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Effects of Cover Crops on Weed Suppression in the Inter-Row Spaces of Citrus Orchards

Running (Short) Title: Cover Cropping in Citrus

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Abstract

A multi-year study was carried out at two citrus groves with mature trees in Southwest Florida in the United States to evaluate the effects of cover cropping on the citrus inter-row as a sustainable weed management strategy in the Florida citrus production system. Two cover crop (CC) mixes (legume + nonlegume species and only non-legume species) were compared with a no-CC grower standard management (GSM) that utilized the herbicide paraquat for weed suppression in the citrus tree inter-row spaces. We gathered data on the biomass and density of both cover crops and weeds, during the spring and summer/fall CC planting seasons throughout the study years. Both mixes of CCs effectively reduced weed density in the citrus inter-row by 58 to 99% (P<0.05), depending on the growing season and study locations, compared to GSM. Additionally, there were no significant differences observed between the different CC mixes. Similarly, both CC mixes reduced the weed biomass by 95 to 99% (P<0.05) in the citrus interrow compared to the GSM. However, weed suppression by CCs varied between growing seasons, mainly due to differences in germination and establishment of the CCs in each season.

Key words: sustainable weed management, weed biomass, weed reduction

Introduction

Florida (FL) was the leading citrus producer in the United States for several decades. However, since 2005, the citrus industry has faced severe challenges due to huanglongbing (*Liberibacter* spp. (HLB), a devastating disease. This has caused production to drop by 80%, moving Florida to second place behind California (USDA, 2023). Prior to the widespread impact of HLB, the citrus industry contributed \$9.3 billion to Florida's economy. Currently, this contribution has decreased to \$2.58 billion (USDA, 2023). HLB affects citrus trees by disrupting their ability to take up water and nutrients, leading to defoliation, fruit drop, root loss, and lower yields (Johnson et al. 2014; Kadyampakeni et al. 2014). In response, citrus producers have implemented various management strategies to maintain production, but these strategies have increased production costs (Singerman and Rogers 2020). As a result there is an ongoing search to find innovative horticultural approaches to sustain citrus production, manage costs, and improve sustainability.

Due to the favorable weather conditions in Florida, weeds consistently germinate and thrive in citrus orchards year-round, requiring efficient weed management approaches (Jhala et al. 2012; Kanissery et al. 2022). Weed proliferation in Florida can pose significant challenges, especially in regions with elevated precipitation and warm, humid temperatures, which provide an ideal environment for weed growth. Young citrus groves typically face greater weed pressure compared to mature ones. The predominant weed issues in Florida citrus orchards involve sedges, grasses, and vines (Jhala and Singh 2012; Kanissery et al. 2022). Weeds in the tree-rows can cause a yield reduction of 23-33% in citrus (Singh and Sharma 2008). Hence, weed management encompasses 14% of the total production cost of citrus in FL (Singerman 2022). Florida citrus growers utilize various strategies, including chemical, physical, and cultural methods, to manage weeds and minimize resource competition in orchards (Buker 2005; Futch 2020). Initial prevention practices involve sanitation and spot spraying to hinder weed proliferation before seed formation or vegetative growth (Kanissery et al. 2022). Chemical control, primarily using herbicides, is prevalent, with selection based on weed species, costs, tree age, and cultivar in the tree rows or areas under citrus canopies. Postemergence and preemergence (or residual) herbicides are applied 2-4 times annually to maintain weed-free areas in the tree rows. However, in the spaces between tree rows (inter-rows or row middles), a strip of vegetation is typically maintained to prevent erosion of the sandy soils. Physical weed management techniques like mowing are common to manage inter-row areas, whereas shallow tillage is less preferred in citrus orchards due to potential damage to fibrous roots responsible for nutrient uptake and risks of soil erosion, particularly in raised-bed orchards. Mowing, performed when vegetation reaches 30-60 cm height, targets tall weeds in inter-rows. To minimize weed growth and reduce the need for frequent mowing, these inter-row areas are treated with herbicides such as glyphosate, paraquat, etc., a method commonly referred to as chemical mowing. This practice is often used in other crops such as blueberries (*Vaccinium corymbosum* L.), pecans [*Carya illinoinensis* (Wangenh.) K. Koch], apples (*Malus pumila* Mill.), and peaches [*Prunus persica* (L.) Batsch] (Besançon and Bouchelle 2023; Buckelew et al. 2018; Faircloth et al. 2007; Kanissery et al. 2022).

Citrus trees are highly vulnerable to competition from the weeds in the tree rows especially during blooming and flushing periods, leading to notable yield losses (Buker 2005). Besides competing for resources, these weeds can also act as hosts for pests and diseases, disrupting production practices and harvest operations (Futch 1997). Weeds in inter-row areas are a major source of seed dispersion and subsequent weed infestation in tree rows (Kanissery et al. 2020). To prevent this spread and minimize weed impact across the orchard, it is crucial to regularly mow these areas or implement alternative weed management techniques such as planting cover crops (CC).

The utilization of CC can bring many advantages to agricultural systems through the reduction of soil erosion, weed suppression, weed seed bank reduction, increase in inorganic nitrogen (N), soil carbon (C) sequestration, increased yields, improved nutrient cycling, nutrient leaching reduction, and improvement of water use efficiency and quality (Conservation Technology Information Center (CTIC) 2017; Dabney et al. 2001; Delgado et al. 2007; Fenton 2017; Schipanski et al. 2014; Scavo et al. 2020). There are several characteristics that a CC should have to provide the most benefits to cash crops. These include quick and easy establishment, rapid biomass production, short growing cycles, low seed cost, and low water requirements (Baligar and Fageria 2007). Careful selection of cover crops is crucial as they may host damaging pests impacting pest management. For instance, according to George et al. (2022), 'young' Asian citrus psyllids (*Diaphorina citri*), which are the primary vectors of the HLB-causing bacterial pathogen in citrus, were observed feeding on specific cover crop species commonly found in Florida citrus orchards. However, 'adult' *D. citri* were unable to feed on

these same cover crops. This indicates that cover crops may serve as alternative hosts for young *D. citri* in citrus orchards. The authors suggest that this could either have a negative impact by increasing the psyllid population in orchards or a positive effect by reducing the number of psyllids that feed on citrus trees.

Although cover cropping for weed suppression is a cultural weed management strategy, the mechanisms of weed suppression by CCs can be either physical or chemical. The degree to which a CC suppresses weeds depends on the CC species, the target weed species, the quantity of biomass produced, and environmental conditions (Liebman and Mohler 2001). Cover crops can suppress weeds while alive or during the decomposition process (Teasdale et al. 2018). When cover crop residue is left on the soil surface, it creates a mulch that reduces weed germination and weed development (Blaise et al. 2020; Kruidhof et al. 2009; Scavo and Mauromicale 2021; Sturm et al. 2016). The mulch from the CC residue reduces light penetration and changes soil temperature, hindering weed seed germination. Living CCs suppress weed seedling emergence, growth, and seed production by competing with the weeds for water, nutrients, light, and space, that otherwise will be available to weeds for establishment (Mennan et al. 2020; Mirsky et al. 2010; Kruidhof et al. 2008; Liebman and Mohler 2001).

Cover crops can also suppress weeds through allelopathy. Allelochemicals can be released while the plant is alive or in the process of decomposition (Liebman and Mohler 2001; Macias et al. 2019; Wezel et al. 2014). Besides weed suppression by competition for resources and through allelopathy, cover crops can affect weed germination and development through nutrient release (Kruidhof et al. 2009; Odhaiambo and Bomke 2001).

Most literature focusing on CCs and their effects on weed management have been primarily developed for annual cropping systems with the implementation of cover crops in the fallow period or off-season of the main crop (Bernstein et al. 2011; Mirsky et al. 2013). However, in the case of perennial crops, CC establishment must be done concurrently with the growing cash crop (Hartwig and Ammon 2002). Effective weed suppression has been demonstrated with CCs in several perennial crops: vineyards (Hartwig and Ammon 2002), hazelnut (Isik et al. 2014), apple orchards (Granatstein and Mullinix 2008), and Tahiti acid lime (Martinelli et al. 2017).

Although the initial grower's interest in CCs for citrus production was to improve soil health conditions for citrus trees, additional benefits such as weed suppression and the addition of N from leguminous cover crops could contribute to lower input costs for herbicides and fertilizer that can offset the cost of cover crop seed and cover crop management. Therefore, to uncover the possibilities of utilizing CCs as a weed management tool in perennial cropping systems like citrus in Florida, we evaluated the impact of CCs on the suppression of weeds in the inter-rows of citrus trees. It was hypothesized that the CC mixes used would reduce weed density and weed biomass in the inter-row spaces between citrus tree rows.

Materials and Methods

Study Site

This study was conducted in two southwest Florida commercial citrus orchards (hereafter referred to as North Grove and South Grove; North Grove lat. 26.50865° N; long. 81.3898° W and South Grove lat. 26.426826° N, long. 81.226163°W). Approximately the distance between both orchards was 32 km, located in Collier County, Immokalee, Florida, US. The trees were 'Valencia' [Citrus xsinensis (L.) Osbeck (pro sp.) [maxima x reticulata; syn. Citrus sinensis [L.] Osbeck)] budded onto Swingle rootstock (C. paradisi Macf × Poncirus trifoliata) and were planted in 1991. The experimental site where the study was conducted exhibited a tropical savanna climate, per Köppen's classification (Köppen, 1936). The highest rainfall is usually recorded between June and October, while the winter season (Nov. to Feb.) and spring season (Mar. to May) are marked by low precipitation levels.

Cover crops were planted with a ten-day interval between the experimental sites (North Grove and South Grove). The weather variables recorded during the study, such as rainfall, temperature, soil temperature at a depth of 10 cm, and evapotranspiration (ET), are shown in the supplementary materials (Figure S1). The soil in the North Grove is classified as the Immokalee series, whereas the South Grove soil is from the Holopaw series (USDA-NRCS 2015).

Experimental Design and Management

The present field study was conducted during the period ranging from August 2018 until April 2022. The treatments were arranged in a randomized block design, with 12 replicates per treatment. Each replicate plot comprised two beds with 52 citrus trees planted per bed. The interrow area width was 3 meters, and the distance from the end of the interrow to the trunk of the citrus tree was 2 meters, as shown in Supplementary Figure. S2 (Brewer et al. 2023). The

spacing between trees within rows was 3.8 meters, while the distance between rows was 7.3 meters in the North Grove. In the South Grove, the spacing between trees within rows was 3.3 meters, and the distance between rows was 6.7 meters.

Two cover crop mixes were evaluated at each orchard: legume + non-legume (LG+NL) and non-legume (NL). Both treatments included the same non-legume cover crop species each planting season, but the LG+NL mix also contained legume species. The cover crops were planted twice a year, in summer/fall and spring (Table 1) (Brewer et al., 2023). The composition of individual cover crop species in the mixes varied over the experimental years due to availability constraints. However, both treatments, NL and LG+NL, primarily included Brassicas and grasses or a combination of Legumes, Brassicas, and grasses.

CCs were planted exclusively in the 3-meter-wide inter-row spaces using a no-till drill, with a 2-meter gap from the end of the inter-row to the trunks of the citrus trees, as shown in Supplementary Figure S2. A no-cover cropped/grower standard management (GSM) was utilized as a control. The GSM/control plots were not seeded with cover crops and adhered to standard grower practices for weed management in the orchard rows. These practices typically involve mowing when the vegetation reaches a height of 30-60 cm and occasionally applying postemergence herbicides to limit vegetation growth and extend the time between mowing. A low dose of paraquat (0.84 kg a.i ha⁻¹) was applied to the inter-row spaces of the GSM plots as part of a chemical mowing strategy to manage vegetation between the citrus tree rows. This sublethal dose aimed to minimize weed growth and reduce mowing frequency, rather than eliminate all weeds, since maintaining a strip of vegetation between rows helps prevent soil erosion. These applications were made quarterly throughout the year. The inter-row spaces in the CC plots, as well as in the GSM plots, did not receive any fertilization or irrigation. A full recommended dose of paraquat (1.86 kg a.i ha⁻¹) was applied to the tree rows of both CC and GSM plots to completely control the emerged weeds and maintain weed-free areas under the canopy. These post-emergence applications were carried out two to three times a year, typically in spring, summer, and fall. Additionally, a pre-emergence application of flumioxazin (0.30 kg a.i ha⁻¹) was carried out in the tree rows of both CC and GSM plots during the summer. Cover crops in the inter-rows of CC plots were terminated by mowing before every successive planting. After mowing, the cover crop residues were incorporated through superficial tillage using the John Deere rotavator (John Deere, Moline, IL) into the topsoil, up to a depth of 10 cm.

Cover Crop and Weed Density Assessment

The effects of legume and non-legume CCs on weed suppression were determined by measuring the density and biomass of CCs and weeds in the inter-row of the orchards. The density of weeds and CCs was measured in the North Grove in March 2019, August 2019, July 2020, and September 2021. Similar measurements were done in the South Grove on the same dates except for the first sampling in March 2019, when no sampling was conducted due to poor germination of the CCs. A 1 m² quadrat was randomly placed at three different locations within each replicate plot in the inter-row to measure the number of weeds and CCs. While weeds and CCs were classified by species whenever possible, their total counts were used for data analysis.

Cover Crop and Weed Biomass Production Assessment

Once a year in Spring, weed and CC biomass collection was conducted in January 2020, February 2021, and January 2022. Using a 0.25 m² square quadrat, 2 samples in each plot were collected from the inter-row. The weeds and CCs were clipped at the soil surface to evaluate above-ground biomass within the quadrat, and individual plants for each weed and cover crop species were sorted and counted (Linares et al. 2008).

Weed and CC biomass was identified by species, and the fresh weight and number of plants per species were recorded. The samples were then placed in separate paper bags by the species and dried in a forced air-drying oven for 72 h at 60°C (Hanlon et al. 1997). After 72 h, the dry weights of the samples were determined. Due to logistical constraints, the collection of weed and cover crop dry weights was not feasible in January 2020.

Statistical Analyses

Response variables were analyzed on a plot mean basis using generalized linear mixed model methodology as implemented in SAS® PROC GLIMMIX (SAS/STAT 15.1; SAS Institute, Cary, NC) using a distribution function suitable for the response variable in question, viz. normal for mass data, and negative binomial for count data. Site, CC, sampling date, and all two and three-way interactions were considered fixed effects. Replicate within site was the sole G-side random effect. The repeated nature of the experiment (measuring the same experimental unit over time) necessitated modeling the residual covariance structure. Based on Akaike's Information Criterion (AICC) and the residual plots, the unstructured covariance was chosen.

Irrespective of the results of the F-tests, the three-way-interaction means were calculated, and CC treatments (LG+NL and NL) were compared to each other and the control through a simple t-test within each site \times sampling date combination. Responses were considered to be statistically meaningful at P-values ≤ 0.05 .

Results and Discussion

Weather Conditions

The average rainfall for the ten days following the planting of the CCs in each orchard (North and South Groves) is shown in Figure 1. Overall, the North Grove experienced slightly higher rainfall during the ten-day period following CC planting compared to the South Grove. Additionally, the two sites faced different drought conditions: the North Grove experienced a moderate drought, while the South Grove was under a severe drought (Supplementary Figure S3) (Data Sources: National Drought Mitigation Center at the University of Nebraska-Lincoln, the United States Department of Agriculture (USDA), and the National Oceanic and Atmospheric Administration (NOAA)). In baseline measurements (data not shown), the North Grove had about 74% higher cation exchange capacity (CEC) and 36% more organic matter (OM) compared to the South Grove.

Cover Crop Density

During the Summer-Fall seasons CC density was significantly influenced by site (P < 0.01), treatment (P < 0.001), sampling date (P < 0.05), and their interactions (P < 0.001) (Supplementary Table S1). Notably, CC density differed significantly between orchards, with variations dependent on the sampling date (Figure 2). The North Grove consistently showed higher CC density on three of four sampling dates (March 2019, August 2019, July 2020) compared to the South Grove. In March 2019, the South Grove had extremely low germination rates, which made sampling impossible; therefore, this data was not included in the study. The North Grove's CC density was 93% and 97% higher for the LG and LG+NL treatments, respectively, in August 2019, and 63% and 73% higher in July 2020. However, in September 2021, the South Grove's CC density surpassed the North Grove's, with 78% and 81% for LG and LG+NL treatments, respectively. It is noteworthy that the North Grove received 63% less rainfall compared to the South Grove for the June 2021 planting (Figure 1). The North Grove's higher

cation exchange capacity (CEC), organic matter content (OM), and higher rainfall may have favored CC germination and establishment. The differences in CC germination and density among orchards may be due to insufficient moisture during establishment (Boyd and Van Acker 2003), poor soil-to-seed contact at planting (Roth et al. 2015), grower management practices (Wilson et al. 2013), and the strong influence of rainfall observed in this study. The findings underscore the need to align CC planting with rainfall patterns in Florida and reveal significant spatial and temporal variability in CC germination and establishment, which poses a challenge for citrus producers. Topography and local conditions are crucial for successful CC growth in Florida, highlighting the importance of site-specific approaches.

The LG+NL treatment exhibited significantly higher CC density in three of the four Summer-Fall seasons. Specifically, in the North Grove, CC density was 30% higher in August 2019, 39% higher in July 2020, and 27% higher in September 2021, compared to the NL treatment. Similarly, in the South Grove, the LG+NL treatment showed 75% higher CC density in August 2019 and 55% higher in July 2020, relative to the NL treatment (Figure 3). No significant difference in CC density was observed between the two treatments in the North Grove in March 2019.

The effect of CC treatment on CC density varied with site and sampling date during the Spring seasons (Supplementary Table S2). Specifically, CC density data collected in Spring (Jan. 2020, Feb. 2021, and Jan. 2022) exhibited distinct patterns depending on the site. In the North Grove, the LG+NL treatment consistently resulted in higher CC density compared to the NL treatment on all sampling dates (19%, 60%, and 45%, respectively) (Figure 3). Conversely, in the South Grove, there was no significant difference in CC density between the CC treatments. It is plausible that less favorable environmental conditions in the South Grove impacted the germination and establishment of legumes, potentially reducing their density and competitive ability. This observation is consistent with Brennan et al. (2009), who reported significant variations in legume competition between two vegetable farms, attributed to differences in organic matter content (3-5% versus 1.2%). Moreover, the North Grove exhibited a baseline organic matter content 36% higher than the South Grove, potentially attributing to the CC performance differences.

In most of the samplings during both Summer and Fall plantings, the LG+NL CC mix exhibited significantly higher CC density, ranging from 19% to 75%, compared to the NL

treatment. The primary distinction between the LG+NL and NL treatments lies in the inclusion of legume species in the former, while the latter does not contain any legume species; both treatments share the same grass and brassica species. Consequently, the disparity in CC density may be attributed to the superior germination and establishment of the legume species and a greater number of units of the small-seeded legume. The Fall season NL treatment was predominantly dominated by Brassica species, particularly daikon radish (Raphanus sativus L. var. Longipinnatus). In one of the NL replicates, daikon radish plants covered 90% of the cover cropped area, with the remaining 10% comprised of other species (grasses). In the NL+LG treatment, daikon radish remained dominant but at a lower percentage, resulting in greater diversity of CC species in these areas, with approximately 18% legumes, 22% grass, and 60% brassica (data not shown). Additionally, due to differences in growth habits, daikon radishes tended to occupy more space than legume species. For example, two months after planting, while one daikon radish plant covered 10% of the area within a quadrat, it took 20 sunn hemp (Crotalaria juncea L.) plants to achieve a similar coverage. This study revealed that the same CC mix, with identical seeding rates and planting equipment, yielded varying CC densities across seasons and sites. This variability in CC density by season and site aligns with the findings reported by Brennan et al. (2009), who noted variations in cover crop dry matter across different years and study locations, attributing this variability to soil and climatic conditions. Although cover crop density is not frequently reported in studies, as shown by these results, it can offer valuable insights into performance variations between sites and improve our understanding of how different cover crop species interact.

Cover Crop Biomass

Cover crop biomass data was collected during the spring only, measuring the biomass of the fall CC planting. Significant three-way interactions among site, treatment, and sampling date were observed for both CC above-ground fresh and dry biomass (P <0.05) (Supplementary Table S3 and S4). In January 2020, cover crop fresh biomass was significantly higher in the LG+NL treatment compared to the NL treatment, but only in the North Grove (34% higher LG+NL treatment) (Supplementary Figure S4). However, no significant difference due to treatment was observed at the other two sampling dates in the North Grove and all three sampling dates in the South Grove. Furthermore, in February 2021, dry biomass was 42% higher with the LG+NL treatment than with the NL treatment in the North Grove, while there was no difference in dry

biomass between the two CC treatments in January 2022 in the North Grove and in both February 2021 and January 2022 in the South Grove (Figure 4). These findings align with previous studies by Brennan et al. (2009) and Linares et al. (2008), which reported CC dry matter differences by year, site, and functional groups in the CC mixture.

The limited significant differences between NL+LG and NL treatments may be attributed to the functional groups used in this study, such as small-seeded legumes (e.g., Crotalaria juncea L.), grasses (e.g., Secale cereale L.), and brassicas (e.g., Raphanus sativus L.). Notably, brassica CCs can produce more biomass and achieve canopy closure earlier in the season than slowergrowing, cool-season legumes (MacLaren et al. 2019). At the time of biomass sampling (84 days after planting), daikon radish dry biomass was 77% higher than that of the legumes, potentially influencing the contribution of dry matter by the legume. The dominance of daikon radish in this study may reflect the early season sampling time, as reported by Brennan et al. (2009). Daikon radish's fast growth and deep root system, which allows access to nutrients and water, could contribute to its higher biomass production compared to legumes, as supported by studies conducted by Teasdale and Mohler (2000) and Fang et al. (2012). In summary, due to its fast growth and deep root system, daikon radish may produce more biomass than legumes in a cover crop mix. Further investigation is needed to understand dry biomass production during the season of different CC species in Florida conditions, including monthly dry biomass measurements, to comprehend the impact of seasonal fluctuations on the biomass accumulation of each species.

Weed Density

In the Summer-Fall planting seasons, there was a significant (P < 0.001) interaction among site, treatment, and sampling on weed density (Supplementary Table S5). The North Grove exhibited lower weed densities with both CC treatments in three of four seasons compared to the GSM (Figure 5). Similarly, in the South Grove, both CCs showed lower weed densities than the GSM in July 2020 and September 2021; however, only the LG+NL effectively suppressed weed density to lower than the GSM in August 2019. Furthermore, the LG+NL and NL treatments reduced weed density by 80% (Mar. 2019), 92% (Aug. 2019), and 97% (Jul 2020) in the North Grove and by 58% (Aug. 2019), 63% (Jul 2020), and 82% (Sep 2021) in the South Grove (Figure 5). Notably, poor weed density suppression with both treatments (LG+NL and NL) in the North Grove at the final sampling date (Sep. 2021) coincided with very low CC

densities for both CC treatments (Figure 3 and Figure 5). Conversely, effective weed suppression in the South Grove for the same season appears to be due to the high CC densities with both the LG+NL and the NL treatments (Figure 3 and Figure 5). In the Spring planting seasons, a significant interaction among site, CC, and sampling date on weed density was observed (Supplementary Table S6). The GSM had higher weed density on the three sampling dates (Jan. 2020, Feb. 2021, Jan. 2022) at both sites than the CC treatments, which were not significantly different from each other (Figure 5). Weed suppression provided by cover crops at the North Grove was 99% for LG+NL and NL (Jan 2020 and Feb 2021), 86% LG+NL and NL (Jan 2022), and for the South Grove weed suppression was 97% LG+NL and NL (Jan 2020), 93% LG+NL and NL (Feb 2021), 91% LG+NL and NL (Jan 2022). The very effective weed density suppression obtained at both orchards corresponded with high cover crop densities with both treatments (Figure 3 and Figure 5). In most of the sampling dates in both orchards, the LG+NL and NL treatments reduced the weed density by 58% to 99% compared to the GSM. This is consistent with previous studies reporting a significantly lower number of weeds in areas covercropped with legumes and non-legume species in mixes compared to the control (Finney et al. 2016; Ranaldo et al. 2019; Smith et al. 2014). Ranaldo et al. (2019) also observed a variation by year in weed suppression in areas planted with CCs. Weed density, biomass, seed bank, and species can vary significantly across years in agricultural systems due to changes in management practices, cultural practices, meteorological conditions, and climate change (Légère et al. 2011; Menalled et al. 2001; Ramesh et al. 2017; Sosnoskie et al. 2009; Teasdale et al. 2018). The CC establishment also appears to be critical since weed densities were unaffected by CC treatment only when CC densities were very low.

Weed Biomass

In the Spring planting season, several factors significantly influenced the above ground weed biomass (fresh weight), including site (P < 0.01), CC treatment (P < 0.001), sampling date (P < 0.01), and their interactions (Supplementary Table S7). Both CC treatments consistently reduced weed fresh biomass compared to the GSM across all sampling dates at both sites, resulting in significant differences (Figure 6). Moreover, the two CC treatments did not exhibit significant differences in fresh biomass values, achieving over 95% weed biomass suppression compared to the control. Regarding above-ground weed dry biomass, CC treatment (P < 0.001), sampling date (P < 0.001), and their interaction were the main significant factors (Supplementary

Table S8). Similar to fresh biomass, the CC treatments resulted in significantly lower weed dry biomass compared to the GSM, with no noticeable differences between the two CC treatments (Figure 4). The impact of the cover crop treatments did not vary by site, as indicated in Supplementary Table S8. The CC treatments achieved weed biomass suppression ranging from 95% to 99%. Both CC treatments consistently demonstrated substantial reductions in weed biomass fresh and dry weight, reaching up to 99% in most sampling points compared to the GSM. This aligns with previous studies (Finney et al. 2016; MacLaren et al. 2019) reporting similar weed suppression levels across different CC mixes, encompassing legumes, grasses, and brassicas in various combinations. The ability of CC species to outcompete weeds can vary, with species such as brassicas and grasses outperforming leguminous species due to their rapid biomass production and resource utilization efficiency (Yu et al. 2015; MacLaren et al. 2019). Furthermore, certain CC species, including daikon radish and buckwheat (Fagopyrum esculentum Moench), present in the cover crop mixes used in this study, can effectively suppress weeds through allelopathic effects (Falquet et al. 2015; Sturm et al. 2018). The creation of a dense canopy and efficient nutrient scavenging by specific CC species can limit the resources available for weed seeds to germinate and thrive, resulting in more effective weed control (Brust et al. 2014).

This study serves as an initial evaluation of cover crops for weed control in Florida citrus production. Our findings indicate that cover crop mixtures containing legumes, brassicas, and grasses, or brassicas and grasses only, can effectively suppress weeds in the inter-row areas of citrus orchards in Florida. Both cover crop treatments significantly reduced weed density and biomass in the inter-row spaces, although their effectiveness varied across different growing seasons due to differences in cover crop germination and establishment. While our study provides valuable insights into using cover crops for weed management in citrus production in southwest Florida, evaluating these cover crops in other citrus-growing regions with different soil types and production conditions will enhance our understanding and help develop more specific, region-tailored recommendations. Further research is also needed to explore additional cover crop species and mixtures beyond those tested in this study. Examining different planting, management, and termination strategies will also help optimize cover cropping in citrus and similar tree production systems.

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Competing Interest

The authors declare none.

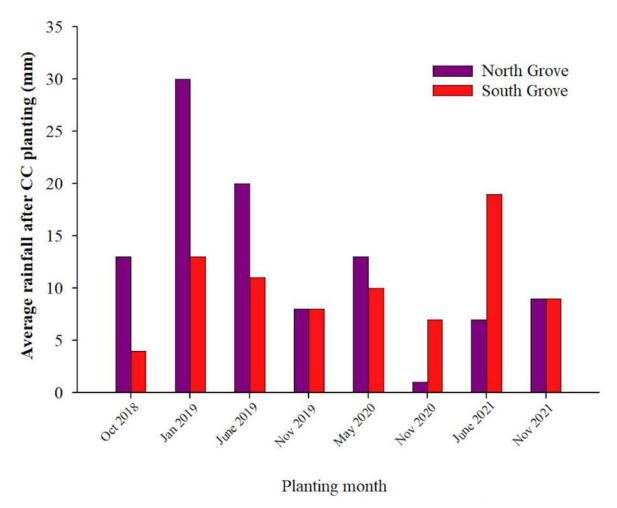


Figure 1. Average rainfall (mm) for the following ten days after cover crop (CC) planting. Cover crop planting was conducted ten days apart; North Grove was planted first, and then South Grove was planted ten days later. Data were obtained from the Florida Automated Weather Network (https://fawn.ifas.ufl.edu/data/) (Brewer et al. 2023).

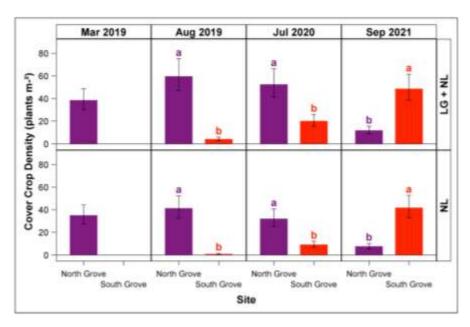


Figure 2. Cover crop density (plants/m²) in the inter-row of citrus trees in March and August 2019, July 2020, and September 2021. Treatments include legume+non-legume (LG+NL) and non-legume (NL). The South Grove results for March 2019 are not shown due to a lack of germination of cover crops at this site. Error bars represent 95% confidence limits based on 12 replicates. Grove (North and South) within a cell sharing a given letter are not statistically different at $P \le 0.05$ based on the least squares difference (LSD) (simple two-sample t-test).

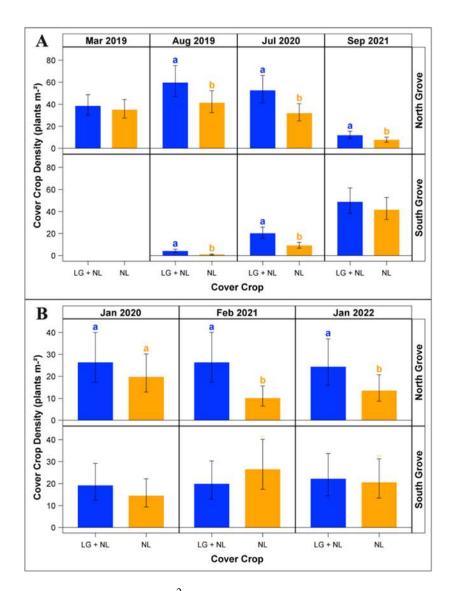


Figure 3. Cover crop density (plants m⁻²) in citrus trees – Summer/Fall data collected in March and August 2019, July 2020, and September 2021 (A). Cover crop density (plants m⁻²) in the inter-row of citrus trees – Spring data collected in January 2020, February 2021, and January 2022 (B). Treatments include legume+non-legume (LG+NL) and non-legume (NL). The South Grove results for March 2019 are not shown due to a lack of germination of cover crops on this site. Error bars represent 95% confidence limits based on 12 replicates. Treatments within a cell sharing a given letter are not statistically different at P<0.05 based on the least squares difference (LSD) (simple two-sample t-test).

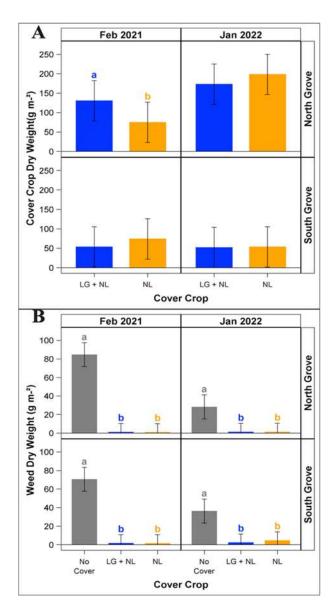


Figure 4. Cover crop above-ground dry biomass (g) (A) and weed above-ground dry biomass (g) (B) of plants sampled within a 1 m² quadrat in the inter-row of citrus trees. Spring data collected in February 2021, and January 2022. Treatments include legume+non-legume (LG+NL), non-legume (NL), and no cover (grower standard management, no cover crop). Error bars represent 95% confidence limits based on 12 replicates. Treatments within a cell sharing a given letter are not statistically different at P<0.05 based on the least squares difference (LSD) (simple two sample t-test).

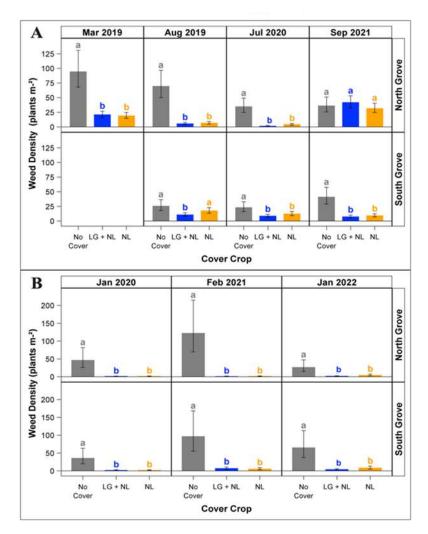


Figure 5. Weed density (plants m⁻²) in citrus trees – Summer/Fall data collected in March and August 2019, July 2020, and September 2021 (A) and Spring data collected in January 2020, February 2021, and January 2022 (B). Treatments include legume + non-legume (LG+NL), non-legume (NL), and no cover (grower standard management, no cover crop). South Grove results for March 2019 are not shown due to a lack of germination of cover crops on this site. Error bars represent 95% confidence limits based on 12 replicates. Treatments within a cell sharing a given letter are not statistically different at P<0.05 based on the least squares difference (LSD) (simple two sample t-test).

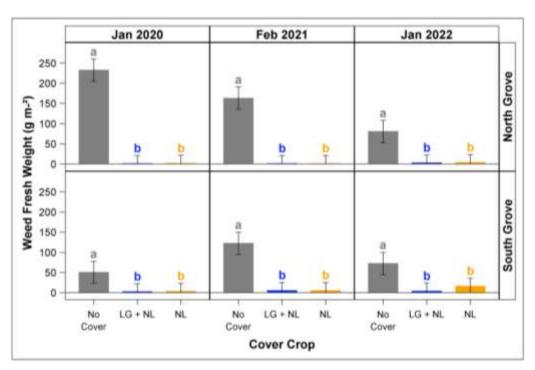


Figure 6. Weed above-ground fresh biomass (g) of plants sampled within a 1 m² quadrat in the inter-row of citrus trees – Spring data collected in January 2020, February 2021, and January 2022. Treatments include legume +non-legume (LG+NL), non-legume (NL), and no cover (grower standard management, no cover crop). Error bars represent 95% confidence limits based on 12 replicates. Treatments within a cell sharing a given letter are not statistically different at P<0.05 based on the least squares difference (LSD) (simple two-sample t-test).

Table 1. List of CCs planted by season and total seeding rate. Two CC treatments were tested: a legume and non-legume mix (LG+NL) and a non-legume mix (NL). Cover crops were planted in 2018, 2019, 2020, and 2021 in Summer/Fall and Spring seasons in the months indicated (Source: Brewer et al., 2023)

Treatment	Category	Oct.	Jan.	June	Nov.	May	Nov.	June	Nov.	Seeding
	of cover	2018	2019	2019	2019	2020	2020	2021	2021	Rate
	crop§									kg ha ⁻¹
		Sunn hemp	Helianthus	Crotalaria	C. juncea,	C. juncea,	C.	C. juncea,	C.	50-100
LG+NL	Legumes	(Crotalaria	annuus L.,	juncea L	S.	S.	juncea,	cowpea	juncea	
		juncea L.),	T. repens		grandiflora	grandiflora	pea	[Vigna		
		vegetable	L., <i>T</i> .		L., A.	, A.	(Pisum	unguiculata		
		hummingbird	incarnatum		vaginalis,	vaginalis,	sativum	(L.) Walp.]		
		[Sesbania	L.		<i>T</i> .	<i>T</i> .	L.)			
		grandiflora			incarnatum,	incarnatum				
		(L.) Poir.],			M.	, <i>M</i> .				
		white			officinalis	officinalis				
		moneywort								
		[Alysicarpus								
		vaginalis (L.)								
		DC.],								
		crimson								
		clover								
		(Trifolium								

incarnatum

L.),

sweetclover

[Melilotus

of ficinal is

(L.) Lam.]

Non-	Daikon radish	R. sativus.,	F.	R. sativus,	R. sativus,	R.	F.	R.	150-200
legumes	(Raphanus	S. cereale,	esculentum,	A. sativa, S.	A. sativa,	sativus,	esculentum,	sativus,	
	sativus L.),	buckwheat	<i>P</i> .	cereale, P.	S. cereale,	A.	U. ramosa,	A.	
	oat (Avena	(Fagopyrum	miliaceum,	miliaceum.	<i>P</i> .	sativa,		sativa,	
	sativa L.), rye	esculentum	browntop		miliaceum.	S.		S.	
	(Secale	Moench)	millet			cereale		cereale	
	cereale L.),		[Urochloa						
	proso millet		ramosa						
	(Panicum		(L.) T. Q.						
	miliaceum L.)		Nguyen]						

	Non-	R. sativus, A.	R. sativus,	F.	R. sativus,	R. sativus,	R.	F.	R.	150-200
NL	legumes	sativa, S.	S. cereale,	esculentum,	A. sativa, S.	A. sativa,	sativus,	esculentum,	sativus,	
		cereale, P.	F.	Р.	cereale, P.	S. cereale,	<i>A</i> .	U. ramosa	<i>A</i> .	
		miliaceum	esculentum	miliaceum,	miliaceum.	<i>P</i> .	sativa,		sativa,	
				U. ramosa		miliaceum	S.		S.	
							cereale		cereale	

[§] The cover crop (CC) seeds were pre-mixed prior to planting.

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