HERE COMES THE SUNN: A COMPREHENSIVE ANALYSIS OF THE COVER CROP SUNN HEMP (*Crotalaria juncea* L.) AND ITS POTENTIAL FOR SEED PRODUCTION AND WEED CONTROL IN PUERTO RICAN AGRICULTURAL SYSTEMS

by

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ABSTRACT

The cover crop *Crotalaria juncea* L. has been recognized for its high biomass production, nitrogen-fixation, and allelopathic properties. However, extensive studies have not been conducted to assess the behavior of different *C. juncea* accessions in Puerto Rico. Studies were conducted from 2008-2009 to analyze the production and response to changes in photoperiod of 16 *C. juncea* accessions, as well as *C. juncea* performance under different planting densities (10, 25 and 40 lb/ac) and apical treatments (no cutting, cutting at 3, 4, and 5 weeks after planting) with respect to their potential for production of biomass, seed, and weed suppression. Results showed that despite photoperiod changes, the accessions ‘Nigeria’, ‘Texas 374’ and ‘T’ai-yang-ma’ have potential for biomass and/or seed production in Puerto Rico. Additionally, the highest biomass and seed yields were obtained with a planting density of 25 lb/ac, and apical cuttings did not improve *C. juncea* yields or weed suppression.

RESUMEN

La cobertura *Crotalaria juncea* L. es reconocida por su alta producción de biomasa, fijación de nitrógeno y propiedades alelopáticas. Sin embargo, no se han realizado investigaciones extensas para evaluar el comportamiento de accesiones de *C. juncea* en Puerto Rico. Se realizaron investigaciones en Lajas, Puerto Rico, durante 2008-2009 para analizar la respuesta a los cambios en fotoperíodo de 16 accesiones de *C. juncea*, y el efecto de densidades de siembra (10, 25 y 40 lb/ac) y cortes apicales (no cortes, cortes a las 3, 4, y 5 semanas después de la siembra) en la producción de biomasa y semilla, así como la supresión de malezas. Los resultados muestran que en diferentes fotoperíodos las accesiones 'Nigeria', 'Texas 374' y 'T'ai-yang-ma' presentan potencial para producción de biomasa y/o semilla en Puerto Rico. En adición, la mayor producción de biomasa y semilla se obtuvo con la densidad de siembra de 25 lb/ac. Los cortes apicales no mejoraron la productividad de *C. juncea* ni su capacidad para suprimir malezas.

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TABLE OF CONTENTS

TABLE LIST

Tables **Page**

FIGURE LIST

Figures **Page**

1 GENERAL INTRODUCTION

The implementation of cover crops in agricultural systems has been widely recognized as a successful tool to help improve productivity while maintaining the integrity of the land. Cover crops can preserve and improve the biological, chemical, and physical properties of the soil, reduce leaching of nutrients and synthetic pesticides, control weed and other pest populations, and resist erosion (Treadwell et al., 2008). Additionally, when used as a green manure, these crops can supply nitrogen and biomass to the soil and reduce the need for synthetic fertilizer amendments.

The production and overuse of synthetic fertilizers can be attributable to a host of detrimental impacts to the environment, including: pollution of ground water, eutrophication of water systems, emission of harmful gases into the ozone, and declines in crop yields. Furthermore, the production processes and transport of these fertilizers are economically inefficient and are highly dependent on non-renewable fossil fuels for their manufacture (Bohlool et al., 1992). Conversion to a cover-cropping system for meeting nitrogen demands can alleviate these effects and ameliorate agricultural systems for a more sustainable future.

The leguminous cover crop sunn hemp (*Crotalaria juncea* L.) has long been documented for its rapid growth, short growing period, capacity for nitrogen fixation, and allelopathic properties (Natural Resources Conservation Service, 1999). In a study of one hundred leguminous green manure crops used in India, sunn hemp was found to be one of the most acceptable to farmers in rice production, for its various beneficial attributes (Vachhani and Murty, 1964). While there has been research conducted on sunn hemp in the United States since the 1930s (Cook and White, 1996) and numerous accessions have been documented internationally, little is known about the production capacities of these accessions. There is also a lack of information regarding ideal planting densities and management practices for optimal production of seed and/or biomass and weed suppression in the southern region of the United States and the

Caribbean. This project seeks to provide a comprehensive study of the phenotypic behavior of various accessions of *C. juncea*, as well as provide an analysis of the effects of different planting densities and apical cuttings when sunn hemp is planted in Puerto Rico.

Originating in India, sunn hemp has been used not only as green manure, but also as a fiber crop, livestock feed, and soil improving crop. Today it can be found worldwide in nations as diverse as Bangladesh, Bhutan, Brazil, South Africa, and the United States. It is a tropical or sub-tropical plant which is adapted to a range of soils and which performs relatively well on poor, sandy soils; however, it has been observed to do best in soils that are well-drained and have a pH between 5 and 7.5 (NRCS, 1999). Sunn hemp seed is set in pods which are green during seed development and are ready for harvest when the pods turn brown and reach the 'rattle stage'. As a fiber crop, it has a broad range of uses and has been utilized in the production of paper, cat litter, twine, marine cordage, fishing nets, and sacking materials (Purseglove, 1981; Cook and White, 1996). It is particularly noted for its strength and resistance to mold, moisture, and salt water. For its application to agricultural systems, *C. juncea* not only provides the benefits of nitrogen fixation and rapid production of biomass, but is also superior to some other cover crops in its ability to resist plant pathogenic nematodes (Wang et al., 2001), control weeds (Linares et al., 2008; Sangakkara et al., 2006), prevent soil erosion (Miller, 1967), and reduce groundwater contamination by atrazine (Potter et al., 2007).

According to the Natural Resources Conservation Service (1999), sunn hemp has the potential to produce more than 5,000 lbs biomass/acre and has the capacity to fix over 100 lbs N/acre in 2-3 months after planting. In comparison, the tropical cover crops sudangrass [*Sorghum bicolor* (L.) Moench] and cowpea [*Vigna unguiculata* (L.) Walp.] produced 4,370 and 2,104 lb biomass/acre, respectively (Wang et al., 2008). Additionally, the above ground nitrogen production of sunn hemp is comparable to, if not higher than, crimson clover (*Trifolium incarnatum* L.) and hairy vetch (*Vicia villosa* Roth), which are commonly used as standards for cover crop research in the southeast United States (Reeves, 1994). Furthermore, while research has shown sunn hemp to produce less biomass and nitrogen than jack bean (*Canavalia ensiformis)* (2,477 kg/ha; 93 kg/ha) and velvet bean (*Mucuna pruriens*) (1,817 kg/ha; 69 kg/ha) when grown in Puerto Rico (Carlo Acosta, 2009), it had the additional attributes of developing a dense canopy cover and allelopathic compounds.

When incorporated into the soil as a green manure, the quantity of nitrogen amended from the sunn hemp residue can greatly reduce or eliminate the need for additional application of nitrogen fertilizers, while the biomass functions to improve the physical and biological quality of the soil. Evidence shows that the decomposition of these residuals releases allelopathic compounds which are toxic to plant-parasitic nematodes (Wang et al., 2004), such as *Meloidogyne* spp., *Rotylenchulus reniformis, Radopholus similis*, *Belonolaimus longicaudatus*, and *Heterodera glycines* (Wang et al., 2002). The presence of sunn hemp may also help to enhance nematode-antagonistic microorganisms (Wang and McSorley, 2004). Thus, the adoption of *C. juncea* can also be used as part of an integrated approach in the management of nematodes.

The allelopathic compounds found in sunn hemp can also be effective in the control of weeds. Typically, cover crops aid in the suppression of weeds through competition for resources, the inhibition of weed germination, phytotoxins, and by creating changes in the soil microclimate. The use of sunn hemp as a cover crop can create unfavorable conditions for weeds through the effect of shading, competition for other resources, and its allelopathic compounds. One of the phytotoxic compounds identified in *C. juncea* is delta-hydroxynorleucine (5-hydroxy-2-aminohexanoic acid) (Pilbeam and Bell, 1979), which has been documented as toxic to lettuce (Wilson and Bell, 1979). Aqueous extracts of the allelopathic compounds in sunn hemp have also been documented to inhibit the germination of goosegrass [*Eleusine indica* (L.) Gaertn] and livid amaranth (*Amaranthus lividus* L.) (Adler and Chase, 2007). In addition to phytotoxicity, it can be proposed that breaking apical dominance early in sunn hemp development can encourage a more rapid canopy closure and possibly augment weed suppression. Increased planting densities of *C. juncea* can also play a similar role in the cultural control of weed populations.

One of the most commercially available varieties of *C. juncea* is ‘Tropic Sun’, which was developed in Hawaii in 1983. This variety, when sown under optimum growing conditions, can produce between 134 and 147 lb N/acre in two months, when planted at a density of 40 kg/ha (Wang and McSorley, 2004); however, the relative cost of ‘Tropic Sun’ seed can act as a deterrent, discouraging farmers from integrating it into their production systems. In 2007, seed price for ‘Tropic Sun’ was $6.90/lb and seed was not commercially available in 2005 and most of 2006 (Klassen, 2007). Prices for *C. juncea* in 2010 ranged from $5.30 to $6.00/lb (Peaceful Valley Farm and Garden Supply, and Adams-Briscoe Seed Company, respectively). Sunn hemp is commonly produced commercially in Hawaii, South America, and South Africa and thus, added shipping costs are to be expected for farmers outside of these regions. Recommended planting densities for use as a cover crop are between 40-60 lb/ac (44.8-67.4 kg/ha) (Wang and McSorley, 2004). Using 2010 prices, this average is about $283/acre for seed, further prohibiting the implementation of *C. juncea* into agricultural systems. Inconsistencies, such as seed availability or lack thereof, serve as a disincentive, causing farmers to potentially dismiss the use of sunn hemp despite its numerous beneficial attributes. The eventual development of local production of sunn hemp seed would help to mitigate costs of seed imports, provide more stability in the market availability of seed, and provide a more locally-adapted product.

The successful production and greater adoption of sunn hemp usage in Puerto Rico would help to stimulate the growing organic farming movement, as well as encourage the development of more sustainable agricultural systems. The overall objectives of this project are to identify the *C. juncea* accessions which would be beneficial for Puerto Rico in terms of biomass and seed production, as well as to determine the optimal cultural management practices to achieve positive results.

2 Assessment of the production and morphology of sixteen *C. juncea* accessions

2.1 INTRODUCTION

While sunn hemp (*Crotalaria juncea* L.) is widely recognized for its rapid biomass production, nitrogen fixation capabilities, and allelopathic properties (NRCS, 1999), lack of seed availability and prohibitively expensive costs create a barrier to its integration as a cover crop and green manure in Puerto Rican agricultural systems. Presently, there are 55 accessions of *C. juncea* in the USDA sunn hemp germplasm collection from various geographical locations, 17 of which are currently available for distribution (ARS, 2010); However, there have been no extensive studies conducted on the flowering patterns, capacity for biomass production, and seed set of these various sunn hemp accessions in Puerto Rico.

Additionally, there is a distinct need for locally-adapted cultivars of *C. juncea*. A well-studied variety such as 'Tropic Sun', which is well-suited for Hawaiian latitudes (approximately 21°N) and climates, may not necessarily perform the same in a different geographic region. Sunn hemp has been characterized for its sensitivity to photoperiod, with a tendency to flower as a response to shorter day length (White and Haun, 1965). Puerto Rico lies at latitude 18°N and thus, the variety 'Tropic Sun' would be expected to respond accordingly to the differences in photoperiod. It is also already known that sunn hemp does not seed well above 30°N latitude. Moreover, while a climate such as that in Florida should be appropriate for sunn hemp seed production, research there has shown that the *C. juncea* plants in this region have a tendency to flower well, but have poor seed production (Wang and McSorley, 2004). Clearly, there is a need for greater knowledge of *C. juncea* accession behavior in Puerto Rico to assess their distinctive potentials for use in tropical agricultural systems and in the possible development of a local sunn hemp seed industry.

The objective of this experiment is to assess the morphology, phenology, and production capacity of sixteen accessions of *C. juncea* when grown under changing photoperiodic conditions in Puerto Rico.

2.2 MATERIALS AND METHODS

The accession trial evaluated 16 accessions of *C. juncea* for their differences in morphology, sensitivity to photoperiod, and capacity for biomass and seed production. The accessions used in this experiment were provided by the USDA Plant Genetic Resources Conservation Unit and originated from a wide variety of geographic locations, including the commercially available ‘Tropic Sun’. These accessions and their origins are displayed in Table 2.1. The individual accessions are hereinafter referred to by their respective Reference ID.

The effect of photoperiod was assessed by implementing three staggered planting dates in each year of the experiment, in May, June, and July of 2008 and 2009. Due to the small quantities of seed provided for these accessions and in order to improve germination rates, the seeds were planted in trays of sterile growing medium and kept under greenhouse conditions. To encourage root nodule formation on the *C. juncea*, seeds were inoculated with *Rhizobium spp.* (Nitragen EL®, EMD Crop BioScience, Milwaukee, Wisconsin) prior to sowing. At three weeks after planting, the seedlings were transplanted to the field. Raised beds were tilled in preparation for the transplant. A completely randomized block design was used, with four replications at each planting date.

Plots consisted of two rows of 8 plants each, for a total of 16 plants/plot; however, in the case of poor germination of the accessions, seedlings were divided equally among the four replications. Plants were spaced 1.5 ft (0.46 m) apart in the rows, with 2 ft (0.61 m) between rows. There was a buffer area of 5 ft (1.52 m) between plots. In 2008, overhead irrigation was utilized to establish the plants during the early stages of development, while drip irrigation was implemented throughout the duration of the experiment in 2009.

Table 2.1. Identification and origin of *C. juncea* accessions evaluated in Lajas, Puerto Rico, 2008-2009

No.	Accession	Origin	Plant ID	Reference ID
1	PI 207 657	Sri Lanka	--	Sri Lanka
2	PI 234 771	Nigeria	--	Nigeria
3	PI 248 491	Brazil	Guizo de Cascavel	Guizo de Cascavel
4	PI 250 485	India	K679	K679
5	PI 250 486	India	K680	K680
6	PI 250 487	India	K681	K681
7	PI 295 851	São Paulo, Brazil	--	São Paulo
8	PI 314 239	Russian Federation	COL NO 524	COL NO 524
9	PI 322 377	São Paulo, Brazil	IRI 2473	IRI 2473
10	PI 337 080	Brazil	--	Brazil 3
11	PI 346 297	Delhi, India	--	Delhi
12	PI 391 567	South Africa	T'ai-yang-ma	T'ai-yang-ma
13	PI 426 626	Pakistan	Sanni	Sanni
14	PI 468 956	Hawaii, United States	Tropic Sun	Tropic Sun
15	PI 561 720	São Paulo, Brazil	IAC-1	IAC-1
16	PI 652 939	Texas, United States	Texas 374	Texas 374

Organic management practices were observed; this included foliar applications of organic, botanical pesticides to control the insect pests *Ceratoma ruficornis* Oliv. and *Diabrotica balteata* Lec. during the primary vegetative growth stages in first two months of the experiment. A neem (*Azadirachta indica*) based pesticide (Aza-Direct™, Gowan Co.®, Yuma, Arizona) and the insecticide/miticide Ecotrol® (EcoSMART Technologies, Inc.®, Franklin, Tennessee) were applied as needed at the recommended rates for field crops. In 2008, weeds were removed manually from within the plots on one occasion shortly after transplant. Two-sided, black and silver polyethylene mulch film was used to mitigate the proliferation of weeds in the experimental planting in 2009. In both years, the areas between plots were kept mowed short to reduce the incidence of weeds and other pests and no additional fertilizers were amended into the soil.

Data was collected to assess the variation in accession morphology, flowering, biomass, and seed production. Accession morphology data was based on the selection of three representative plants from each plot for data collection. For each planting date, the plant height and width was recorded by measuring the primary stalk and widest point of the three selected plants at 2 and 4 months after planting (MAP). Leaf area was determined by collecting 5 leaves from each plot during the time of flowering and measuring the area with a LiCor® surface-area meter. Flowering data were collected on a weekly basis. Accessions were considered to have flowered when there were flowers present on 50% of the plants in a plot. Pods were harvested at 'rattle stage' from three representative plants per plot. The 'rattle stage' refers to the point at which the pods have begun to dry, change in color from green to brown, and the seeds can be heard rattling within the pod when shaken. Seed data collected consisted of: number of pods/plant, number of seeds/pod (based on the average number of seeds in 20 pods), total seed weight/plant, and relative moisture. Biomass data were taken post-harvest from one plant/plot cut at soil level and oven dried. Additionally, the putative primary pollinators and principal pests were collected and identified.

The data collected from this experiment were assessed to determine the effect of planting date on accession morphology and productivity using an analysis of variance (ANOVA) for a split-split-plot design [Duncan multiple range test (α=0.05), unless otherwise noted]. The statistical program Infostat (Di Rienzo et al., 2009) was used to identify the potential of each accession for biomass and seed production in Puerto Rico.

The analyses were defined considering the experimental year as the whole plot, planting date as the sub-plot, and replications as the sub-sub-plot.

Data from accessions which had poor germination or from those which failed to thrive in the field, resulting in insufficient or inaccurate samples, were removed from the data set before analysis. These removed accessions were: ‘São Paulo’, ‘Brazil 3’, ‘Delhi’, and ‘IAC-1’. Additionally, the accessions ‘Nigeria’ and ‘Guizo de Cascavel’ (in 2008), and ‘Tropic Sun’ and ‘Texas 374’ (in 2009) were taken out of the data set during one year of the experiment. Observational data for these accessions is noted when applicable.

2.3 RESULTS AND DISCUSSION

2.3.1 *Accession morphology*

Plant height

An analysis of variance for the height of each accession over the three planting dates was conducted for each year of the experiment. Since height was taken over time (2 and 4 MAP), the split-split-plot was designed so that planting date was considered the whole plot, replication as the sub-plot, and data collection date as the sub-sub-plot. The results showed that, in both 2008 and 2009, there were significant differences among accessions, as well as an effect of planting date on height performance. These differences were also dependent on the number of months after planting.

In 2008, results showed that at two months after planting, heights were significantly greater in all accessions planted in July, and the shortest when planted in June. The average heights measured at 2 MAP for each accession and at each planting date are shown in Figure 2.1. At this stage in the experiment, distinct differences can be observed as an effect of the date of planting on plant growth. The accession ‘T’ai-yang-ma' was consistently among the tallest, with an average height of 1.14 m. In comparison, 'Tropic Sun' has been described as reaching a height of 1.22 m at 2 MAP (NRCS, 1999). 'K679' ranked as one of the shortest, at an average of 0.82 m. In addition, 'K679' showed the least effect of planting date, noted by the proximity of the data points and overlap of the standard error bars, implying that it may be less sensitive to changes in environmental conditions with respect to height.

'Tropic Sun' was comparable in height to 'T'ai-yang-ma' in the May and July plantings; however, had relatively poor growth when planted in June. In the early stages of the experiment in 2008, Green iguanas (*Iguana iguana*) became a noted pest species. The *I. iguana* showed a particular preference for feeding on the 'Tropic Sun' and 'São Paulo' accessions, which may have been cause for the differences observed at 2 MAP in the June trial. This is supported by the reduction in variation observed at 4 MAP (Figure 2.2), when the plants were generally more established and *I. iguana* presence was less pronounced.

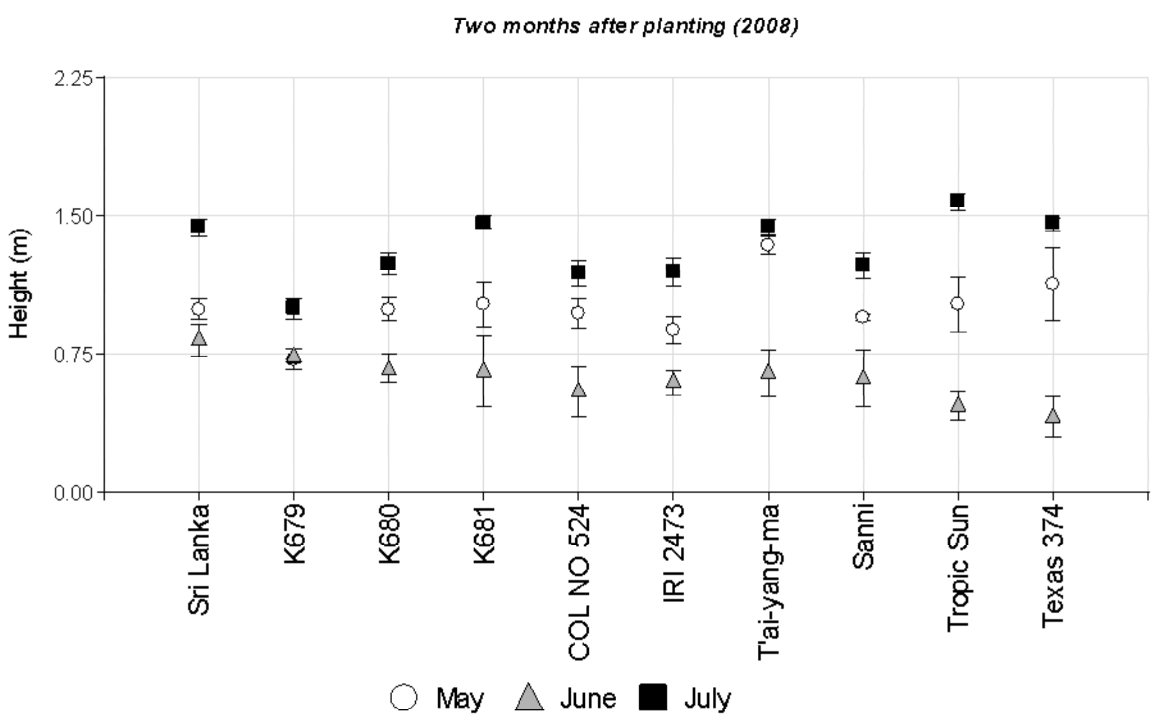

Figure 2.1. Average height of *C. juncea* accessions by planting date, measured at 2 months after planting in Lajas, Puerto Rico (2008)

At four months after planting, there was a reduced effect of planting date on *C. juncea* height and greater differences in height observed between accessions. Figure 2.2 illustrates the height differences between accessions and the respective effects of planting date, measured at 4 MAP. Again, 'T'ai-yang-ma' and 'Tropic Sun', along with 'Sri Lanka', 'K681', and 'Texas 372', represent the tallest (averaging between 1.26-1.78 m). These values are slightly lower than the 1.83 m average height which has been previously documented for 'Tropic Sun' (NRCS, 1999). 'K679' remained significantly shorter (with an average of 0.85 m) than the other accessions during each planting trial.

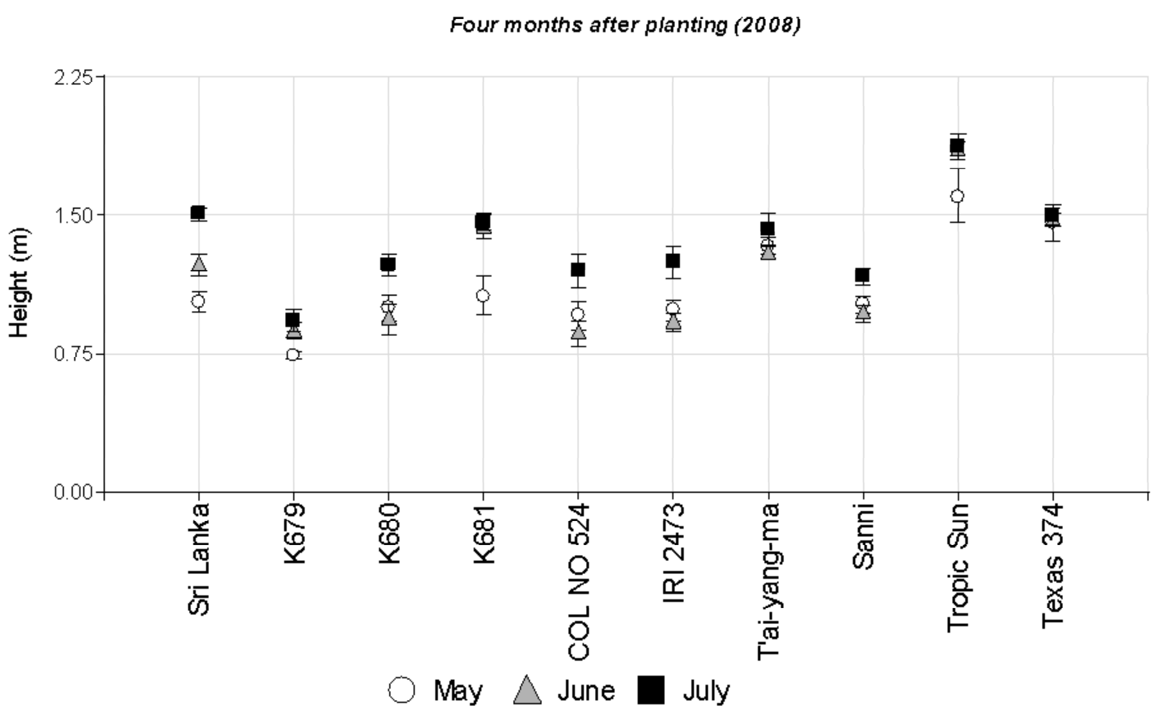

Figure 2.2. Average height of *C. juncea* accessions by planting date, measured at 4 months after planting in Lajas, Puerto Rico (2008)

Planting trials in 2009 reflected similar differences between planting dates and accessions as those present in 2008. At 2 MAP, the May planting presented the shortest heights per accession, while the measurements taken from the June trial showed to be among the tallest in each accession (Figure 2.3). This may indicate that environmental factors, such as photoperiod and precipitation have an impact on the growth rate of *C. juncea* (White and Haun, 1965). 'T'ai-yang-ma' and 'Sri Lanka' were significantly the tallest at each planting date (averaging 1.13 and 1.31 m, respectively). In the May and June plantings, 'K679' was the shortest (with an average of 0.96 m), while in the July planting there was no clear height differentiation.

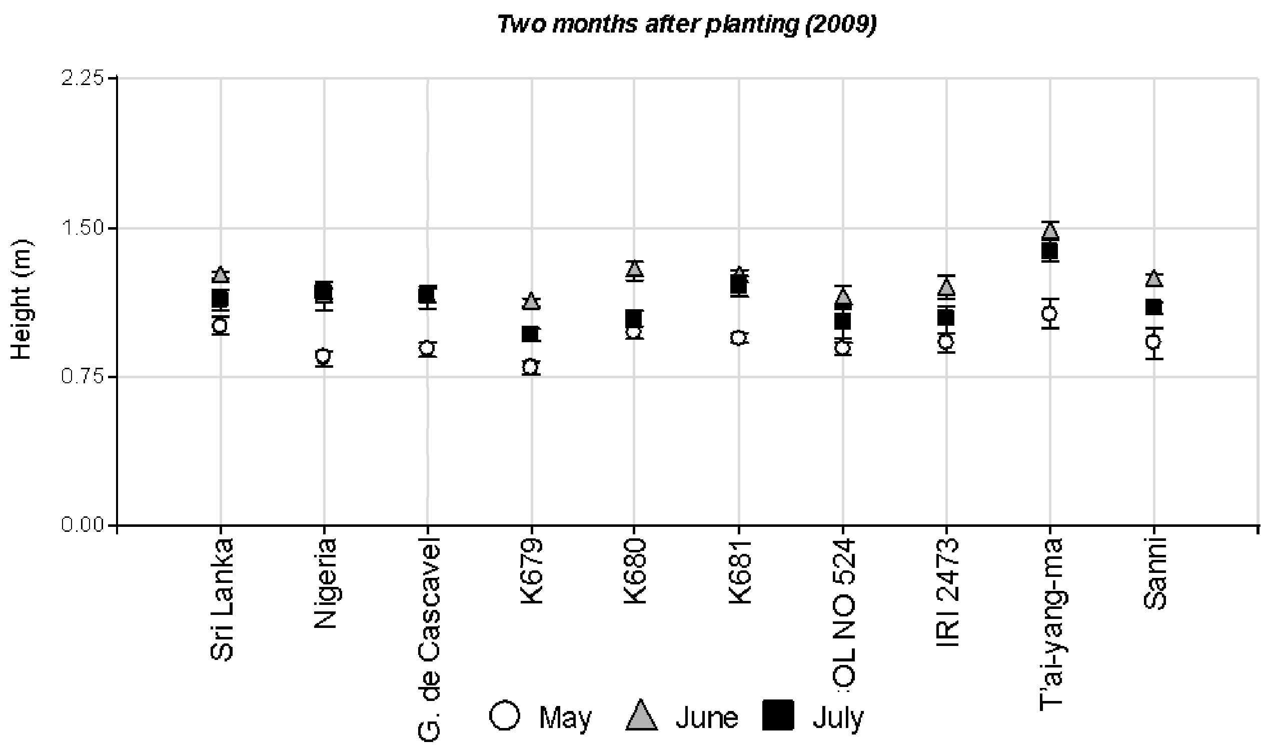

Figure 2.3. Average height of *C. juncea* accessions by planting date, 2 months after planting (2009)

At four months, the differences between the heights of the accessions in the second year of the experiment become more prevalent and there was less correlation between height and planting date (Figure 2.4). The trials in 2009 resulted in significantly taller 4 MAP height averages in each planting from the accessions 'Nigeria', 'Guizo de Cascavel', and 'T'ai-yang-ma' (1.92, 1.91, and 1.71 m, respectively). 'Nigeria' and 'Guizo de Cascavel' were not included in the 2008 analysis, due to low germination and insufficient sample size. The shortest plants, on average, were from the accessions 'K679', 'COL NO 524', and 'IRI 2473' (1.18, 1.22, and 1.21 m, respectively).

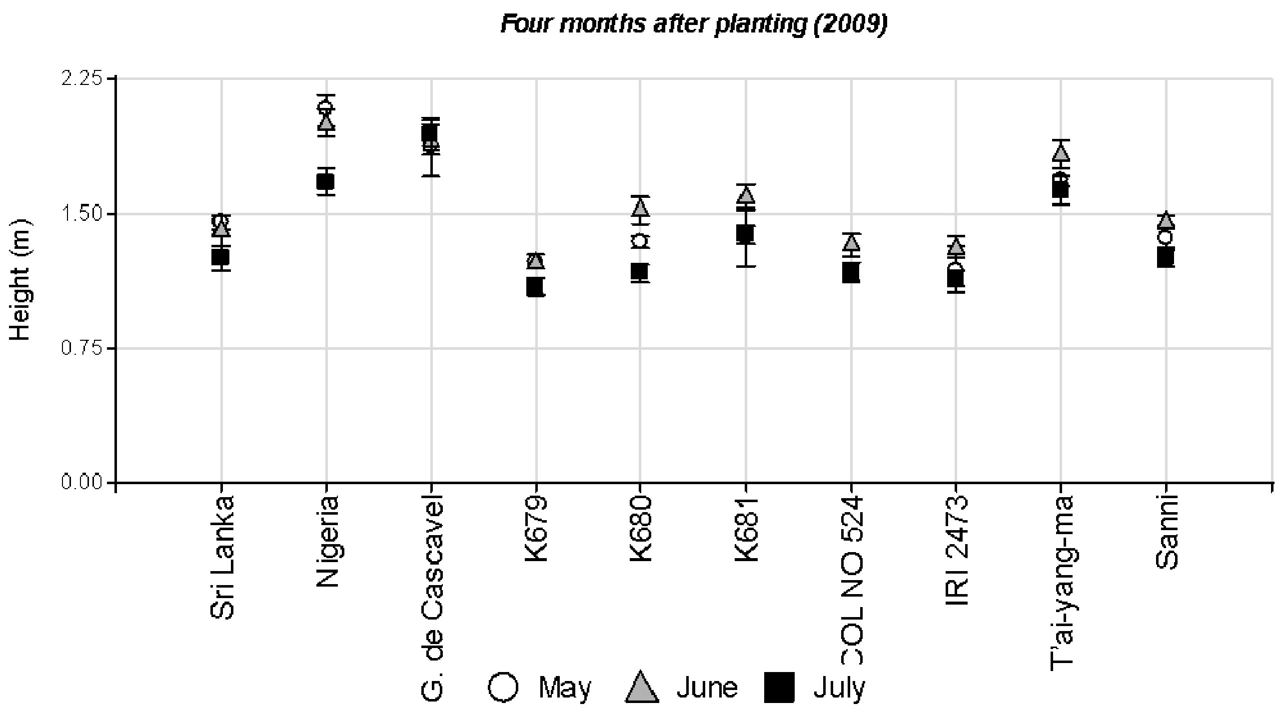

Figure 2.4. Average height of *C. juncea* accessions by planting date, 4 months after planting, in Lajas Puerto Rico (2009)

In many cases, the heights of the sunn hemp from the July planting in 2009 are significantly shorter at 4 MAP. This contrasts previous observations which show this planting to generally produce taller plants. Higher productivity is to be expected in the July plantings, as a result of the shortened photoperiod present during the vegetative growth stages. One potential reason for this disparity is the increased pressure from pest populations in the July planting, which was observed to occur as the May and June plantings reached senescence and insect pests migrated to the remaining vegetative replications. A noted *Crotalaria* spp. pest, *Utetheisa bella* L. (Seale et al., 1957) and *Utetheisa ornatrix* L. were the most prominent and problematic pests (discussed in further detail in Section 2.3.4).

The relatively small effect of planting date in 2009 may have been a result of the adjustments made to the methodology, such as the installation of drip irrigation and the implementation of polyethylene mulch film. Drip irrigation systems can be more precise in regulating water to the field, while the mulch would have reduced the effect of competition with weeds. Reducing environmental stresses such as these could result in higher productivity and less susceptibility to seasonal changes.

In both years, the effect of planting date on the height of the accessions is diminished at 4 MAP, as compared to 2 months. This is evidenced in the reduced number of significant differences between treatments. Therefore, one could conclude that planting date is most relevant to plant height during the earlier stages of plant development.

Plant diameter

The analysis of variance showed that there was no effect of planting date or accession on plant diameter in 2008. This may be a result of the previously mentioned environmental stressors which were present in 2008, but mitigated by changes in methodology in 2009. A statistical summary of plant diameter by accession for 2008 is located in Appendix B (Table B1).

In 2009, differences were present at two months after planting; however, there were no significant differences at four months. This reflects the observation made earlier, which mentioned that planting date may have a greater effect on the accessions during the primary stages of vegetative growth, and have a reduced effect as the accessions shift towards reproductive growth. At 2 MAP, a trend is visible among the accessions, which shows that the diameter of each accession planted in May as the smallest, and the diameters of those accessions planted in July to be among the largest (Figure 2.5). The exception to this is the accession ‘K679’, in which the June planting had the greatest values. Additionally, the July planting showed that the accessions ‘Nigeria’, ‘Guizo de Cascavel’, and ‘T’ai-yang-ma’ to be significantly narrower than the other accessions. These three accessions were noted in the discussion on plant height to be among the tallest. This suggests that there may be a correlation between taller accessions and a narrow plant diameter. In general, the June planting showed no distinctive effect with respect to the other planting dates.

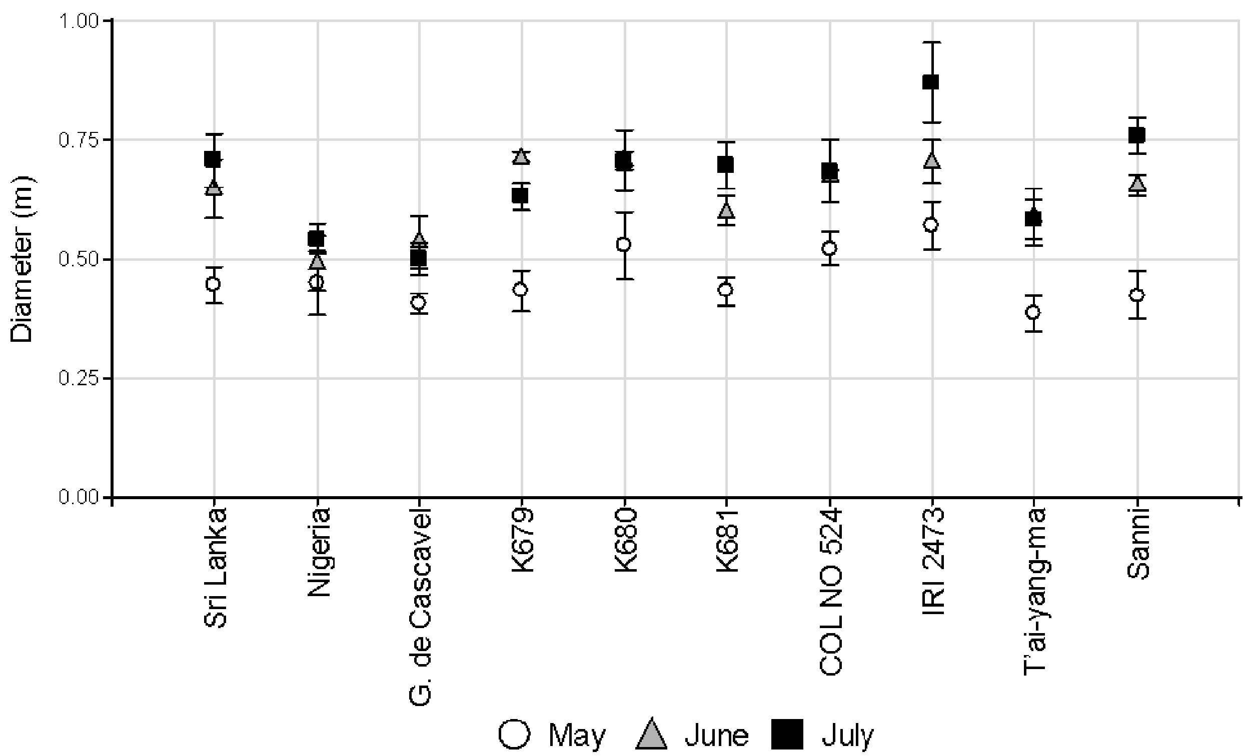

Figure 2.5. Plant diameter by accession and planting date, at 2 months after planting (2009)

Leaf area

An analysis of variance (LSD Fisher, α=0.05) showed a wide variation in leaf area in 2009, which was not affected by planting date, nor was there an interaction between planting date and accession. The largest leaf area was present in the accessions 'São Paulo', 'Nigeria', and 'IAC-1', with an average of 37.5, 38.0, and 39.6 cm²/leaf, respectively. Accessions 'Delhi' and 'K679' had the smallest leaf area, with averages of 14.3 and 14.5 cm²/leaf, respectively. The average leaf area for each accession is represented in Figure 2.6.

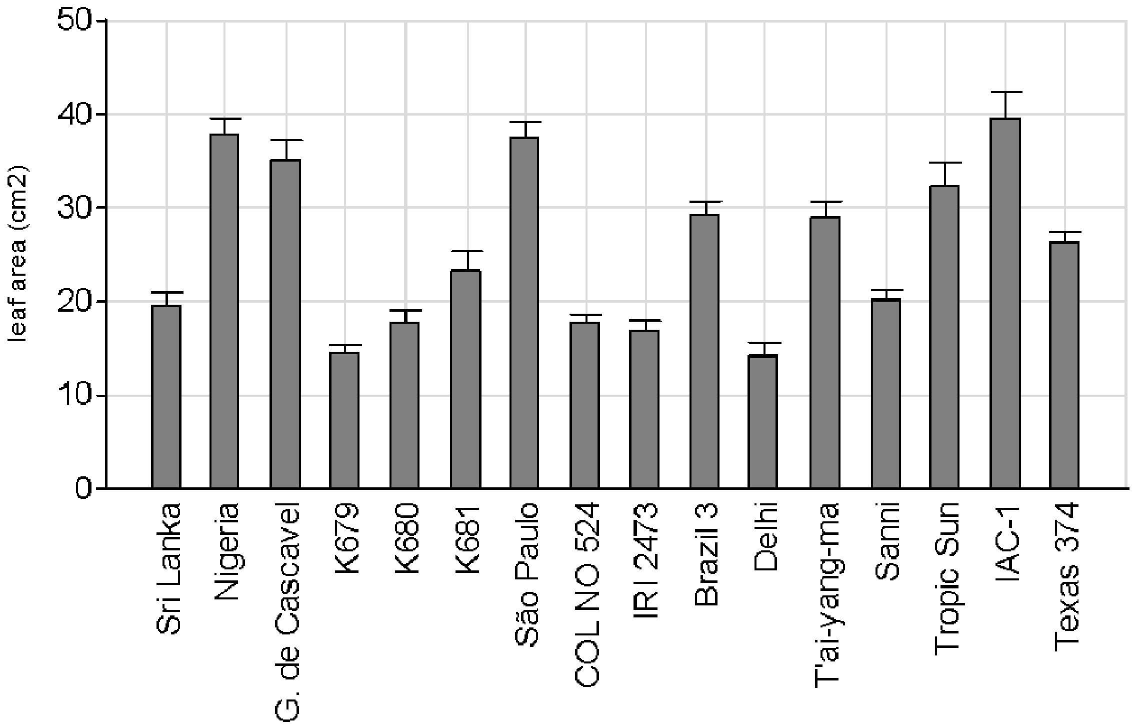

Figure 2.6. Average leaf area (cm²/leaf) by accession in 2009, Lajas. Puerto Rico

2.3.2 *Flowering and sensitivity to photoperiod*

Assessment of the time of flowering for each accession showed wide variation and reflected a photoperiodic response with respect to planting date for selected accessions. Figure 2.7 shows the relationship between the average number of days to flowering, DTF, for each accession and planting date, in both 2008 and 2009. In the 2008 experiment, some accessions did not have a sufficient number of surviving plants to accurately determine flowering during a particular planting date. These occasions are left blank in the figure and occurred as follows (expressed as accession/planting date): 'Guizo de Cascavel'/July, 'Brazil 3'/June, and 'IAC-1'/June/July. The remaining fields which show 0 DTF indicate that flowering did not reach 50 percent before the termination of each trial at 150 days.

The analysis of variance expressed a significant difference between DTF amongst accessions, as well as an interaction between accession and planting month. In the figure, a high variation between the DTF in May, June, and July for each accession indicates that there was a significant effect of planting date on the number of days to flowering. There were four such accessions which were determined to have significant differences in both years. These were identified as 'Nigeria', 'Guizo de Cascavel', 'São Paulo', and 'Tropic Sun'. Accessions with significant differences between DTF and planting date are highlighted in italics in Figure 2.7. The accessions 'Nigeria', 'Guizo de Cascavel', and 'São Paulo' did not flower when planted in May and June, and had an average of 84, 43, and 81 DTF, respectively, when planted in July. The accession 'IAC-1' also did not flower when planted in May 2009, and failed to produce a sufficient number of plants to assess in 2008. 'Tropic Sun' reflected a significantly lower number of DTF when planted in July (72 DTF), as compared with May and June of both years (102 and 95 DTF, respectively). Additionally, the July 2008 planting of 'Texas 374' resulted in significantly fewer DTF than the trials planted in the previous two months. While the remaining accessions showed some differences in DTF between planting times, these differences were negligible (≤10 days) when compared with the approximate differences of 30 days in the more widely varying accessions.

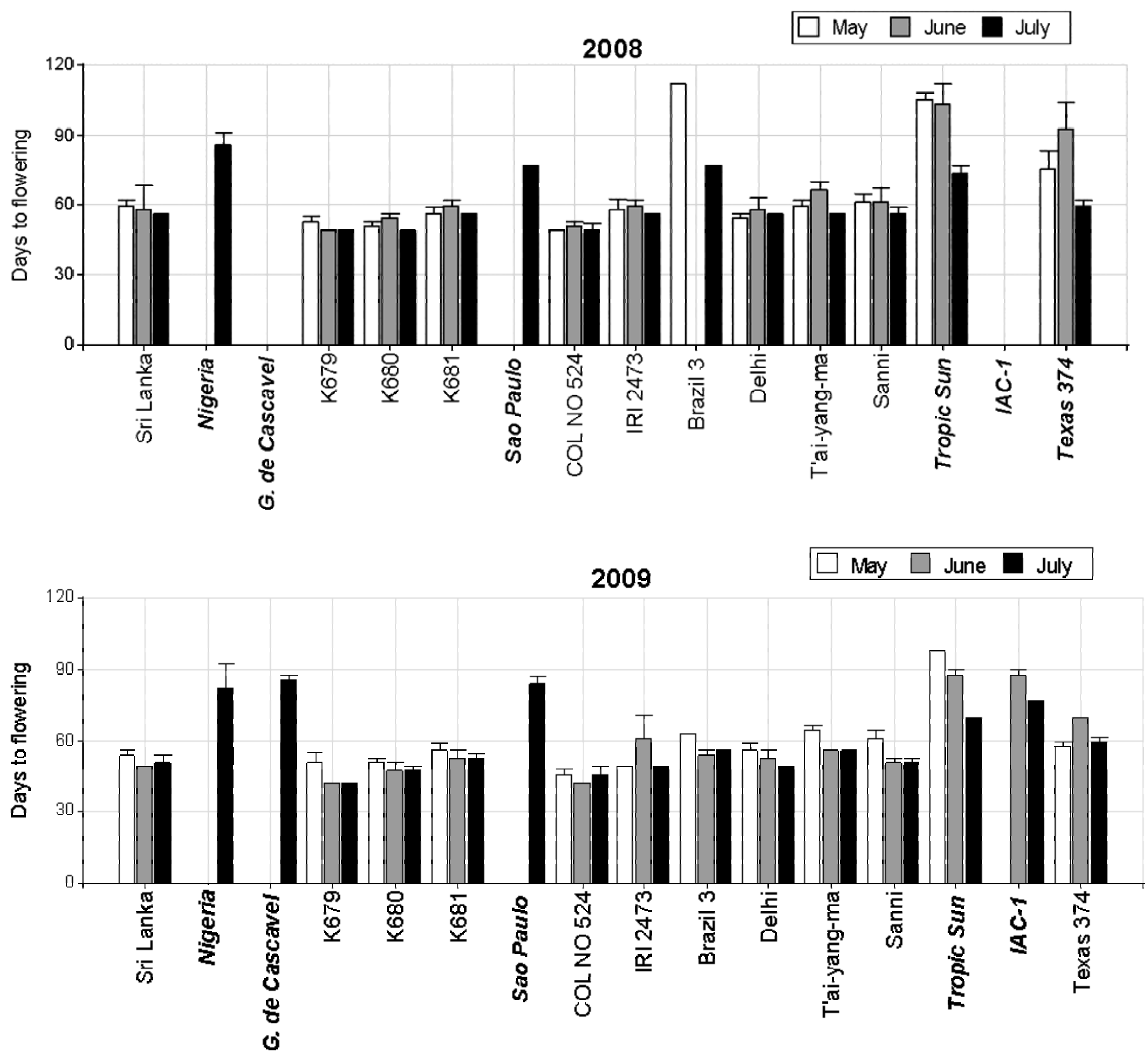

Figure 2.7. Days to 50 percent flowering for *C. juncea* accessions, categorized by planting month (2008 and 2009)

With respect to the changes in photoperiod throughout the duration of each planting time, it should be recognized that the summer solstice [June 20 and 21 in 2008 and 2009, respectively (US Naval Observatory)] occurred after the May, but before the June plantings of each year were transplanted to the field. Therefore, during approximately the first 40 days of the May trials, the photoperiod was progressively getting longer. Meanwhile, the June trials initially experienced approximately two weeks of lengthening photoperiod, and the July trials experienced a steadily shortening day length throughout. As previously mentioned, sunn hemp has been known to be highly susceptible to photoperiod and favors shorter day lengths to induce flowering (White and Haun, 1965). The more photoperiodic-sensitive accessions would be expected to reflect these differences in photoperiod between planting times by delaying flowering until exposure to favorable day lengths occurred, or by failing to initiate florescence. Accordingly, the accessions 'Nigeria', 'Guizo de Cascavel', 'São Paulo', 'Tropic Sun', and 'IAC-1' can be considered as highly sensitive to photoperiod, as they each reflected a delay or failure to flower in response to early exposure to longer photoperiods (during the May and June plantings). Moreover, accessions such as 'Tropic Sun' and 'IAC-1' showed a shortened DTF response to the July plantings, as compared with May and June.

This is important to consider when selecting an accession for developing seed production and in scheduling planting dates for optimized flowering. Ideal seed production varieties would not only exhibit desirable morphological qualities, but would also predictably initiate florescence within a reasonable period after planting. To maximize the potential for number of growing seasons per year in a tropical area such as Puerto Rico, an accession which is less sensitive to changes in photoperiod would also be ideal. Therefore, accessions such as 'Nigeria', 'Guizo de Cascavel', 'São Paulo', 'Tropic Sun', and 'IAC-1' would not be recommended for seed production based on their flowering tendencies alone.

2.3.3 *Biomass production*

A split-plot design was used to assess the differences in biomass production (g dry weight) among the *C. juncea* accessions. The results of an analysis of variance (Duncan, α=0.05) show there to be a significant difference of biomass between the accessions; however, there was no interaction effect of planting date on production of the accession. Figure 2.8 shows the average biomass by accession with the combined data for 2008 and 2009. Since biomass samples were only taken from the June and July plantings in 2008, this figure shows the averages for only these two plantings. The accessions highlighted in black are those which statistically produced the most biomass, 'Nigeria', 'São Paulo', and 'Tropic Sun'. The average biomass produced per plant for these accessions was 182.7, 200.7, and 195.5 g, respectively. The accession displayed in white ('K679') produced the least biomass per plant (37.6 g). Additionally, the accessions 'Guizo de Cascavel', 'IAC-1', and 'Texas 374' produced moderately high levels of biomass, ranging on average between 115.2-143.1 grams of biomass per plant. A statistical summary of the biomass production for all *C. juncea* accessions is located in Appendix B (Table B3).

An interaction of year and planting month was present between the June and July plantings. Figure 2.9 shows that, in 2008, biomass production was significantly higher when planted in July, as compared to the June planting. In 2009, there was no statistical difference between planting months. The difference in production by year could be attributable to damage of the plants from the aforementioned pest *Utetheisa* spp., which was particularly prominent in the July planting of 2009.

An additional analysis was conducted to determine if there were any significant differences in 2009 between the three planting dates. The results reflected no additional interaction of planting date and accession when the three plantings were analyzed together.

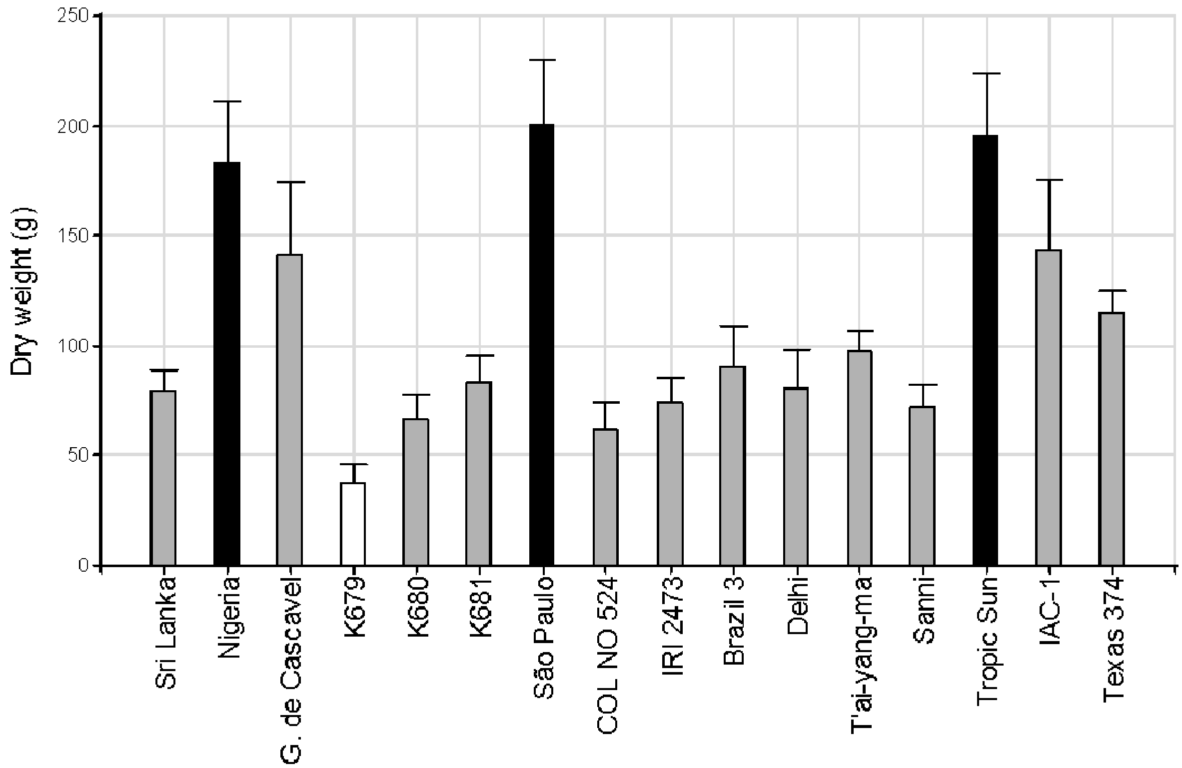

Figure 2.8. Biomass production per plant categorized by *C. juncea* accession, 2008 and 2009 combined, Lajas, Puerto Rico

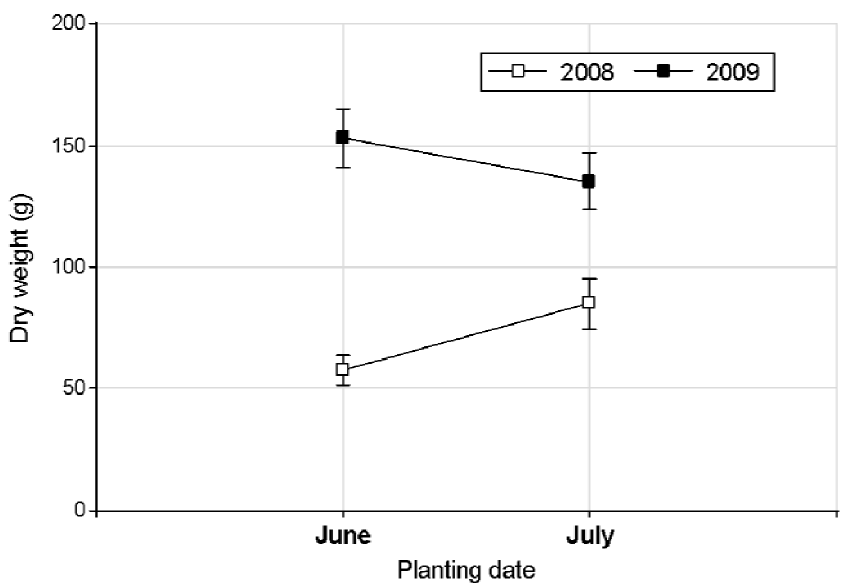

Figure 2.9. Effect of interaction between year and planting date on accession biomass production

The accessions with high to moderately high biomass production are the same as those which were identified as highly sensitive to photoperiod: 'Nigeria', 'São Paulo', 'Tropic Sun', 'Guizo de Cascavel', and 'IAC-1'. While biomass production is a crucial trait in assessing the potential for an accession to be used as a green manure and cover crop, the accession's capacity for flowering and subsequent seed production are attributes which are essential in creating a sustainable sunn hemp production system.

2.3.4 *Seed yield*

The analysis of variance showed that there were significant differences present in seed production amongst the *C. juncea* accessions. No relationship between accession and time of planting was evident; however, Figure 2.10 illustrates that there was an interaction effect between experimental year and the time of planting. In the May and June plantings, there was significantly less seed produced in 2008 as compared to 2009, whereas no difference occurred between years of the July plantings. An improved methodology of the accession plantings in 2009, including the use of polyethylene mulch film for weed suppression and the installation of a drip irrigation system, may have accounted for the large increase in seed production during the May and June trials of the second year. Meanwhile, the large incidence of pests and the deterioration of said drip irrigation system in the July 2009 trial may have been the basis for the observed decline in seed yield.

Field observations noted that, in 2009, there was an increased presence of the pests *U. bella* and *U. ornatrix*, specifically during the July planting. This occurred as a result of the *Utetheisa* spp. increasing in population in the field during the three consecutive sunn hemp trials and converging in the area of the July trial as the May and

June trials reached senescence and could no longer provide a source of vegetation and refuge.

The *Utetheisa* spp. typically feed on *Crotalaria* spp. foliage as young larvae, before boring into the pods and consuming the young seeds (Eisner, 2003). This would have affected both the plants' ability to produce seed, due to the loss of foliage and related photosynthetic production capacities, and would have directly impacted seed yield from the loss of consumed seeds. Because of the proportion of empty pods encountered in the harvest as a result of the *Utetheisa* spp., estimates for seed yields in 2009 were used to conduct the ANOVA. These estimates were calculated based on the number of pods harvested, the average number of seeds/pod for each accession, and the average weight per 100 seeds for each accession.

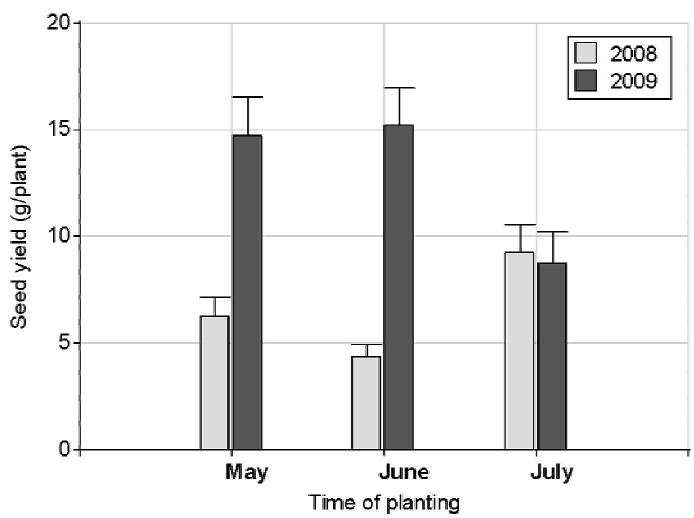

Figure 2.10. Interaction effect of year and time of planting on *C. juncea* seed yield (g/plant) in Lajas, Puerto Rico

A high incidence of weed presence within the plots in 2008 would have created a proportionately high competition for resources, which may have been a factor in the lower seed production rates for that year. This effect was mitigated in 2009 by the implementation of a polyethylene mulch film and the installation of a drip irrigation system. However, by the later stages of the July 2009 trial, the irrigation tubing had begun to deteriorate and leak, possibly resulting in reduced water flow to that replication.

The primary pollinators observed in the field were the common honey bee, *Apis mellifera* L., and the carpenter bee, *Xylocopidae mordax*. Pollination may have played a role in seed production rates; however, no studies were conducted to assess if there were any differences in the prevalence of these pollinators over the duration of the two years.

The average seed production (g/plant) per accession, categorized by planting time, for both 2008 and 2009 is represented in

Figure 2.**11**. Accessions which produced seed in fewer than three representative plants per plot were removed from the data set and reflect a value of zero in the figure. These removed accessions were ‘Guizo de Cascavel’ and ‘São Paulo’ and were considered as having no seed yield. The accessions ‘Nigeria’ and ‘Brazil 3’ reflect seed yields in one year, however, did not produce sufficient viable plants to have a yield in the other. Both ‘Tropic Sun’ and ‘IAC-1’ had few plants per plot in each year of the experiment.

The relatively high variation which occurred across accessions, planting times, and years creates a difficulty in identifying the highest producing accessions; however, it is noteworthy to mention, that ‘T’ai-yang-ma’ produced the highest average seed yield (24 g/plant) in the July planting of 2008. Additionally, this accession appeared to thrive during the July planting of 2009, when most other accessions were experiencing a drop-off in seed production due to the aforementioned pest pressures. Although this was not statistically the highest yield of 2009, ‘T’ai-yang-ma’ performed at a level significantly higher than the other accessions in the same planting time.

The accessions previously identified as having relatively high biomass production and sensitivity to changes in photoperiod (‘Nigeria’, ‘Guizo de Cascavel’, ‘São Paulo’, ‘IAC-1’, ‘Tropic Sun’, and ‘Texas 374’) predominantly did not produce significant seed yields. However, the June and July plantings for ‘Nigeria’ and all plantings of ‘Texas 374’ in 2008 produced moderate seed yields as compared with the other accessions during the same planting time and year. The respective seed yield averages for ‘Nigeria’ were 7.1 and 6.6 g/plant, and for ‘Texas 374’ were 8.8, 7.3, and 16.9 g/plant.

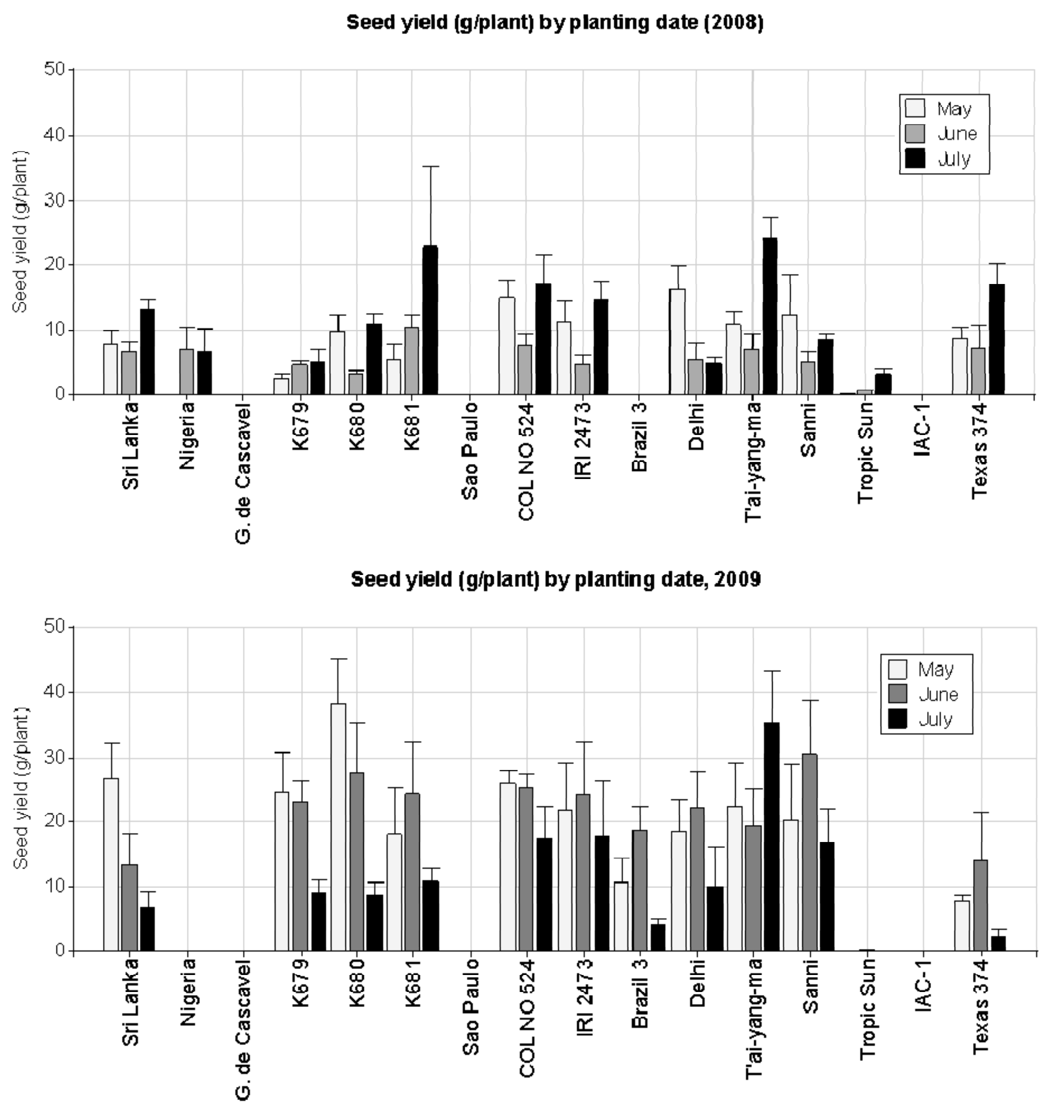

Figure 2.11. Average seed production (g/pl) for *C. juncea* accessions at three planting dates in 2008 and 2009 in Lajas, Puerto Rico

2.4 CONCLUSIONS

In sum, the accessions represented a wide variety of morphological and phenological attributes. The accessions identified for producing high biomass were characterized as tall and narrow, the most prominent of which were ‘Nigeria’, ‘São Paulo’, and ‘Tropic Sun’; however, these same accessions were also identified for their photoperiodic sensitivity, resulting in a greater number of days to flowering when exposed to longer day lengths. The accessions ‘Guizo de Cascavel’, ‘IAC-1’, and ‘Texas 374’ produced moderately high levels of biomass and reflected similar parallels in respect to flowering and photoperiod. A relationship was also noted between these accessions and poor seed production, often resulting in little to no seed yield. Consequently, while these accessions would be ideal for use as a green manure, owing to their biomass production capacities, the extent of this study showed them to lack the potential for the development of a strong *C. juncea* seed production industry. The exceptions were ‘Texas 374’ and ‘Nigeria’, which produced average seed yields comparable to the other accessions when exposed to the longer photoperiods which were present during the June and July plantings in 2008. In the case of ‘Nigeria’, this accession failed to produce sufficient plants for harvest during the 2009 trials. While ‘Texas 374’ produced a harvest in 2009, seed yields for from each planting time were significantly lower than average. Nonetheless, the results of this study provide sufficient evidence for both the ‘Texas 374’ and ‘Nigeria’ accessions to merit further research.

Although the accession ‘T’ai-yang-ma’ produced only average levels of biomass with respect to the other accessions studied, its low sensitivity to photoperiod and distinctive seed production characteristics make it another good candidate for future research. Interestingly, while the other accessions responded to the environmental stresses and pest pressures in the July planting of 2009 with reduced seed yields, ‘T’ai-yang-ma’ appeared to thrive, out performing all of the other accessions during the same trial. Additionally, this accession was one of the highest seed producers among all three trials in 2008.

Future studies should include trials of these three accessions during seasons with shorter photoperiods and under different environmental conditions than were defined in the parameters of this research. Furthermore, crosses between these accessions should be studied to assess the potential for developing a hybrid variety with the favorable characteristics of each of these accessions.

3 Evaluation of the effects of planting density and apical cutting treatments on *C. juncea* production

3.1 INTRODUCTION

The use of sunn hemp (*Crotalaria juncea* L.) as a cover crop and green manure has been internationally documented over the years and has been touted for its rapid biomass production, capacity for nitrogen fixation (NRCS, 1999), a swell as for its allelopathic effect on weeds (Pilbeam and Bell, 1979) and plant-parasitic nematodes (Wang et al., 2004). However, there have been no comprehensive studies conducted in Puerto Rico to assess appropriate planting densities and cultural management practices to achieve desired weed suppression, and biomass and seed production effects.

Sunn hemp has been found to function in weed suppression through the physical effects of shading and from the release of allelopathic compounds into the surrounding soil. A study of aqueous extracts of these compounds have been shown to inhibit germination (Adler and Chase, 2007) and create a toxic environment (Wilson and Bell, 1979) for weed species. Increased planting densities of *C. juncea* would likely increase both the physical effects of shading and the allelopathic effects on soil microclimate.

Inducing branching through pruning has been suggested as one way to stimulate and improve seed production (Abdul-Baki et al., 2001), while also potentially increasing the shading effect on weed populations. It has also been proposed that sunn hemp growth can be stimulated by breaking apical dominance early in development, encouraging a more rapid canopy closure and increasing the effect of shading on weeds. The objective of this experiment was to determine the effects of planting density and apical cutting treatments on seed and biomass production of *C. juncea,* as well as its effect on weed suppression in the field.

3.2 MATERIALS AND METHODS

This planting density/apical cutting experiment was carried out over two years, 2008 and 2009, using ‘T’ai-yang-ma’, a South African cultivar of *C. juncea.* This accession was selected based on its availability in the international market at the time of the experiment. The field study was conducted at the University of Puerto Rico Agricultural Experiment Station in Lajas, Puerto Rico. The experiment studied the effects of three planting densities (10, 25, and 40 lbs *C. juncea* seed per acre), and four apical cutting treatments. The apical cuts consisted of manually removing the top 1-2 inches from the primary stem of each plant in the plot and occurred at 3, 4, and 5 weeks after planting. A group with no cutting treatment was used as a control. For simplification purposes, these treatments will hereinafter be referred to as NC (no cut), 3W, 4W, and 5W. There were a total of 12 treatments, as seen in Table 3.1.

The experiment was first planted on June 19, 2008 in tilled beds using 25x6 ft (7.62x1.82 m) plots, with an 8 ft (2.43 m) buffer between plots. This was replicated on April 22, 2009 using slightly smaller plots of 20x6 ft (6.10x1.82 m) and an 8 ft buffer zone. The sunn hemp seeds were inoculated with *Rhizobium* spp. (Nitragen EL®, EMD Crop BioScience, Milwaukee, Wisconsin) on the day of planting to improve root nodule formation and seeded by hand in four evenly-spaced rows within each plot. A completely randomized block design with four replications was used. Irrigation was utilized at the start of the experiment to improve germination and to help establish the plants during the early stages of development. Overhead irrigation was used in 2008, while drip irrigation was utilized in 2009. Organic management practices were observed; this included foliar applications of organic, botanical pesticides to control the insect pests *Ceratoma ruficornis* Oliv. and *Diabrotica balteata* Lec. during the primary vegetative growth stages in first two months of the experiment. A neem (*Azadirachta indica*) based pesticide (Aza-Direct™, Gowan Co.®, Yuma, Arizona) and the insecticide/miticide Ecotrol® (EcoSMART Technologies, Inc.®, Franklin, Tennessee) were applied as needed at the recommended rates for field crops. The areas between plots were frequently mowed to manage weeds, while the area within the plot remained undisturbed so that the allelopathic effect of *C. juncea* on weed suppression could be accurately assessed.

Table 3.1. *C. juncea* planting density and apical cutting treatments, Lajas, Puerto Rico, 2008 and 2009

	Planting Density		**Apical Cut**
No.	**lb/ha**	**kg/ha**	**weeks after planting**
1	10	11.2	NC (no cut)
2	10	11.2	3W
3	10	11.2	4W
4	10	11.2	5W
5	25	28.0	NC
6	25	28.0	3W
7	25	28.0	4W
8	25	28.0	5W
9	40	44.8	NC
10	40	44.8	3W
11	40	44.8	4W
12	40	44.8	5W

Data were collected to establish the differences in biomass production, plant morphology, canopy cover, seed production, and weed suppression. These attributes were studied by collecting data from four plants in each plot, which were representative of the overall form present in the plot. The data collected included height and width (taken at 2, 3, 4, and 5 months after planting, MAP). Weed data were collected on one occasion in 2008 (2.5 MAP) and on two occasions in 2009 (1 and 2 MAP). On each date, the data were collected from a representative area of 1.5 ft^2 (0.46 m^2) in each plot.

Weeds were cut at soil level and categorized by grass, broad leaf, and sedge. They were oven dried and weighed by category. In 2008, pods were harvested at the ‘rattle stage’ from four representative plants. The ‘rattle stage’ refers to the point at which the pods have begun to dry, change in color from green to brown, and the seeds can be heard rattling within the pod when shaken. Due to low levels of fruit set in 2009, all pods from one row in each plot were harvested. Yield data were based on the number of pods/plant, number of seeds/pod (based on the average number of seeds in 20 pods), and total seed weight/plant. Biomass was collected two times during each year of the experiment, once during flowering and again after harvest. On each occasion, four representative plants were cut at soil level and oven dried before they were weighed. In addition, estimates were calculated for the number of plants per hectare for each planting density. This was calculated based on the averages of data collected for the number of plants in one row of each plot.

The data collected from this experiment were analyzed using an analysis of variance (ANOVA) for a split-split-plot design [Duncan multiple range test (α=0.05), unless otherwise noted] using the statistical program Infostat (Di Rienzo et al., 2009) to identify the potential of each planting density/apical cutting treatment for biomass, seed production, and weed suppression in Puerto Rico.

3.3 RESULTS AND DISCUSSION

3.3.1 *Effect of treatments on morphology of* C. juncea

Plant height

Using the average heights collected from each treatment at 2, 3, 4, and 5 MAP, an analysis of variance showed that there was a significant interaction between planting density and apical cutting, as well as a significant difference in height over time in both

2008 and 2009. The results of the density/apical cutting interaction proved to be disordinal and therefore the singular effects of density or apical treatment alone could not be clearly inferred from the data.

Figure 3.1 represents the average height in 2008 expressed over time for each planting density and is separated by apical cutting treatment to show the effect of the interaction. Apical treatment did not cause significant differences in height among the three planting densities at both 2 and 5 MAP, indicating that the effect of the interaction between planting density and apical cutting was present only during the period of 3 and 4 months after planting. This is supported by statistical analysis for 2008, which demonstrates that the most prevalent differences occurred during the third and fourth month after planting. It should be noted that, the 2 MAP measurements were recorded only shortly after the final apical cuttings were performed and this may account for the lack of differences at that point in time.

The planting density 10 lb/ac had significantly less growth than both 25 and 40 lb/ac at 3 and 4 month, while with apical cuttings at 4 WAP, the 25 lb/ac density has the lowest performance during the same months. The effect of cutting the *C. juncea* apex at 3 or 4 weeks after planting appears to have a varied effect, signifying that while there is a distinct interaction occurring between density and apical cutting, a trend cannot be clearly delineated. Additionally, the analysis of variance showed that the combination of 10 lb/ac-3W treatments was consistently the shortest and 25 lb/ac-3W was among the tallest across each of the 12 treatments in the experiment. The mean values for the 10 lb/ac-3W and 25 lb/ac-3W treatments at the earliest measured date (2 MAP) were 1.69 and 1.92 m, respectively, while the plant heights at the final date (5 MAP) were 1.92 and 2.19 m, respectively. A more detailed statistical summary of these significant interactions is represented in Appendix B (Table B2). The apical treatment 5W was comparable to the control (NC), and while both showed growth in height over time, neither presented a clear effect of treatment.

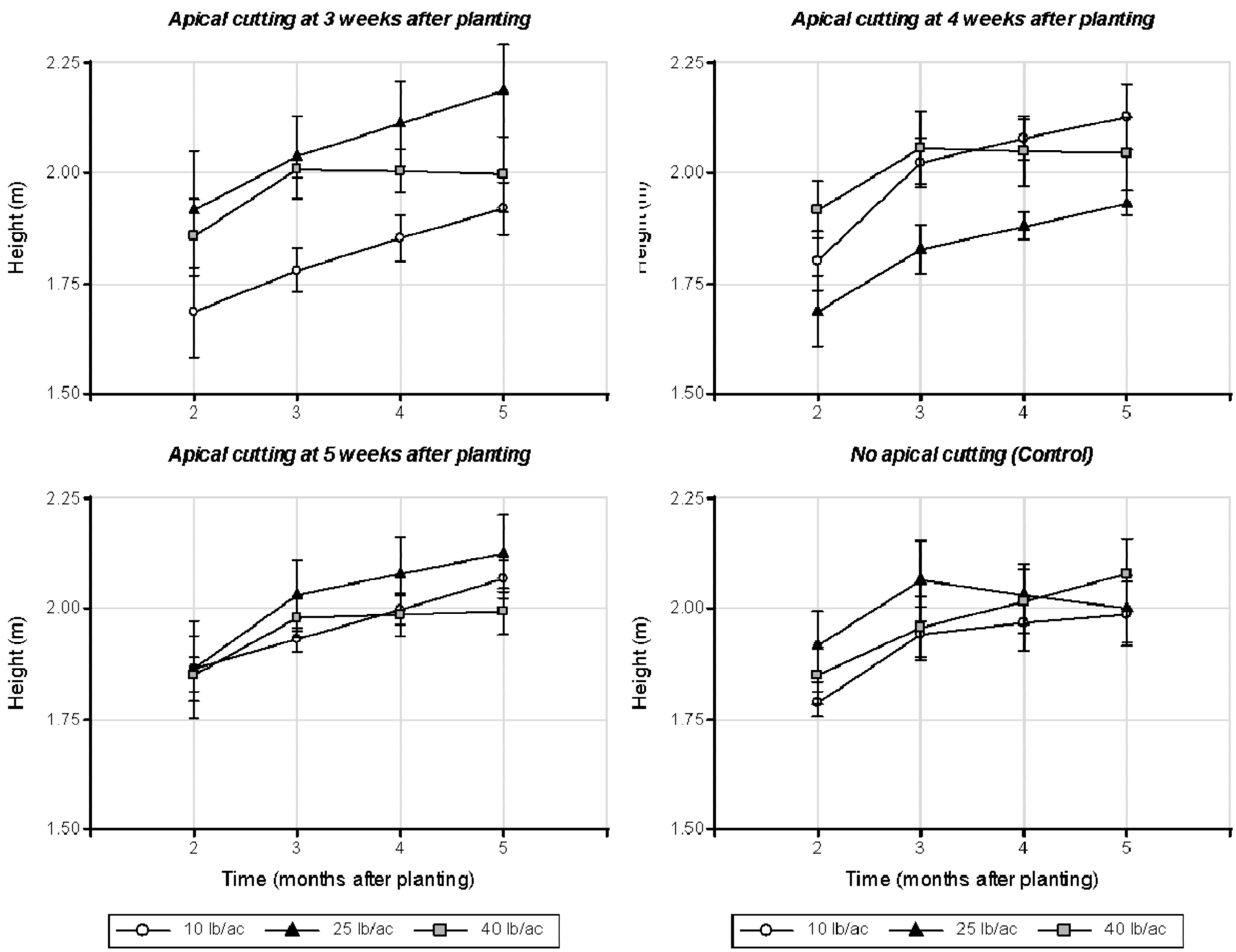

Figure 3.1. Effect of apical cutting treatment and planting density in *C. juncea* on plant height over time in Lajas, Puerto Rico (2008)

In 2009, the interaction between apical cutting and planting density was also significant (Figure 3.2). Plant heights at 2 MAP were relatively similar across treatments. Additionally, there was less variation present within the data set; However, beginning at 3 MAP, differences are observed between plant heights. When the apical cutting occurred at 3 weeks after planting, the 10 lb/ac density was significantly shorter than 25 lb/ac (at 4 MAP) and 40 lb/ac (at 5 MAP). Similar to the results in 2008, this effect was reversed in the 4W treatments, where 25 lb/ac became the density with the least height (at 3 and 4 MAP), when it was significantly smaller than the 10 lb/ac density. Also, as in 2008, the density of 10 lb/ac with a 3W treatment presented among the lowest values, along with 40 lb/ac-4W. The average heights of these treatments ranged 1.26-2.25 m from 2 to 5 MAP. On the other end of the scale, 25 lb/ac-NC represented the tallest treatment, with a range of 1.53-2.43 m (2-5 MAP).

Overall, there was a greater change in growth through the duration of the experiment in the second year, with heights at 2 MAP averaging shorter and heights at 5 MAP developing taller than those in the previous year. Since the two experiments were planted during different seasons (June in 2008 and April in 2009), there are a number of environmental factors which may have played a role in causing these changes, including photoperiod, precipitation, and ambient air temperature, among others.

Planting density alone did not have an effect on height; therefore, the overall effect of apical cutting treatment over time for each year can be demonstrated, as seen in Figure 3.3. While it appears that there is a disordered interaction between the 3W and 4W treatments in both years, this difference was not found to be significant. However, it can be observed that there is an additive effect on height when comparing the 5W cuttings with the 3W and 4W cutting treatments. This additive effect is more clearly defined in 2009.

It should be noted that, in 2008, the initial planting was met with some difficulty. During this year, a lack of rainfall and irrigation during the first week after planting resulted in uneven germination rates which were evident in the field. After the first few weeks and sufficient irrigation, the plots appeared to have recovered; however, there may have been residual effects from this initial period of plant stress. The implementation of drip irrigation during the germination period and first month of growth in 2009 reduced the impact of these environmental stressors and resulted in the more clearly defined responses to the applied treatments.

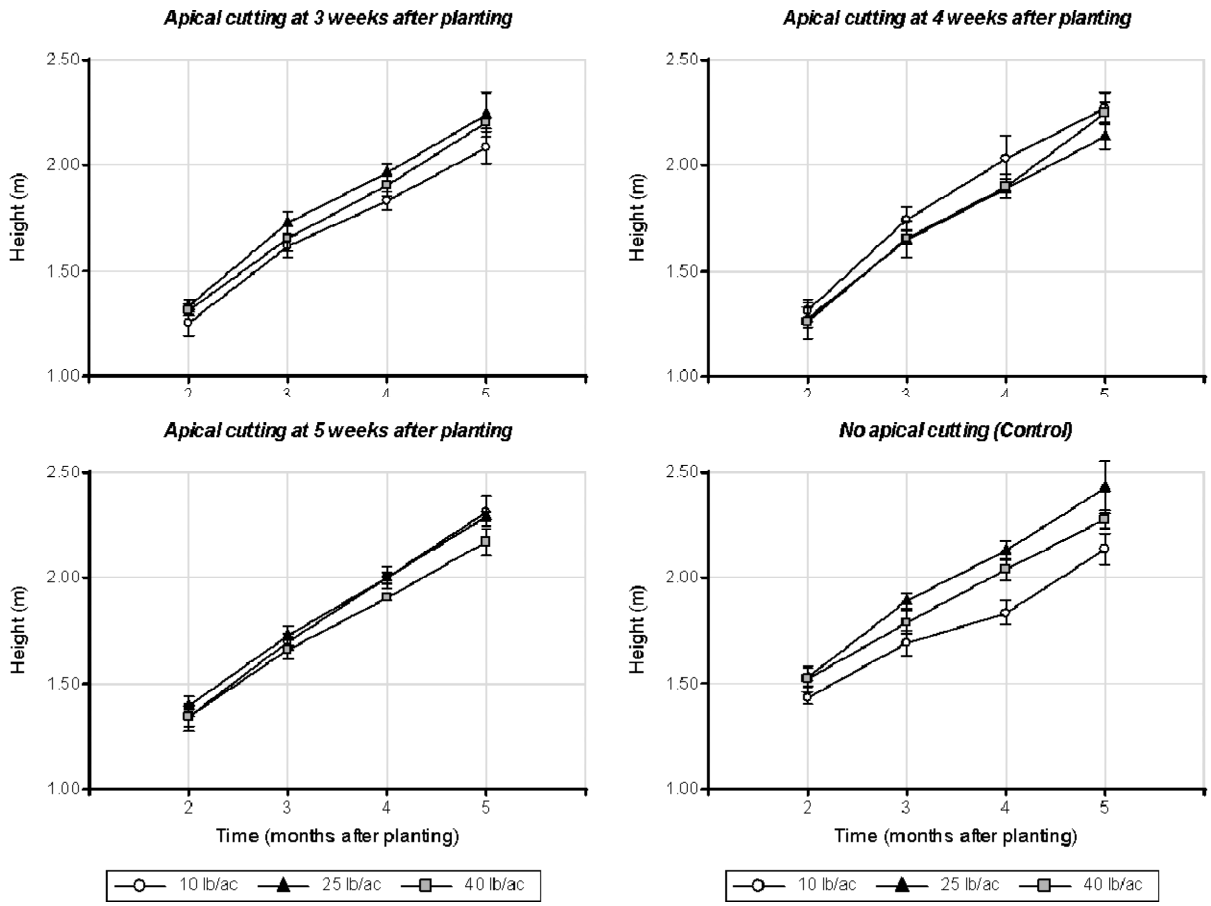

Figure 3.2. Effect of apical cutting treatment and planting density in *C. juncea* on plant height over time in Lajas, Puerto Rico (2009)

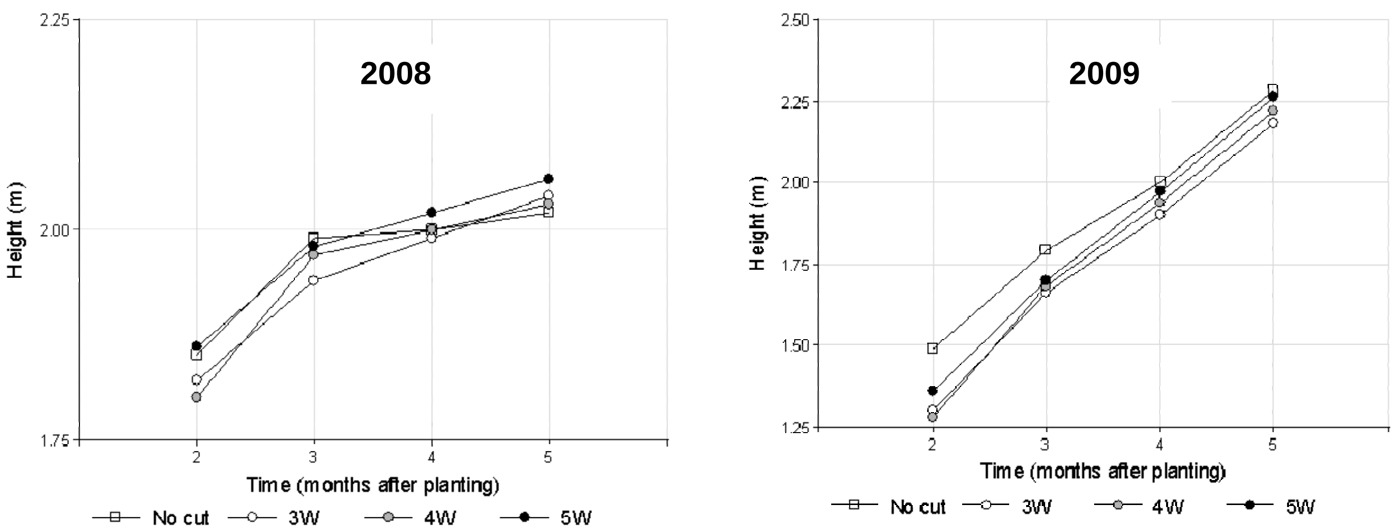

Figure 3.3. Effect of apical cutting on *C. juncea* height over time in 2008 and 2009 in Lajas, Puerto Rico

3.3.2 *Weed Suppression*

In both years of the experiment, the same weed species were observed. The primary grasses were identified as: *Digitaria sanguinalis* L., *Echinochloa colona* L., and *Sorghum halepense* L. The main broadleaf species present were: *Amaranthus dubius* Mart., *Ipomoea setifera*, *I. tiliacea* Willd., *Phyllanthus niruri* L., and *Trianthema portulacastrum* L. Only one species of sedge was noted, *Cyperus rotundus* L.

Since the effect of *C. juncea* on weed suppression under the treatments of planting densities and apical cutting was assessed on different occasions in each year, samples from 2008 and 2009 were analyzed separately. In 2008, there was no effect of planting density, apical cutting treatment, or interaction found in relation to weed suppression at 2.5 months after planting. The weed sampling from 2009 showed that there was a significant increase in the overall presence of grass, broadleaf, and sedge populations between 1 and 2 months after planting (

Figure 3.4). The grasses were present in the highest proportion in comparison with broadleaf and sedges and showed considerable growth between one and two MAP. This is primarily attributable to the presence of *S. halepense*, or Johnson grass, which is characterized by its rapid growth rate and seed spread (NRCS, 2010). The grasses were followed by broadleaf weeds and sedges as the next most populous weed categories. While there was no effect of the *C. juncea* treatments on the presence of grass or sedge populations, broadleaf weeds were shown to be significantly suppressed at 2 months after planting at a density of 40 lb/ac (Figure 3.5). The 10 and 25 lb/ac densities were not shown to be significantly different.

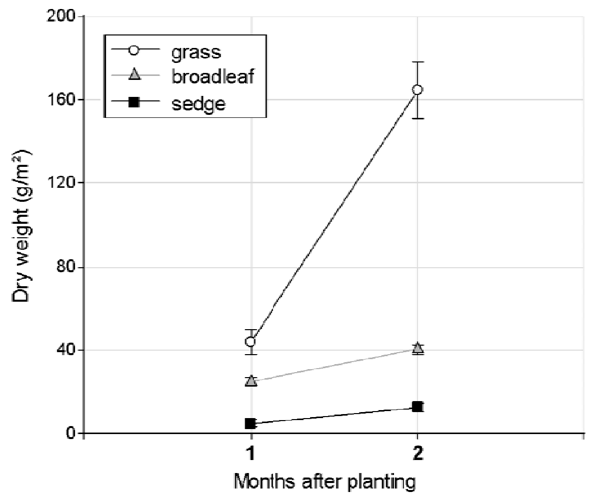

Figure 3.4. Weed presence (g/m²) in *C. juncea* by grass, broadleaf, and sedge at 1 and 2 months after planting in Lajas, Puerto Rico (2009)

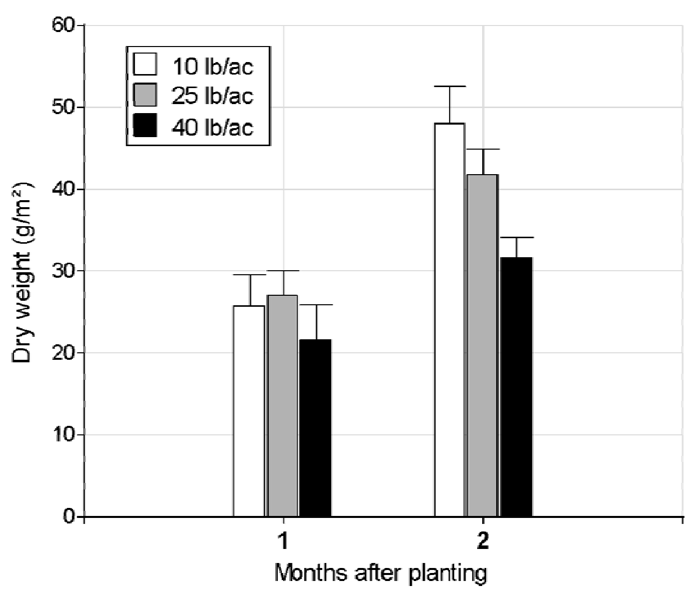

Figure 3.5. Broadleaf weed presence (g/m²) by *C. juncea* planting density at 1 and 2 months after planting in Lajas, Puerto Rico (2009**)**

3.3.3 *Biomass production*

Production of biomass was assessed at the time of flowering and post-harvest in both years. For 2008, these samples were collected at 23 and 26 weeks after planting, respectively. However, in 2009, biomass collections occurred at 17 and 29 WAP. While *C. juncea* flowered earlier in the second year, there was a twelve week period between the sampling dates at flowering and post-harvest. In contrast, there was only a three week separation between sampling dates in 2008. The longer photoperiod which was present as a result of planting in April (2009) as compared to June (2008) may have been a factor in prolonging pod set in the second year. Additionally, differences in precipitation and environmental conditions were observed between the two years, and pest pressures were considerably increased during 2009. Figure 3.6 shows biomass production (kg/ha dry weight) at the two sampling stages for each year. This reflects a considerable difference between biomass produced in each year, as well as a significant increase in biomass between flowering and post-harvest in 2008, and a decrease which occurs in 2009. The decrease may have been a result of the plants in 2009 having begun senescence at the time of post-harvest sampling, in addition to damage from pests.

The analysis also showed that there was a significant effect of planting density in both years; however, there was neither an effect of apical cutting treatment nor an interaction between density and cutting. Despite the differences between the two years, both reflect significantly less biomass production at the 10 lb/ac density, as compared with the 25 and 40 lb/ac densities, which were not found to be statistically distinct from one another. Average biomass production in 2008 for the 25 and 40 lb/ac densities were, respectively, 29,973 and 29,144 kg/ha at flowering, and 41,438 and 48,080 kg/ha post-harvest. At 10 lb/ac, there was an average biomass production of 14,564 kg/ha at flowering and 20,965 kg/ha post-harvest. In 2009, the 25 and 40 lb/ac densities produced 7,288 and 8,491 kg/ha at flowering, and 5,043 and 6,003 kg/ha post-harvest, respectively. The lowest producing planting density, 10 lb/ac, yielded 4,211 kg/ha at flowering, and 3,209 kg/ha at post-harvest.

For use as a green manure, biomass production levels would be most significant at the time of florescence, when the nitrogen levels in *C. juncea* would be ideal for incorporation into the soil (Treadwell and Alligood, 2008). The outcome of the biomass analysis indicates that, of the three planting densities, 25 lb/ac would be ideal from an agricultural standpoint. As previously mentioned, the cost of *C. juncea* seed can be prohibitively expensive, and while planting seed at 25 lb/ac produced significantly more biomass than 10 lb/ac, the difference was negligible when compared with 40 lb/ac. Apical cutting treatments showed no effect with regard to biomass production; however, planting season and the respective changes in environmental conditions may play a significant role in biomass productivity.

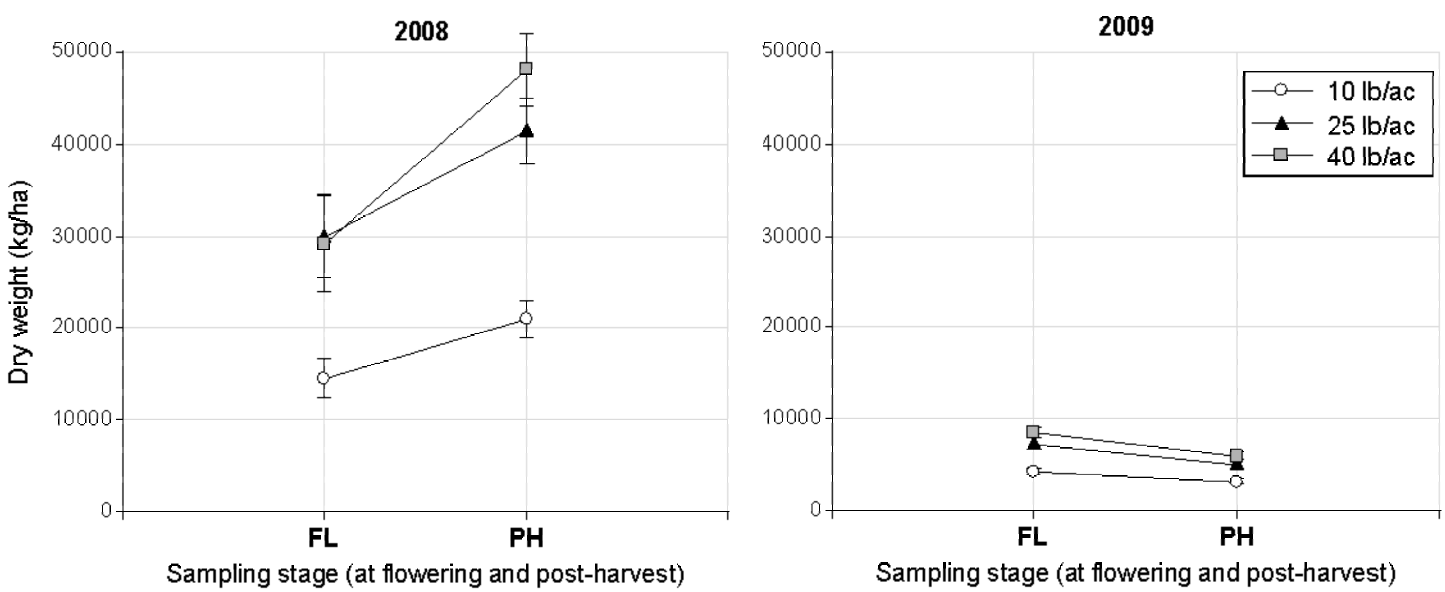

Figure 3.6. Biomass (kg/ha) by *C. juncea* planting density at flowering and post-harvest in 2008 and 2009

3.3.4 *Seed yield*

Results showed that there was a significant divide between seed yields in 2008 and 2009 (Figure 3.7), however, there was a distinct pattern of interaction between planting density and the time of apical cutting which was nevertheless reflected in both years of the experiment. The stark differences in yield are most likely a result of the change in planting season (June in 2008 compared with April in 2009) and their respective differences in environmental conditions, as well as the additional pest pressures which were present in 2009. In both years, the primary pollinators observed in the field were the common honey bee, *Apis mellifera* L., and the carpenter bee, *Xylocopidae mordax*.

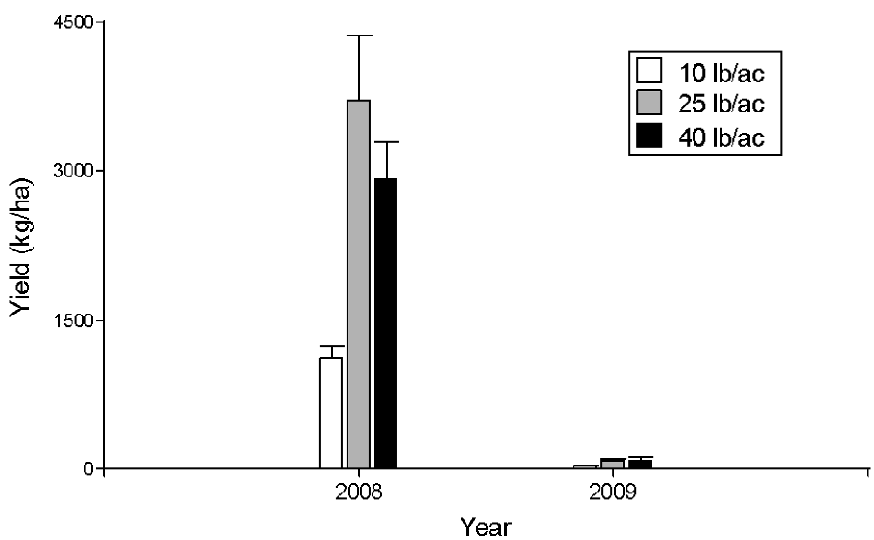

Figure 3.7. Average *C. juncea* seed yield (kg/ha) for each planting density by year, in Lajas, Puerto Rico

The interactions for planting density and apical cutting treatments during each year are displayed in Figure 3.8. Additionally, a complete statistical summary is available in Appendix B (Table B4). Seed yields when the *C. juncea* was planted at 10 lb/ac were shown to be low in all apical cutting treatments and were not significantly different from one another. The average yields at 10 lb/ac were 1123.7 and 31.9 kg/ha in 2008 and 2009, respectively. The figure shows that when apical cuttings were conducted at 3 and 4 weeks after planting at a density of 25 lb/ac, seed yields were significantly higher than all of the apical treatments at 10 lb/ac; however, the effect of these treatments was not significantly different from the control (no cut). Cuttings at 25 lb/ac which were applied at 5 weeks produced yields similar to those in the 10 lb/ac group. The seed yield at 40 lb/ac had the opposite effect with respect to apical cutting. The analysis showed that cuttings applied at 3 and 4 weeks after planting resulted in moderately low yields (1938.3 kg/ha in 2008 and 57.2 kg/ha in 2009), which were statistically similar to those seen in all cutting treatments at 10 lb/ac and the 5W cutting at 25 lb/ac. Additionally, the NC and 5W treatments resulted in relatively high yields at 40 lb/ac (averaging 3895.6 and 118.3 kg/ha in 2008 and 2009, respectively), which were comparable to the high yields of the 25 lb/ac NC, 3W, and 4W treatments.

In sum, a 10 lb/ac planting density resulted in low seed yields in all treatments. At 25 lb/ac, apical cuttings at 5 weeks after planting proved to be detrimental to seed yields, while at 40 lb/ac, the same apical treatment showed results statistically similar to the NC control. Conversely, at a density of 25 lb/ac, the 3W and 4W treatments were not statistically different from the control, while these apical treatments significantly reduced yields when applied to 40 lb/ac plots.

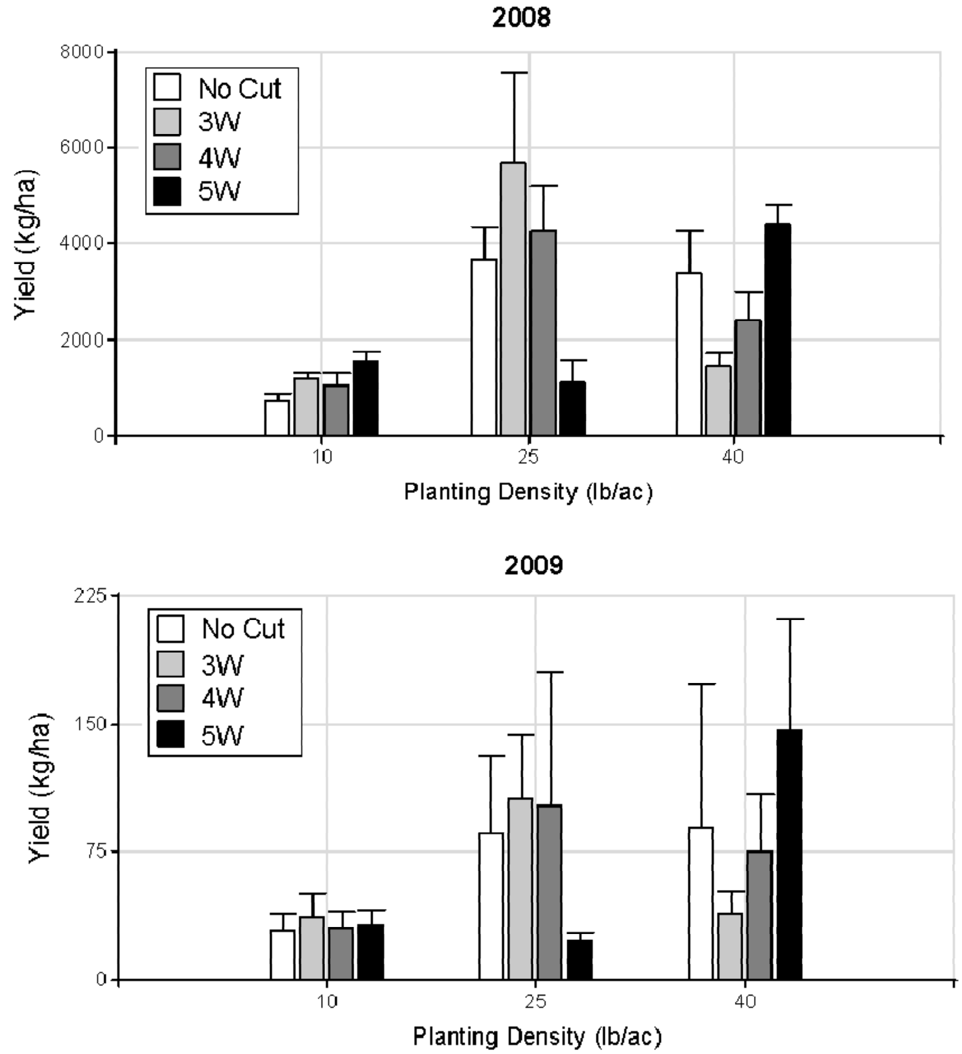

Figure 3.8. *C. juncea* seed yield (kg/ha) by planting density and apical cutting treatment for 2008 and 2009

3.4 CONCLUSIONS

In general, the difference in time of planting between the two years of the experiment played a significant role in the production of biomass and seed. From the results of an analysis of variance for seed production in Chapter 2 (Section 2.3.4), planting time is shown to have a strong effect on the accession 'T'ai-yang-ma' with relation to seed production. A relationship is shown between exposure to a shorter photoperiod and an increase in seed production. This correlation is reflected in the differences in seed yield between the experiments planted in June 2008, as compared with April 2009. In 2009, the *C. juncea* would have been exposed to approximately two months of increasing daylength before the summer solstice, when the photoperiod would have begun to decrease. In contrast, the experiment in 2008 was planted one day before the summer solstice (USNO, 2009).

The optimal planting density with respect to both biomass and seed production proved to be 25 lb/ac with no apical cut. Although planting *C. juncea* at 40 lb/ac and applying a 5W apical cutting treatment resulted in similar seed yields, it would be considerably more costly with respect to the greater seed inputs and labor required for the apical cutting treatment. Similarly, the assessment of biomass production did not reflect any effect of apical cut and a planting density of 40 lb/ac did not produce a significantly greater amount of biomass than at 25 lb/ac. Planting at 10 lb/ac produced significantly less biomass and seed than planting at 25 lb/ac NC. Furthermore, while apical cutting did have an effect on height, these differences were not reflected in biomass and seed production, nor were they significant in weed suppression. In conclusion, and within the parameters of this study, planting *C. juncea* at 25 lb/ac proved to be both productive and cost efficient.

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Appendix A. List of Abbreviations

3W	Apical cut at 3 weeks after planting
4W	Apical cut at 4 weeks after planting
5W	Apical cut at 5 weeks after planting
ANOVA	Analysis of variance
DTF	Days to 50 percent flowering
MAP	Months after planting
NC	No apical cut (Control)
WAP	Weeks after planting

Appendix B. Statistical Summary Tables

Table B1. Plant diameter by accession in 2008

Plant ID	Mean (m)	S.D.	Minimum	Maximum
COL NO 524	0.67	0.24	0.17	1.07
IRI 2473	0.54	0.23	0.09	0.94
K679	0.52	0.20	0.22	1.04
K680	0.58	0.23	0.08	1.09
K681	0.56	0.26	0.09	1.05
Sanni	0.62	0.23	0.21	1.07
Sri Lanka	0.59	0.21	0.07	0.99
T'ai-yang-ma	0.62	0.23	0.21	1.13
Texas 374	0.51	0.18	0.16	0.82
Tropic Sun	0.60	0.24	0.25	1.24

Table B2. Significant planting density/cutting interactions for height (m) in 2008

Density	Cut	MAP	Mean (m)	S.D.	Minimum	Maximum
10	3	2	1.69	0.20	1.52	1.98
10	3	3	1.78	0.10	1.71	1.93
10	3	4	1.85	0.10	1.74	1.99
10	3	5	1.92	0.12	1.77	2.05
25	3	2	1.92	0.26	1.59	2.16
25	3	3	2.04	0.19	1.78	2.23
25	3	4	2.11	0.19	1.87	2.34
25	3	5	2.19	0.21	1.95	2.46 .

Table B3. Average biomass production (g dry wt/plant) by C. juncea *accession for June and July plantings, 2008 and 2009 combined*

acc	Plant ID	n	Mean	S.D.	Minimum	Maximum
1	Sri Lanka	16	79.58	38.57	10.90	152.41
2	Nigeria	16	182.67	116.24	0.00	405.00
3	G. Cascavel	16	141.91	129.49	0.00	410.00
4	K679	16	37.60	35.27	0.00	131.00
5	K680	16	66.48	46.62	12.70	153.00
6	K681	16	82.89	49.85	0.00	170.00
7	São Paulo	16	200.74	117.69	0.00	374.00
8	COL NO 524	16	61.84	47.01	4.70	184.00
9	IRI 2473	16	74.05	45.95	21.40	165.00
10	Brazil 3	16	90.88	73.02	0.00	200.49

11	Delhi	16	80.85	68.32	0.00	224.98
12	T'ai-yang-ma	16	96.98	39.41	28.00	160.00
13	Sanni	16	72.53	39.20	15.70	135.00
14	Tropic Sun	16	195.48	113.13	41.73	425.00
15	IAC-1	16	143.16	127.81	0.00	380.00
16	Texas 374	16	115.23	40.57	39.40	171.00

Table B4.Average seed yield (kg/ha) for planting density and apical cutting treatments, categorized by year

2008

density	cut	n	Mean	S.D.	Minimum	Maximum
10	0	4	738.94	257.83	432.53	1029.86
10	3	4	1179.00	269.82	793.53	1366.41
10	4	4	1029.00	535.09	496.82	1743.24
10	5	4	1547.76	429.92	906.19	1796.87
25	0	4	3687.90	1335.56	2108.99	5374.26
25	3	4	5715.86	3718.16	1700.81	10593.57
25	4	4	4276.78	1865.01	1766.29	6276.92
25	5	4	1126.30	913.47	581.48	2492.08
40	0	4	3397.71	1813.74	1144.66	5373.19
40	3	4	1458.46	541.54	974.93	2235.01
40	4	4	2418.15	1161.74	1389.42	3645.23
40	5	4	4393.55	875.24	3669.95	5561.29

2009

density	cut	n	Mean	S.D.	Minimum	Maximum
10	0	4	29.28	17.89	0.01	50.50
10	3	4	36.46	27.24	7.40	72.71
10	4	4	29.93	20.38	9.58	53.99
10	5	4	32.00	18.84	15.24	57.03
25	0	4	86.42	89.17	24.38	216.81
25	3	4	106.23	74.44	63.13	217.25
25	4	4	101.88	158.85	15.24	340.02
25	5	4	22.09	11.72	11.32	38.75
40	0	4	89.68	168.10	2.18	341.76
40	3	4	39.29	23.98	7.84	65.74
40	4	4	75.10	68.27	0.00	155.86
40	5	4	146.83	127.94	24.38	258.17

Table B5. Average days to flowering (DTF), by accession, planting month (1=May, 2=June, 3=July), and year

Plant ID	month	year	Var.	n	Mean	S.D.	Min.	Max.
Brazil 3	1	2008	DTF	4	112.00	0.00	112.00	112.00
Brazil 3	1	2009	DTF	4	63.00	0.00	63.00	63.00
Brazil 3	2	2008	DTF	4	0.00	0.00	0.00	0.00
Brazil 3	2	2009	DTF	4	54.25	3.50	49.00	56.00
Brazil 3	3	2008	DTF	4	77.00	0.00	77.00	77.00
Brazil 3	3	2009	DTF	4	56.00	0.00	56.00	56.00
COL NO 524	1	2008	DTF	4	49.00	0.00	49.00	49.00
COL NO 524	1	2009	DTF	4	45.50	4.04	42.00	49.00
COL NO 524	2	2008	DTF	4	50.75	3.50	49.00	56.00
COL NO 524	2	2009	DTF	4	42.00	0.00	42.00	42.00
COL NO 524	3	2008	DTF	4	49.00	5.72	42.00	56.00
COL NO 524	3	2009	DTF	4	45.50	7.00	42.00	56.00
Delhi	1	2008	DTF	4	54.25	3.50	49.00	56.00
Delhi	1	2009	DTF	4	56.00	5.72	49.00	63.00
Delhi	2	2008	DTF	4	57.75	10.50	49.00	70.00
Delhi	2	2009	DTF	4	52.50	7.00	42.00	56.00
Delhi	3	2008	DTF	4	56.00	0.00	56.00	56.00
Delhi	3	2009	DTF	4	49.00	0.00	49.00	49.00
G. de Cascavel	1	2008	DTF	4	0.00	0.00	0.00	0.00
G. de Cascavel	1	2009	DTF	4	0.00	0.00	0.00	0.00
G. de Cascavel	2	2008	DTF	4	0.00	0.00	0.00	0.00
G. de Cascavel	2	2009	DTF	4	0.00	0.00	0.00	0.00
G. de Cascavel	3	2008	DTF	4	0.00	0.00	0.00	0.00
G. de Cascavel	3	2009	DTF	4	85.75	3.50	84.00	91.00
IAC-1	1	2008	DTF	4	0.00	0.00	0.00	0.00
IAC-1	1	2009	DTF	4	0.00	0.00	0.00	0.00
IAC-1	2	2008	DTF	4	0.00	0.00	0.00	0.00
IAC-1	2	2009	DTF	4	87.50	4.04	84.00	91.00
IAC-1	3	2008	DTF	4	0.00	0.00	0.00	0.00
IAC-1	3	2009	DTF	4	77.00	0.00	77.00	77.00
IRI 2473	1	2008	DTF	4	57.75	8.81	49.00	70.00
IRI 2473	1	2009	DTF	4	49.00	0.00	49.00	49.00
IRI 2473	2	2008	DTF	4	59.50	4.04	56.00	63.00
IRI 2473	2	2009	DTF	4	61.25	20.11	49.00	91.00
IRI 2473	3	2008	DTF	4	56.00	0.00	56.00	56.00
IRI 2473	3	2009	DTF	4	49.00	0.00	49.00	49.00
K679	1	2008	DTF	4	52.50	4.04	49.00	56.00
K679	1	2009	DTF	4	50.75	8.81	42.00	63.00
K679	2	2008	DTF	4	49.00	0.00	49.00	49.00
K679	2	2009	DTF	4	42.00	0.00	42.00	42.00
K679	3	2008	DTF	4	49.00	0.00	49.00	49.00
K679	3	2009	DTF	4	42.00	0.00	42.00	42.00

K680	1	2008	DTF	4	50.75	3.50	49.00	56.00
K680	1	2009	DTF	4	50.75	3.50	49.00	56.00
K680	2	2008	DTF	4	54.25	3.50	49.00	56.00
K680	2	2009	DTF	4	47.25	6.70	42.00	56.00
K680	3	2008	DTF	4	49.00	0.00	49.00	49.00
K680	3	2009	DTF	4	47.25	3.50	42.00	49.00
K681	1	2008	DTF	4	56.00	5.72	49.00	63.00
K681	1	2009	DTF	4	56.00	5.72	49.00	63.00
K681	2	2008	DTF	4	59.50	4.04	56.00	63.00
K681	2	2009	DTF	4	52.50	7.00	49.00	63.00
K681	3	2008	DTF	4	56.00	0.00	56.00	56.00
K681	3	2009	DTF	4	52.50	4.04	49.00	56.00
Nigeria	1	2008	DTF	4	0.00	0.00	0.00	0.00
Nigeria	1	2009	DTF	4	0.00	0.00	0.00	0.00
Nigeria	2	2008	DTF	4	0.00	0.00	0.00	0.00
Nigeria	2	2009	DTF	4	0.00	0.00	0.00	0.00
Nigeria	3	2008	DTF	4	85.75	10.50	77.00	98.00
Nigeria	3	2009	DTF	4	82.25	20.90	56.00	105.00
Sanni	1	2008	DTF	4	61.25	6.70	56.00	70.00
Sanni	1	2009	DTF	4	61.25	6.70	56.00	70.00
Sanni	2	2008	DTF	4	61.25	11.95	49.00	77.00
Sanni	2	2009	DTF	4	50.75	3.50	49.00	56.00
Sanni	3	2008	DTF	4	56.00	5.72	49.00	63.00
Sanni	3	2009	DTF	4	50.75	3.50	49.00	56.00
Sao Paulo	1	2008	DTF	4	0.00	0.00	0.00	0.00
Sao Paulo	1	2009	DTF	4	0.00	0.00	0.00	0.00
Sao Paulo	2	2008	DTF	4	0.00	0.00	0.00	0.00
Sao Paulo	2	2009	DTF	4	0.00	0.00	0.00	0.00
Sao Paulo	3	2008	DTF	4	77.00	0.00	77.00	77.00
Sao Paulo	3	2009	DTF	4	84.00	5.72	77.00	91.00
Sri Lanka	1	2008	DTF	4	59.50	4.04	56.00	63.00
Sri Lanka	1	2009	DTF	4	54.25	3.50	49.00	56.00
Sri Lanka	2	2008	DTF	4	57.75	20.90	28.00	77.00
Sri Lanka	2	2009	DTF	4	49.00	0.00	49.00	49.00
Sri Lanka	3	2008	DTF	4	56.00	0.00	56.00	56.00
Sri Lanka	3	2009	DTF	4	50.75	6.70	42.00	56.00
T'ai-yang-ma	1	2008	DTF	4	59.50	4.04	56.00	63.00
T'ai-yang-ma	1	2009	DTF	4	64.75	3.50	63.00	70.00
T'ai-yang-ma	2	2008	DTF	4	66.50	7.00	56.00	70.00
T'ai-yang-ma	2	2009	DTF	4	56.00	0.00	56.00	56.00
T'ai-yang-ma	3	2008	DTF	4	56.00	0.00	56.00	56.00
T'ai-yang-ma	3	2009	DTF	4	56.00	0.00	56.00	56.00
Texas 374	1	2008	DTF	4	75.25	15.52	63.00	98.00
Texas 374	1	2009	DTF	4	57.75	3.50	56.00	63.00

Texas 374	2	2008	DTF	4	92.75	22.41	70.00	112.00
Texas 374	2	2009	DTF	4	70.00	0.00	70.00	70.00
Texas 374	3	2008	DTF	4	59.50	4.04	56.00	63.00
Texas 374	3	2009	DTF	4	59.50	4.04	56.00	63.00
Tropic Sun	1	2008	DTF	4	105.00	5.72	98.00	112.00
Tropic Sun	1	2009	DTF	4	98.00	0.00	98.00	98.00
Tropic Sun	2	2008	DTF	4	103.25	17.50	77.00	112.00
Tropic Sun	2	2009	DTF	4	87.50	4.04	84.00	91.00
Tropic Sun	3	2008	DTF	4	73.50	7.00	63.00	77.00
Tropic Sun	3	2009	DTF	4	70.00	0.00	70.00	70.00