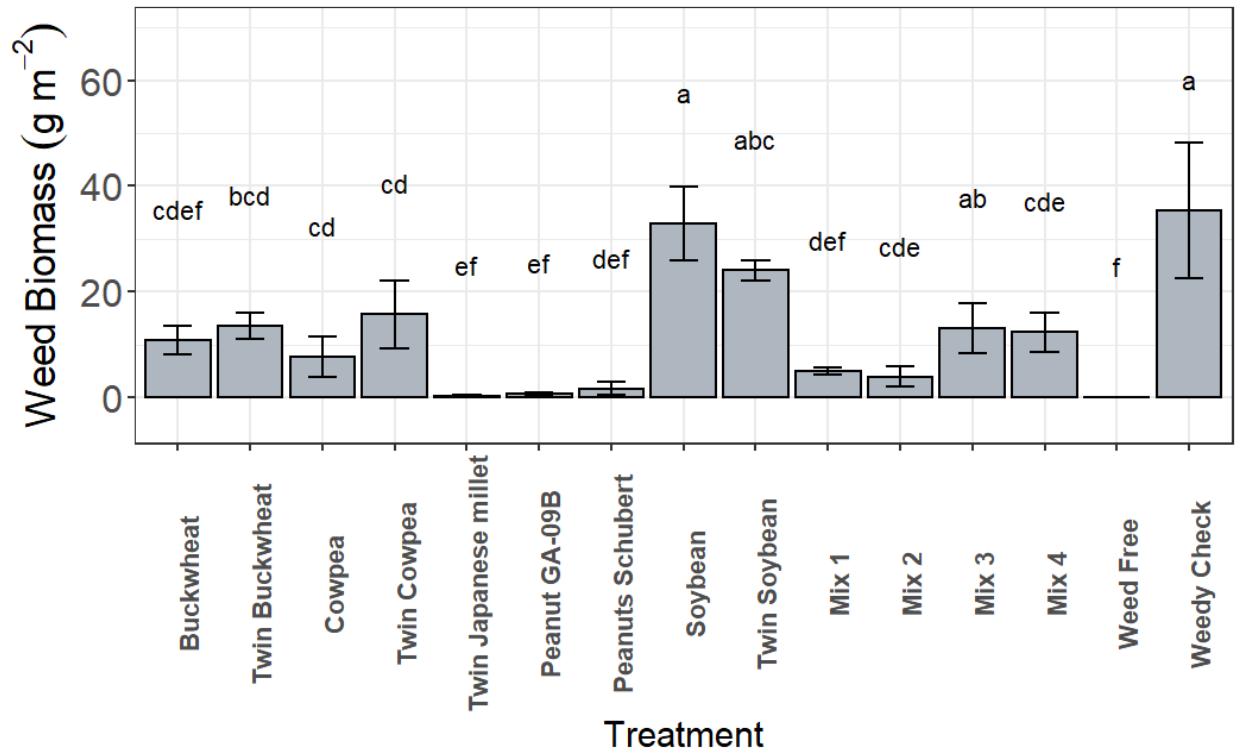


\*Living mulches were planted in both single-row and twin-row patterns; twin rows were planted at the same rate as the single rows.

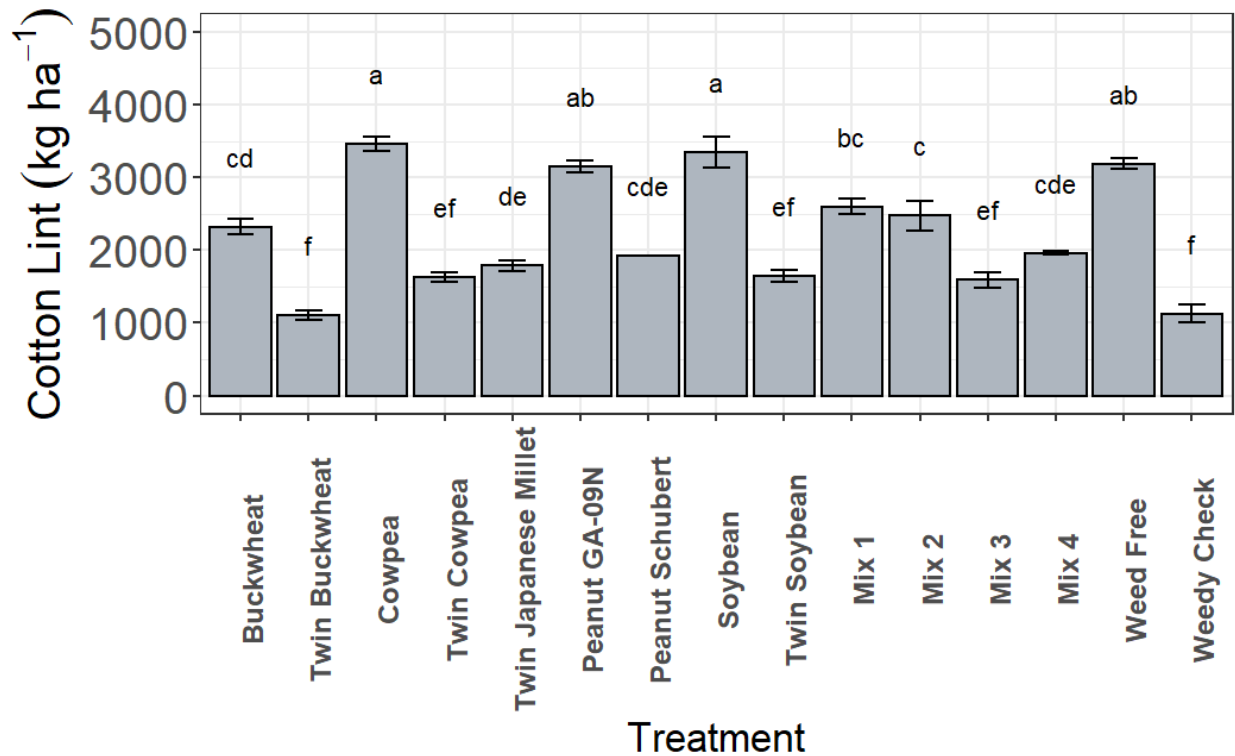
+Living mulches planted only in twin-rows.



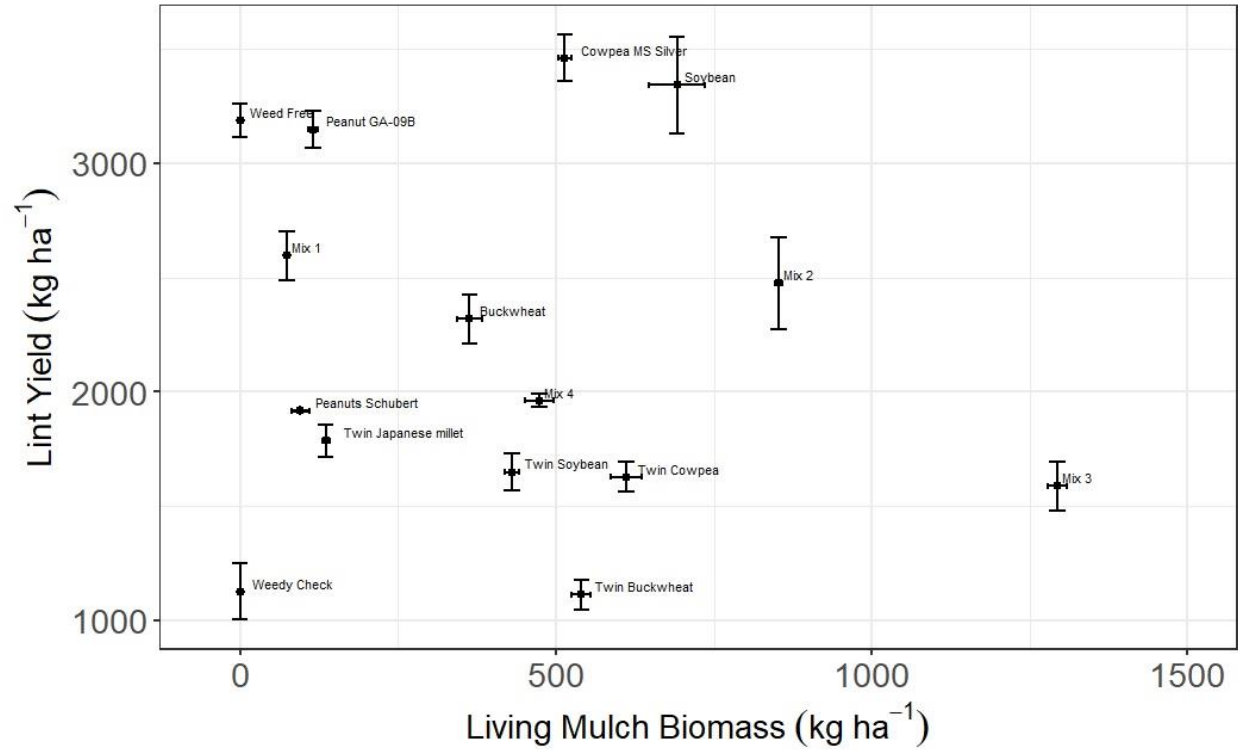
**Figure 15.** Groundcover (%) provided by different living mulch species observed on June 27, 2021, before the first square stage in cotton. A rating of 0 corresponded to no living mulch groundcover and 100 corresponded to living mulch completely covering the ground. Different letters above the standard error bars indicate significant differences based on the Fisher's protected Least Significant Difference (LSD) test ( $\alpha=0.05$ ).



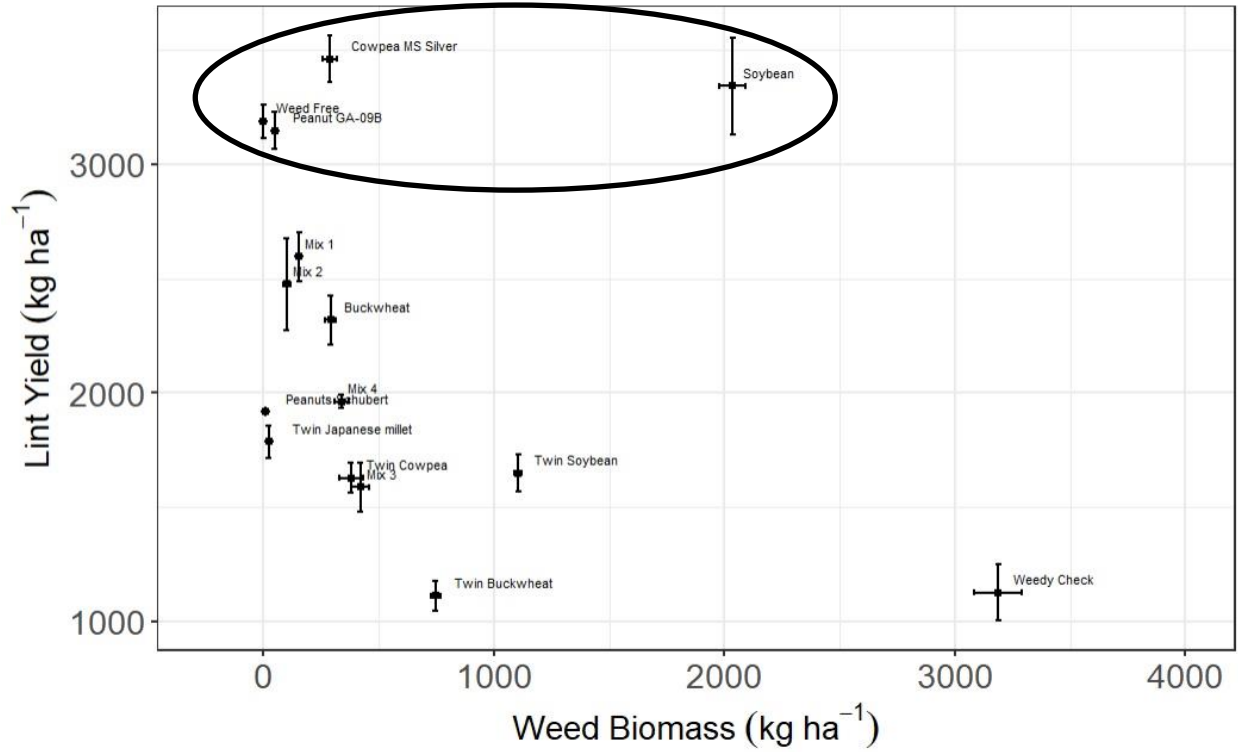
**Figure 16.** Weed biomass ( $\text{g m}^{-2}$ ) as influenced by living mulch treatment, observed on 20 July 2021. Different letters above the standard error bars indicate significant differences based on the Fisher's protected Least Significant Difference (LSD) test ( $\alpha=0.05$ ).



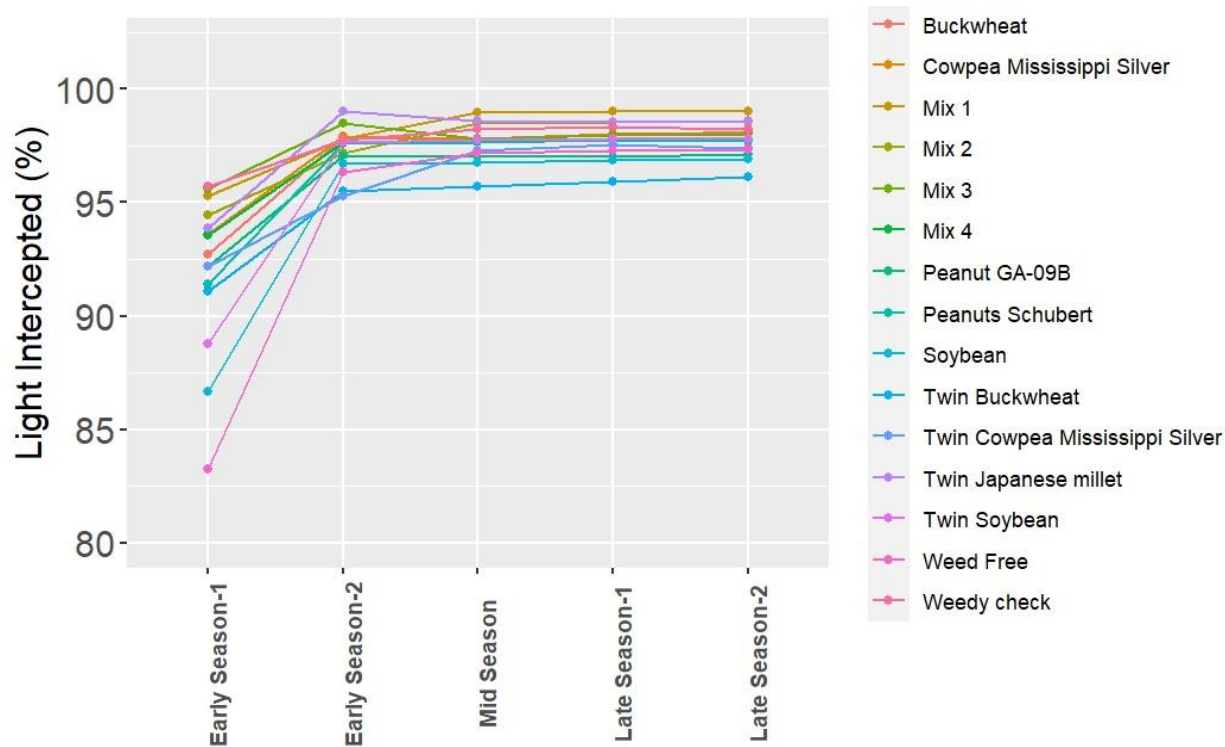
**Figure 17.** Cotton lint yield as influenced by living mulch treatment observed at cotton harvest in 2021. Different letters above standard error bars indicate significant differences based on the Fisher's protected Least Significant Difference (LSD) test ( $\alpha=0.05$ ).



**Figure 18.** Cotton lint yield (kg ha<sup>-1</sup>) observed at cotton harvest relative to living mulch biomass (kg ha<sup>-1</sup>) production, observed on 20 July 2021.

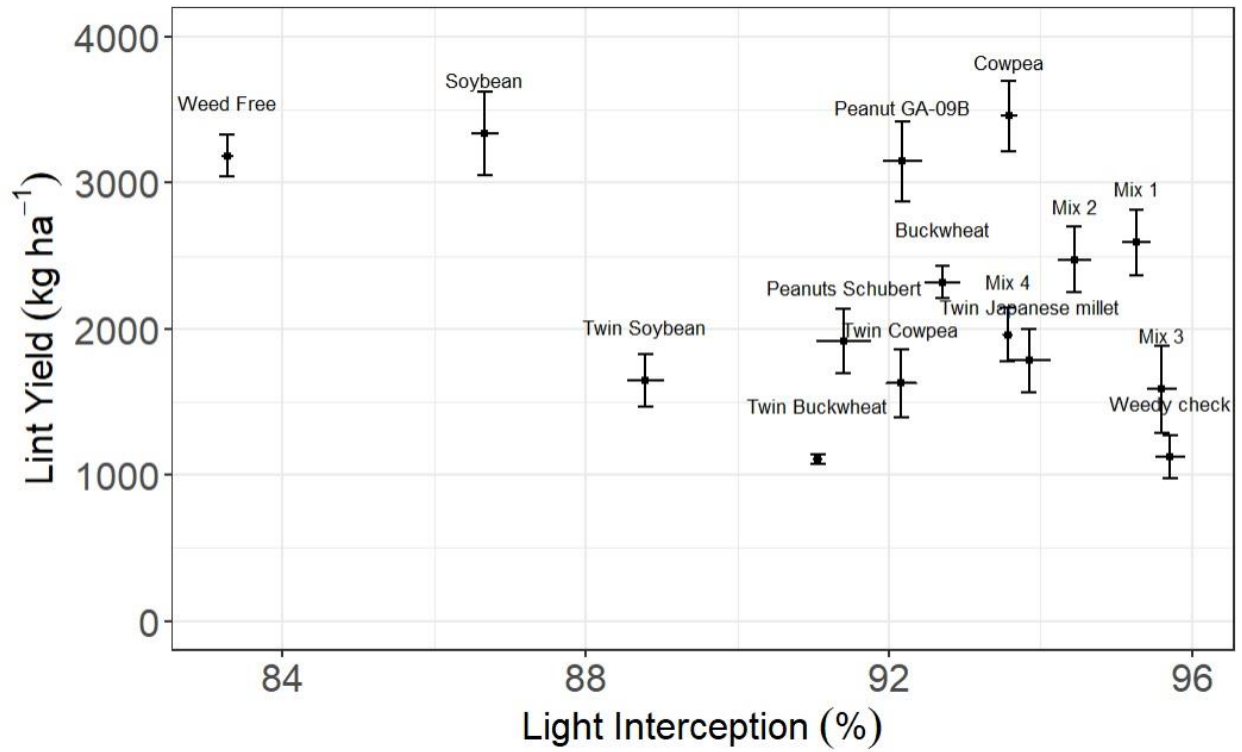


**Figure 19.** Cotton lint yield (kg ha<sup>-1</sup>) observed at cotton harvest relative to weed biomass (kg ha<sup>-1</sup>) production observed on 20 July 2021. The best-performing treatments, cowpea MS silver, peanut GA-09B, and soybean, are circled at the top of the graph.



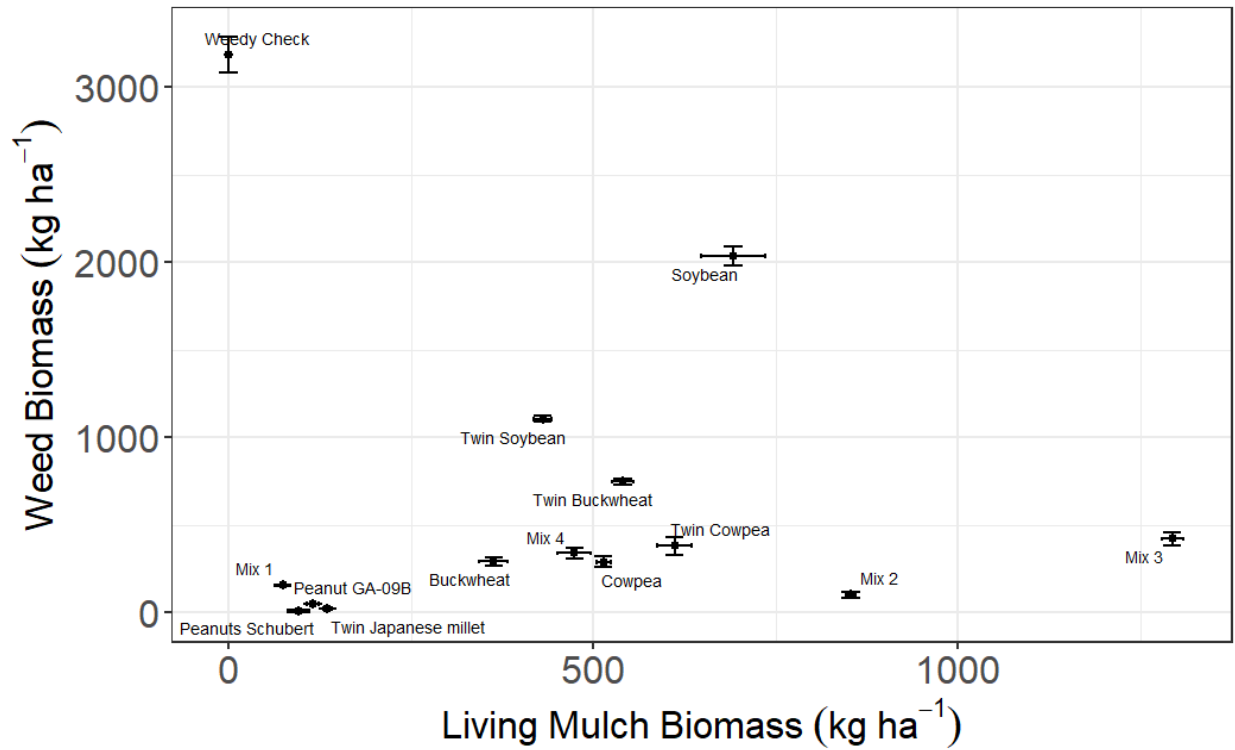
**Figure 20.** The percentage of photosynthetically active radiation intercepted by the living mulch canopy throughout the cotton growing season.

Early season-1 : 7/2/2021, Early season-2: 7/16/2021, Mid season: 7/30/2021, Late season-1: 8/13/2021, Late Season-2: 8/27/2021.

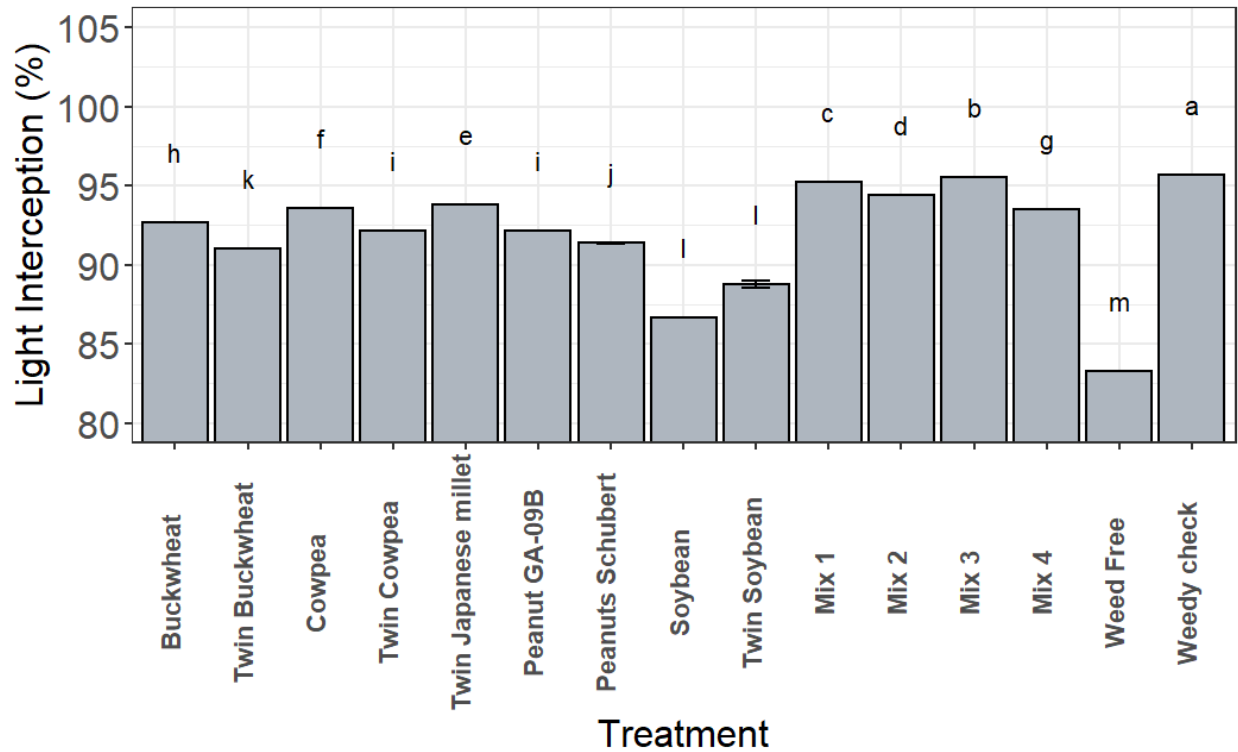


**Figure 21.** Cotton lint yield (kg ha<sup>-1</sup>) relative to the percentage of photosynthetically active radiation (PAR) intercepted by the living mulch canopy, observed on July 2, 2021, with cotton at the first-square stage.





**Figure 22.** Weed biomass (kg ha<sup>-1</sup>) relative to living mulch biomass (kg ha<sup>-1</sup>), observed on July 20, 2021, with cotton at the first-flower stage. Species in the lower left corner of the graph led to low weed biomass even with low living mulch biomass production. These include Mix 1, Peanut GA-09B, Peanut Schubert, and Twin Japanese millet.



**Figure 23.** Percent of photosynthetically active radiation (PAR) intercepted from above cotton canopy to ground level observed on July 2, 2021. Different letters above the standard error bars indicate significant differences based on the Fisher's protected Least Significant Difference (LSD) test ( $\alpha=0.05$ ).



**Figure 24.** A twin-row cowpea Mississippi Silver living mulch plot (left photo taken on July 1, 2021, 47 days after cotton emergence with cotton before the first square, right photo taken on July 15, 2021, 61 days after cotton planting, with cotton at the first square stage).



**Figure 25.** A buckwheat living mulch plot (left photo taken on July 1, 2021, 47 days after cotton emergence with cotton before the first square, right photo taken on July 15, 2021, 61 days after cotton planting, with cotton at the first square stage).

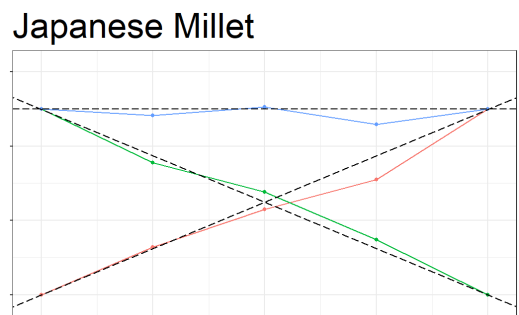
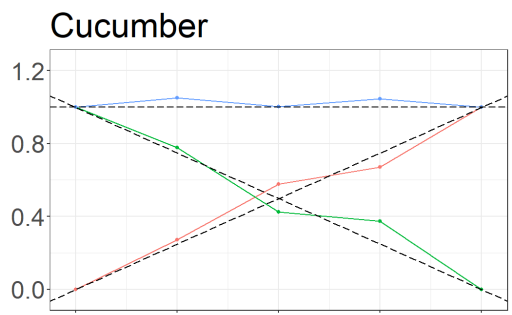
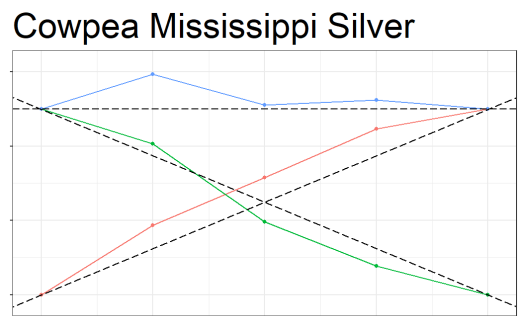
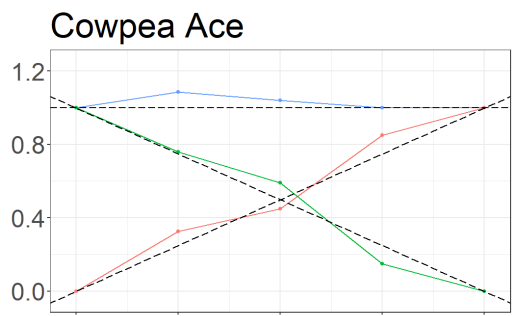
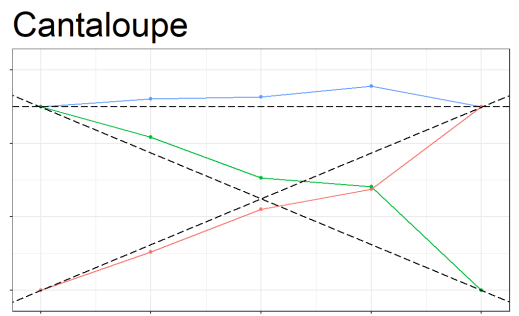
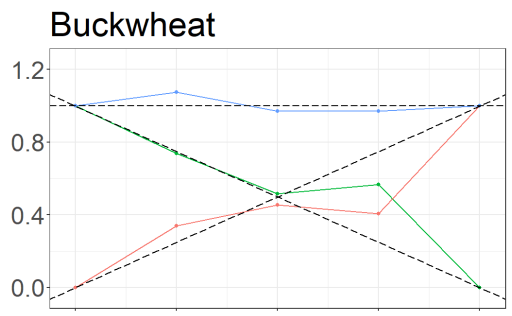
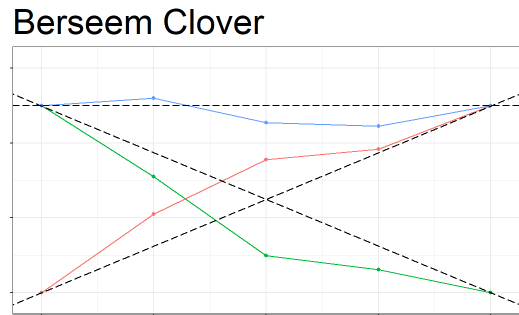
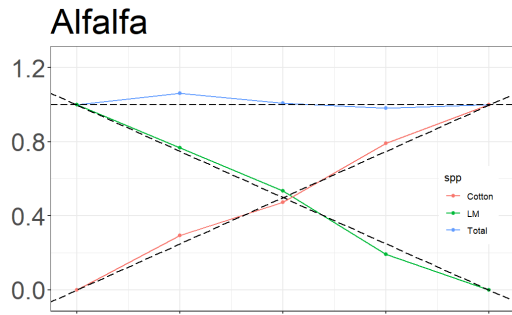


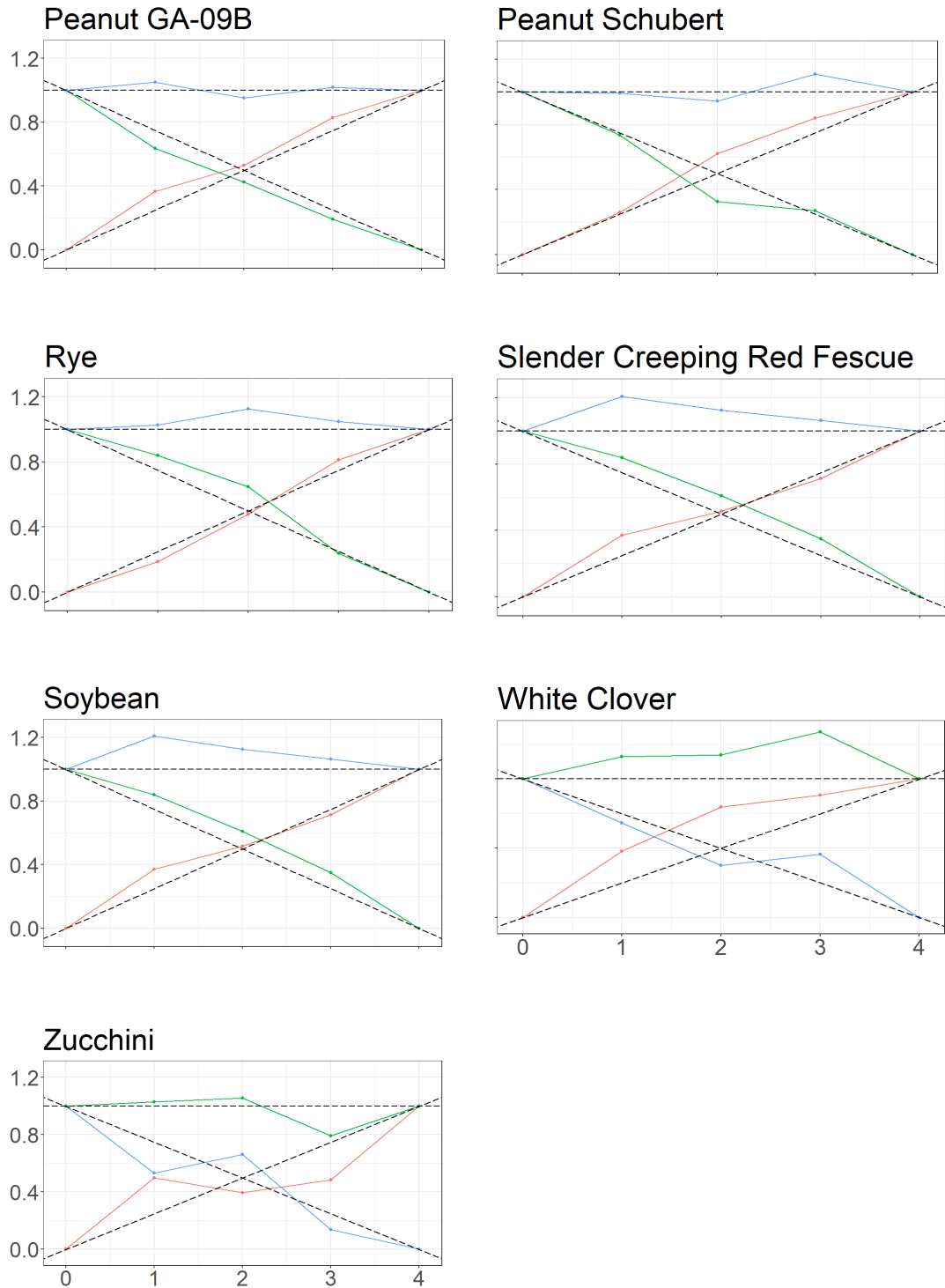
**Figure 26.** Peanut GA-09B living mulch plot (left photo taken on July 1, 2021, 47 days after cotton emergence with cotton before the first square, right photo taken on July 15, 2021, 61 days after cotton planting, with cotton at the first square stage).



**Figure 27.** A Mix 3 living mulch plot (left photo taken on July 1, 2021, 47 days after cotton emergence with cotton before the first square, right photo taken on July 15, 2021, 61 days after cotton planting, with cotton at the first square stage). Mix 3 consisted of sorghum-sudangrass, pearl millet, cowpea, and sunnhemp.

**Relative Yield**



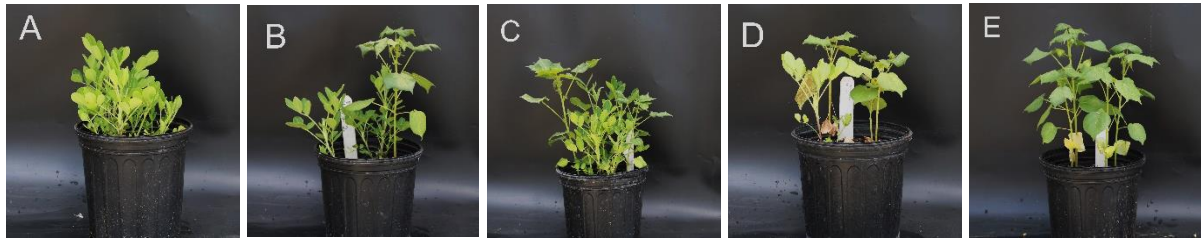


**Figure 28.** Relative biomass yield of each living mulch species and cotton in each competitive ratio, observed at biomass harvest. Each number on the X axis refers to the number of cotton plants in the replacement series. Relative yield is expected to follow the dashed black lines in each graph.

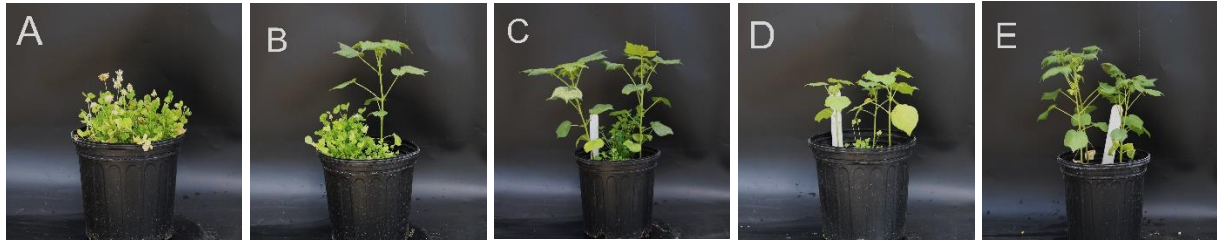




**Figure 29.** Soybean and cotton plants established in different ratios. (A, 4:0; B, 3:1; C, 2:2; D, 1:3; and E, 0:4 for soybean and cotton, respectively) in the replacement series experiment conducted in the greenhouse (Photo taken at biomass harvest 10 weeks after planting).



**Figure 30.** Peanut Schubert and cotton plants established in different ratios. (A, 4:0; B, 3:1; C, 2:2; D, 1:3; and E, 0:4 for peanut and cotton, respectively) in the replacement series experiment conducted in the greenhouse (Photo taken at biomass harvest 10 weeks after harvest).



**Figure 31.** White clover and cotton plants established in different ratios. (A, 4:0; B, 3:1; C, 2:2; D, 1:3; and E, 0:4 for white clover and cotton, respectively) in the replacement series experiment conducted in the greenhouse (Photo taken at biomass harvest 10 weeks after harvest).

## CHAPTER IV: CONCLUSIONS AND SUMMARY

### 4.1 Conclusions

In the organic cotton study, results consistently showed that tillage could not be replaced by cover crops alone. Tillage is a powerful tool in organic production that can only be replaced by the implementation of multiple weed control measures, if replaced at all. Without any cultivations in organic cotton, there was a catastrophic crop failure due to extreme weed pressure. A single cultivation event made the difference between complete crop failure and producing satisfactory yield in organic cotton.

Cover crops differed as expected in their ability to suppress weeds throughout the season and their impact on cotton yield. Cover crops that performed the best with regard to cotton yield and weed biomass suppression were purple-top turnip and oat. Further research should investigate more planting rates for cover crops in organic production and add in more alternatives to tillage in MT treatments, such as more frequent vinegar applications and additional flaming events.

In the living mulch field study, light interception, weed biomass, and living mulch biomass production showed some promise in identifying suitable living mulch species and mixes, but there were only a few consistent trends across living mulch species. Three treatments showed promise based on cotton yield and weed biomass production: twin row cowpea Mississippi Silver, soybean, and peanut GA-09B.

Living mulch candidate species were not all able to effectively suppress weeds and thereby failed to maintain cotton yield. Further research is required to investigate multiple factors of managing living mulches, for example, planting cotton and living mulches on the same date.

Planting living mulches earlier in the growing season may allow for cool season living mulches to perform better.

Greenhouse results did not entirely match field results in several promising living mulch species, potentially because of a late living mulch planting date. When planted in equal proportions, several living mulch species and cotton outperformed the expected cotton biomass production, most notably alfalfa, cowpea Ace, cowpea Mississippi Silver, cucumber, and Japanese millet. Living mulch biological characteristics such as heat tolerance and water usage should be considered when planting, and future research should investigate additional planting rates of living mulches in a field production setting.

## **4.2 Summary**

Current cotton production systems need additional forms of weed control to be developed. Organic production is dependent on tillage and cultivation for weed control and will need several “little hammers” to replace tillage. The potential for increased soil health when reducing tillage in organic systems is high and should be a priority for organic farmers and researchers. Cover crops make a significant contribution to weed control in organic systems but cannot be the only form of weed control in a successful organic production system.

Living mulches have the potential to be another tool for cotton farmers but must be able to suppress weeds while preserving cotton yields. We understand the need for more tools for farmers, and more research should be conducted on additional planting dates relative to cotton planting. Changing the planting date of living mulches has the potential to completely change the dynamics of living mulch, weeds, and cotton interactions.

Developing new weed control methods is essential to protect crops from weed competition. The tools investigated here are not new but have not been implemented to their full strength and may be able to significantly improve integrated weed management in cotton.

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