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Evaluation of Goji Berry (Lycium barbarum L.) Cultivars and Air Root Pruning for Edible Greens Production in the Midwest

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EVALUATION OF GOJI BERRY (*LYCIUM BARBARUM* L.) CULTIVARS AND AIR ROOT PRUNING FOR EDIBLE GREENS PRODUCTION IN THE MIDWEST

A Master's Thesis

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By

Jesse Lee Carroll

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EVALUATION OF GOJI BERRY (*LYCIUM BARBARUM* L.) CULTIVARS AND AIR ROOT PRUNING FOR EDIBLE GREENS PRODUCTION IN THE MIDWEST

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ABSTRACT

Goji berry plants (*Lycium barbarum* L.) are grown across the globe for their berries and the health benefits that come from eating those berries. However, in Ningxia, China, Goji berry plants are also grown as a source of edible greens. In order to harvest the young green shoots, the plants are subjected to intense pruning and kept as a small, low-growing shrub. There is little information on this method of Goji berry management and greens production in the United States. The purpose of this research is to determine if Goji berry cultivars grown for berry production in the United States can survive and grow under the intense pruning used for Goji berry greens production. This research will also examine the effect of air root pruning on potted Goji berry plants grown in greenhouse conditions, as well as transplant success into field conditions and subsequent plant vigor and survival. Three cultivars, 'Big Lifeberry', 'Vermillion Sunset', and 'Sweet Lifeberry' were evaluated in this experiment. Forty plants of each cultivar were purchased from Stark Bros Nursery in Louisiana, MO. Twenty plants from each cultivar were potted in air root pruning containers and the other 20 plants were potted into standard non-air root pruning containers. In May 2018, eight plants of each cultivar (four plants from each treatment) were harvested and their rootballs were rinsed, weighed, and scanned for total root length, volume, average diameter, and number of root tips. The remaining plants were transplanted into the field in a complete randomized design with four replications, six plots, and four plants per plot. When new shoots were harvested for greens, fresh weight, dry weight, number of shoots, total shoot length, and average shoot length, were measured per plant. Few differences of rooting characteristics and greens production were found between the air root pruned and non-air root pruned plants. The cultivars exhibited late spring and early summer growth, reduced growth in the middle of summer, and the most abundant growth early fall through late fall. Of the three cultivars evaluated, 'Big Lifeberry' produced consistently greater yields of greens throughout the growing season.

KEYWORDS: *Lycium barbarum*, Goji Berry, greens, air root pruning, Midwest, Solanaceae

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This thesis would not have been possible without your constant, unfailing love and support. I am truly lucky to have you as parents. Not a day goes by where I fail to realize how much you have done for me, Stella, Amanda, Dannay, Shane, and so many others. You are both truly amazing.
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INTRODUCTION

The Goji Berry

The Goji berry (*Lycium barbarum* L.), popular among many Asian countries, is a deciduous shrub that produces a bright orange-red berry (Donno et al., 2015). The Goji berry has many different names. In China, it is referred to as the Goji berry because the Chinese character "gou" is used to write the name. This character is also used as a character for the English word, "wolf". Hence, the *L. barbarum* plant is also known as the wolfberry plant in the United States of America, China, and other countries.

While the specific location of the Goji berry's origin is unknown, and few studies have been conducted to determine this location, it is currently hypothesized that the genus *Lycium* originated in North or South America, and *L. barbarum* originated in Eurasia (Fukuda et al., 2000). The study conducted by Fukuda used chloroplast DNA sequences to study the phylogenetic relationships, create a cladogram, and determine the origin of the genus *Lycium*. Divergence times were estimated for each region of the world using molecular clock estimations. The results of this study were not able to determine whether the genus originated in North or South America, but, it was concluded that the genus *Lycium* originated in the New World and the center of diversification was South America (Fukuda et al., 2000). It was also concluded that intercontinental dispersal events by birds and ocean and air currents likely played significant roles in the global distribution of the *Lycium* genus. The estimated global distribution began in the New World, then made its way to South Africa and the Pacific Islands. This study also concluded that *L. barbarum* is native to Eurasia, and all species present in Eurasia and Australia originated from the region of South Africa (Fukuda et al., 2000).

According to the United States Department of Agriculture Natural Resources Conservation Service (USDA NRCS Plant Data Team), *L. barbarum* has been introduced in forty-six of the forty-eight consecutive United States. The NRCS also lists *Lycium halimifolium* as a synonym for *L. barbarum.* The hardiness zones listed for *L. barbarum* range from zone two to zone seven (Demchak, 2016).

While originally grown as a health food in Asia, *L. barbarum* and its berry are now known worldwide for its health benefits, including a high fiber content, potassium, magnesium, iron, vitamin E, vitamin C, carotenoids, and beta-carotene (Niro et al., 2017). In China, *L. barbarum* is grown not just for the berries, but also as a source of edible greens. These greens are used for several different purposes. They are added to soups, salads, steeped in hot water for tea, or served as a side course. However, there is very little published information on the methods used to grow *L. barbarum* as a source of greens, or even how to cultivate the plant for berry production in the United States of America. China is the main exporter of Goji berry products, generating $120 million in 2010 using 82,000 ha to produce Goji berry fruits (Donno et al., 2015). The Ningxia Hui region and the Xinjiang Uyghur region are the main producers of Goji berries (Donno et al., 2015). Street markets across these regions can be found selling fresh Goji berries, dried Goji berries, and many other Goji berry products. Developing a method of production for *L. barbarum* plants in the United States of America could help increase access to the health benefits of the Goji berry.

Lycium is a genus found in the Solanaceae family (Aronson, 2006). This family includes many edible plants such as tomatoes, potatoes, eggplant, and peppers. This family also includes many toxic plants such as nightshade and jimson weed, both of which are deadly to humans if consumed. The *L. barbarum* plant has been consumed for over 2500 years with no toxicity being reported (Donno et al., 2015).

The *L. barbarum* plant is a perennial, woody shrub. It can grow up to 3 m high and has alternate, lanceolate leaves (Donno et al., 2015). New growth from this plant is green and tender, but as it matures, the stems become woody and produces thorns. If the plant is used in greens production, it is important to harvest the shoots before they mature to avoid woody stems and thorns. When growing *L. barbarum* for greens production, only the tender, new shoots are harvested (Crawford, 2012).

There are many uses for the fruits and leaves of *L. barbarum.* Fresh leaves can be used in food dishes such as soups and stir-fry (Crawford, 2012). The fruits are squeezed for their juice, or sold fresh, or dried (Donno et al., 2015). Not only is the *L. barbarum* plant used as a food source, but the fruits and dried leaves have been used for medicinal purposes and herbal teas in China and East Asia for many years (Dong et al., 2009). The dried fruits of *L. barbarum* have been incorporated into an herb formula that has been used as a medicine for many years (Donno et al., 2015). *L. barbarum* leaves are believed to have therapeutic benefits because they contain a high level of a flavonoid known as rutin, which has been shown to help control free radicals that are associated with causing many diseases (Dong et al., 2009). Many studies also indicate effects on many other qualities of health such as aging, fatigue, metabolism, and antioxidant intake (Donno et al., 2015). Growing *L. barbarum* greens would allow access to the health benefits and nutritional value the plants have to offer.

Little is known about the methods used to grow *L. barbarum* for greens or berries in the United States. There are no cultivars or varieties in the United States that are used for greens production. There are very few nurseries that sell *L. barbarum* plants in the United States for berry production. Many of the nurseries that do grow and sell the Goji berry do not know the specific variety or cultivar of the plant they sell. The reason for much of this confusion is due to another species known as *L. chinense*. This species is almost identical to *L. barbarum* both in anatomy and tissue structure (Donno et al., 2015). The only way to be confident of the species of the plant requires DNA analysis (Donno et al., 2015).

Goji Berry Production

There is limited available information on Goji berry production methods. However, in a personal interview with a Goji berry breeder and greens production expert from Ningxia Forestry Institute (Ningxia, China), XiongXiong Nan, common production methods for berry production in China were discussed. He stated that when produced for the berries, *L. barbarum* does not need intense pruning. The plant produces multiple berries on indeterminate shoots. In commercial production, the plants are limited to one main shoot which is allowed to grow to approximately 180 cm in height and pruned by a method known as tip-pruning. Tip pruning allows the branching shoots of the plant to reach a certain length, usually 45-60 cm in length. Once the shoots reach this length, they are pruned back by two to three nodes. This method of pruning induces branching, allowing for increased production while keeping the plant at a manageable size. Some form of a trellis system must be used to keep the plant upright. Each plant can be staked individually or a wire trellis system can be used to stabilize the plants. If grown from seed, the Goji berry plant does not normally bear fruit until the second year of growth and each year the fruit is produced on the new growth. Berries can be harvested 35-40 days after flowering, which, in the Midwest, occurs in mid to late July.

Goji Berry Greens Production

There is also extremely limited available information on Goji berry greens production. XiongXiong Nan provided detailed cultural methods for greens production in China and adaptations to production methods for the Midwest region. The Goji berry plant has an extremely vigorous growth habit that makes it a good candidate for greens production. In Ningxia, China, plants that will be used in greens production are started in a greenhouse, cut back to 5 cm in height and transplanted into the field. The normal spacing used in China for Goji greens production is 20 cm between plants, and 70 cm between rows.

XiongXiong Nan also stated that Goji berry plants that are grown for greens must be maintained as a small, low growing shrub. The new shoots of the plant are pruned every four to six days to maintain this growing habit, and to harvest the Goji berry greens. Harvesting for Goji berry greens targets tender, herbaceous, new shoot growth. When the Goji berry shoot begins to lignify, it is no longer suitable for greens production. The *L. barbarum* plant produces thorns as it lignifies which is also unsuitable for greens production. The Goji berry plants are pruned throughout the growing season. If production slows, or after the plants enter dormancy, the Goji berry plants are cut back to 5 cm in height.

Plants that are used for Goji berry greens production can live for three years under the correct management methods. Annual fertilization can help increase vegetative production of Goji berry greens, and according to XiongXiong Nan, fertilization studies are currently being conducted in Ningxia, China. The plants also need supplemental irrigation during dry periods, as greens production could slow down or stop completely during drought stress. For cold stress, the plants generally overwinter with few problems, but new growth is susceptible to frost and cold damage. However, XiongXiong also stated that because of their vigorous growth habits, plants are not negatively affected if cold damage occurs.

Lignification is a physiological process that reinforces the new herbaceous growth of a plant. The process of lignification emerged in higher plants around 430 million years ago, which allowed these plants to grow upright in terrestrial habitats (Boudet, 1999). Lignin is incorporated into the cell walls of new growth which, in turn, increases the structural rigidity and durability of the plant cells (Lee et al., 2007). Lignification is a process that is subjected to modulation at different times during the normal growth of a plant, as well as in response to different environmental stress factors, such as pathogens, and UV radiation (Boudet, 1999). However, lignification of new shoots is undesirable in greens production because lignified shoots are inedible. Knowing what triggers lignification can help producers avoid this process, and possibly increase greens production.

In a study conducted by Lee et al. (2007), white clover was subjected to water-deficit stress to observe its effects on lignification and peroxidases, the enzymes most involved in lignification biosynthesis. White clover was transplanted into pots, which were watered to field capacity for the first two weeks after transplanting, then the daily irrigation was split into two treatments, 50 ml of water per pot for the control, and 5 ml for the water-deficit treatment (Lee et al., 2007). An increase in lignin production was observed after day 14 in the water-deficit treated clover. This led to the conclusion that water-deficit stress increases the production of lignin, and therefore can be considered a trigger of lignification (Lee et al., 2007). Lignification production can also be triggered by a plant's response to disease, such as around wounds infected with Tobacco Necrosis Virus (Conti et al., 1990).

Air Root Pruning

Standard potting containers often produce kinked or deflected roots (root binding) which lead to restricted growth (Marshall and Gilman, 1998). A technique known as air root pruning have been shown to prevent root-binding. Air root pruning is accomplished using a special pot designed with slits, or holes on the sides and/or bottom that allow the roots to be exposed to air (Whitcomb, 1982). When the roots are exposed to air without adequate humidity, the root tips die. The death of the primary root tips promotes the branching of secondary roots and also reduces root binding (Huang and Liang, 1988). This technique helps increase the surface area, total length, and number of root tips in the root systems by promoting secondary branching.

Air root pruning has led to increased transplant establishment for papaya (*Carica papaya*) seedlings, higher crop quality, increased average leaf number, and increased yield (Huang and Ai, 1992). The accelerated establishment of transplants into the field was due to the increased number of root tips that form when air root pruning is implemented (Whitcomb, 1982). Air root pruning has been shown to increase transplanting success in the field in water stress conditions. Marshall and Gilman (1998) found that after red maple (*Acer rubrum*) had been grown in air root pruning pots, it exhibited less water stress when transplanted into the field after 12 weeks of infrequent irrigation. The low stress levels were thought to have occurred due to the difference in root growth that was experienced in the different containers (Marshall and Gilman, 1998). Also, Huang and Liang (1988) reported significantly higher number of leaves produced in glossy privet (*Ligustrum lucidum*) and papaya (*C. papaya*) plants compared to the non-air root pruned plants. Increased leaf production, increased plant vigor after transplant, and reduced water stress are all desired when growing *L. barbarum* for greens.

Phytohormones, or plant hormones, hold an important role in shaping the architecture of a plant in response to variable environmental conditions (Růžička et al., 2009). Pruning treatments affect the growth habit and shape of the plants in many ways. There is the immediate effect of physically removing branches, stems, leaves, or roots, but this action also triggers physiological responses from the plant. Pruning stimulates the hormonal responses that signal a plant to regenerate the missing vegetation. These responses are believed to have evolved from the plant's need to stay alive and reproduce even when damaged or consumed by the local fauna. Auxin and cytokinin are the two main hormones involved in the responses associated with vegetation regeneration. Together these hormones regulate root and shoot regeneration after pruning, environmental damage, or a foraging event.

Auxin and cytokinin are controlled mainly by a plant's root and shoot meristem. The removal of these meristems illicit a response that induces secondary branching in the roots and shoots (Müller and Leyser, 2011). Auxin and cytokinin work together to regulate the primary and secondary growth of a plant. Auxin promotes the development of primary and lateral (secondary roots), as well as many other plant organs. Cytokinin regulates the development of these plant hormones by controlling the growth of the primary and lateral roots (Růžička et al., 2009). Specifically, cytokinin inhibits the growth of lateral roots by modulating several auxin carriers which regulate the cell-to-cell transport of auxin (Růžička et al., 2009). This process ensures that the primary root growth is promoted more than the lateral root growth. However, the root apical meristem is known to be the site of cytokinin synthesis, and when this meristem is pruned, cytokinin is no longer produced by that root apical meristem (Aloni et al. 2006). This allows the growth of the secondary roots to continue because the roots are no longer suppressed by cytokinin.

Air root pruning induces a hormonal response where the synthesis of cytokinin by the root apical meristem is suppressed, allowing secondary branching, regulated by auxin, to occur. This secondary branching has been shown to have a positive effect on transplant success, survivability, and increased vegetative production.

Hypothesis

I hypothesize that if I measure the fresh weight, dry weight, length, surface area, average diameter and number of root tip in the of root systems of Goji berry plants treated with and without air root pruning, these measures of the root systems will be found to be greater with air root pruning. I hypothesize that *L. barbarum* can survive and produce under the severe pruning method that is required when producing Goji berry greens. The purpose of this research is to examine the potential of greens production with different cultivars of *L. barbarum*; 'Big Lifeberry', 'Vermillion Sunset' and 'Sweet Lifeberry', grown for berries in the United States, as well as whether a method known as air root pruning helps to increase transplant success and early season growth of the three formerly mentioned cultivars.

MATERIALS AND METHODS

Root Pruning Study

Goji berry plants (*L. barbarum*) were purchased from Stark Bros Nursery in Louisiana, Missouri on September 20th, 2017. Forty of each cultivar 'Big Lifeberry', 'Sweet Lifeberry', and 'Vermillion Sunset' were used in the root pruning experiment. Plants were acclimated to greenhouse conditions (Karls Hall, Missouri State University campus, Springfield, Missouri) for one month. On October 31st, 2018, twenty plants from each cultivar were potted in 3.8 L Rootmaker™ root pruning pots (Lacebark, Inc., Stillwater, Oklahoma), and 20 from each cultivar were potted in standard, 3.8 L Gro Pro™ (Hawthorne Gardening Company, Vancouver, Washington) non-air root pruning pots. Sun Gro™ Professional Growing Mix (Sun Gro Horticulture, Agawam, Massachusetts) was used for transplanting media. Three grams of Osmocote (The Scotts Company LLC, Marysville, Ohio) 14-14-14, a slow-release granular fertilizer, was top-dressed in each pot after transplanting.

While in the greenhouse, the plants were watered as needed, every one to three days. Once a week, during active growth, Nature's Source (Ball DFP LLC, Sherman, Texas) 10-4-3 professional plant food liquid fertilizer was applied using a Dosatron D14MZ2 (Dosatron International, Inc, Clearwater, Florida) at a rate of 14 g/min and an injection rate of 1:500. The *L. barbarum* plants were sprayed with Pyrethrin (Bonide Products Inc., Oriskany, New York) a rate of 0.5 oz/ gal to control aphids on November 21st and 28th, and December 02nd, 19th, and 21st of 2017. The plants were sprayed with Azatin XL (OHP Inc., Bluffton, South Carolina) at a rate of 2.4 oz/15 gal for aphid control on October 18th and November 5th of 2017. They were sprayed with EnstarAQ (Wellmark International, Schaumburg, Illinois) at a rate of 16 oz/50 gal for aphid control on November 29th of 2017, and were sprayed with Phyton 35 Fungicide (Phyton Corporation, Bloomington, Minnesota) at a rate of 2 oz/15 gal for powdery mildew on November 1st of 2017. The plants were sprayed with 3336 Fungicide at a rate of 2 oz/gal for powdery mildew on February 8th and 28th of 2018, and a Pyrethrin TR (BASF Corporation, Research Triangle Park, North Carolina) fogger (containing 2 oz of insecticide) was used for aphid control on March 11th, 2018. Safer Insecticidal Soap (Woodstream Coropration, Ltitz, Pennsylvania) was used to control spidermites at 0.6 oz/0.5 gal on April 26th. On May 22th, 2018, four air root pruned plants and four non-air root pruned plants from each cultivar (24 plants total) were randomly selected and set aside for the root pruning harvest. The remaining 96 plants were pruned back to 10 cm above the crown in preparation for transplanting into the field.

Four air root pruned and four non-air root pruned plants from each cultivar were harvested for root measurements from June 7 through June 19, 2018. Each cultivar was washed and scanned within 24 hours of starting the washing process. During harvest, shoots were removed from the root system and the roots were soaked in tap water until the potting mix loosened. The root system was then cleaned in four separate tap water baths. Plants were rolled in the first wash until the majority of the pearlite was separated, collected, and removed. Plants were moved to the second wash and agitated to release the remaining pearlite and loosen the rootball. The third wash was used to further clean the root systems and separate the rootball, then the rootball was moved to the final wash to collect small root fragments and remove any remaining debris. Clean rootballs were blotted with paper towels and excess moisture was air dried from the root system before fresh weight was measured. Rootballs were then positioned next to a meter stick, photographed, and the length of the intact root segments were recorded. The rootballs were then placed in a tray of water to preserve them until they were scanned.

The cleaned rootballs were scanned using a Regent scanner (Regent Instruments Inc., Quebec City, Canada) and analyzed using WhinRhizo™ software (Regent Instruments Inc., Quebec City, Canada) (Figure 1). Each rootball was clipped and separated into large continuous fragments in transparent trays filled with water. The roots were teased with a small brush so that the roots overlapped as little as possible. A scan of each tray was taken in both greyscale and color (Figure 1). The roots were then removed from the tray and placed in a labelled paper bag and dried in a forced air oven at 46 ˚C. After all moisture was removed from the roots, dry weights were recorded, and the root samples were stored in boxes. After all scans had been collected, WinRhizo™ software was used to measure total root length, root surface area, average root diameter, total root volume, and number of root tips (Figure 1).

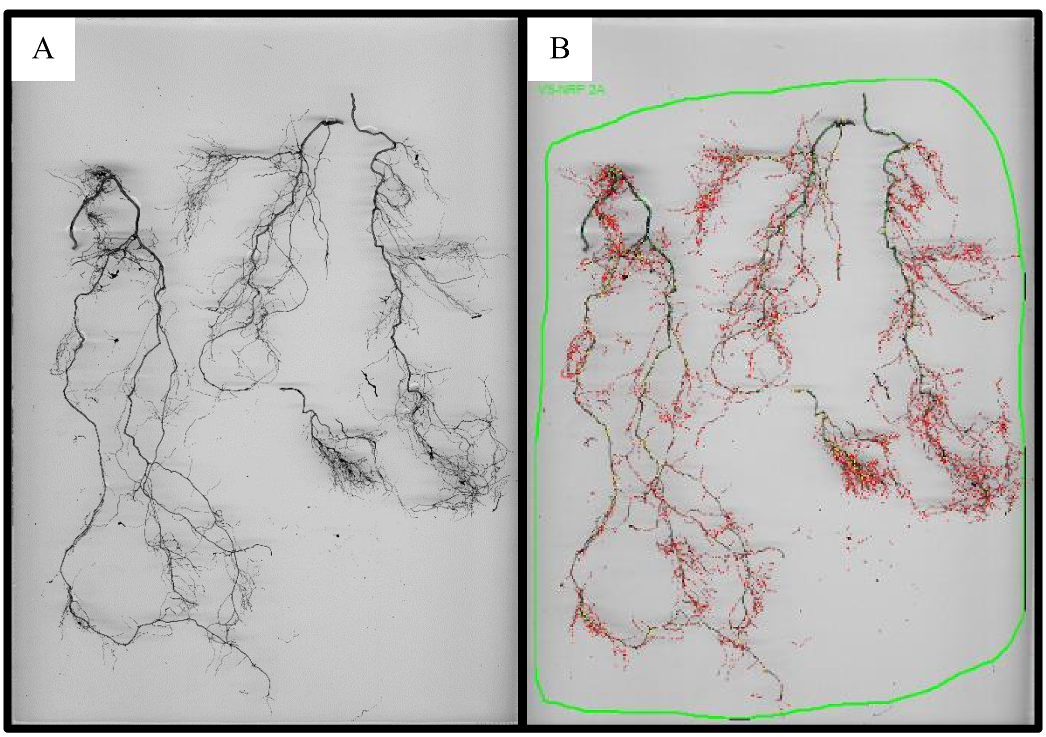

Figure 1. Greyscale scan of partially separated rootball (A). WinRhizo™ analysis of partially separated rootball (B).

Goji Berry Greens Study

The field site was located at the Darr Agriculture Center in Springfield, MO on a Newtonia silt loam, 1 to 3% slope with a pHs of 5.8, 2.4% organic matter, 96 kg/ha of phosphorous, 300 kg/ha of potassium, 2087 kg/ha of calcium, and 212 kg/ha of magnesium. The soil had a neutralizable acidity of 2.2 meq/100g and a cation exchange capacity of 9.6 meq/100g (University of Missouri Soil Testing Lab, Columbia, Missouri). A 26 m by 3 m plot was tilled twice to prepare the ground. On May 23th, 2018, the soil was mounded to 0.2 m in height, creating a 0.6 m wide, 26 m long row. Osmocote slow-release fertilizer (14-14-14) was spread over the row totaling a rate of 80 kg/ha. Drip tape, with 20 cm spaced emitters and a flow rate of 0.95 L/hour, was centered on the mound. Five oz woven weed barrier, 1.22 m wide, was rolled and cut to the 26 m length. One edge of the weed barrier was buried before transplanting.

Ninety-six *L. barbarum* plants were laid out in plots, each plot being a group of four plants of the same cultivar and treatment type, according to a complete randomized design plot map (Figure 2). The design included three cultivars, two treatments, and four replications, totaling 24 plots. Each plot was spaced 40 cm apart from one another. The four plants within each plot were spaced 20 cm from one another.

For each plot, four plants were placed in a trench with half the rate of Osmocote. Drip tape emitters were lined up with plant bases and the trench was backfilled with soil. The remaining Osmocote was top-dressed. The weed barrier was cut so that it fit around the plants and it was then secured with metal stakes. The remaining edge of the weed barrier was buried, leaving a 0.6 m width of fabric exposed (Figure 3). Rainfall was measured with a range gauge placed at the south end of the plots, and plants were irrigated between rainfall events to maintain soil moisture (Table 1).

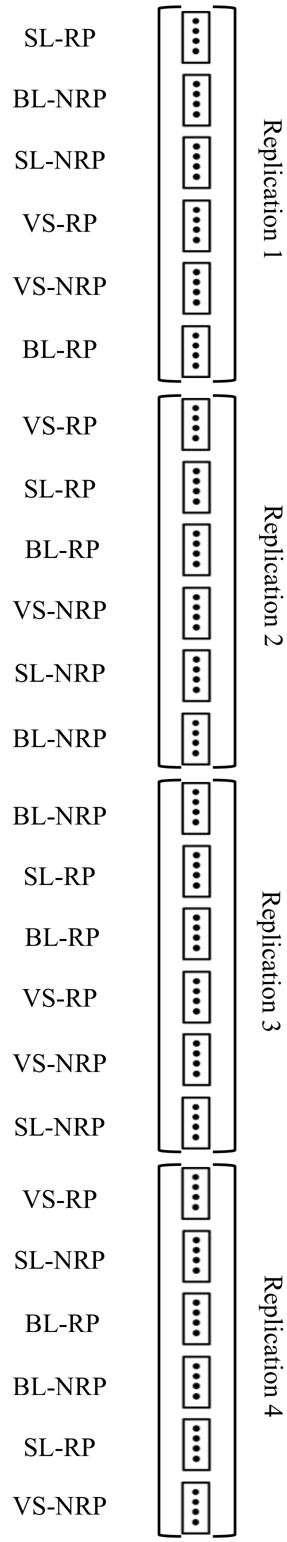

Figure 2. Field site plot map (BL = 'Big Lifeberry', SL = 'Sweet Lifeberry', VS = 'Vermillion Sunset', NRP = non-root pruned, RP = root pruned).

Figure 3. Goji berry field site after planting.

When the majority of the new, tender shoots reached a suitable length (at least 4 cm), the greens from each plant were harvested. Harvests were conducted on June 11th, June 20th, July 2nd, July 13th, August 13th, August 22nd, August 31st, September 10th, September 19th, September 26th, and October 10th. At each harvest, a greens shoot was separated at the point where stem lignification was apparent. To identify the point of lignification, new shoots were pushed toward the ground until the stem no longer bent easily. The new shoot was harvested at the node above this point (Figure 4).

Table 1. Rainfall (R) and Irrigation (I) rates over the 2018 growing season.

Date	Rainfall(R)/Irrigation(I) (cm)	Date	Rainfall(R)/Irrigation(I) (cm)
May 23rd	3.15 (I)	August 15th	1.27 (R)
June 1st	3.15 (I)	August 16th	0.51 (R)
June 4th	3.15 (I)	August 20th	0.25 (R)
June 10th	3.15 (I)	August 21st	0.25 (R)
June 12th	1.27 (R)	August 23rd	0.64 (R)
June 14th	4.45 (R)	August 29th	6.32 (I)
June 17th	3.15 (I)	August 29th	0.25 (R)
June 20th	2.03 (R)	August 30th	6.35 (R)
June 23rd	3.15 (I)	September 7th	0.76 (R)
June 24th	1.91 (R)	September 9th	2.03 (R)
June 29th	3.15 (I)	September 17th	3.15 (I)
July 4th	3.15 (I)	September 22nd	1.52 (R)
July 8th	3.15 (I)	September 26th	0.76 (R)
July 11th	3.15 (I)	October 7th	2.54 (R)
July 16th	3.15 (I)	October 10th	1.27 (R)
July 17th	1.91 (R)	October 12th	3.81 (R)
July 19th	0.64 (R)	October 13th	0.25 (R)
July 24th	3.15 (I)	October 14th	0.38 (R)
July 26th	3.15 (I)	October 15th	1.27 (R)
July 30th	3.15 (I)	October 20th	0.64 (R)
August 3rd	3.15 (I)	October 26th	1.27 (R)
August 6th	3.15 (I)	October 31st	1.02 (R)
August 7th	0.20 (R)	November 1st	0.25 (R)
August 8th	1.52 (R)	November 2nd	2.03 (R)
August 10th	6.99 (R)	November 3rd	1.78 (R)
August 13th	0.76 (R)	November 4th	0.25 (R)
August 14th	0.25 (R)		

For each plant, the harvested greens were placed in labelled paper bags until fresh weight was collected. The greens were removed from the paper bag and fresh weight was recorded at the field site. Each shoot was measured to the nearest 0.25 inch in length (later converted to cm), and total number of shoots were recorded for each plant. Shoots were then placed back in the paper bags and placed in a forced air oven to dry at 46 ˚C. Once fully dried, shoot dry weights were measured and samples were boxed and placed in storage.

Figure 4. Example of identifying where to clip new shoots for greens harvest.

Data and Statistics

Data that were collected per plant included, root fresh weight, root dry weight, total root length, root surface area, average root diameter, total root volume, number of root tips, new shoot fresh weight, new shoot dry weight, total number of shoots, and average shoot length. The experiment was considered a complete randomized design. This model was used to test for statistical significance of root pruning or cultivar using a general linear model (PROC GLM) in SAS v. 9.4 (SAS Institute Inc., Cary, North Carolina). Main plot effects were root pruning and cultivar. Root pruning experiment data were analyzed using a one-way ANOVA within cultivar only. Greens experiment data were analyzed using a two-way ANOVA between treatment and cultivar within and across harvests. Effects and interactions were considered significant when means differed at $p<0.05$. Tukey's pairwise comparison was used to separate means.

RESULTS

Root Pruning

At harvest, rootballs of plants grown in standard pots had more visible roots at the base of the pot than the rootballs of plants grown in root pruned pots (Figure 5). However, compared to plants grown in standard pots, root pruning pots had no effect on root fresh weight, dry weight or any of the measurements obtained from the WinRhizo™ analysis in ‘Big Lifeberry’ and ‘Vermillion Sunset’ (Table 2). For ‘Sweet Lifeberry’, plants grown in standard pots were greater in root fresh weight and average root diameter than plants in root pruned pots, however no other differences were found (Table 2). In the field, differences in greens production between non-air root pruned plants and root pruned plants only occurred in the first harvest (June 11, 2018). ‘Vermillion Sunset’ was the only cultivar exhibiting differences in greens production, with non-air root pruned plants producing greater new shoot fresh and dry weights than root pruned plants (Figure 6). ‘Big Lifeberry’ and ‘Sweet Lifeberry’ showed no statistical difference in greens production between root pruned and non-air root pruned plants in the first harvest (Figure 7).

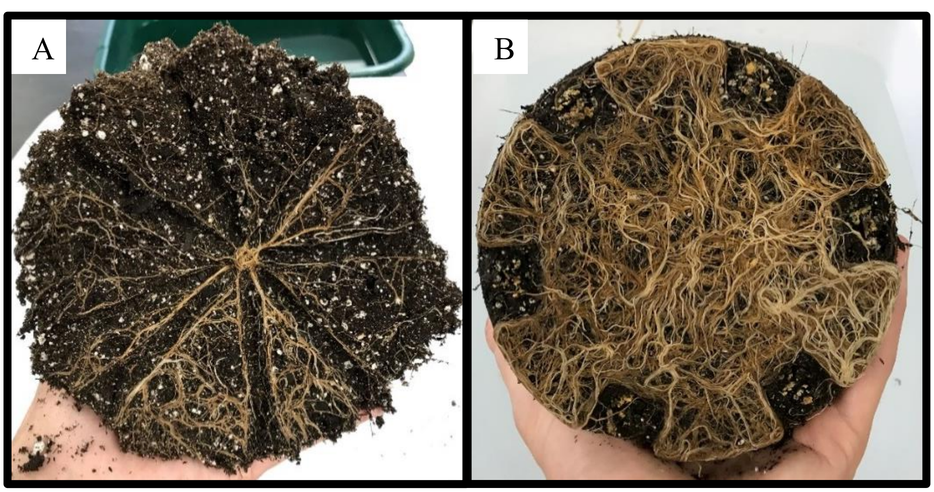

Figure 5: ‘Big Lifeberry’ rootball base after growing in air root pruning pot (A) and after growing in non-air root pruning pot (B).

Table 2. Root system characteristics of three Goji berry cultivars grown in root pruning pots and standard pots.

Cultivar	Treatment	Fresh Weight (g/plant)	Dry Weight (g/plant)	Length (cm/plant)	Root Surface Area (cm^2/plant)	Average Diameter (cm^2/plant)	Root Volume (cm^3/plant)	Root Tips (tips/plant)
Big Lifeberry	RP	49.99±5.09	11.60±1.13	9047.10±1171.34	397.76±51.66	1.86±0.30	13.84±1.87	42092±4882
	NRP	65.56±7.42	14.37±1.29	9806.66±432.61	414.72±12.66	2.42±0.20	13.86±0.39	40113±4376
Vermillion Sunset	RP	41.55±3.35	7.94±0.86	7378.13±2082.03	213.64±58.29	1.27±0.18	4.91±1.31	37785±12279
	NRP	32.79±1.73	6.85±0.59	7212.62±1471.29	219.03±49.09	1.21±0.10	5.31±1.28	25460±5555
Sweet Lifeberry	RP	24.90±1.25b	6.06±0.46	4047.65±598.26	183.63±23.52	1.14±0.13b	6.64±0.86	23061±4715
	NRP	36.47±3.98a	6.96±0.93	4481.52±355.10	199.30±10.57	1.68±0.08a	7.05±0.30	28740±1083
ANOVA F Statistic (and p value)								
Big Lifeberry		2.99 (0.1343)	2.62 (0.1564)	0.37 (0.5653)	0.10 (0.7606)	2.37 (0.1746)	0.00 (0.9929)	0.09 (0.7730)
Vermillion Sunset	df 7	5.39 (0.0593)	1.09 (0.3369)	0.00 (0.9503)	0.01 (0.9458)	0.07 (0.7990)	0.05 (0.8371)	0.84 (0.3957)
Sweet Lifeberry		7.69 (0.0323)	0.75 (0.4183)	0.39 (0.5558)	0.37 (0.5655)	12.2 (0.0130)	0.20 (0.6274)	1.38 (0.2848)

Column values are means ± SE, n=4. Within cultivar, column means not followed by the same letter are statistically different ($p<0.05$, Tukey's Pairwise Comparisons).

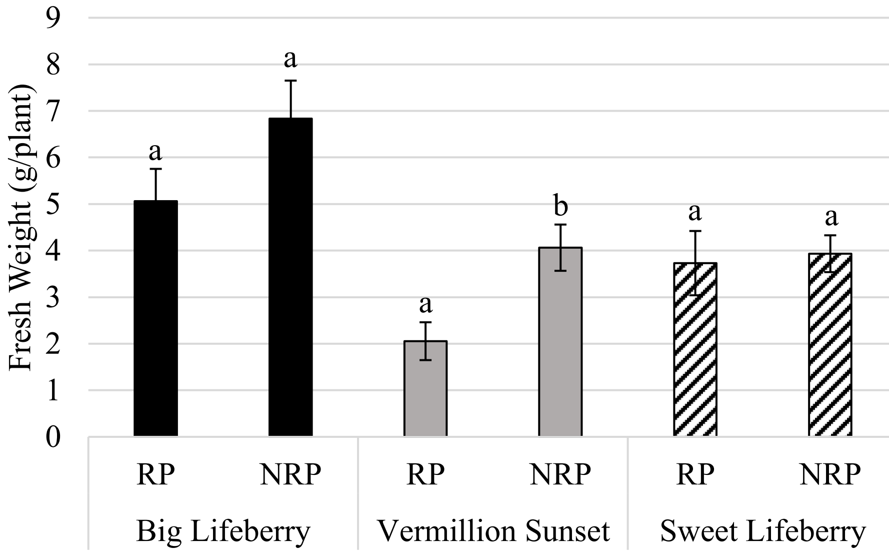

Figure 6. Fresh weight per plant production by treatment over growing season. Cultivar means ± SE, n=16. Within cultivar, values not followed by the same letter are significantly different (p< 0.05, Tukey's pairwise comparisons).

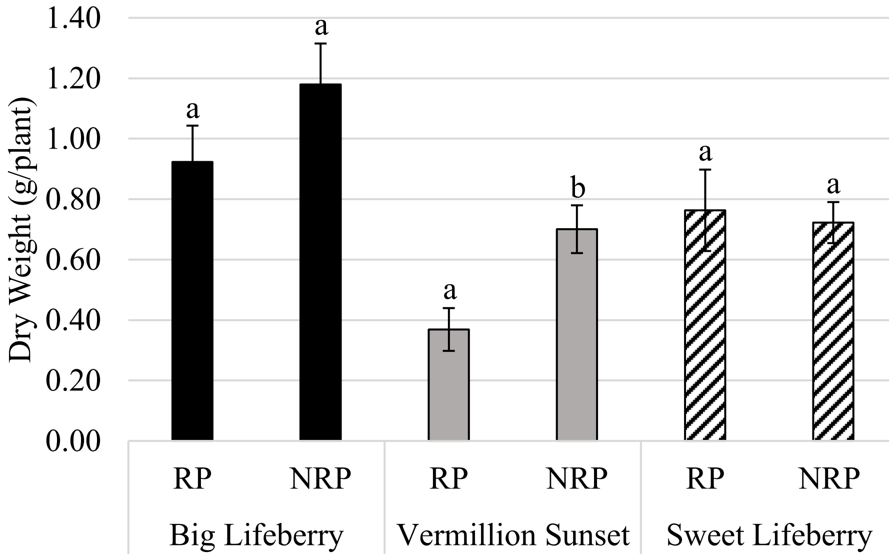

Figure 7. Dry weight per plant production by treatment over growing season. Cultivar means ± SE, n=32. Within cultivar, values not followed by the same letter are significantly different (p< 0.05, Tukey's pairwise comparisons).

Goji Berry Greens Production

All three cultivars, 'Big Lifeberry', 'Vermillion Sunset', and 'Sweet Lifeberry', produced greens at first three harvest on June 11th, 2018. However, during the harvests conducted from July 13th to August 13th, the regrowth, exhibited by fresh weight produced by all plants, was extremely slow contributing to a "summer slump" (Figure 8). However, the plants rebounded and produced the greatest amount throughout the rest of the growing season. From the harvest conducted on July 13th, to the harvest conducted on September 10th, 'Big Lifeberry' fresh weight production increased four-fold, 'Vermillion Sunset' fresh weight increased three-fold, and 'Sweet Lifeberry' increased two-fold (Figure 8). From July 13th to September 10th, 'Big Lifeberry' dry weight production increased three-fold and 'Vermillion' Sunset' and 'Sweet Lifeberry' increased two-fold. 'Big Lifeberry and 'Sweet Lifeberry' maintained a consistent increase in fresh weight (Figure 8), dry weight (Figure 9), number of shoots (Figure 10), total shoot length (Figure 11), and average shoot length (Figure 12) across this timeframe as well. This was not the case for 'Vermillion Sunset', as the amount of fresh weight and dry weight fluctuated from harvest to harvest (Figures 8 and 9). 'Big Lifeberry' and 'Sweet Lifeberry' also maintained a consistent decrease in fresh weight, dry weight, number of shoots, total shoot length, and average shoot length production at the end of the growing season on September 19th, September 26th, and October 8th (Figure 8).

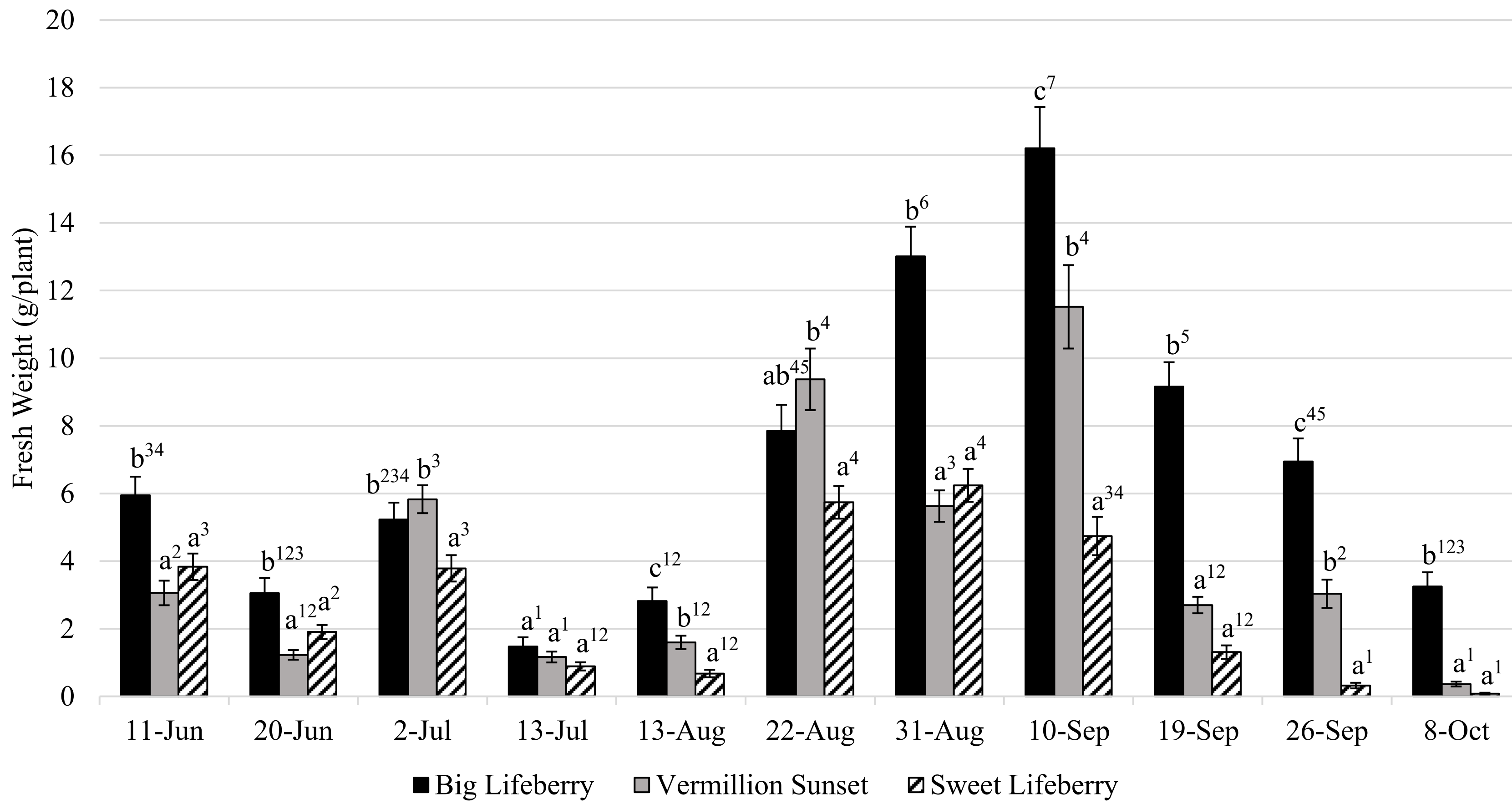

Figure 8. Fresh weight per plant production over growing season. Values are cultivar means ± SE, n=32. Within harvest, between cultivars, values not followed by the same letter (a, b, c, …) are significantly different ($p < 0.05$, Tukey's pairwise comparisons). Within cultivar, between harvests, values not followed by the same superscript number ($^{1, 2, 3, \ldots}$) are significantly different ($p<0.05$, Tukey's pairwise comparisons).

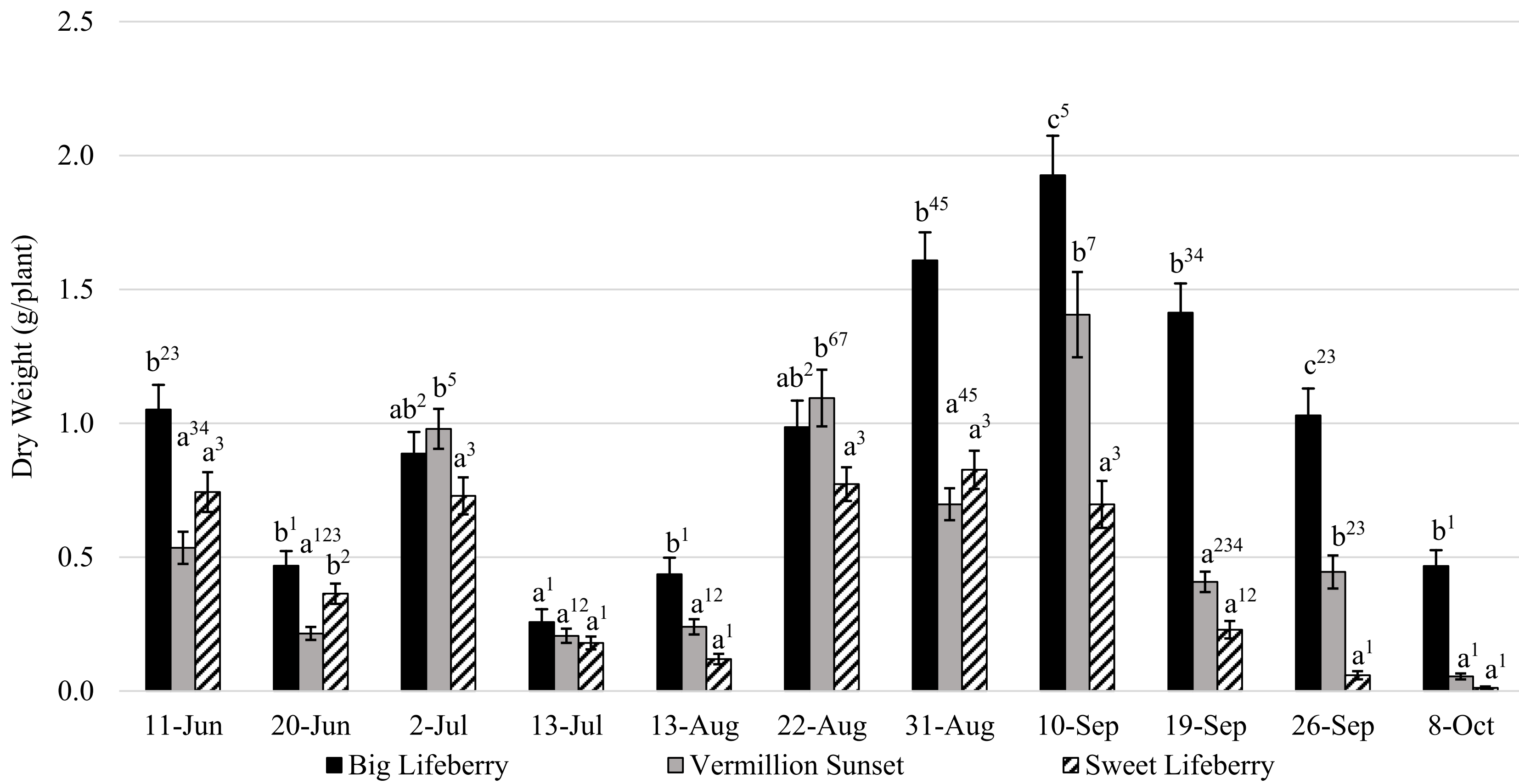

Figure 9. Dry weight per plant production over growing season. Values are cultivar means $\pm$ SE, n=32. Within harvest, between cultivars, values not followed by the same letter (a, b, c, …) are significantly different (p< 0.05, Tukey's pairwise comparisons). Within cultivar, between harvests, values not followed by the same superscript number ($^{1, 2, 3, \ldots}$) are significantly different (p<0.05, Tukey's pairwise comparisons).

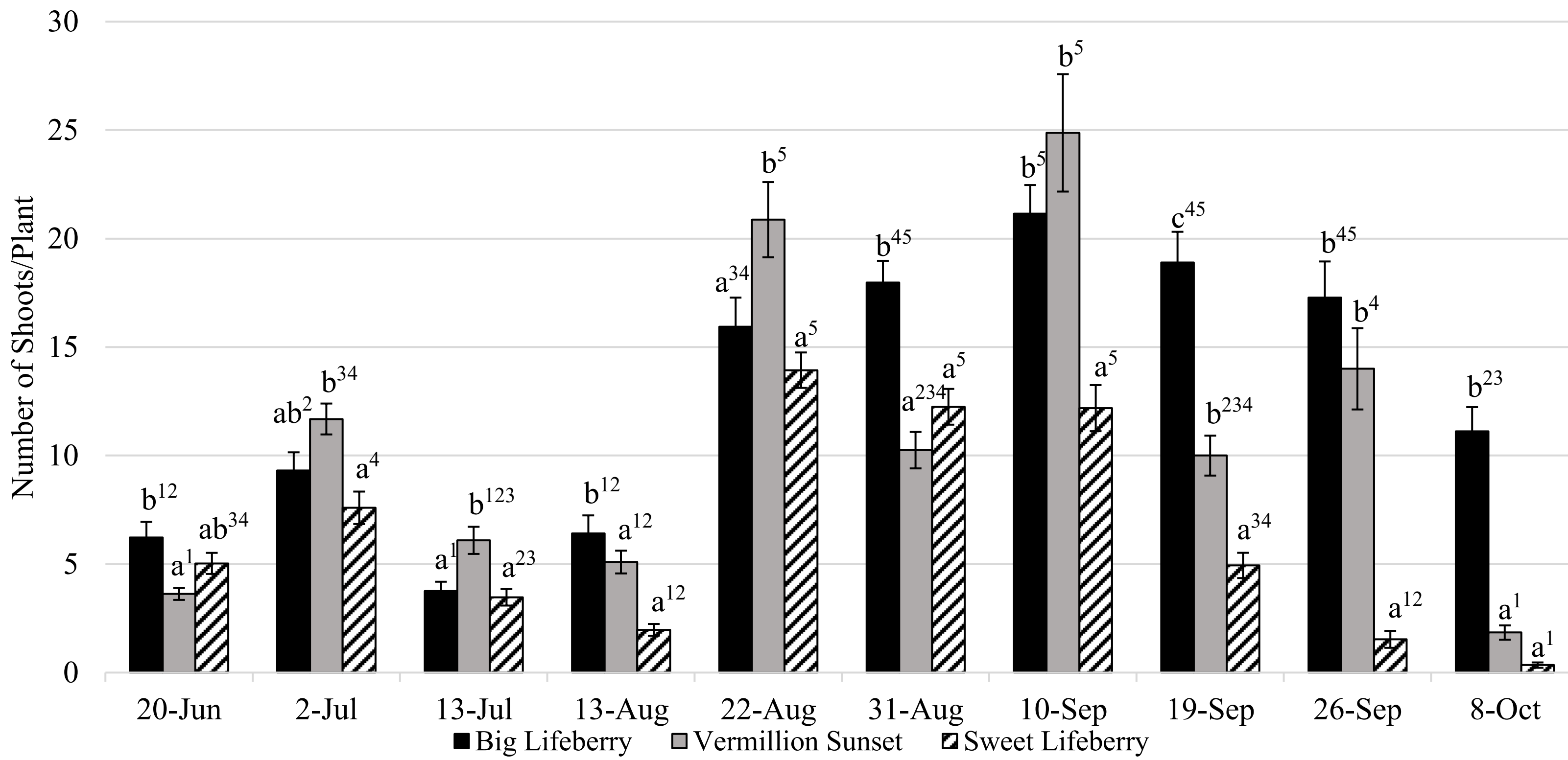

Figure 10. Number of shoots per plant produced over growing season. Values are cultivar means ± SE, n=32. Within harvest, between cultivars, values not followed by the same letter (a, b, c, …) are significantly different ($p < 0.05$, Tukey's pairwise comparisons). Within cultivar, between harvests, values not followed by the same superscript number ($^{1, 2, 3, \cdots}$) are significantly different ($p<0.05$, Tukey's pairwise comparisons).

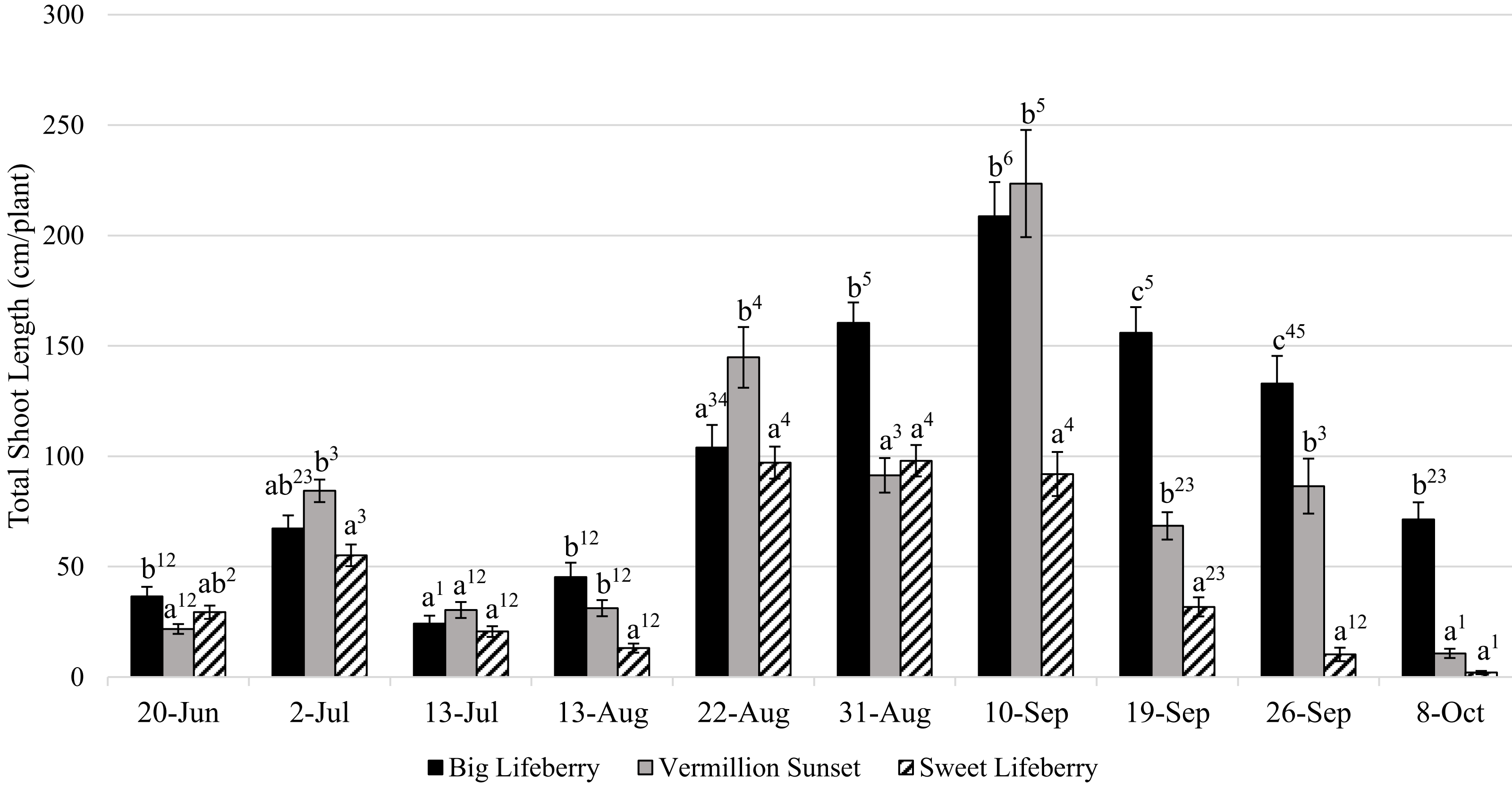

Figure 11. Total shoot length per plant produced over growing season. Values are cultivar means ± SE, n=32. Within harvest, between cultivars, values not followed by the same letter (a, b, c, …) are significantly different ($p < 0.05$, Tukey's pairwise comparisons). Within cultivar, between harvests, values not followed by the same superscript number ($^{1, 2, 3, \ldots}$) are significantly different ($p<0.05$, Tukey's pairwise comparisons).

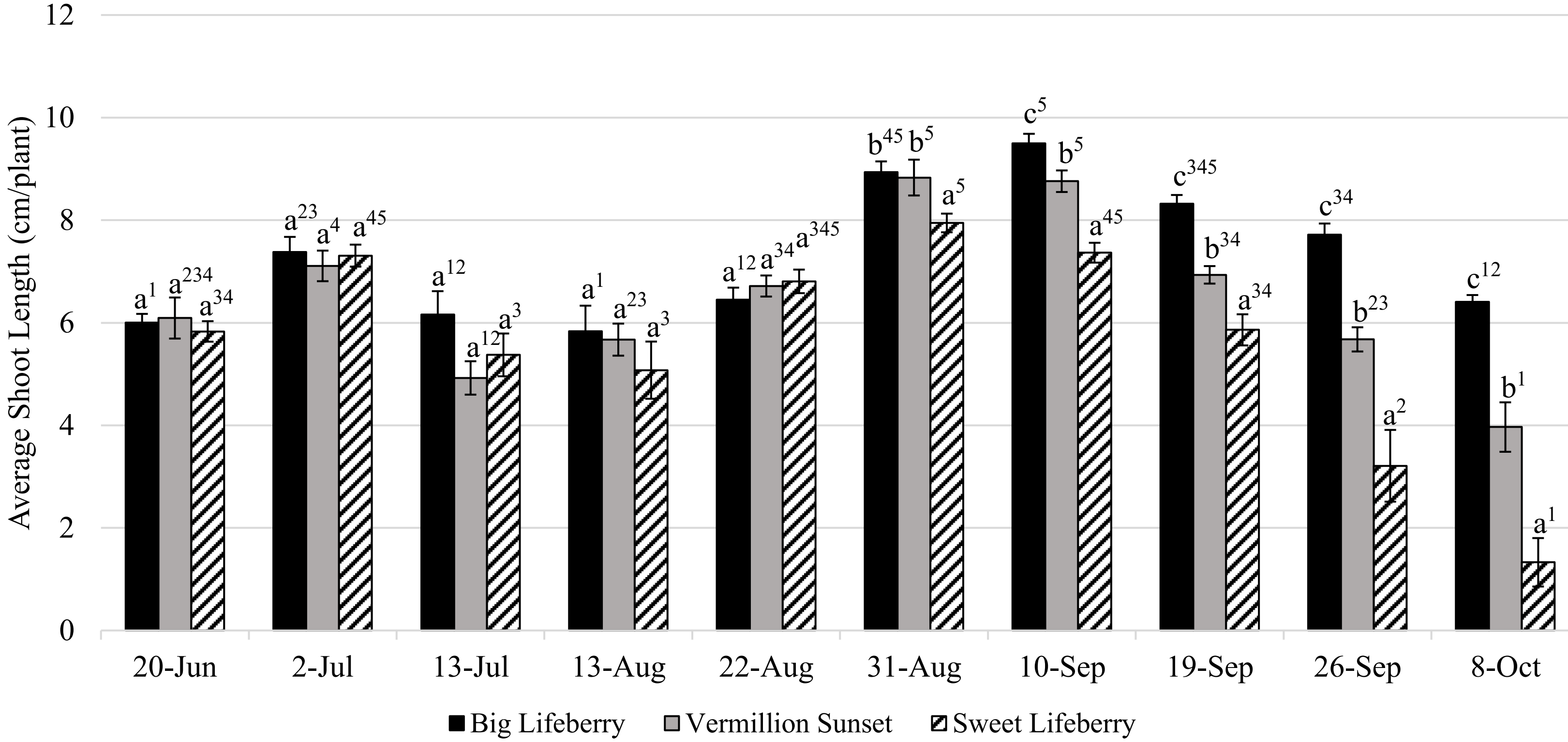

Figure 12. Average shoot length per plant production over growing season. Values are cultivar means ± SE, n=32. Within harvest, between cultivars, values not followed by the same letter (a, b, c, …) are significantly different ($p< 0.05$, Tukey's pairwise comparisons). Within cultivar, between harvests, values not followed by the same superscript number ($^{1, 2, 3, \cdots}$) are significantly different ($p<0.05$, Tukey's pairwise comparisons).

'Big Lifeberry' produced the highest amounts of fresh weight per plant compared to 'Vermillion Sunset' and 'Sweet Lifeberry' within a majority of the harvests including June 11th, June 20th, August 13th, August 31st, September 10th, September 19th, September 26th, and October 8th of 2018 (Figure 8). In general, 'Vermillion Sunset' produced greater fresh weight than 'Sweet Lifeberry' over the growing season, including harvests conducted on July 2nd, August 13th, August 22nd, September 10, and September 26, 2018 (Figure 8). 'Big Lifeberry' produced a significantly higher total fresh weight per plant when compared to 'Vermillion Sunset' and 'Sweet Lifeberry' over all harvests conducted in 2018 (Figure 13). These differences in fresh weight production contributed to 'Big Lifeberry' producing 49% more fresh weight than 'Vermillion Sunset', and 87% more fresh weight than 'Sweet Lifeberry' over the entire growing season (Figure 13). Also, 'Vermillion Sunset' produced 43% more fresh weight than 'Sweet Lifeberry' over the entire growing season (Figure 13).

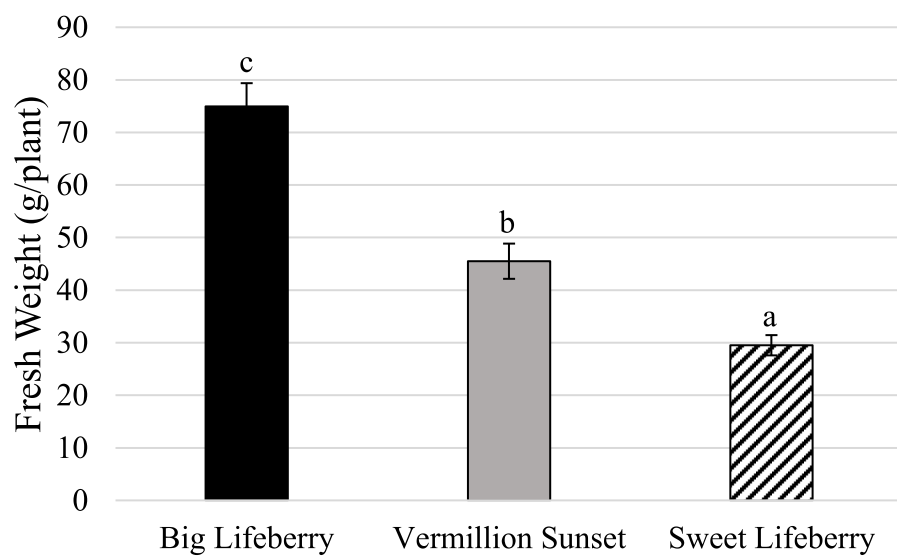

Figure 13. Total greens fresh weight production over growing season. Cultivar means $\pm$ SE, n=32. Within cultivars, values not followed by the same letter are significantly different ($p < 0.05$, Tukey's pairwise comparisons).

'Big Lifeberry' produced the greatest amounts of dry weight per plant compared to 'Vermillion Sunset' and 'Sweet Lifeberry' during the harvests conducted on June 11th, August 13th, August 31st, September 10th, September 19th, September 26th, and October 8th of 2018 (Figure 9). In general, 'Vermillion Sunset' produced greater dry weight than 'Sweet Lifeberry' over the growing season within the harvests conducted on July 2nd, August 22nd, September 10th, and September 26th of 2018 (Figure 9).

'Big Lifeberry' also produced a significantly higher total dry weight per plant when compared to 'Vermillion Sunset' and 'Sweet Lifeberry' over all harvests conducted in 2018 (Figure 14). However, 'Vermillion Sunset' and 'Sweet Lifeberry did not produce significantly different amounts of dry weight per plant over all harvests conducted in 2018 (Figure 14). 'Big Lifeberry produced 51% more dry weight than 'Vermillion Sunset', and 76% more dry weight than 'Sweet Lifeberry' over the entire growing season (Figure 14). 'Vermillion Sunset' also produced 28% more dry weight than 'Sweet Lifeberry' over the entire growing season (Figure 14).

'Big Lifeberry' produced a higher number of shoots per plant compared to 'Vermillion Sunset and 'Sweet Lifeberry' within the harvests conducted on August 13th, August 31st, September 19th, and October 8th of 2018 (Figure 10). While 'Vermillion Sunset' produced a significantly higher number of shoots per plant compared to 'Big Lifeberry' and 'Sweet Lifeberry', within the harvests conducted on July 13th, and August 22nd of 2018 (Figure 10).

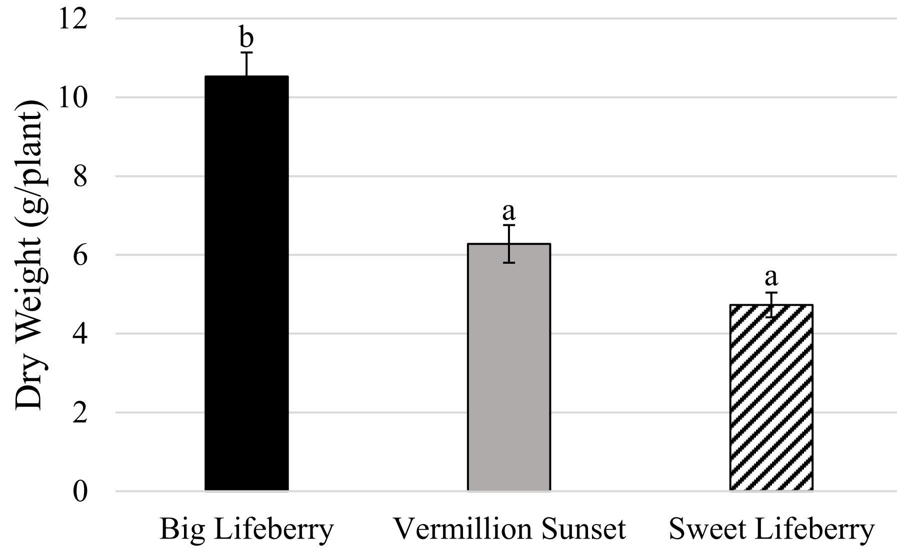

Figure 14. Total greens dry weight per plant production over growing season. Cultivar means ± SE, n=32. Within cultivars, values not followed by the same letter are significantly different ($p < 0.05$, Tukey's pairwise comparisons).

'Big Lifeberry' produced the highest total shoot length compared to 'Vermillion Sunset' and 'Sweet Lifeberry' within the harvests conducted on August 31st, September 19th, September 26th, and October 8th of 2018 (Figure 11). However, within the harvest conducted on August 22nd, 2018, 'Vermillion Sunset' produced a significantly higher total shoot length per plant when compared to 'Big Lifeberry' and 'Sweet Lifeberry' (Figure 11). 'Vermillion Sunset' also produced a significantly higher total shoot length per plant than 'Sweet Lifeberry', within the harvests that took place on August 13th, September 19th, and September 26th of 2018 (Figure 11). There was a four-fold increase of total shoot length produced by 'Big Lifeberry' and 'Vermillion Sunset' from the lowest harvest, July 13th, 2018, to the most productive harvest, September 10th, 2018. 'Sweet Lifeberry' only increased two-fold between these same harvests (Figure 11).

There was no significant difference in average shoot length per plant between 'Big Lifeberry', 'Vermillion Sunset', and 'Sweet Lifeberry within the harvests conducted on 'June 20th, July 2nd, July 13th, August 13th, and August 22nd of 2018 (Figure 12). 'Big Lifeberry produced the highest average shoot length within the harvests conducted on September 10th, September 19th, September 26th, and October 8th of 2018 (Figure 12). During these same harvests, 'Vermillion Sunset' produced a higher average shoot length than 'Sweet Lifeberry' (Figure 12). However, 'Big Lifeberry' and 'Vermillion Sunset' were not significantly different in average shoot length per plant but, produced a significantly higher average shoot length when compared to 'Sweet Lifeberry' within the harvest conducted on August 31st, 2018 (Figure 12). Average shoot length per plant did not vary more than 2.5 cm in any cultivar across the harvests conducted on June 20th, July 2nd, July 13th, August 13th, and August 22nd of 2018 (Figure 12). Across all harvests, 'Big Lifeberry' did not vary more than 4 cm, 'Vermillion Sunset' did not vary more than 5 cm, and 'Sweet Lifeberry' did not vary more than 7cm (Figure 12).

DISCUSSION

Root Pruning

Contrary to the literature, root pruning did not increase root fresh weight, root dry weight, total root length, root surface area, average root diameter, or number of root tips in the Goji berry rootballs that were subjected to this treatment. In fact, in the two instances where a significant difference was noted, root pruning actually decreased these measures when compared to non-air root pruning treatment (Table 2). It is important to note though, that this root pruning experiment was conducted for only eight months. Most root pruning studies that were reviewed took approximately two years to complete (Huang and Liang, 1988). Also, during the time these plants spent in the greenhouse, malfunctions in the heating system, as well as multiple pest and disease problems including; aphids, white flies, mealybugs, and powdery mildew, stunted growth and induced premature leaf drop. When the amount of fresh weight and dry weight of greens produced by root pruned plants was compared to non-air root pruned plants, the treatment effect was only significant in 'Vermillion Sunset' during the first harvest (Figures 6 and 7). It is important to note that again, non-air root pruned plants out-produced root pruned plants in this case as well. These results could be due to the vigorous growth habit of the Goji berry plant, making any pruning treatment obsolete after the first few months of growth. The decrease in fresh weight and dry weight of greens produced by the root pruned plants could also be explained by the insufficient amount of time spent in the root pruning pots. In summary, while there were some statistical differences noted, overall no difference between root pruning and non-air root pruning treatments was found.

Goji Berry Greens Production

Greens production was achieved with all three cultivars used in this experiment. Over all of the harvests conducted during the growing season, ‘Big Lifeberry’ out produced both ‘Vermillion Sunset’ and ‘Sweet Lifeberry’ in fresh weight and dry weight (Figure 13). There were some harvests in which ‘Vermillion Sunset’ out produced “Big Lifeberry’ and ‘Sweet Lifeberry’ in the number of shoots produced and the total length of shoots produced (Figure 11). However, it is important to note that during these harvests, ‘Big Lifeberry’ showed no statistical difference in fresh weight and dry weight of greens produced (Figures 8 and 9). This indicates that while ‘Vermillion Sunset’ produced a larger amount of longer shoots, ‘Big Lifeberry produced more robust, herbaceous shoots. The shoots of ‘Big Lifeberry’ were more desirable for greens production because of their quality. Also, over the majority of the harvests, ‘Big Lifeberry’ consistently produced higher average shoot lengths when compared to ‘Vermillion Sunset’ and ‘Sweet Lifeberry’ (Figure 12). The consistency of production of ‘Big Lifeberry’ can be noted throughout all measurements taken over the growing season (Figures 8 and 9). While greens production from ‘Vermillion Sunset’ and ‘Sweet Lifeberry’ slowed down at the end of the growing season, ‘Big Lifeberry’ continued to produce high-quality greens. ‘Vermillion Sunset’ did produce well throughout the growing season, but the quality of greens this cultivar produced was not desirable. The shoots were slender, short, and flimsy. ‘Sweet Lifeberry’ had poor performance in greens production compared to the other cultivars. It was noted that ‘Sweet Lifeberry’ plants were flowering and producing fruit throughout the season. The lack of greens production from this cultivar could be attributed to energy shifting toward reproductive growth, rather than vegetative. In summary, Goji berry greens can be produced in the Midwest, with most production occurring in late spring and early to late fall. ‘Big Lifeberry’ seems to be the best candidate for greens production from the three cultivars studied because of its consistent and high yielding production of robust, herbaceous shoots.

Future Research

Air root pruning may have an effect on early season growth of the Goji berry greens, but this was not identified in this study. Air root pruning could also help to increase greens production during drought or the summer months. An air root pruning study that allowed enough time for root growth to fill the pots, and used plants with similar cutting/seeding dates would reduce the variability and may give a clearer answer to the questions posed by this research.

Knowing that Goji berry greens can be successfully produced in the Midwest is only the first step. This research has provided a starting point for future projects. Further research is needed to evaluate the Goji berry plants' winter survival rate, as well as second season production. Second season production evaluations can be done using the same plants and methods that were used in this research. Increasing the sample size, number of cultivars, and adjusting the harvest intervals to accommodate the different growth speeds of each cultivar could also provide interesting and important information for greens producers.

While it is possible to produce greens in the Midwest, it would be important to analyze the greens for dietary nutrition content, as well as for toxic compounds. While no toxic compounds have been reported in *L. barbarum* leaf tissue, knowing that the cultivars used in this experiment also possess no toxins would ensure food safety for consumers. A consumer evaluation of cultivar tastes and opinion of edible Goji green products would also be useful in future studies.

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