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Phenotypic Characterization of 16 Accessions of Sunn Hemp in Florida

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ABSTRACT

Adoption of sunn hemp (*Crotalaria juncea* L.) as a cover crop has been limited primarily due to the availability of seed sources, leading to high seed costs and unreliable supplies. Seed production in Florida with the commercially available sunn hemp cultivar Tropic Sun has been largely unsuccessful. An experiment was designed to evaluate 16 accessions of sunn hemp for potential commercial seed production in Florida at three planting dates (May, June, July). Vegetative and reproductive characteristics were evaluated to provide baseline information for future development of a cultivar of sunn hemp that could produce seed in Florida with desirable cover crop attributes. The results indicate that the sunn hemp accessions could be separated into two groups. Regardless of planting date, accessions in Group 1 (PI 234771, PI 248491, PI 295851, PI 337080, PI 468956, PI 561720, PI 652939) displayed desirable cover crop characteristics, such as high biomass production and leaf area. Accessions in Group 1 had a short-day flowering response and produced few to no pods and seeds. Group 2 accessions (PI 207657, PI 250485, PI 250486, PI 250487, PI 314239, PI 322377, PI 346297, PI 391567, PI 426626) were less sensitive to photoperiod, were generally smaller plants because of earlier transition to reproductive growth, and produced more seedpods and seeds. Seed production was generally better when seeds were sown at the earliest date. It appears that in Florida, sunn hemp accessions that are capable of flowering in summer rather than fall have greater potential for seed production.

Core Ideas

- Based on vegetative and reproductive characteristics sunn hemp accessions could be divided into two groups.
- Group 1 accessions produce more shoot biomass and have a short-day flowering response.
- Flowering occurs in fall when effective pollinators are absent.
- Group 2 accessions are less sensitive to photoperiod.
- Group 2 accessions flower in summer when effective pollinators occur and successfully set seed.

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Sunn hemp (*Crotalaria juncea* L.) is a leguminous cover crop that is capable of providing multiple ecosystem services and economic benefits. In India, it is second to kenaf (*Hibiscus cannabinus* L.) in importance as a bast fiber crop, in addition to being one of the most widely grown green manures in the tropics (Purseglove, 1974; White and Haun, 1965). Sunn hemp rapidly establishes canopy closure, competing with weeds, and can produce 5.9 to 7.6 Mg ha^{-1} of biomass in 12 to 14 wk (Balkcom and Reeves, 2005; Mansoer et al., 1997) while also producing 120 to 144 kg ha^{-1} of N in 9 to 14 wk (Balkcom and Reeves, 2005; Mansoer et al., 1997). Additionally, a commercially available cultivar of sunn hemp, Tropic Sun, demonstrates resistance and suppression of root-knot nematode populations and can be used as an alternative to nematicides (Rotar and Joy, 1983).

Although cover crops such as sunn hemp can provide farming system benefits, disadvantages include costs and management of the additional crop in the farming system (Teasdale, 1996). These costs can include seeding and the cost of termination and incorporation of the cover crop. Recently, renewed interest in sunn hemp as a cover crop in the United States created an imbalance between supply and demand of sunn hemp seed. This imbalance also contributed to the high cost of sunn hemp seed due to limited seed sources (Abdul-baki et al., 2001). The limitation in seed availability has been reported since 1946 as being the major factor in the prevention of widespread use of sunn hemp as a soil-improving crop (McKee et al., 1946).

Currently, seed production areas limit the amount of available sunn hemp seed and control the market price of seed (Abdul-baki et al., 2001). Developed by the University of Hawaii and the USDA–NRCS, the cultivar Tropic Sun has historically been the focus for commercial seed production (Abdul-baki et al., 2001; USDA, 1983). Although Tropic Sun grows well in the southeastern United States, the environmental conditions for seed production cannot be met in subtropical regions where the first fall frost prevents effective seed set (White and Haun, 1965). Commercial seed production of sunn hemp occurs primarily in tropical locations where

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Abbreviations: PD, planting date.

photoperiods that are inductive to flowering are abundant and frost is not an issue. Expansion of sunn hemp seed production to a domestic location, such as southern Florida, could create a local seed source to meet market demands while simultaneously lowering the cost of seed (Abdul-baki et al., 2001). Identifying sunn hemp germplasm that is better adapted for seed production than Tropic Sun in the continental United States could also encourage greater use of sunn hemp as a cover crop and potentially as a seed crop. Two new cultivars of sunn hemp, AU Durbin and AU Golden, which were selected from USDA germplasm accessions PI 207657 and PI 322377, respectively, are capable of producing viable seed in Alabama (Mosjidis, 2014).

Seeds of 16 accessions of sunn hemp from the USDA Plant Genetic Resources Conservation Unit (Griffin, GA) collection were available in sufficient quantity for evaluation. The objective of this research was to evaluate these sunn hemp accessions for vegetative and reproductive characteristics for cover crop and commercial seed production potential in Florida. It was hypothesized that at least one accession would show potential for seed production in Florida while maintaining the beneficial qualities of a cover crop.

MATERIALS AND METHODS

The experiment was conducted between May and December in 2008 at Rosie's Organic Farm, Gainesville, FL, and was repeated in 2009 at a different location on the same farm. The experimental design was a split-plot with main plots arranged in a randomized complete block with four replications. The main plot treatments consisted of three planting dates (PDs)—May (PD1), June (PD2), and July (PD3)—to determine the effect of planting date on vegetative and reproductive parameters. The main plots' size was 5.6 m by 18.3 m. The subplot treatments were 1.4 m by 4.58 m and consisted of 16 accessions of sunn hemp (Table 1), and each subplot had a target population of 20 plants. Because seed was limited (100 seeds per accession per planting date), scarification and transplanting were used to maximize germination and survival. Seeds were hot water scarified by dipping in boiling water for 5 s and inoculated with cowpea-type *Rhizobium* (Nitragin, Milwaukee, WI) before seeding. Seeds were then planted into Jiffy peat pots (5.72 cm by 5.72 cm) with organic potting mix (Fafard, Apopka, FL) in a greenhouse. Seeding occurred on 5 May, 5 June, and 7 July in 2008 and on 1 May, 1 June, and 1 July in 2009. Seedlings were watered daily by hand. Seedlings were transplanted by hand into two rows spaced 45.72 cm apart on 1-m bed centers approximately 3 wk later (26 May, 25 June, and 23 July in 2008 and 26 May, 25 June, and 23 July in 2009). No fertilizer or irrigation was used in the field. Lodging was addressed by performing a tomato weave through the plants using twine and 1.8-m bamboo stakes placed between every other plant.

Vegetative Phenotypic Characteristics

Two months after seeding, six plants from each plot (24 plants total per accession per planting date) were randomly selected for data collection. Plant height was measured from the soil surface to the top leaf on the tallest branch, and the numbers of branches were determined. One leaf from the main stem of each sample plant was harvested, and a leaf scan was taken using an area meter (LI-3100; LI-COR Bisosciences, Lincoln, NE) to determine leaf area.

At 4 mo after seeding, primary lateral branches greater than 1 m in length were counted. Photosynthetically active radiation and leaf area index were measured using an AccuPar Ceptometer (Decagon Devices; Pullman, WA) at 4 mo after seeding. Readings were taken at midday with no cloud cover. An unobstructed reading was taken from above the sunn hemp canopy if possible; if not, an unobstructed reading was taken at 1 m above the ground. Below-canopy readings were taken by inserting the probe parallel to the sample plants. Five months after seeding, the shoots of sample plants were harvested at the soil surface from each plot. The samples were oven dried at 120°F for at least 72 h, and dry weights were taken. Final

Table 1. List of the 16 accessions from the USDA–ARS evaluated for their vegetative and reproductive phenotypes in Gainesville, FL, in 2008 and 2009.

Accession	USDA PI no.†	Other names	Country of origin
1	207657‡	–	Sri Lanka
2	234771	–	Nigeria
3	248491	Guizo de Cascavel	Brazil
4	250485	K679	India
5	250486	K680	India
6	250487	K681	India
7	295851	–	Brazil, São Paulo
8	314239	Col No 524	former Soviet Union
9	322377‡	IRI 2473	Brazil, São Paulo
10	337080	–	Brazil
11	346297	–	India, Delhi
12	391567	T'ai-yang-ma	South Africa
13	426626	Sanni	Pakistan
14	468956	Tropic Sun	United States
15	561720	IAC-1	Brazil, São Paulo
16	652939	Texas 374	United States, Texas

† USDA plant introduction number.

‡ Cultivars AU Durbin and AU Golden were selected from base populations of PI 207657 and PI 322377, respectively.

harvests were on 28 Aug., 29 Oct., and 24 Nov. 2008 for the first, second, and third planting dates, respectively. In 2008 earlier harvests were required due to tropical storm Fay and an early frost on 29 October. In 2009, final harvests were on 29 September, 3 November, and 2 December for the first, second, and third planting dates, respectively.

Reproductive Phenotypic Characterization

Days from seeding to date of first open flower were recorded. At 2 mo after seeding, the number of open flowers per plant was determined. At 4 mo after seeding, the number of branches with flowers and/or seedpods was determined. Seedpods were harvested from the same six sample plants used to assess vegetative parameters. All seed data were collected per six plants rather than by individual plant. Dried seedpods were harvested and counted at rattle-stage at weekly intervals, and seed weights were determined.

Data Analysis

All statistical analyses were performed using SAS statistical software, versions 9.0 and 9.2 (Cary, NC), using the MIXED, GLIMMIX, GLM, CORR, and IML procedures. Before analysis, square root transformations were performed on quantitative data to approximate normality. Photosynthetically active radiation penetrating the canopy was expressed as a fraction of unobstructed PAR measured at 1 m aboveground. A logit transformation was applied to PAR readings to better approximate normality.

Analysis of variance was performed for each variable of interest with effects for accession, year, planting date, and a planting date by accession interaction. For each response variable, the mean value for each accession was estimated from the ANOVA model after correcting for the year and planting date effects. Pairwise means comparisons were conducted for each response using the step-down Student–Newman–Keuls method to control the false discovery rate at the 0.05 level of significance. To assess multivariate differences across accessions when simultaneously considering multiple variables, Hotelling's T^2 test of multivariate mean equality was used. To account for multiple testing (120 tests for each pair of accessions), we control the false discovery rate using the Benjamini–Hochburg procedure. To interpret the results, graphics were constructed by connecting the accessions that were not significantly different (Fig. 1).

RESULTS AND DISCUSSION

Vegetative Parameters

A Hotelling's t test was conducted using the parameters of plant height, total number of leaves per plant, and leaf size at 2 mo after seeding; total branches (primary) at 4 mo after seeding; and plant weight at final harvest. Based on these parameters, Fig. 1 was created by using lines to connect accessions that were not significantly different from one another. The length of the line does not reflect the level of separation of the accessions. These vegetative parameters separated the 16 accessions into two groups, which were consistent with field observations. Accessions in Group 1 (accessions 2, 3, 7, 10, 14, 15, and 16) demonstrated erect growth, larger leaves, higher plant biomass, and fewer branches than accessions in Group 2 (1, 4, 5, 6, 8, 9, 11, 12, and 13).

High biomass accumulation (plant dry weight) (Fig. 2) and canopy closure (indicated by branching and leaf size) are important characteristics for the use of sunn hemp as a cover crop. Accessions in Group 1 displayed these desirable characteristics and could be used as cover crops. Group 1 included the commercially available Tropic Sun cultivar (accession 14; PI 468956), which is often used as a cover crop in the United States and which has demonstrated the desirable characteristics described above.

The vegetative data support the prediction of White and Haun (1965) that early-yielding varieties of sunn hemp, such

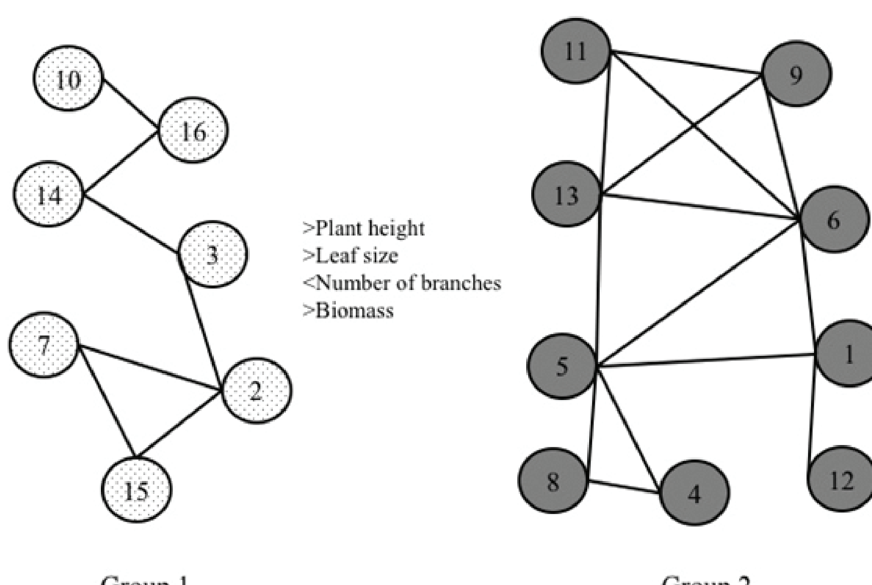

Fig. 1. Results for Hotelling's test for vegetative data of sunn hemp (2008–2009). Variables included height (2 mo after seeding [MAS]), total leaves (2 MAS), leaf area (4 MAS), total number of lateral branches (4 MAS), and plant weight (5 MAS). Accessions connected by a line are not significantly different from one another at α = 0.05; however, the length of the line does not represent the level of separation of the accessions.

Table 2. Average vegetative and reproductive characteristics of 16 sunn hemp accessions planted at three planting dates in Gainesville, FL, in 2008–2009.

Planting date†	Plant weight 5 MAS‡§	Days to first flower§	Mature seed pods§	Mature seed weight§
	g			g
PD1	603.3a	56b	15.1a	8.1a
PD2	377.3b	61a	5.3b	3.3a
PD3	230.7c	56b	11.3ab	8.2a

† PD1, May; PD2, June; PD3, July.

‡ Months after seeding.

§ Means included four replications of 16 accessions with six plants (384 plants) for each planting date. Means followed by the same letter were not significantly different at $\alpha = 0.05$.

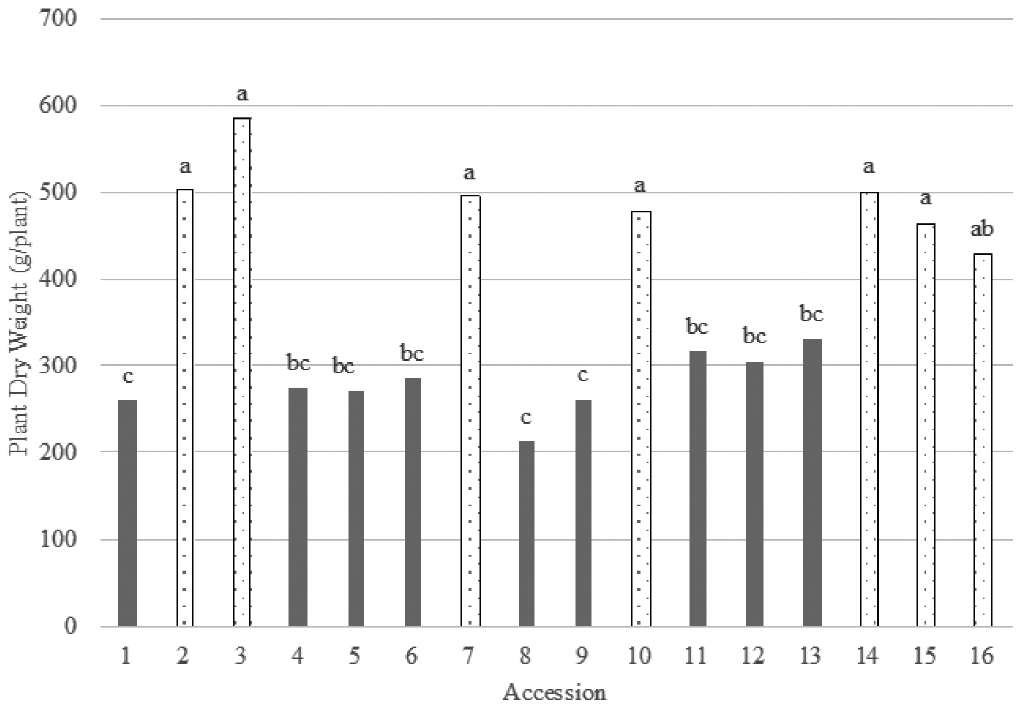

Fig. 2. Average weight of each plant at harvest (5 mo after seeding) from 16 sunn hemp accessions evaluated in Gainesville, FL (2008–2009). Accessions with the same letter were not significantly different at $\alpha = 0.05$. Accessions with dotted bars were from Group 1 and solid bars from Group 2.

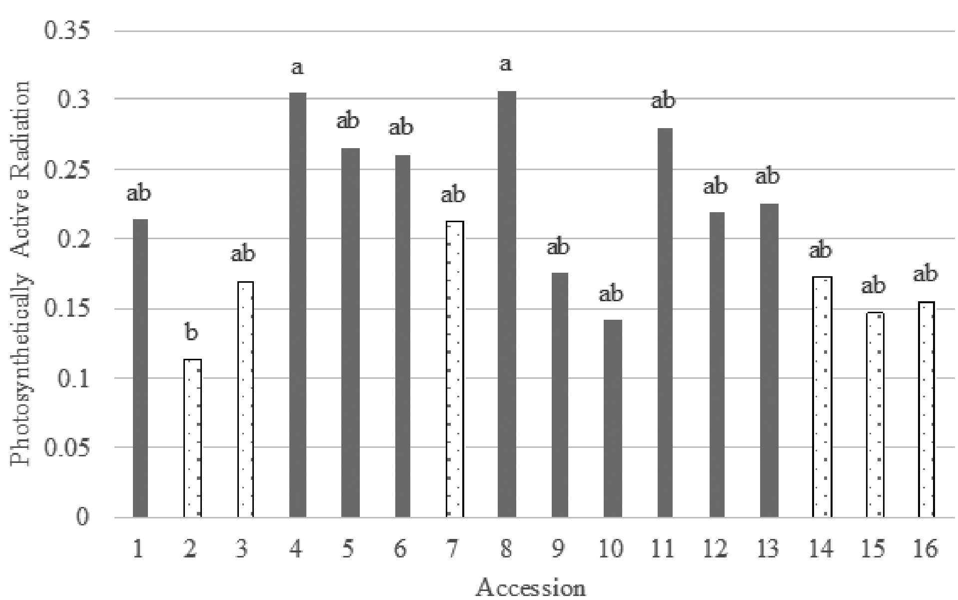

Fig. 3. Photosynthetically active radiation penetrating the canopies of 16 sunn hemp accessions 4 mo after seeding. Data were logit transformed for analysis. Accessions with the same letter were not significantly different at $\alpha = 0.05$. Accessions with dotted bars were from Group 1 and solid bars from Group 2.

as those in Group 2, would not produce as much biomass as late-maturing varieties (Group 1 accessions) (Fig. 2). In addition to the differences observed among accessions, planting date also induced differences in plant weight (Table 2). Highest (603 g), intermediate (377 g), and lowest plant biomass (231 g) were obtained with PD1, PD2, and PD3, respectively (Table 2). Plants from PD1 were exposed to the greatest number of days exceeding 13 h, allowing for significant biomass accumulation, especially in accessions (Group 1) that require short days for flower bud initiation and to transition from vegetative to reproductive growth. By the last planting date (July), daylength had dropped to less than 13 h, which is inductive to flowering (Nanda, 1962) for the daylength-sensitive accessions (such as those in Group 1). These photoperiods would have shortened the vegetative growth period of plants seeded after June, thereby reducing the total plant biomass for these planting dates.

Photosynthetically active radiation readings taken at 4 mo after seeding indicated few significant differences among accessions (Fig. 3), most likely due to the wide plant spacing used in this study. Planting density of sunn hemp affects the height and formation of primary and secondary branches, thereby affecting both canopy closure and flowering (Rotar and Joy, 1983). Lower planting densities are used for seed production (Rotar and Joy, 1983), and seed yield was of particular interest in the present study. However, because seed production is intended to facilitate use as a cover crop, promising accessions should be evaluated at higher planting densities. To determine the effect of earlier branching on weed suppression, accessions from Group 2 should be planted at cover crop seeding densities to determine their phenotypic expressions under these seeding rates.

Reproductive Parameters

Accessions categorized into Group 2 based on the vegetative parameters were the accessions that also produced flowers and seeds during the experimental period. Accessions in Group 2 (1, 4, 5, 6, 8, 9, 11, 12, and 13) flowered from 49 to 65 d after seeding, whereas accessions from Group 1 (2, 3, 7, 10, 14, 15, 16) flowered 65 d after seeding and later (with the exception of accession 2 at PD1), whereas some (7 and 15) did not flower at all during the experimental period (Fig. 4). Previous observations recognize the photoperiod sensitivity of sunn hemp for flower initiation (Nanda, 1962; White and Haun, 1965).

The accessions in Group 1 seem to be photoperiod sensitive because these accessions were late to produce flowers, regardless of the planting date (Fig. 4). The mean number of days to first flower was longest at PD2 and shortest at PD1 and PD3 (Table 2). During the experimental period in Florida, the longest days were in June, which may have influenced the flowering of accessions planted at this time. Nanda (1962) observed that sunn hemp did not become inductive to flower bud initiation until photoperiods decreased below 13 h. A study conducted by Keatinge et al. (1998) indicated that the earliest days to flowering in sunn hemp occurred at 38 d after planting under daylengths of 11.5 h. In the present study, accessions from Group 1 followed this pattern and did not flower until August, at the earliest, when daylength dropped below 13 h. However, accessions from Group 2 appear to be either daylength neutral or less sensitive to photoperiod and flowered as early as June.

Accessions from Group 2 had more branches with flowers and with both flowers and pods at 4 mo after seeding than accessions from Group 1 (Fig. 5). The higher numbers of flowers coincided with the earliness of flowering of some accessions as well as the seed production that follows. Because flowers of sunn hemp appear on the terminal racemes of secondary branches, flower number is greatly related to branching (Nanda, 1962; Purseglove, 1974). At 2 mo after seeding (averaged across three planting dates), accessions from Group 1 had fewer primary branches per plant than accessions from Group 2 (Table 3). The increase in branching may be an influential factor for the higher number of flowers in accessions

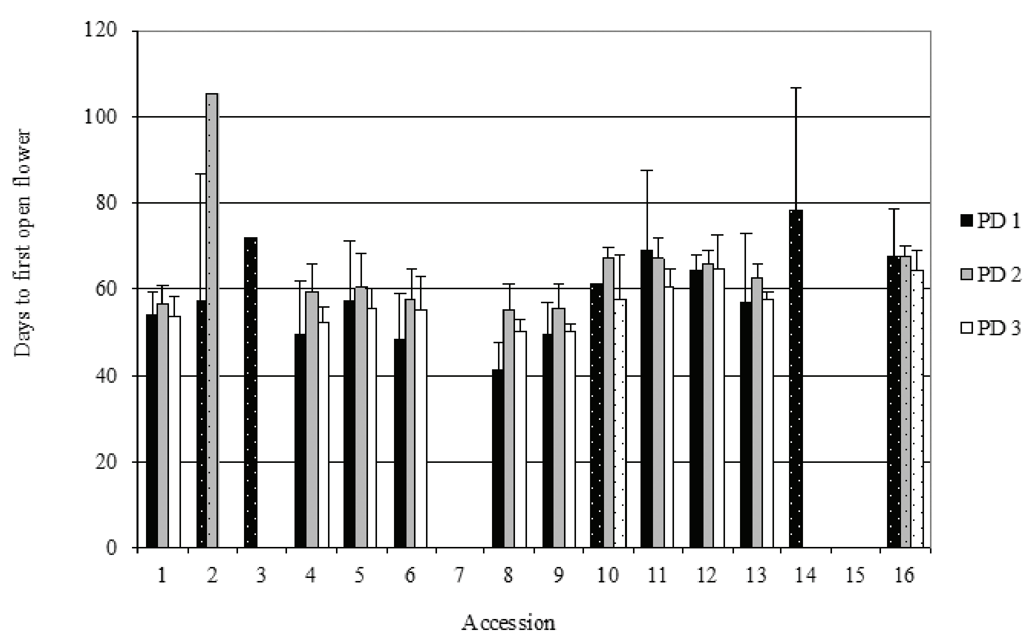

Fig. 4. Days to first open flower by planting date (PD) of 16 sunn hemp accessions in Gainesville, FL. PD1 = May, PD2 = June, and PD3 = July compared using standard error bars. Accessions with dotted bars were from Group 1 and solid bars from Group 2.

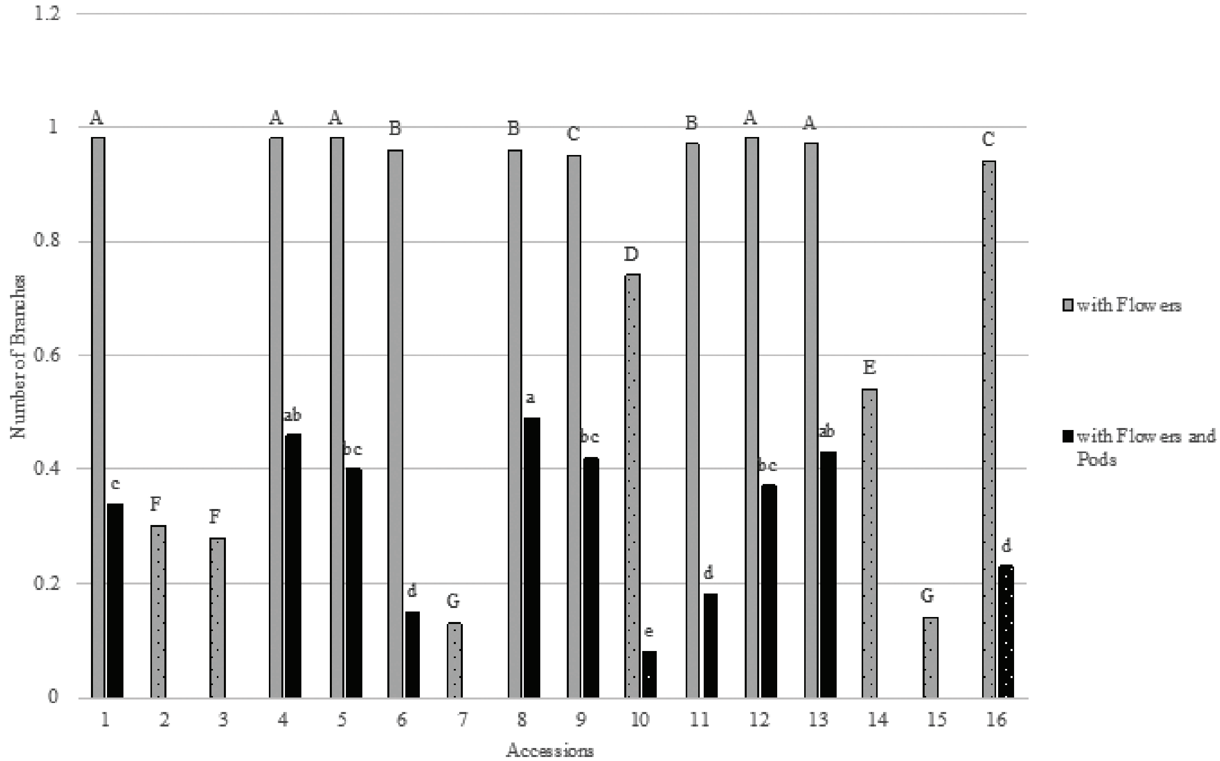

Fig. 5. Proportion of branches with flowers and of branches with flowers and pods to total number of branches of 16 sunn hemp accessions evaluated in Gainesville, FL (2008–2009) at 4 mo after seeding. Accessions with no pods were eliminated from the evaluation (2, 3, 7, 14, 15). Data were logit transformed for analysis. Accessions with the same letter were not significantly different at $\alpha = 0.05$. Accessions with dotted bars were from Group 1 and solid bars from Group 2.

from Group 2 (Table 3). Abdul-baki et al. (2001) proposed that there is a positive correlation between branching and flower number and reported an increase in flower number when the main stems of sunn hemp were cut at 90 cm.

Accessions 1, 4, 5, 6, 8, 9, 10, 11, 12, 13, and 16 produced mature pods and seed during the experimental period (Table 3). Accessions 8, 9, and 13 had the most mature pods per plant (Table 3). Considering the total amount of potential seed production from these accessions, these could potentially be used for future work that explores the seed production of sunn hemp in Florida. Consistent with our findings, accessions 1 and 9 were used as base populations for the selection of the commercial cultivars AU Durbin and AU Golden, respectively, which are capable of producing seed within the continental United States (Mosjidis, 2014).

The first planting date could be appropriate for seed production of sunn hemp in north-central Florida. The second planting date (June) did not produce as many mature and immature seedpods as the first planting date and could be recommended for growers who want to avoid seed set and only want to use sunn hemp as a cover crop. The third planting date includes some risk due to the potential loss of the crop to an early frost. Balkcom et al. (2011) recommended planting AU Golden sunn hemp as a cover crop immediately after the cash crop rather than delaying 2 wk to obtain the highest yields of biomass and nitrogen in Alabama.

Although there was profuse flowering in several accessions, production of mature seedpods was variable. If the flower and mature seedpod data in Table 3 are used to calculate percent pod set for the five accessions with 20 or more flowers per plant at 2 mo after planting, accessions 1, 4, 5, 8, and 9 resulted in 44, 39, 64, 45, and 109% pod set, respectively. A significant factor to consider in discussion of these data is effective pollination of sunn hemp. To have good and consistent seed set, a large insect, such as a bee, is needed to depress the keel of the flower and stimulate the stigmatic surface (Morris and Kays, 2005). Large bees from the *Xylocopa* or *Megachile* genera are capable of stimulating the stigmatic surface (Purseglove, 1974). Keatinge et al. (1998) investigated the effects of photoperiod and temperature on flowering and reproduction. They observed a lack of seed production in all photoperiod treatments, despite flowering in sunn hemp, even after 173 d. They attributed the lack of fruit set in sunn hemp to reproductive problems within a new environment where there was an absence of appropriate pollinating insects. Mansoer et al. (1997) observed flowering in sunn hemp at 35 to 49 d after planting in Alabama, but no seedpods were produced before the first frost killed the cover crop, despite a mid-April planting date. The lack of seed production might also be attributed to the lack of pollinators, as observed by Keatinge et al. (1998). Researchers at other locations in Florida investigating the seed production of sunn hemp have encountered issues with pollination, noting the presence of honeybees but also their inability to pollinate sunn hemp effectively (USDA–NRCS, 2000).

In the present study a large, non-native species of bee (*Megachile sculpturalis* Smith) was observed at Rosie's Organic Farm at the beginning of July and reported in Hall and Ascher (2010), which is sufficiently large and has appropriate anatomy to be an effective pollinator for sunn hemp (H. Glenn Hall, personal communication, 2008). Additionally, *Xylocopa*

Table 3. Number of branches, flowers, mature seed pods, and seed weight of 16 accessions of sunn hemp averaged across three planting dates evaluated in Gainesville, FL (2008–2009).

Accession	Group	No. of branches 2 MAS†‡§	No. of flowers 2 MAS†‡§	No. of mature pods¶	Seed weight¶
					g
1	2	9.8ab	41.2bc	18cdef	4.70abc
2	1	3.6c	4de	–	–
3	1	4.3c	0.7e	–	–
4	2	7.8b	77.3b	30bcd	5.41abc
5	2	9.8ab	20.4cd	13cdef	2.10bc
6	2	12.6a	8.8de	5cdef	1.11bc
7	1	2.5c	0.9e	–	–
8	2	9.3ab	194.4a	88a	13.95a
9	2	12.9a	62.4bc	68ab	12.33a
10	1	9.4ab	0e	2f	0.46c
11	2	12.9a	0.2e	3f	0.36c
12	2	9.2ab	0.9e	23cde	5.26abc
13	2	11.6a	2.6e	32abc	6.80ab
14	1	6b	0.6e	–	–
15	1	2.8c	0.8e	–	–
16	1	6.5b	1.6d	1f	0.00c

† Months after seeding.

‡ Data were square-root transformed for analysis.

§ Accessions with the same letter were not significantly different at $\alpha = 0.05$.

¶ Accessions without values (2, 3, 7, 14, and 15) did not produce seed pods and therefore were not included in the analysis (0 values).

virginica L., a large carpenter bee native to North America, was reported to be present at the location of our experiment during August. *Xylocopa virginica* activity in Florida occurs from April to August and from November to January (Grissell et al., 2014). Therefore, we propose that accessions in Group 2 (day-length-neutral sunn hemp plants) are capable of setting seed in Florida because they produce flowers during the summer when two potential pollinators are active. Group 1 or short-day sunn hemp accessions flower in fall when these pollinators are absent. Commercial bumble bees (*Bombus impatiens* Cresson; Koppert Biological Systems, Inc., Howell, MI) visited flowers of a short-day sunn hemp variety but did not result in seed set (Cho et al., 2015).

Future research in Florida could use some sunn hemp accessions for breeding to develop a cultivar of sunn hemp to be used for domestic commercial seed production, as was done in Alabama (Mosjidis, 2014). Although the Group 2 accessions are capable of seed production in Florida, they produce less biomass than accessions in Group 1. Biomass production influences many of the farming system benefits that a cover crop provides, such as the amount of nitrogen produced, weed suppression from canopy closure, and biomass available for organic mulch or use as a green manure. Within the seed-producing (Group 2) accessions, seed production was likely limited due to lack of sufficient numbers of effective pollinators at flowering. Gerling and Hefetz (1989) indicate a possible role for *Xylocopa* spp. for pollination of agricultural crops in which the honey bee (*Apis mellifera* L.) is ineffective if natural populations can be managed. A citizen scientist project in Florida illustrated that solitary bees, including *M. sculpturalis* and *Xylocopa* spp., will utilize various types of man-made tubular habitats (Graham et al., 2014). Further research should address whether providing nesting sites for solitary bees will improve the pollination of summer-flowering sunn hemp accessions. These accessions should also be evaluated for efficacy of weed suppression and provision of other farming system services, such as nitrogen accumulation under cover crop planting densities. This study characterized 16 accessions of sunn hemp for vegetative and reproductive qualities and provides information that can be used for breeding and/or selection to develop cultivars of sunn hemp that can produce seed in Florida and that possess desirable cover crop attributes.

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