Journal of Agricultural Science; Vol. 9, No. 1; 2017
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Year-Round Lettuce (*Lactuca sativa* L.) Production in a Flow-Through Aquaponic System

Gaylynn E. Johnson[1], Karen M. Buzby[2], Kenneth J. Semmens[3,4] & Nicole L. Waterland[1]

[1] Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV, USA

[2] Department of Civil and Environmental Engineering, West Virginia University, Morgantown, WV, USA

[3] Division of Animal and Nutritional Sciences, West Virginia University, Morgantown, WV, USA

[4] Aquaculture Research Center, Kentucky State University, Frankfort, KY, USA

Correspondence: Nicole L. Waterland, Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV, 26506, USA. Tel: 1-304-293-2969. E-mail: nicole.waterland@mail.wvu.edu

Received: October 21, 2016 Accepted: November 18, 2016 Online Published: December 15, 2016

doi:10.5539/jas.v9n1p75 URL: http://dx.doi.org/10.5539/jas.v9n1p75

The research is financed by the U.S. Department of Agriculture Special Grant Agreement 2010-34386-21745 and West Virginia University Agriculture and Forestry Experimental Station (Hatch Grant WVA00640). Scientific Article No. 3292 of the West Virginia Agricultural and Forestry Experiment Station, Morgantown.

Abstract

Aquaponics is the combination of hydroponics and aquaculture that sustainably produces both animal and plant food products. Soluble nutrients are released into water by the fish providing nutrition for plant growth. Lettuce (*Lactuca sativa* L.) is one of the most popular vegetables grown in aquaponic systems. In this experiment, the feasibility of year-round lettuce production utilizing a cold water flow-through aquaponic system (FTS) growing trout (*Oncorhynchus mykiss*) in a high tunnel was evaluated. A high tunnel is a greenhouse-like facility constructed with polyethylene covering a metal frame which extends the growing season and protects the crop from cold temperatures. The average night air temperature inside the high tunnel during winter in Wardensville, WV was 2.9±3.4 °C and it helped extend the growing period into the fall and winter. Results from this pilot scale experiment showed the potential for year-round lettuce production in an FTS. Average yield (fresh harvest weight per tray) in the spring season was the highest, while productivity (average yield per week) during the summer season was higher than that in spring. During the extended growing seasons (fall and winter), more than a quarter (30.6%) of the total lettuce production was obtained. The yield per unit area (7.4 kg m^{-2}) from our pilot study was significantly higher than that from the reported average field production (3.1 kg m^{-2}) in the U.S. except California and Arizona where year-round production of lettuce occurs. To compensate for lower lettuce yields during cold seasons, high value crops requiring less nutrients and tolerant to the colder environment may be considered.

Keywords: aquaculture, flow-through aquaponics, high tunnel, lettuce, trout

1. Introduction

Aquaponics refers to any agricultural system that integrates aquaculture (rearing fish) with hydroponics (producing plants without soil). Soluble nutrients are released into water by the fish providing nutrition for plant growth while plants remove compounds such as ammonia that may impact growth and health of the fish. Traditionally, aquaponics has combined recirculating aquaculture system (RAS) with plant production. Nutrient availability in RAS has been shown to be similar to that in nutrient rich hydroponic systems (Rakocy, Masser, & Losordo, 2006). Recently, Buzby et al. (2016) demonstrated that a cold water flow-through aquaculture production system may also be used to produce aquaponic crops. Flow-through aquaculture systems differ from RAS in that water is not reused or recycled and nutrients do not accumulate over time. Nutrient concentrations in the flow-through aquaculture system are substantially lower than that in RAS (Buzby & Lin, 2014). In addition, water temperature is dependent on water source and tends to be cooler in spring fed flow-through aquaculture systems than that in RAS (Losordo, Masser, & Rakocy, 1992).

The plant production component in an aquaponic system utilizes hydroponic technology. Hydroponic systems employ different arrangements to provide nutrients to the plants' roots. Among them, raft or deep water culture (DWC), nutrient film technique (NFT) and media-filled beds (MFB) are commonly practiced methods in hydroponics (Love, Uhl, & Genelloa, 2015; Love et al., 2014; Resh, 2013; Saavas, 2002). In a DWC system, plants are grown in the polystyrene trays or rafts that float on top of the water. Roots of plants grow into the water to take up the nutrients. DWC systems require a large volume of water in order for the root system to be submerged to the nutrient solution. In our system DWC was used in a high tunnel.

A high tunnel is a tunnel-shaped enclosed structure constructed with polyethylene, polycarbonate, plastic or fabric over a metal or wooden frame which is easier and more economical to construct compared to building a greenhouse. The use of a high tunnel not only protects the crop from harsh environments, but also extends the growing season to produce cold-tolerant crops such as lettuce (Lee, Liao, & Lo, 2015). Cold-tolerant crops are able to grow during winter months for both a longer cropping season and a winter market (Borrelli, Koenig, Jaeckel, & Miles, 2013). Crop production in high tunnels can exploit market conditions where supplies are limited and prices are high (Castoldi, Bechini, & Ferrante, 2011). Production of several vegetables and herbs including basil (Adler, Harper, Takeda, Wade, & Summerfelt, 2003; Rakocy, Bailey, Shultz, & Thoman, 2004), cucumber and herbs (Savidov, Hutchings, & Rakocy, 2007), and lettuce and tomato (Rakocy, Hargreaves, & Bailey, 1993) have been reported in aquaponic systems. Recently, performance of a wide variety of food crops including lettuce, herbs, Asian greens and vegetables was evaluated in a flow-through aquaponic system (Buzby et al., 2016).

Lettuce is one of the most popular crops grown aquaponically. Lettuce is genetically diverse and comes with variety of shapes, colors and textures (Kim, Moon, Tou, Mou, & Waterland, 2016). Lettuces rank first in the United States in total value of production of fresh market vegetables (United States Department of Agriculture, 2016). National consumption, on average, exceeded 23 lbs per person annually between 1980 and 2010 (United States Department of Agriculture, 2014). Lettuce production is limited by temperature. If air temperature exceeds 30/16 °C day/night, the crop will develop tipburn, produce premature flower stalks (bolting), and form loose heads (Fukuda et al., 2011). Thus, lettuce is a good candidate for a cold water flow-through aquaponic system (FTS).

Aquaponic production of crops has gained popularity and crop production in RAS is well documented (Losordo et al., 1992; Ido, 2016; Martins et al., 2010; Rakocy et al., 2004; Rakocy, 2007). However, there is limited information regarding crop production, especially year-round lettuce production in an FTS. In this study, the feasibility of year-round aquaponic lettuce production using effluent from an FTS in a high tunnel was evaluated. The cultivar 'Red Sails' was chosen because it resists bolting and grows well in cold environments (Park Seed, 2016).

2. Materials and Methods

2.1 Facility

Experiments were conducted in a 7.9 m × 14.6 m × 3.7 m tall high tunnel covered in 6 mil (0.15 mm thick) clear greenhouse plastic located in Wardensville, West Virginia (394′32″N, 7835′40″W). Ventilation in the high tunnel was provided by rolling up the side walls approximately 1.4 m and removing the end walls. A shade cloth cover was put on the high tunnel on June 29th and removed October 1st to reduce light intensity and moderate air temperature inside the high tunnel. The end and side walls were closed in mid-October. Three channels (13.7 m × 2.7 m) were constructed of dry stacked concrete block on a fine gravel base and then covered with a 45 mil, heavy-duty, black plastic pond liner (Firestone Pondgard, ethylene propylene diene monomer (EPDM), Model PG 5000; Indianapolis, IN, USA) liner. The block was stacked such that the channel was 30.5 cm deep.

2.2 Growth Conditions

The high tunnel maintained the inside temperature above outside temperatures during the entire year. This was especially apparent during the late fall through winter season (Figure 1). Average night temperatures inside the high tunnel remained above the freezing point, which allowed lettuce to grow without being damaged during the winter months. Water temperature of the spring fed system was relatively constant through the year (12.1-13.8 °C) and represented a heat source during the cold winter season.

Effluent from rainbow trout (*Oncorhynchus mykiss*) reared in a spring fed flow-through system in a nearby structure was used as the sole water and nutrient source in this study. Trout effluent was pumped to the high tunnel and distributed through a PVC manifold. Flow of effluent into each channel was independently controlled with a ball valve. Water depth was maintained at 23 cm with a standpipe drain.

'Red Sails' lettuce (*Lactuca sativa* L. var. *crispa* 'Red Sails') were grown in this experiment from seed purchased from Johnny's Selected Seeds (Winslow, ME, USA). Lettuce plants were grown in 128-cell styrofoam Speedling (67.6 cm × 34.6 cm; Speedling, Inc., Ruskin, FL) trays and vermiculite (Therm-O-Rock East Inc., Grade 3A; New Eagle, PA, USA) was used as the growing medium. Seeds were sown directly into the trays using a vacuum seeder. After seeds were sown, the trays were placed directly into the channels. Nine trays (3 × 3) as a block were placed at the top of the channel next to the influent. Initially, the remainder of the channel was filled with empty trays. An additional block of trays was sown and placed closest to the inlet next to the previous block of trays and placement of trays was repeated weekly until the channel was full. Each channel held maximum of 45 trays (five blocks of nine trays). Lettuce production was evaluated for an entire year from December 1, 2011 through December 21, 2012.

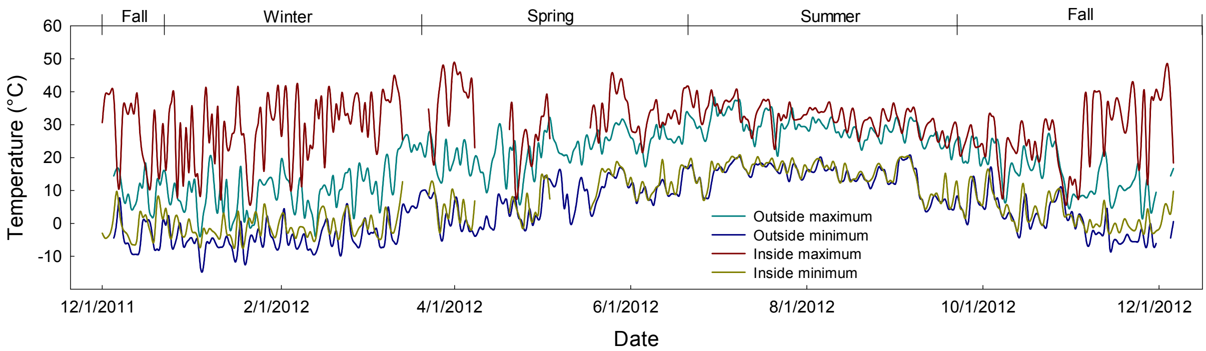

Figure 1. Daily maximum and minimum temperatures outside and inside of the high tunnel

Note. Maximum and minimum temperatures outside the high tunnel were obtained from National Oceanic and Atmospheric Administration (Wardensville in Hardy County).

2.3 Stand Establishment, Average Yield and Productivity

Stand establishment (SE), the ability of the seedlings to survive after germination, was determined two weeks after seeds were sown. The SE was defined as the percentage of visible seedlings at least 5 mm tall in a tray. Lettuce was considered ready to harvest when it reached an average height of 12.7-15.2 cm as measured from the surface of media to the top of the tallest leaf (United States Department of Agriculture, 2013). The trays of lettuce were harvested using an electric fillet knife which was drawn across the top of the tray severing the shoot from the roots. All lettuce in a block of nine trays were harvested at the same time and considered one harvest. Total fresh weight of all lettuce combined from nine trays was defined as harvest biomass. Average yield was determined as the average fresh harvest weight per tray. Seasonal yield was determined as the total fresh weight harvested in each season. Productivity was determined on a seasonal basis as the average yield per week.

2.4 Water Quality

Water samples were collected from the end of each channel in acid washed plastic bottles every two weeks. A 2 L bottle was used for TSS and a 250 ml bottle for the nutrient analyses. As soon as water samples were collected, they were immediately placed on ice. Upon transfer to the lab, samples were stored at 4 ºC until they were analyzed. The samples were analyzed according to methods delineated by APHA (1995) for total ammonia nitrogen (TAN) (4500-NH_3 colorimetric method), nitrite (4500-NO_2) colorimetric method), nitrate (4500-NO_3 cadmium reduction colorimetric method), phosphate (4500-P ascorbic acid method) and total suspended solids (TSS) (2540D). Samples were analyzed within 24 hours of collection and then frozen. Nitrite concentrations never exceeded 0.02 mg L^{-1}, were often less than 0.01 mg L^{-1}, and they are not reported. Concentration of nitrate was determined within 30 days of collection.

2.5 Statistical Analysis

Temperature, light intensity and crop production data were analyzed by season. The seasons were defined as: spring (March 20-June 19), summer (June 20-September 21), fall (September 22-December 19) and winter (December 20-March 19).

Experimental design was randomized complete design. Stand establishment (SE), average yield and productivity values are the means of three replications (n = 3). Analysis of variance (ANOVA) was performed with PROC

GLM (generalized linear model) by SAS version 9.3 (SAS Institute, Inc., Cary, NC). Differences were determined using Tukey's significance test at $P \leq 0.05$.

3. Results

3.1 Environmental Conditions

The experiment was conducted in a high tunnel where air temperature inside the high tunnel was expected to be higher than outside, especially during the colder seasons (Table 1 and Figure 1). During the cold seasons (fall and winter), the average night temperature inside high tunnel was above the freezing point, allowing lettuce growth throughout the year. Once there was no danger of frost, the sides of the high tunnel were rolled up and the plastic was removed from the end walls. Additionally, shade cloth was placed over the high tunnel during the summer months. Both measures prevented air temperatures within the high tunnel from greatly exceeding the outside temperatures. Maintaining passive ventilation during the spring and summer and closing the high tunnel during fall and winter reduced the seasonal difference in daytime air temperatures (Figure 1).

Light intensity, measured as photosynthetic photon flux density (PPFD), was significantly higher in the spring than the other seasons. Installation of the shade cloth reduced light intensity during the summer months to levels similar to fall and winter (Table 1). Daily light integral, the integrated light intensity, was significantly greater during the spring than the other seasons. Water temperature was measured three times in December (12.1±0.05 °C), January (13.1±0.05 °C) and February (13.8±0.05 °C).

Table 1. The seasonal average of day and night temperatures, photosynthetic photon flux density (PPFD), and daily light integral (DLI) in the high tunnel

Season	Temperature (°C)		PPFD (μmol m^{-2} s^{-1})^z	DLI (mol m^{-1} d^{-1})^y
	Day	Night		
Spring	24.5±8.7	11.4±5.6	652.8a	31.8a
Summer	27.1±5.9	17.4±4.1	345.6b	17.0b
Fall	18.4±9.7	5.5±5.5	346.6b	12.3b
Winter	18.3±11.4	2.6±4.5	383.5b	15.4b

Note. ^{z}Photosynthetic photon flux density (PPFD) is a measure of the number of photons in the 400-700nm range of the visible light spectrum that fall on a square meter of target area per second.

^{y}Daily light integral (DLI) is a measure of the amount of photosynthetically active radiation photons received each day in a square meter.

Different letter down the column indicate significant difference by Tukey's significance test at $P \leq 0.05$.

3.2 Stand Establishment, Average Yield and Productivity

Stand establishment was not significantly different among summer, fall and winter seasons. In spring, however, stand establishment was generally low for each sowing including very poor germination for the 11th harvest (49.7%). After the shade cloth was installed, SE was improved. Overall, stand establishment averaged 83.5±11.6% for the year (Table 2).

Lettuce was harvested on 30 occasions; ten harvests each in spring and summer, seven harvests in fall and three harvests in winter (Table 2). The number of harvests was dependent on the growth rate of the lettuce. Growth rates were highest in summer when it took 4.6 weeks to achieve harvestable size and lowest in winter when it took 9 weeks.

Total annual yield was 644.0 kg with significant differences among all four seasons ($P \leq 0.001$). The highest yields occurred in the spring with an average yield of nearly 700 g per tray and a seasonal yield of 250.4 kg. Yield in summer was also higher than that in colder seasons with an average yield of 622.0 g per tray and a seasonal yield of 196.5 kg. Yields in fall and winter were lower with an average yields of 520.8 g and 435.6 g per tray, respectively. Average yield per harvest was 25.0, 19.7 and 21.4 kg for spring, summer and fall, respectively, while 15.7 kg of lettuce was harvested during winter. Nearly 70% of lettuce production (446.9 kg) was obtained from the spring and summer seasons.

Because there was a significant difference in growing period ($P \leq 0.001$) among seasons, productivity (g produced per week per tray) was determined. While yield was the greatest in spring, productivity was highest in summer (139.2 g/wk/tray) and increased more than 30% compared to that in spring. Productivity was higher in spring (105.5 g/wk/tray) than fall (85.3 g/wk/tray) and winter (48.7 g/wk/tray). Productivity during summer was nearly three times greater than in winter.

Table 2. Seasonal standard establishment, number of harvest times, yield, productivity of lettuce.

Season	Standard establishment (%)	No. of harvest times	Seasonal yield (kg)	% of total yield	Yield/harvest (kg)	Average yield (g/tray)	Growing period (weeks)	Productivity (g/tray/week)
Spring	78.4b[z]	10	250.4	38.9	25.0	699.5a	6.6 b	105.5b
Summer	86.7a	10	196.5	30.5	19.7	622.0b	4.6 c	139.2a
Fall	85.1a	7	150.0	23.3	21.4	520.8c	6.6 b	85.3c
Winter	86.6a	3	47.0	7.3	15.7	435.6d	9.0 a	48.7d
Significance	***	N/A	N/A	N/A	N/A	***	***	***

Note. [z]Values are the means of three replications (n = 3).

Different letters down the column indicate significant difference by Tukey's significance test at $P \leq 0.05$.

NA, not applicable.

*** Significant at $P \leq 0.001$.

3.3 Water Quality

The average influent nutrient and TSS concentrations were 0.34 mg L^{-1}, 0.30 mg L^{-1}, 0.17 mg L^{-1} and 1.95 mg L^{-1} for TAN, NO_3^{-1}, phosphate (PO_4^{-3}) and TSS, respectively. There were significant seasonal differences in influent and effluent concentration for all nutrients and the influent TSS ($P \leq 0.01$) (Table 3). Influent TAN concentrations ranged between 0.22 and 0.40 mg L^{-1} with the highest concentrations in summer. The concentration of TAN in effluent during the summer season was the highest (0.35 mg L^{-1}) and the lowest in fall (0.21 mg L^{-1}). The seasonal differences in the quantity of TAN removal were significant ($P \leq 0.001$). During spring and summer removal of TAN was significantly higher than that in fall and winter.

Influent nitrate concentrations ranged between 0.29 and 0.41 mg L^{-1} and the highest concentrations of nitrate was detected in winter (0.41 mg L^{-1}), while there was no difference among other seasons. Removal of nitrate was low all year ranging from -0.02 to 0.02 mg L^{-1} with no significant difference among seasons. Influent phosphate concentrations ranged between 0.14 and 0.17 mg L^{-1} and generally were low during fall and winter compared to spring and summer. There was minimal removal during spring and summer and no detectable level of removal was observed during the fall and winter. Influent TSS concentration during summer (2.25 mg L^{-1}) was higher than that in fall (1.55 mg L^{-1}) that was the lowest among the four seasons. There were significant differences in influent concentration among seasons, however, amount of TSS removed was not significantly different.

Table 3. Seasonal nutrient removal of total ammonia nitrogen (TAN), nitrate (NO_3^{-1}), and phosphate (PO_4^{-3}) and total soluble solids (TSS) in flow-through aquaponic system by lettuce[z]

Season	TAN (mg L^{-1})			NO_3^{-1} (mg L^{-1})			PO_4^{-3} (mg L^{-1})			TSS (mg L^{-1})		
	Influent	Effluent	Removal[y]	Influent	Effluent	Removal	Influent	Effluent	Removal	Influent	Effluent	Removal
Spring	0.34b	0.30ab	0.04ab	0.30b	0.29b	0.01	0.17a	0.16a	0.01a	1.95ab	1.2	0.75
Summer	0.40a	0.35a	0.05a	0.29b	0.27b	0.02	0.17ab	0.16a	0.01ab	2.25a	1.25	1.00
Fall	0.22d	0.21c	0.01bc	0.30b	0.28b	0.02	0.14c	0.14b	0.00c	1.55b	1.11	0.44
Winter	0.28c	0.28b	0.00c	0.41a	0.43a	-0.02	0.15bc	0.15ab	0.00bc	1.79ab	1.27	0.52
Significance	***	***	***	***	***	NS	***	**	***	*	NS	NS

Note. [z]Values are the means of three replications (n = 3).

[y]Removal concentration was calculated by subtracting effluent concentration from influent concentration.

Different letters down the column indicate significant difference by Tukey's significance test at $P \leq 0.05$.

NS, not significant.

*, **, *** Significant at $P \leq 0.01$, 0.05 or 0.001, respectively.

4. Discussion

4.1 Stand Establishment, Yield and Productivity

The seeds of 'Red Sails' lettuce were able to germinate and establish well throughout the year including during the cold seasons in FTS (Table 2). However, it took longer until harvest during the cool seasons (fall and winter) and fewer harvests were made compared to the warm seasons (spring and summer). In other systems lettuce seeds are usually sown in a germination area and seedlings were transplanted to the aquaponic facility. In our study seeds were directly sown into the tray. Direct sowing method can be beneficial because it saves labor, time and space that are associated with germination and transplanting, however, it could cause lower SE, because 100% seed germination can't always be guaranteed and more often than not there will be empty cells in a tray. Empty cells can't be refilled after seeds were directly sown into the tray and it could result in lower SE as observed during the spring season in our study.

Seasonal average yield of 'Red Sails' lettuce was varied. Average yield in spring was the greatest followed by summer, fall and winter in descending order. In spring average yield was higher than in summer likely due to cooler temperature and higher light intensity (Table 1). Nutrients in the aquaculture wastewater are unlikely to be a major factor influencing seasonal average yield. Although the concentration of TAN during the summer was higher than that in the spring, removal of TAN was not different between the spring and summer (Table 3). Additionally, there was no difference in nitrate, phosphate and TSS concentration in either influent or effluent. During the fall and winter seasons, the average yields were lower than warmer seasons, most likely due to lower night temperatures and the low concentration of TAN (both seasons), nitrate (winter only), and potentially phosphate. It should be noted that in summer two harvests were discarded because lettuce appeared to be damaged by stress and became unmarketable. Summer yield would have been higher if there were no such incident. The exact cause for the damage was not known.

Seasonal yields of lettuce were variable across season with the greatest yield in spring higher than in summer. In spring, temperatures were moderate, and light intensity and DLI were the highest while in summer temperatures were higher and light intensity was comparable to fall and winter due to the shade cloth. Particularly, SE was lower in spring than that in other seasons, but the seasonal yield and average yield in the spring harvests were highest, indicating that SE alone may not be a definite indicator of the crop production. Additionally, higher intensity of light during the spring season might have negatively affected SE. The lower yield during cold seasons, although SE was similar to that in warm seasons, might have been caused by not only cold air temperatures, but also limited supply of nutrients (Table 3).

During the summer months, lettuce is not generally grown in the field because elevated temperatures and long day photoperiod stimulate bolting and reduce lettuce production (Simko, Hayes, Mou, & McCreight, 2014). For the opposite reason in the cold season, lettuce is not grown in the field because the air and soil temperatures are too low for crop production in most temperate regions. Generally, water keeps relatively constant temperature compared to air. In our FTS, spring water provided steady root-zone temperature for lettuce production year-round. Root-zone temperature significantly affects plant production (Gosselin & Trudel, 1985). Moderate root-zone temperature might have mitigated the adverse effect of either elevated and decreased air temperature during the summer and winter, respectively, and helped lettuce to grow in a cold water FTS.

Growing period until harvest varied among seasons. During the summer, lettuce was ready to be harvested in less than five weeks (Table 2) compared to longer than six weeks in spring and fall. Elevated temperature and/or adequate light intensity, considering similar amounts of nitrogen and phosphate supply or removal in FTS during the spring and summer, could have accelerated plant growth and consequently shortened harvest time in the summer. Additionally, lower night time temperatures in the spring (11.6 °C) than the summer (17.6 °C) might have contributed to a longer growing period in the spring and resulted in lower productivity, although with same reason average yield were higher in spring. During the extended growing seasons (fall and winter), harvest time was longer and fewer harvests were made compared to the warm seasons (spring and summer), potentially due to significantly lower night temperature and lower level of nutrient caused by slow growth rate or low number of fish in the aquaculture system.

During the warm seasons (spring and summer), the temperature both inside and outside the high tunnel was often higher than the optimum temperature range (16-20 °C) for lettuce production. Lettuce is prone to heat stress and often bolts, i.e. produces flowers prematurely. Bolting occurs when lettuce is grown under elevated temperature and high light intensity, and it diminishes the marketability of the lettuce (Zhao & Carey, 2009). However, bolting was not observed during the year-round production in a cold water FTS. Shading during the summer season, in conjunction with cool water temperatures may have helped prevent bolting by reducing the temperature and light intensity inside the high tunnel.

4.2 Water Quality

The only nutrient source supporting lettuce production was the nutrients coming from the aquaculture facility. The aquaculture effluent nutrient concentrations were low and frequently there was little difference in concentration between the effluent entering the channels and that leaving them. Nonetheless, this system was able to produce lettuce at comparable rates to soil based systems. This may be due in part to the high delivery rates of effluent to the hydroponic channels. At a flow rate of 76 liter min^{-1} an average of 25.8 mg L^{-1}, 22.8 mg L^{-1} and 12.9 mg L^{-1} of TAN, nitrate and phosphate, respectively, was delivered to the top of the channel. Due to the conveyor production system used, the smallest plants received the most concentrated nutrients yet would have the lowest uptake due to low biomass.

Nutrient availability may have been greater than the measured concentrations entering the channels due to decomposition and mineralization of accumulated solids (uneaten fish food and feces) in the bottom of the channel. No attempt was made to remove the solids comprised of vermiculite and particulate organic material that accumulated in the channel. The aquaculture facility does have quiescent zones at the end of each raceway section, however they do not trap all of the solids. The entrained solids deposit mostly in the upper third of the channel and represent the largest particles. The deposition is apparent from the reduction in TSS concentrations at the end of the channel. Accumulated solids then decompose creating an unmeasured nutrient source.

TAN removal was higher than nitrate or phosphate removal and varied seasonally (Table 3). The highest removal rates occurred during spring and summer when the lettuce was growing the fastest. TAN removal was substantially greater than nitrate removal. Buzby and Lin (2014) determined that lettuce grown in this cool water flow-through aquaponic system has a strong preference for TAN. Xu et al. (1992) determined that ammonium was the preferred nitrogen source when N concentrations were low while nitrate was preferred when N concentrations were high. Low water temperatures may also have played a role in the preference for TAN. Multiple studies, reviewed by von Wirén et al. (1997) have demonstrated that low temperatures generally increased the reliance of plants on ammonium as a mineral N source.

Lower removal rates for PO_4^{-3} may be related to the N:P ratio which was 6.6. Koerselman and Meuleman (1996) determined that for most plants an N:P ratio of less than 14 is indicative of N limitation. Buzby and Lin (2014) determined that this system was strongly N limited and that additions of ammonia increased PO_4^{-3} removal rates. It is unclear whether the slightly higher TAN concentrations in summer increased PO_4^{-3} removal or if it was due to increased growth rates of lettuce.

Concentrations of all the soluble nutrients in the flow-through raceway effluent were substantially lower than nutrient concentrations found in either hydroponic culture or in effluent from recirculating fish culture. Despite the low nutrient concentrations, no nutrient deficiencies were observed and all plants appeared healthy, even those at the end of the channel where nutrient concentrations would be the lowest. This may be due to the flow-through nature of the system where plants were supplied with a continual nutrient supply. In flow-through culture, fresh effluent, with its full complement of nutrients derived from both the source water and fish culture, is delivered to the plant growing channel. In a recirculating system nutrients are removed with each pass through the system and those that are not replaced by inputs from fish culture can become limited, which may cause an imbalance of nutrients leading to nutrient deficiencies (Rakocy et al., 2006).

4.3 Lettuce Production

In our study, lettuce was produced in 115.9 m^2 high tunnel (26' × 48' or 1,268 ft^2) using three of four available channels and it produced 5.6 kg m^{-2} (644 kg/115.9 m^2) of lettuce. If all four channels were used, the estimated total yield per m^2, by adding 33% yield to the total production, would have been 7.4 kg m^{-2}, which is significantly higher than average field lettuce production in the U.S. in 2012 (3.1 kg m^{-2}, based upon 40.3, 27.3 and 35.6 t ha^{-1} for iceberg, leaf and romaine lettuce production, respectively) (Simko et al., 2014; Galinato & Miles, 2013), and the soil based high tunnel in West Virginia (4.9 kg m^{-2}) (personal communication, L.W. Jett). Combined yield during the spring and summer in FTS was 446.9 kg or 3.9 kg m^{-2}, which was higher than field lettuce production per area. Lettuce production during the extended seasons (197.0 kg or 1.7 kg m^{-2}) could make up almost 54.4% of the yearly field production.

In a 128-cell tray (67.6 cm × 34.6 cm) with annual average SE of 83.5%, 107 lettuce plants would be expected to grow in a tray or 166 plants per m^2 (based upon 107 lettuces per tray, 45 trays in a channel, four channels in the facility of 115.9 m^2). The density of lettuce grown in a tray was about 26 times higher than the crop densities in the field production (6.4 plants m^{-2} based upon 26,000 heads per acre) (Galinato & Miles, 2013) and 3 times higher than the soil based high tunnel (53.8 plants m^{-2} based upon 0.2 ft^2 per plant in West Virginia) (personal communication, L.W. Jett). High density lettuce production requires high level of nutrients. In an FTS, the content of nutrients was generally limited and supplied at a lower level, although there was a moderate increase of TAN in the summer and nutrient was constantly supplied at a low level. Insufficient nutrients in the wastewater in an FTS might have limited lettuce growth. The average fresh weight of a single lettuce plant grown in a tray was assumed to be about 44.6 g m^{-2} throughout the year, based upon the annual yield 7.4 kg m^{-2} from estimated 166 lettuces per m^2, was significantly low compared to lettuce grown in the field (484.4 g m^{-2}; 3.1 kg per 6.4 heads m^{-2}) at maturity. Depending on the configuration of a tray manufactured for a hydroponic system, up to 128 lettuces can grow in one tray. Using a tray with smaller cells, it is recommended to produce baby or spring mix lettuces instead of fully grown loose leaf or whole head lettuce. There is no defined size for baby or spring mix vegetables. Usually, baby lettuces are lettuces with four to six true leaves and spring mix refers to mixture of baby sized leafy vegetables. A higher number of lettuce can be produced for a shorter growing period in a tray with smaller cells. Bulk production of loose leaf lettuce or baby lettuce for salad processing, food service or value added products would be a better choice if the scale of lettuce production and marketing strategy fit such operation (Simko et al., 2014). It is a growing trend that farmers do not grow head lettuce to their full mature size because lettuce often needs to be immediately packaged in a fixed size of a carton for 24 heads at the production site (Simko et al., 2014). In addition, multiple research studies indicated that premature lettuces such as microgreen and baby greens contained more nutritious phytochemicals and demand for miniature sized lettuce is increasing (Kim et al., 2016; Pinto, Almeida, Aguiar, & Ferreira, 2015; Xiao et al., 2015; Ascensión, Luna, Selma, Tudela, Abad, & Gil, 2012; Martínez-Sánchez et al., 2012).

5. Conclusions

The present study has demonstrated that year-round lettuce production using an FTS inside a high tunnel was not only feasible, but also better than the field or soil based high tunnel production based upon yield per area. However, yield during the winter months was low and needs to be improved. To further increase the yield of lettuce during winter, low tunnels within a high tunnel and solar powered supplemental lighting systems such as low energy light emitting diode (LED) lighting system can be installed to enhance low light conditions and increase photosynthetic activity during cold seasons to a certain extent. Depending on the goals of the grower, a selection of different plant species that are more tolerant to a cold growing environment such as cole crops (a group of vegetables in the Brassica family) could be another option for consideration. Conversely, some growers may wish to shut down an aquaponic operation during the winter months.

Acknowledgements

We would like to thank Matthew Ferrell and Gene Jacobs for their assistance with maintaining the aquaculture system.

References

Adler, P. R., Harper, J. K., Takeda, F., Wade, E. M., & Summerfelt, S. T. (2000). Economic evaluation of hydroponics and other treatment options for phosphorus removal in aquaculture effluent. *HortScience, 35*, 993-999.

APHA (American Public Health Administration). (1995). *Standard methods for the examination of water and wastewater* (19th ed.). American Public Health Administration, Washington, D.C.

Ascensión, M.-S., Luna, M. C., Selma, M. V., Tudela, J. A., Abad, J., & Gil, M. I. (2012). Baby-leaf and multi-leaf of green and red lettuces are suitable raw materials for the fresh-cut industry. *Postharvest Biology and Technology, 63*, 1-10. https://doi.org/10.1016/j.postharvbio.2011.07.010

Borrelli, K., Koenig, R. T., Jaeckel, B. M., & Miles, C. A. (2013). Yield of leafy greens in high tunnel winter production in the Northwest United States. *HortScience, 48*(2), 183-188.

Buzby, K. M., & Lin, L. (2014). Scaling aquaponic systems: Balancing plant uptake with fish output. *Aquacultural Eng., 63*, 39-44. https://doi.org/10.1016/j.aquaeng.2014.09.002

Buzby, K. M., Waterland, N. L., Semmens, K. J., & Lin, L.-S. (2016). Evaluating aquaponic crops in a freshwater flow-through fish culture system. *Aquaculture, 460*, 15-24. https://doi.org/10.1016/j.aquaculture.2016.03.046

Castoldi, N., Bechini, L., & Ferrante, A. (2011). Fossil energy usage for the production of baby leaves. *Energy, 36*, 86-93. https://doi.org/10.1016/j.energy.2010.11.004

Fukuda, M., Matsuo, S., Kikuchi, K., Kawazu, Y., Fujiyama, R., & Honda, I. (2011). Isolation and functional characterization of the FLOWERING LOCUS T homolog, the *LsFT* gene, in lettuce. *Journal of Plant Physiology, 168*, 1602-1607. https://doi.org/10.1016/j.jplph.2011.02.004

Galinato, S. P., & Miles, C. A. (2013). Economic profitability of growing lettuce and tomato in western Washington under high tunnel and open-filed production systems. *HortTechnology, 23*(4), 453-460.

Gosselin, A., & Trudel, M.-J. (1985). Influence of root-zone temperature on growth, development and yield of cucumber plants cv. Toska. *Plant and Soil, 85*, 327-336. https://doi.org/10.1007/BF02220188

Ido, S. (2016). Growth models of gilthead sea bream (*Sparus aurata* L.) for aquaculture: A review. *Aquacultural Engineering, 70*, 15-32. https://doi.org/10.1016/j.aquaeng.2015.12.001

Jett, L. W. (2016). *Personal communication*. April 8, 2016.

Kim, M. J., Moon, Y., Tou, J. C., Mou, B., & Waterland, N. L. (2016). Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). *J. of Food Composition and Analysis, 49*, 19-34. https://doi.org/10.1016/j.jfca.2016.03.004

Lee, A.-C., Liao, F.-H., & Lo, H.-F. (2015). Temperature, daylength, and cultivar interact to affect the growth and yield of lettuce grown in high tunnels in subtropical regions. *HortScience, 50*(10), 1412-1418.

Losordo, T. M., Masser, M., & Rakocy, J. (1992). *Recirculating aquaculture tank production systems: An overview of critical considerations* (No. 451). Southern Regional Aquaculture Center, SRAC Publication. Retrieved from http://university.uog.edu/cals/people/pubs/aquac/451recag.pdf

Love, D. C., Fry, J. P., Genello, L., Hill, E. S., Frederick, J. A., Li, X., & Semmens, K. (2014). An international survey of aquaponics practitioners. *Plos One, 9*(7), e102662. https://doi.org/10.1371/journal.pone.0102662

Love, D. C., Uhl, M. S., & Genelloa, L. (2015). Energy and water use of a small-scale raft aquaponics system in Baltimore, Maryland, United States. *Aquacultural Engineering, 68*, 19-27. https://doi.org/10.1016/j.aquaeng.2015.07.003

Martínez-Sánchez, A., Lunaa, M. C., Selma, M. V., Tudela, J. A., Abad, J., & Gil, M. I. (2012). Baby-leaf and multi-leaf of green and red lettuces are suitable raw materials for the fresh-cut industry. *Postharvest Biol. and Tech., 63*, 1-10. https://doi.org/10.1016/j.postharvbio.2011.07.010

Martins, C. I. M., Eding, E. H., Verdegem, M. C. J., Heinsbroek, L. T. N., Schneider, O., Blancheton, J. P., … Verreth, J. A. J. (2010). New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacult. Eng., 43*(3), 83-93. https://doi.org/10.1016/j.aquaeng.2010.09.002

National Oceanic and Atmospheric Administration (Wardensville in Hardy County). (2016). Retrieved April 1, 2016, from https://www.ncdc.noaa.gov/cdo-web/search

Park Seed. (2016). Retrieved May 9, 2016, from http://parkseed.com/red-sails-lettuce-seeds/p/05153-PK-P1/

Pinto, E., Almeida, A. A., Aguiar, A. A., & Ferreira, I. M. P. L. V. O. (2015). Comparison between the mineral profile and nitrate content of microgreens and mature lettuces. *Journal of Food Composition and Analysis, 37*, 38-43. https://doi.org/10.1016/j.jfca.2014.06.018

Rakocy, J. E. (2007). Aquaponics: Vegetable hydroponics in recirculating systems. In M. B. Timmons & J. M. Ebeling (Eds.), *Recirculating aquaculture systems* (2nd ed., pp. 631-672). Cayuga Aqua Ventures, Ithaca, NY.

Rakocy, J. E., Bailey, D. S., Shultz, R. C., & Thoman, E. S. (2004). Aquaponic production of tilapia and basil: Comparing a batch and staggered cropping system. *Acta Hort. (ISHS), 648*, 63-69. https://doi.org/10.17660/ActaHortic.2004.648.8

Rakocy, J. E., Hargreaves, J. A., Bailey, D. S. (1993). *Nutrient accumulation in a recirculating aquaculture system integrated with hydroponic vegetable production*. Paper Presented at the American Society of Agricultural engineers Conference, Spokane WA, June 21-23, 1993.

Rakocy, J. E., Masser, M. P., & Losordo, T. M. (2006). *Recirculating aquaculture tank production systems: Aquaponics–integrating fish and plant culture* (No. 454, pp. 1-16). Southern Regional Aquaculture Center, SRAC Publication. Retrieved from https://srac.tamu.edu/

Resh, H. M. (2013). *Hydroponic Food Production* (7th ed.). CRC Press, Boca Raton, FL.

Saavas, D. (2002). Nutrient solution recycling. In D. Saavas & H. Passam (Eds.), *Hydroponic production of vegetables and ornamentals* (pp. 299-343). Embryo Publications, Athens, Greece.

Savidov, N., Hutchings, E., & Rakocy, J. E. (2007). Fish and plant production in a recirculating aquaponic system: an new approach to sustainable agriculture in Canada. *Acta Hortic., 742*, 209-213. https://doi.org/10.17660/ActaHortic.2007.742.28

Silva, E. C., Maluf, W. R., Leal, N. R., & Gomes, L. A. A. (1999). Inheritance of bolting tendency in lettuce *Lactuca sativa* L. *Euphytica, 109*, 1-7. https://doi.org/10.1023/A:1003698117689

Simko, I., Hayes, R. J., Mou, B., & McCreight, J. D. (2014). Lettuce and Spinach. In S. Smith, B. Diers, J. Specht, & B. Carver (Eds.), *Yield gains in major U.S. field crops* (pp. 53-85). American Society of Agronomy, Madison, WI. https://doi.org/10.2135/cssaspecpub33.c4

U.S. Department of Agriculture. (2013). Western Region SARE Research Innovations. *Organic winter production scheduling in unheated high tunnels in Colorado*. U.S. Dept. of Agr., Washington, D.C.

U.S. Department of Agriculture. (2014). National Statistics Service. *2012 Census of Aquaculture*. U.S. Dept. of Agr., Washington, D.C.

U.S. Department of Agriculture. (2016). National Agricultural Statistics Service. *Vegetables 2015 summary*. U.S. Dept. of Agr., Washington, D.C. Retreived from http://usda.mannlib.cornell.edu/usda/current/VegeSumm/VegeSumm-02-04-2016.pdf

von Wirén, N., Gazzarrini, S., & Frommer, W. B. (1997). Regulation of mineral nitrogen uptake in plants. *Plant and Soil, 196*, 191-199. https://doi.org/10.1023/A:1004241722172

Xiao, Z., Lester, G. E., Park, E., Saftner, R. A., Luo, Y., & Wang, Q. (2015). Evaluation and correlation of sensory attributes and chemical compositions of emerging fresh produce: Microgreens. *Postharvst Biology and Technology, 110*, 140-148. https://doi.org/10.1016/j.postharvbio.2015.07.021

Xu, Q. F., Tsai, C. L., & Tsai, C. Y. (1992). Interaction of potassium with the form and amount of nitrogen nutrition on growth and nitrogen uptake of maize. *J. of Plant Nutri., 15*, 23-33. https://doi.org/10.1080/01904169209364299

Zhao, X., & Carey, E. E. (2009). Summer production of lettuce, and microclimate in high tunnel and open field plots in Kansas. *HortTechnology, 19*(1), 113-119.