HortScience 60(1):73–80. 2025. https://doi.org/10.21273/HORTSCI18276-24

# Evaluation of Legume Cover Crop Species for Citrus Production in Southeast Florida

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*Keywords.* *Citrus* spp., cover crops, Leguminosae, nitrogen fixation

***Abstract.*** **Because of the low fertility soils native to southeast Florida and the high nutrient demand of citrus trees under citrus greening—an endemic bacterial disease impacting nutrient uptake—growers are returning to the use of cover crops to improve soil fertility. Cover crops, specifically legumes, can improve soil nitrogen (N) availability because of their ability to fix N from the atmosphere. More citrus growers in southeast Florida are growing cover crops; however, there is a lack of recent research of suitable legume species and their impact on soil N cycling. To address this gap in the literature, six different treatments consisting of five legume species monocultures and one fallow plot (control) were organized into a completely randomized design. The experiment was conducted twice under warm and cool season conditions, and each treatment was replicated five times ($n = 5$), for a total of 30 experimental plots. Legume species were hairy indigo (*Indigofera hirsuta*), sunn hemp (*Crotalaria juncea*), cowpea (*Vigna unguiculata*), alfalfa (*Medicago sativa*), and aeschynomene (*Aeschynomene americana*). Biomass production, N concentration, and nodule characteristics as well as their impact on soil N were measured over a 1-year span in both warm and cool seasons. Overall, both cowpea and hairy indigo produced more biomass and, as a result, higher tissue N compared with those of the other legumes at both 60 and 150 days after planting. However, no impact on soil N was observed. Additionally, all legumes were unable to survive in the cool season, resulting in no measurable biomass at 150 days after planting. These results are relevant for citrus growers aiming to enhance soil fertility through cover crops in southeast Florida. While several legume options are available for the warm season (e.g., cowpea, hairy indigo, and sunn hemp), suitable legume species for the cool season have yet to be identified.**

Leguminous plants play a crucial role in cover crop mixtures because of their unique ability to symbiotically fix atmospheric nitrogen ($N_2$) (Blesh 2018). These plants form symbiotic relationships with *Rhizobium* bacteria, providing carbohydrates to the bacteria in exchange for nitrogen (N) (Wang 2019; Willems 2006). This symbiotic relationship occurs when the bacteria infect root hairs, thus inducing the formation of nodules, where they convert atmospheric N to ammonia ($NH_3$) (Franche et al. 2009; Mahmud et al. 2020; Raza et al. 2020).

When terminated, legumes decompose, releasing N into the soil. Thus, growing and then terminating legumes can produce a significant amount of N, potentially reducing the amount of N fertilizer needed to support crop production (Jensen et al. 2020; Magdoff and van Es 2021). Additions of N to the sandy and low-fertility soils commonly found along the east coast of Florida can be of great benefit to the region's citrus industry. These additions could help maintain adequate levels of nutrients for fruit production, which is increasingly challenging and expensive, especially in the age of the devastating disease citrus greening [also known as huanglongbing (HLB)] (Dong et al. 2021; Mattos et al. 2020). The disease greatly reduces root mass, thus increasing instances of nutrient deficiencies, which growers manage by increasing nutrient application rates and frequencies (Kadyampakeni and Chinyukwi 2021; Tardivo et al. 2024). Consequently, this increased fertilizer usage has reduced the operational profitability of citrus growers in the region (Bassanezi et al. 2021; Li et al. 2020).

Depending on planting density and growth stage, citrus trees require an estimated 28.02 to 224.17 $kg \cdot ha^{-1}$ of N per year (Obreza et al. 2020). Cowpea (*Vigna unguiculata*) can fix 100 to 150 $kg \cdot ha^{-1}$ of N, while sunn hemp (*Crotalaria juncea*) can fix 84 to 100 $kg \cdot ha^{-1}$ of N (Das et al. 2021). In Florida, a 7-year study by Stokes et al. (1932) found that *Crotalaria striata* produced an average of 5570 $kg \cdot ha^{-1}$ of air-dried residues, which returned an estimated 121.05 $kg \cdot ha^{-1}$ of N to the soil. Additionally, Stokes et al. (1932) found that cowpeas produced an average of 2316 $kg \cdot ha^{-1}$ of air-dried residues, which returned 38.1 $kg \cdot ha^{-1}$ of N to the soil.

Although the practice of growing and terminating legumes species to add organic material to the soil and supply trees with N has been known to citrus growers in Florida since the late 1800s, its popularity greatly declined in the mid-20th century because of the wide-scale adoption of synthetic fertilizers (Hallman et al. 2024; Hume 1904). Because of issues with soil fertility, tree health, and economic challenges associated with citrus greening, growers have expressed a renewed interest in growing legume cover crops to improve soil N availability (Chakravarty et al. 2023). However, to improve soil N availability, sufficient legume biomass and nodulation (Denton et al. 2017; Kebede 2021), which can be challenging on the sandy, low-fertility soils native to southeast Florida, must be produced.

Because both biomass and nodulation are directly dependent on environmental factors such as soil type and moisture, geographic location plays a major role in the suitability of legume cover crops (Buetow et al. 2017). In Florida, differences in soil type, rainfall, and temperature among the eastern Flatwoods and other regions, such as the central ridge, cause certain legumes to be suitable for one area but not for another.

Currently, management recommendations for legume cover crop species selection are based on research nearly 100 years old and/or trials that were primarily conducted in central regions of Florida (Hallman et al. 2024; Stokes et al. 1932). A recent study by Grabowski and Williams (2020) compared biomass production of 16 different warm-season species and cultivars of legumes and found that sunn hemp consistently produced higher biomass compared with that of all other legumes tested. In 2017, for example, sunn hemp produced 24,386 $kg \cdot ha^{-1}$ of dry matter, thus greatly outperforming other common

Received for publication 21 Oct 2024. Accepted for publication 14 Nov 2024.
Published online 13 Dec 2024.
This research was part of Lukas Hallman's doctoral dissertation and was funded by the UF/IFAS Citrus Initiative and the UF/IFAS College of Agricultural and Life Sciences Dean's Award, which was awarded to Lukas Hallman. We thank Randy and Xander Burton at the UF/IFAS Indian River Research and Education Center for their assistance with farm equipment and operations.
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legumes such as 'Iron Clay' cowpea and velvet bean (*Mucuna pruriens*), which only produced 9421 $kg \cdot ha^{-1}$ and 4966 $kg \cdot ha^{-1}$, respectively. However, it is important to note that this study was conducted at the USDA Plant Material Center in Brooksville, FL, USA, which is located on Florida's west coast (lat. 28°37′43″N, long. 82°20′51″W), and focused solely on warm-season legumes.

In addition to the lack of recent research of warm-season legumes in southeast Florida, there is a lack of published data of suitable cool-season legumes for this region. The standard cover crop practice for citrus growers in Florida is to plant two rotations of cover crops per year (Chakravarty and Wade 2023). A warm-season cover crop is planted in June and terminated in approximately October or November, and a cool-season cover crop is planted between October and December and terminated between April and June (Campbell and Treadwell 2021). Importantly, the N demand of citrus trees is highest during flowering and fruit set, which usually occur between February and June (Obreza et al. 2020). A cool-season legume terminated during this time could provide a substantial amount of N when the citrus trees need it most. In response to the lack of cool-season legume choices, some growers have planted warm-season legumes, such as sunn hemp and cowpeas, during the cool season, with mixed results.

Because of the overall lack of recent research of legume cover crops for citrus production systems in southeast Florida, a legume species field trial was established. The specific goals were to evaluate legume(s) for both the warm and cool seasons by measuring biomass, legume nodulation, and N concentration, and to measure the impact of these legumes on N concentration in the soil.

## Materials and Methods

*Site description and climate data.* The experiment was conducted at the University of Florida's Institute of Food and Agricultural Sciences (UF/IFAS) Indian River Research and Education Center (IRREC), which is located in Fort Pierce, FL, USA (lat. 27°26′02″N, long. 80°26′49″W). The site was a former citrus grove and contains raised beds, which are standard for citrus production in southeast Florida. The soil was classified as Pineda sand, which is loamy, siliceous, hyperthermic Arenic Glossaqualfs (Watts et al. 1980). Initial soil samples were collected on 15 May 2023 to measure nutrient concentrations of the site (Table 1). Average daily temperature and precipitation data were collected from an onsite Davis Vantage Pro2 weather station (Fig. 1) (Davis Instruments Hayward, CA, USA). Historical temperature and precipitation data for Fort Pierce, FL, USA, were obtained from the National Oceanic and Atmospheric Administration National Weather Service online database climate database (https://www.weather.gov).

*Treatments and experimental design.* Six different treatments consisting of five legume species monocultures and one fallow plot (control) were organized into a completely randomized design. Each treatment was replicated five times, for a total of 30 experimental plots. Each plot measured 6.09 m (length) × 3.70 m (width). The plot size was selected to match the industry standard dimension of row middles in commercial citrus groves. Legume species grown were hairy indigo (*Indigofera hirsuta*), sunn hemp (*Crotalaria juncea*), cowpea (*Vigna unguiculata*), alfalfa (*Medicago sativa*), and aeschynomene (*Aeschynomene americana*). Legume species were selected based on local grower input, historical use in the citrus industry, and seed availability.

Table 1. Initial soil measurements ± standard error of the nutrient concentration, organic matter, cation exchange capacity (CEC), and pH of the experimental site ($n$ = 30) collected before the start of the study on 15 May 2023.

| Soil parameter | Initial soil measurements |
|---|---|
| Total nitrogen (mg/kg) | 800.00 ± 100.00 |
| Nitrate ($kg \cdot ha^{-1}$) | 1.27 ± 0.73 |
| Ammonium ($kg \cdot ha^{-1}$) | 1.18 ± 0.58 |
| Phosphorus ($kg \cdot ha^{-1}$) | 38.31 ± 10.01 |
| Potassium ($kg \cdot ha^{-1}$) | 55.36 ± 15.40 |
| Magnesium ($kg \cdot ha^{-1}$) | 63.78 ± 8.80 |
| Organic matter (%) | 0.68 ± 0.13 |
| CEC (cmol/kg) | 3.55 ± 0.47 |
| pH | 5.79 ± 0.35 |

*Legume management practices.* The legumes were planted twice per year (Jun and Nov 2023) and grown for 5 months. Before planting, the plots were tilled using a Maletti rotary tiller (Scandiano, RE, Italy) to a depth of 2.54 cm. Hairy indigo, sunn hemp, cowpea, and aeschynomene seeds were then inoculated with Exceed Superior Legume Inoculant containing *Rhizobium leguminosarum* biovar *viceae*, *Bradyrhizobium* sp. (Vigna), and *Rhizobium leguminosarum* biovar *phaseoli*, at the manufacturer's recommended rate (Visjon Biologics, Henrietta, TX, USA). Alfalfa seeds came precoated with inoculant.

Seeds were then spread at the species-recommended rate provided by UF/IFAS

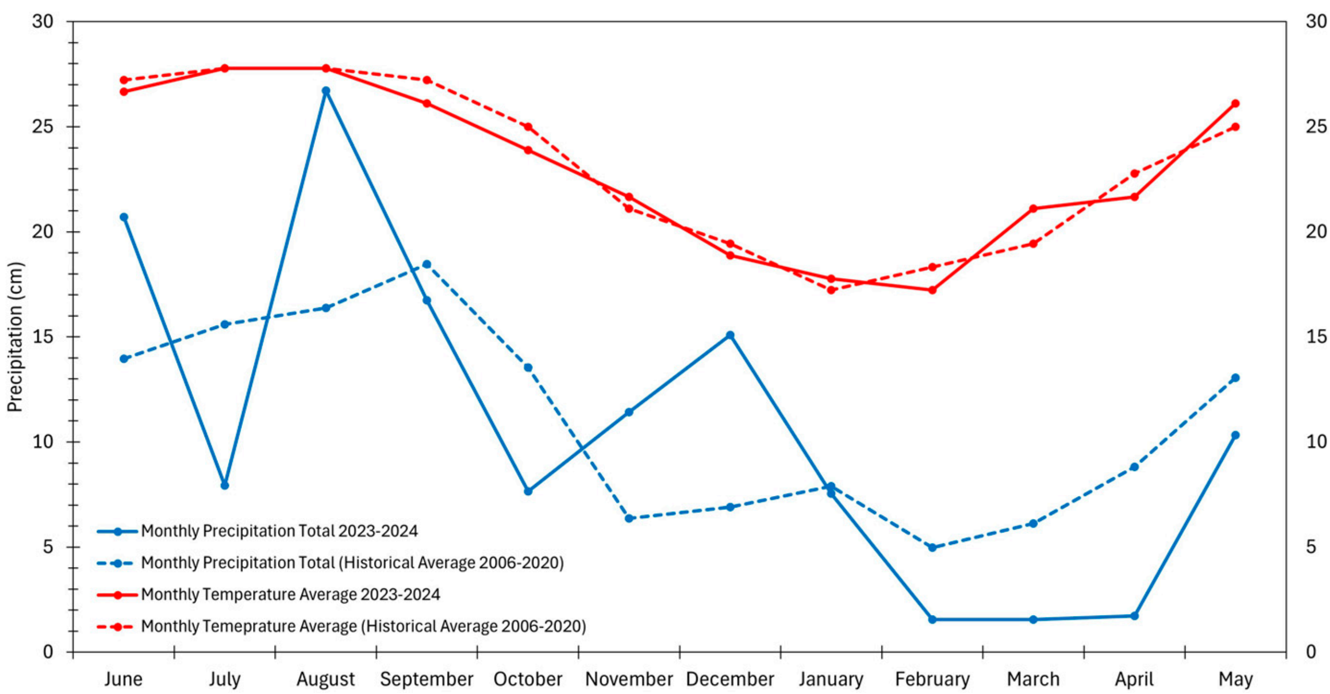


Fig. 1. Monthly precipitation totals (blue line) and monthly temperature average (red line) taken at the experimental site located at the University of Florida's Institute of Food and Agricultural Sciences (UF/IFAS) Indian River Research and Education Center in Fort Pierce, FL, USA. The historical monthly precipitation total (average total from 2006 to 2020; blue dotted line) and historical monthly temperature average (red dotted line) of Fort Pierce, FL, USA, were provided by National Oceanic and Atmospheric Administration (NOAA).

Table 2. Recommended legume seeding rates from the University of Florida Institute of Food and Agricultural Science (UF/IFAS) and Hancock Seed Company as well as the rate used in the study.

| | Rate ($kg \cdot ha^{-1}$) | | |
|---|---|---|---|
| Legume species | UF/IFAS | Hancock Seed Company | Rate used |
| Aeschynomene | 22.42–28.02 | 28.02–39.23 | 28.02 |
| Alfalfa | 20.18–22.42 | 22.42–28.02 | 22.42 |
| Cowpea | 33.63–56.04 | 56.04–84.06 | 67.25 |
| Hairy indigo | 5.35–11.21 | 5.60–9.00 | 9.00 |
| Sunn hemp | 33.63–56.04 | 33.63–56.04 | 44.83 |

(Rich et al. 2003; Seepaul et al. 2023) and the seed provider (Table 2) using a Scotts Turf Builder EdgeGuard Mini 15-lb broadcast fertilizer spreader (Scotts Miracle-Gro Company, Marysville, OH, USA) and then gently raked by hand. Once planted, the treatments were not irrigated or fertilized to reduce cover crop management costs and to make them economically feasible. At the end of each season, the legumes were terminated, and debris was incorporated into the soil via disking (Table 3). The described methods for planting, management, and termination of legumes reflected the local industry cover crop management practices (Hallman et al. 2024).

*Legume biomass.* Legume biomass was measured 2 months after planting and 1 d before termination. A sampling frame measuring 0.61 × 0.61 m was randomly thrown into each plot, and all aboveground legume biomass and belowground legume biomass within the frame were harvested. A shovel was used to gently lift the entire legume from the soil, keeping the root mass intact. Fresh weight of the aboveground and that of the belowground components were measured and nodulation data were collected. The samples were then dried at 60 °C for 5 d, and dry weight was recorded. Finally, a subsample of the biomass was analyzed to determine the nutrient concentration. Any plants within the plot other than the legume species planted were considered weeds and discarded.

*Soil nitrogen concentration.* Soil samples were collected in the center of each experimental plot using a one-piece soil auger (One-Piece Auger model #400.48; AMS, Inc., American Falls, ID, USA) with a diameter of 7 cm and depth of 10 cm. Four cores from each plot were collected and thoroughly mixed to create one representative soil sample per plot. Soil samples were then dried at 60 °C for 3 d and analyzed to determine soil nitrate and total N.

Soil nitrate was analyzed using the cadmium reduction method (Huffman and Barbarick 1981). Briefly, 3 g of CaO and 50 mL of distilled water were mixed and used to extract nitrate from 25 g of soil. The solution was shaken for 15 min and then filtered. Then, 0.5 mL of filtrate was diluted to 50 mL with $NH_4Cl$-EDTA solution. Next, 25 mL of the diluted sample was poured into a column at a flow rate of 7 to 10 mL per minute, and the remaining 25 mL of the diluted sample was added to the column. Then, 15 mL of the leachate was collected, and 1 mL of color reagent was added. The transmittance of the solution was measured at 540 nm, and the concentration was determined from a standard nitrate curve.

Total soil N was measured using the dry combustion procedure (Matejovic 1997). Samples were milled and then dried at 105 °C for 3 h. Then, 1 g of soil per sample was loaded into tin capsules and combusted using a CNS-2000 analyzer (LECO Corp., St. Joseph, MI, USA). Total soil N was reported as a percentage of weight.

*Legume nitrogen concentration and production.* The N concentration of the legumes was measured twice per season. A subsample of roots and leaves was taken from the biomass sampling and used for nutrient analysis. Plant samples were collected from each experimental plot and dried at 80 °C for 72 h. Plant material was then ground to pass through a 1-mm mesh screen, and 5 mL of $HNO_3$ was added. Samples were then heated to 95 °C for 90 min, and 4 mL of 30% $H_2O_2$ was added. After 20 min of cooling, 50 mL of deionized water was added to each sample (Isaac and Johnson 1985). An analysis of the N concentration was conducted using inductively coupled argon plasma emission spectrophotometer (Spectro Ciros CCD, Fitzburg, MA, USA). Based on the legume N percentage and biomass from aboveground and belowground components, total legume N production was estimated using the following formula:

$$\text{Total N (kg/ha)} = \frac{\text{Biomass yield} \times \% \text{ N}}{100}.$$

*Legume nodulation and activity.* Root nodulation data were collected from the legume biomass samples 60 and 150 d after planting. The roots were washed to remove loose soil and debris. Nodules were separated from the roots, counted, and weighed. Root nodulation was measured as the nodule count per gram of dry weight of the root. Once weighed, 10 nodules were checked for internal color to classify N fixation activity following the procedure of Kasper et al. (2019). Pink, red, or brown nodules were considered active, whereas any other color was considered inactive.

*Statistical analysis.* A Kruskal-Wallis test was used to evaluate the effect of legume species on legume biomass, nodulation, nodule weight, nodule activity, and N concentration. When a significant difference between means was detected ($P < 0.05$), Dunn's post hoc test was used to make pairwise comparisons between treatments. A two-way analysis of variance was conducted to assess the effects of legume species, time, and species × time on soil nitrate and total N levels. When significant differences were detected, Tukey's honest significant difference post hoc test was performed to evaluate pairwise comparisons of the factor. All statistical analysis was conducted using R (R version 4.3.3).

Table 3. Management and sampling schedule of the field trials.

| Season | Time | Management and sampling activity | Description |
|---|---|---|---|
| Warm season | 10 Jun 2023 (planting) | Site preparation, initial soil sampling, and warm season planting | • The raised beds were sprayed with glyphosate to kill perennial turfgrass and weeds and then tilled. • Four soil cores were collected from the center of each plot. • Legume seeds were inoculated and broadcasted into designated plots. |
| | 9 Aug 2023 (60 d after planting) | Biomass collection and soil sample collection | • Four soil cores were collected from the center of each plot and legume biomass subsamples were collected. |
| | 7 Nov 2023 (150 d after planting) | Biomass sampling and legume termination | • Legume biomass subsamples were collected. • Legumes were terminated via tilling. |
| | 17 Nov 2023 (10 d after termination) | Soil sample collection | • Four soil cores were collected from the center of each plot. |
| Cool season | 17 Nov 2023 (planting) | Site preparation, initial soil sampling, and cool season planting | • Four soil cores were collected from the center of each plot. • The cool season seeds were inoculated then broadcasted into designated plots. |
| | 16 Jan 2024 (60 d after planting) | Biomass sampling and soil sample collection | • Four soil cores were collected from the center of each plot. • Legume biomass subsamples were collected. |
| | 15 Apr 2024 (150 d after planting) | Biomass sampling and legume termination | • Legume biomass subsamples were collected. • Legumes were terminated via tilling. |
| | 25 Apr 2024 (10 d after termination) | Soil sample collection | • Four soil cores were collected from the center of each plot. |

Table 4. Legume dry weight biomass (kg·ha$^{-1}$) ± standard error of five replicates at 60 and 150 d after planting in both warm and cool seasons.

| | Warm season biomass (kg·ha$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 60 d after planting | | | 150 d after planting | | |
| | Aboveground | Belowground | Total | Aboveground | Belowground | Total |
| Aeschynomene | 118.94 ± 19.28 b | 125.40 ± 45.69 | 244.33 ± 60.29 b | 26.91 ± 12.03 b | 80.73 ± 32.96 b | 107.64 ± 44.22 b |
| Alfalfa | – | – | – | – | – | – |
| Cowpea | 4709.04 ± 1795.04 a | 487.05 ± 115.30 | 5196.09 ± 1898.19 a | 107.64 ± 26.91 ab | 2260.34 ± 727.53 a | 2367.98 ± 720.28 a |
| Hairy indigo | 3525.06 ± 907.95 a | 559.17 ± 135.37 | 4084.22 ± 986.45 a | 242.18 ± 50.34 a | 3390.51 ± 1214.93 a | 2489.07 ± 689.33 a |
| Sunn hemp | 1614.53 ± 1208.65 ab | 1752.30 ± 1585.36 | 609.48 ± 518.78 ab | 188.36 ± 117.29 ab | 1103.26 ± 675.94 ab | 1291.62 ± 792.10 ab |
| | Cool season biomass (kg·ha$^{-1}$) | | | | | |
| | 60 d after planting | | | 150 d after planting | | |
| | Aboveground | Belowground | Total | Aboveground | Belowground | Total |
| Aeschynomene | 11.36 ± 7.37 | 13.02 ± 8.70 | 24.38 ± 15.97 | – | – | – |
| Alfalfa | – | – | – | – | – | – |
| Cowpea | 41.01 ± 30.73 | 15.71 ± 14.27 | 56.72 ± 44.77 | – | – | – |
| Hairy indigo | – | – | – | – | – | – |
| Sunn hemp | 72.12 ± 34.64 | 43.05 ± 20.88 | 115.17 ± 54.60 | – | – | – |

Different letters within columns and sampling times indicate significant differences calculated using Dunn's post hoc test. – indicates insufficient legume biomass for sample collection.



## Results

*Legume biomass.* Significant differences in legume biomass were observed between species 60 d after planting during the warm season sampling period (Table 4). Both cowpea and hairy indigo produced more aboveground biomass (190% and 186%) compared with that of aeschynomene. Similarly, cowpea and hairy indigo produced more total biomass (186% and 177%) compared with that of aeschynomene. No significant differences were observed in belowground biomass between any of the species at either time point. Significant differences were observed between the aboveground, belowground, and total biomass between legume species 150 d after planting during the warm season. Hairy indigo produced more aboveground biomass (160%) compared with that of aeschynomene. Additionally, both cowpea and hairy indigo produced more belowground biomass (186% and 191%) and total biomass (182% and 183%) compared with those of aeschynomene. Alfalfa germination was unsuccessful and did not produce biomass in any of the plots throughout the warm growing season.

During the cool growing season, no differences were observed between aboveground, belowground, and total biomass 60 d after planting. During the 150-d sampling period after planting, no legume biomass was detected in any of the plots.

*Legume nodulation, nodule weight, and nodule activity.* Variation in nodulation was observed at both 60 and 150 d after planting during the warm season (Table 5). At both time points, aeschynomene had significantly higher nodulation (201.13 nodules/g of root) than that of cowpea (3.44 nodules/g of root) and sunn hemp (1.99 nodules/g of root). No significant differences were observed in nodulation 60 d after planting in the cool season. No nodulation data were collected 150 d after planting in the cool season because of the lack of biomass.

No significant differences in nodule weight were observed during the warm season. However, 60 d after planting in the cool season, sunn hemp had significantly greater average nodule mass (7.27 mg) compared with that of aeschynomene (0.01 mg). No nodulation data were collected 150 d after planting in the cool season because of the lack of biomass.

No significant differences in nodule activity were observed at either time point during the warm season. The nodule activity ranged 60% to 77% of active nodules at 60 d after planting and then dropped to 13% to 40% of active nodules at 150 d after planting. During the cool season, sunn hemp and aeschynomene nodule activity ranged from 44% to 62%, which was significantly higher compared with that of cowpea which had only 5% active nodules. No nodule activity data were collected 150 d after planting in the cool season because of the lack of biomass.

*Legume nitrogen concentration.* No significant differences in leaf N concentrations were measured between legume species at 60 and 150 d after planting during the warm season. The N concentration ranged from 2.5% to 3.5% at 60 d after planting and from 2.05% to 2.40% at 150 d after planting (Table 6). No significant differences were observed in leaf N concentrations 60 d after planting in the

Table 5. Legume nodulation measurements ± standard error of five replicates. Data comprise the number of nodules per gram of root, nodule weight (mg), and % of nodule activity at 60 and 150 d after planting in both warm and cool seasons.

| | Warm season | | | | | |
|---|---|---|---|---|---|---|
| | 60 d after planting | | | 150 d after planting | | |
| | Nodulation (no./g of root) | Nodule weight (mg) | Activity (%) | Nodulation (no./g of root) | Nodule weight (mg) | Activity (%) |
| Aeschynomene | 201.13 ± 96.88 a | 2.25 ± 0.18 | 60.0 ± 8.37 | 92.17 ± 18.54 a | 0.57 ± 0.37 | 13.33 ± 3.33 |
| Alfalfa | – | – | – | – | – | – |
| Cowpea | 3.44 ± 1.74 b | 12.57 ± 8.99 | 76.0 ± 8.12 | 1.47 ± 0.12 bc | 9.94 ± 2.42 | 26.67 ± 12.02 |
| Hairy indigo | 10.23 ± 3.88 ab | 2.27 ± 2.13 | 77.5 ± 6.29 | 5.43 ± 4.20 ab | 3.98 ± 2.60 | 22.0 ± 8.60 |
| Sunn hemp | 1.99 ± 1.97 b | 11.53 ± 8.97 | 75.0 ± 5 | 0.68 ± 0.59 c | 6.09 ± 5.53 | 40.0 ± 10.0 |
| | Cool season | | | | | |
| | 60 d after planting | | | 150 d after planting | | |
| | Nodulation (no./g of root) | Nodule weight (mg) | Activity (%) | Nodulation (no./g of root) | Nodule weight (mg) | Activity (%) |
| Aeschynomene | 93.24 ± 24.16 | 0.01 ± 0.0002 b | 44 ± 5.10 a | – | – | – |
| Alfalfa | – | – | – | – | – | – |
| Cowpea | 52.16 ± 31.30 | 2.66 ± 2.49 ab | 5 ± 2.89 b | – | – | – |
| Hairy indigo | – | – | – | – | – | – |
| Sunn hemp | 121.18 ± 14.59 | 7.27 ± 3.02 a | 62.5 ± 8.54 a | – | – | – |

Different letters within columns and sampling times indicate significant differences calculated using Dunn's post hoc test. – indicates insufficient legume biomass for sample collection.

Table 6. Legume nitrogen (N) (%) concentration ± standard error of five replicates: aboveground and belowground N (%) and total fixed N (kg·ha$^{-1}$) at 60 and 150 d after planting in both warm and cool seasons.

| | Warm season | | | | | |
|---|---|---|---|---|---|---|
| | 60 d after planting | | | 150 d after planting | | |
| | Aboveground N (%) | Belowground N (%) | Total N (kg·ha$^{-1}$) | Aboveground N (%) | Belowground N (%) | Total N (kg·ha$^{-1}$) |
| Aeschynomene | 2.52 ± 0.80 | 2.09 ± 0.06 a | 5.78 ± 1.79 b | 2.40 ± 0.19 | 1.58 ± 0.18 | 3.41 ± 0.46 b |
| Alfalfa | – | – | – | – | – | – |
| Cowpea | 3.53 ± 0.14 | 1.62 ± 0.08 b | 173.08 ± 46.70 a | 2.24 ± 0.05 | 1.39 ± 0.12 | 46.70 ± 16.90 a |
| Hairy indigo | 3.42 ± 0.34 | 1.79 ± 0.06 ab | 163.30 ± 32.01 a | 2.19 ± 0.20 | 1.39 ± 0.10 | 52.02 ± 14.90 a |
| Sunn hemp | 2.69 ± 0.57 | 1.17 ± 0.24 b | 125.59 ± 78.86 ab | 2.05 ± 0.19 | 0.88 ± 0.07 | 36.48 ± 2.74 ab |
| | Cool season | | | | | |
| | 60 d after planting | | | 150 d after planting | | |
| | Aboveground N (%) | Belowground N (%) | Total N (kg·ha$^{-1}$) | Aboveground N (%) | Belowground N (%) | Total N (kg·ha$^{-1}$) |
| Aeschynomene | 2.36 ± 0.04 | 1.89 ± 0.06 | 0.73 ± 0.34 | – | – | – |
| Alfalfa | – | – | – | – | – | – |
| Cowpea | 3.22 ± 0.825 | 1.70 ± 0.17 | 4.45 ± 2.38 | – | – | – |
| Hairy indigo | – | – | – | – | – | – |
| Sunn hemp | 3.18 ± 0.62 | 2.00 ± 0.08 | 5.78 ± 2.08 | – | – | – |

Different letters within columns and sampling times indicate significant differences calculated using Dunn's post hoc test. – indicates insufficient legume biomass for sample collection.

cool season. Not enough biomass was harvested 150 d after planting in the cool season to conduct a nutrient analysis. Significant differences in the belowground N concentration were observed 60 d after planting in the warm season. Aeschynomene had a significantly higher root N concentration (2.09%) compared with that of cowpea (1.62%) and sunn hemp (1.17%).

Significant differences in legume total N were detected at both 60 and 150 d after planting in the warm season. Both cowpea and hairy indigo produced significantly more N compared with that of aeschynomene at both time points. At 60 d after planting, cowpea produced 173.08 kg·ha$^{-1}$ of N and hairy indigo produced 163.30 kg·ha$^{-1}$ of N, whereas aeschynomene only produced 5.78 kg·ha$^{-1}$ of N. At 150 d after planting, cowpea produced 46.60 kg·ha$^{-1}$ of N and hairy indigo produced 52.02 kg·ha$^{-1}$ of N, while aeschynomene only produced 3.41 kg·ha$^{-1}$ of N. No significant differences were measured in the cool season.

*Soil nutrients.* No significant differences in soil nitrate were detected between treatments at planting, 60 d after planting, and 10 d after termination during the warm season. However, nitrate levels varied significantly over time within treatments (Fig. 2A). Cowpea had significantly higher soil nitrate levels 10 d after termination compared with planting, and hairy indigo had significantly higher nitrate levels 10 d after termination compared with 60 d after planting. The time × treatment interaction was not significant. No significant differences in soil nitrate were detected between treatments and time during the cool season (Fig. 2B).

No differences in total soil N were detected between treatments in the warm season or the cool season. In the warm season, time significantly impacted soil N (Fig. 3A and B). In all treatments, total N decreased between planting and 60 d after planting and then increased 10 d after termination. The time × treatment interaction was not significant.

## Discussion

To significantly improve soil N using legumes, sufficient biomass must be produced. For example, research by Finney et al. (2016) found that biomass production had a positive relationship ($R^2$ = 0.53) with soil N retention. Cover crop biomass (including legumes) is a function of many factors such as water availability, soil quality, genetics, and management (Brennan and Boyd 2012; Moore and Mirsky 2020; Ruis et al. 2019). In this study, legumes such as cowpea, hairy indigo, and sunn hemp produced sufficient biomass to establish a stand in the warm season, as expected, because all three of these species have been used in cover crop mixtures in Florida for more than a century

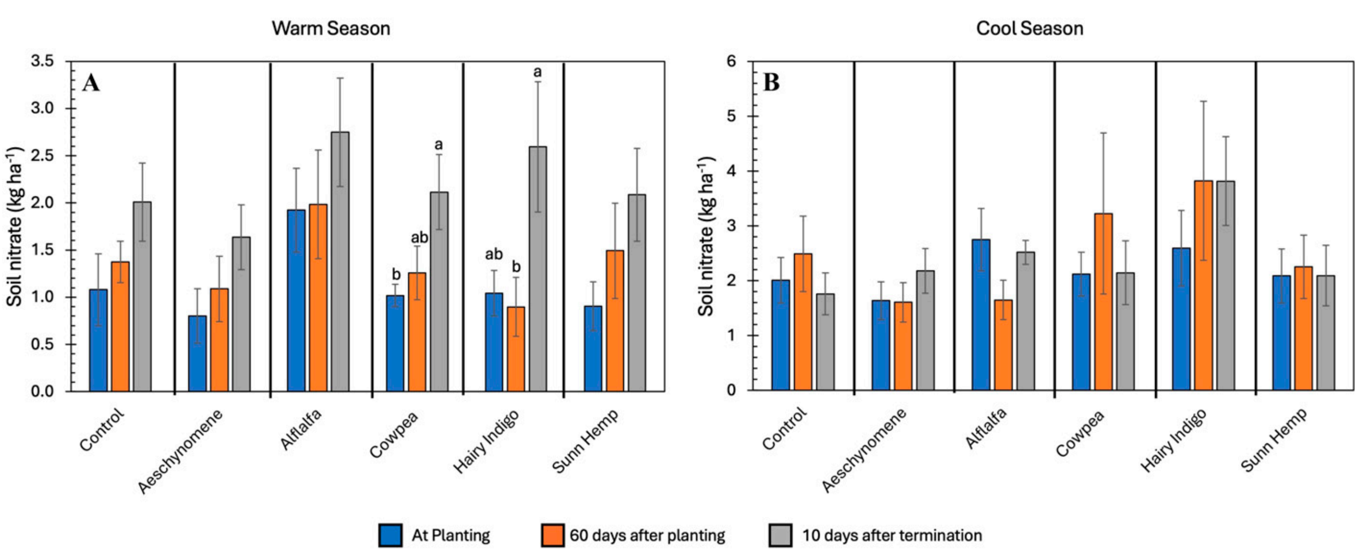


Fig. 2. Soil nitrate concentrations for the warm season (**A**) and cool season (**B**) collected at planting, 60 d after planting, and 10 d after termination. During the warm season, planting occurred on 10 Jun 2023; during the cool season, planting occurred on 17 Nov 2023. Different letters indicate significant differences ($P \leq 0.05$) in mean soil nitrate at different time points within each treatment. Bars represent ± standard error of five replicates.

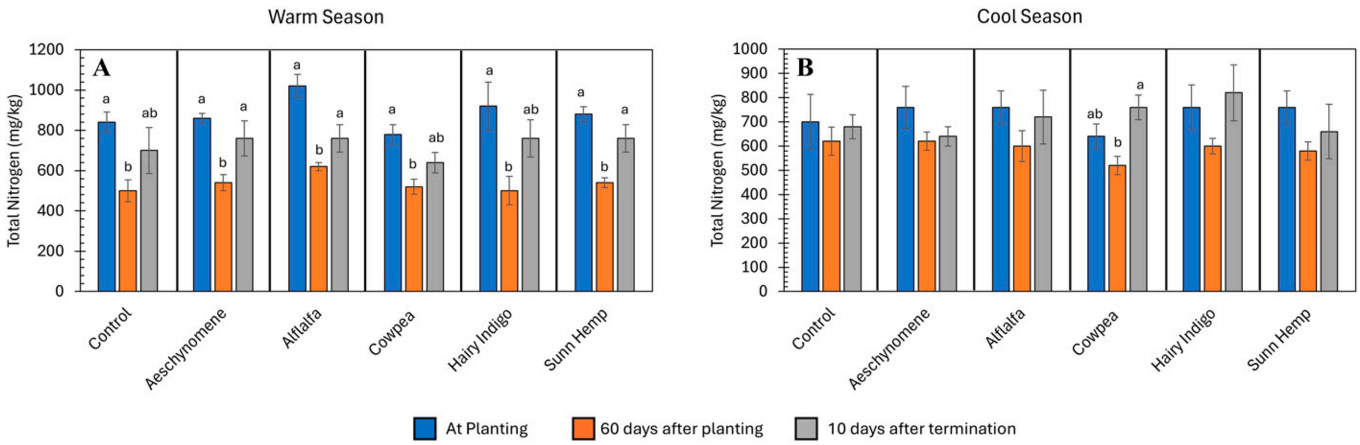


Fig. 3. Total soil nitrogen concentrations collected at planting, 60 d after planting, and 10 d after termination in both the warm season (**A**) and the cool season (**B**). The warm season planting occurred on 10 Jun 2023, and the cool season planting occurred on 17 Nov 2023. Different letters indicate significant differences ($P \leq 0.05$) in mean soil total nitrogen at different time points within each treatment. Bars represent ± standard error of five replicates.

(Grabowski and Williams 2020; Hallman et al. 2024; Hume 1904; Stokes 1925; Stokes et al. 1932). Adequate precipitation was likely the driving factor for biomass production in the warm season (Fig. 1). However, even the increased precipitation was likely insufficient for aeschynomene and alfalfa, which have higher water requirements (Tobisa et al. 2014). Although biomass production was low for aeschynomene and nonexistent for alfalfa in this trial, these species have been successfully grown in soils with higher water-holding capacity in southeast Florida (Dubeux et al. 2022; Kalmbacher et al. 1993).

The inability to achieve a consistent stand of all legume species during the cool season highlights the challenges that citrus growers face when attempting to implement cover crops in their groves. The major limitation for legume growth in the cool season is the lack of rain (Brewer et al. 2023; Hallman et al. 2024). Despite receiving rainfall soon after seeding in November and early December, the months from January to April received insufficient rainfall (Fig. 1). Additionally, compared with the historical average, Feb 2024 through May 2024 were particularly dry. Combined with the sandy, low-fertility soil typical of the region, this led to severe water stress and the eventual death of the legumes.

In addition to biomass, legumes must have active nodules to convert atmospheric N to plant available forms of N (Dubach and Russelle 1994). The number of nodules and their activity are generally controlled by environmental factors such as water availability and temperature (Mortier et al. 2012). Therefore, stressed plants and/or plants at the end of the growing season should have a smaller number of nodules and lower nodule activity (Lumactud et al. 2023; Matamoros et al. 1999; Ramos et al. 1999). Unexpectedly, aeschynomene produced a higher number of nodules despite having low biomass. This may be attributed to genetic differences because some legume species are more prone to producing higher nodule counts than others (Dhillon et al. 2022). Additionally, aeschynomene had a higher root N concentration compared with that of cowpea and sunn hemp, likely because of its greater nodulation. However, no differences were observed in the aboveground N concentration.

The absence of differences in soil nitrate and total N concentration among treatments was likely caused by a combination of insufficient biomass production and the timing of the soil N sample collection relative to when N was released from legume debris. Although cowpea and hairy indigo produced a significant amount of biomass after 60 d after planting in the warm season, samples were collected approximately 4 months before termination. At 150 d after planting sampling, the approximately 2000 $kg \cdot ha^{-1}$ of biomass from these legumes, along with the 46 and 52 kg of N, may not have been enough to affect soil N levels. Furthermore, because all legumes were unable to survive in the cool season after 150 d after planting, increases in soil N were unlikely to be observed.

Soil sampling was conducted 10 d after legume termination. This brief interval was selected because of the rapid decomposition anticipated when the plant debris are incorporated into the soil via tilling, in combination with the climate conditions in southeast Florida (Dorissant et al. 2022; Garzon et al. 2023). Warm soil temperatures, high soil moisture, and the impact of tilling are all known to speed up plant decomposition and, thus, N mobilization (Drinkwater et al. 2000; Poffenbarger et al. 2015). Research by Nyabami et al. (2023) found that in the humid and warm conditions of south Florida, plant debris lost more than 60% of the N concentration and 50% mass after the first month of decomposition. Although the final soil sampling in each season was conducted within 10 d of termination, the cooler and drier weather in November (at the end of the warm season) could have slowed decomposition. As a result, no impacts on the soil were detected within our sampling period. This is supported by research conducted by Zhou et al. (2011), who found legume cover crop treatments had lower net N mineralization rates in an arid environment, which indicated lower N availability in the short term.

In conclusion, cowpea and hairy indigo outperformed other legumes in biomass production and N fixation during the warm season. However, no legumes produced sufficient biomass by the end of the cool season, and no effects on soil N concentration were observed throughout the study. These results are relevant for citrus growers aiming to enhance soil fertility through cover crops in southeast Florida. Although several legume options are available for the warm season (e.g., cowpea, sunn hemp, and hairy indigo), suitable legume species for the cool season have yet to be identified. Future research should focus on evaluating the biomass production of a wider range of legume species. Additionally, studies should explore the effects of different termination methods and their timing and correlate them with different legume species and soil N concentrations.

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