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# Comparison of Two Harvest Methods for Lettuce Production in an Aquaponic System

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**Abstract**

Aquaponics is an integrated food production technology of aquaculture and hydroponics. Lettuce (*Lactuca sativa* L.) is an economically important vegetable crop that can be grown aquaponically. In addition to selecting the right choice of lettuce cultivars, developing an optimal harvest strategy could increase lettuce production. Lettuce production using two harvest methods, Cut-and-Come-Again (CC) and Once-and-Done (OD), was evaluated using 'Red Sails' lettuce in a flow-through aquaponic system rearing trout. With the CC method continual harvesting was possible on a weekly basis after the initial harvest, while it took five weeks for each harvest using the OD method. The total yield of lettuce by the CC method was 6.7 kg from 9 trays, while 22.6 kg of lettuce was harvested by the OD method using 54 trays. In harvests by the OD method, 6 times as many seeds were sown compared to the CC method. The average yield per tray harvested by the CC method (744.4 g/tray) was 78% higher than that by the OD method (418.5 g/tray) because the CC method used 6 times less trays. Productivity, calculated by the average yield per growing week, of the two harvest methods at the first harvest was similar, but 4.8 times higher in the CC method than in the OD method at the second harvest due to the shorter harvest time. However, visual and decay ratings of lettuce harvested by the CC method began to decline afterwards. Together, the OD method after two consecutive harvests by the CC method would help growers to obtain increased yield of quality lettuce.

**Keywords:** aquaculture, aquaponics, flow-through system, harvest method, high tunnel, lettuce, trout

## 1. Introduction

Aquaponics is a synergistic combination of fish farming (aquaculture) and soilless plant production in a nutrient solution (hydroponic) and is a potentially sustainable method of food production (Love et al., 2014). In an aquaponic system, the effluent containing nutrients generated from fish production passes through the root zone of the plants (Rakocy, 2007; Buzby & Lin, 2014). In turn, plants are able to utilize the soluble nutrients in the aquaculture effluent for growth. As the fish digests proteins, ammonia ($NH_3$) is excreted into the water. During a process called nitrification, bacteria utilize this nutrient and convert it from ammonium ($NH_4^+$) into nitrite ($NO_2^{-1}$) and then to nitrate ($NO_3^{-1}$) (Rakocy, Masser, & Losordo, 2006; Boyd & Tucker, 2014).

Many economically important vegetables and flowering plants can utilize $NH_4^+$ and $NO_3^{-1}$ for growth (Rana et al., 2011). Among the most commonly grown plants are leafy greens such as lettuce and herbs (J. Oziel & T. Oziel, 2013). Lettuce and herbs make excellent crops because of their high market prices and short production cycles (Rakocy et al., 2006). These crops also have modest nutritional requirements and are well adapted to aquaponic systems (Blidariu & Grozea, 2011).

In this study, a cold water (14-15 °C) flow-through aquaponic system (FTS) was utilized. An FTS involves the continuous flow of water through a tank or a raceway (Adler, Harper, Takeda, Wade, & Summerfelt, 2000; Snow, Anderson, & Wootton, 2012; Soderberg, 1995). These systems are the most commonly used for producing salmonids such as rainbow trout (*Oncorhynchus mykiss*) (Snow et al., 2012). Clean water is typically directed through a channel where the fish are retained and fed (Blidariu & Grozea, 2011). Waste products are carried away in the flow and the effluent is discharged directly into a receiving body of water (Viadero, Cunningham, Semmens, & Tierney, 2005). Unlike recirculating aquaculture systems, flowing water aquaculture systems do not concentrate nutrients through water reuse so the effluent is characterized by high volume and low nutrient content (Hinshaw & Fornshell, 2002).

To produce crops in nutrient limited water, a selection of crops requiring a low level of nutrient for growth is important. One of the low nutrient demanding vegetables is lettuce. Lettuce is also a cool season crop with an optimal temperature ranging from 13 to 16 °C, but can tolerate temperatures as low as -2 °C (Borrelli, Koenig, Jaeckel, & Miles, 2013). As a cool season crop, lettuce is a good candidate for production in a cold water FTS. The cultivar 'Red Sails' was selected in this study because it performed well in previous study utilizing an FTS (Buzby & Lin, 2014; Buzby, West, Waterland, & Lin, 2016b).

In addition to crop selection for FTS, harvest methods can affect the harvestable yield and the timing of harvest. Lettuce is usually destructively harvested with the entire above ground portion of the plant being removed. In order to obtain a second crop the plants have to be resown and the process starts over again. Alternatively, multiple harvests with one time sowing would allow speedy harvest and conservation of resources and time. In this study, two harvest strategies were evaluated: Cut-and-Come-Again (CC) and Once-and-Done (OD). With the CC strategy, all plants in the system are sown at one time, but multiple harvests are made until the crop is no longer productive. In the CC method, only the aerial parts of the lettuce are harvested, leaving the meristem intact, allowing for continued growth. Because there is no need to constantly resow seeds in CC, the CC method could be potentially beneficial for growers by reducing the space needed for production, labor and material costs (seed, media, etc). In contrast, the OD strategy requires more seeds that need to be planted for each harvest.

Development of an effective harvest strategy can help to increase crop yield and efficiency by reducing the period of time it takes to grow a crop to harvestable size. With the CC method, the intact meristem remained after the initial harvest enabled lettuce to regrow quicker than lettuce from the new batch of seeds. The objective of this research was to evaluate lettuce production under two harvest methods (CC and OD) in an FTS. To the best of our knowledge, this is the first study evaluating harvest methods of lettuce production in an FTS.

## 2. Materials and Methods

### *2.1 Facility*

The experiment was conducted in a high tunnel (HT; 7.9 m wide × 12.2 m long) located at Wardensville, West Virginia (394′32″N, 7835′40″W) between May 16th and July 25th, 2013. The HT housed 13 beds (1.2 m wide × 2.4 m long × 26.7 cm deep per bed) constructed with 1.9 cm plywood. Each bed was subdivided into three channels (38.1 cm wide × 2.4 m long per channel). The channels were lined with 20 mil white polyethylene liner (Raven Industries' Dura-Skrim R-Series, Model R20WW; Sioux Falls, SD, USA). Each channel held three floating trays (34.3 cm wide × 66.7 cm long) (Speedling, Inc., Model 32; Ruskin, FL, USA). Aquaculture effluent entered through a valve at the inlet of each channel and drained at the outlet. Water depth was maintained at 23 cm via a standpipe. Influent velocity was adjusted to 0.012 m$^3$ min$^{-1}$ or 10 L min$^{-1}$ with 9.5 mm aperture as described in Dyer (2006). There were sixteen fish tanks (3.82 m$^3$ per tank) in the aquaculture facility. The total number and weight of trout (*Oncorhynchus mykiss*) were 5,520 and 2.8 tons, respectively, during the period of experiment. The average fish density was 90 fish/m$^3$.

### *2.2 Growing Environment and Water Sample Analyses*

Environmental conditions in the HT were continually measured over the course of the experiment. The average day and night air temperature inside the HT was 25.2/17.7 ± 4.0/3.3 °C day/night (mean ± SD). A black shade cloth was installed to reduce the effect of elevated temperatures and excessive light exposure in the HT. Average light intensity was 310.3 µmol m$^{-2}$ s$^{-1}$. Water temperature was also monitored with probes submerged in the water. Average water temperature was 19.2 ± 3.7 °C (mean ± SD). Electrical conductivity (EC), pH and dissolved oxygen (DO) were measured every two weeks. Average pH, EC and DO were 7.0 ± 0.08, 0.13 ± 0.01 mS cm$^{-1}$ and 8.0 ± 0.7 mg L$^{-1}$, respectively.

Water samples for mineral analysis and total suspended solids (TSS) were also taken every two weeks and analyzed according to methods delineated by the American Public Health Association (APHA, 1995) for nutrient content (4500-$NH_3$, phenate method, 4110-$NO_2$ ion chromatography with direct conductivity detection and 4500-P ascorbic acid method) and TSS (2540D). Nutrients measured included total ammonia nitrogen (TAN), nitrite ($NO_2^{-1}$), nitrate ($NO_3^{-1}$), and phosphate ($PO_4^{-3}$). Total suspended solids consisted of nominal particle size of ≥ 1.2 µm.

*2.3 Plant Material*

Seeds of red leaf lettuce 'Red Sails' (*Lactuca sativa* L. var. *crispa*) were purchased from Johnny's Selected Seeds (Winslow, ME, USA) and were vacuum-seeded in 128-cell trays (Speedling, Inc., Model 32; Ruskin, FL, USA) filled with vermiculite (Therm-O-Rock East Inc., Grade 3A; New Eagle, PA, USA) as substrate.

*2.4 Harvest Methods*

Seeds for the first harvests of CC and OD were sown at the same time (Week 0). The CC method was sown only once (CC), while seeds for OD (OD 1-6) were sown six times every week for six weeks to coincide harvest time with CC (Figure 1). OD 2-6 were sown in a staggered method by one week intervals. Lettuce was harvested six times each in both methods until the lettuce in the CC treatment was no longer productive. A total of 24 channels in eight beds were used; three channels for a control, another three channels for the CC harvest method, and 18 channels for the OD harvest method. Each channel contained three trays and each treatment (CC and OD 1-6) had a total of nine trays for three replications (n = 3). The trays in control channels contained only vermiculite without plants to evaluate nutrient removal by the vermiculite substrate. Trays were rotated every week to avoid preferential nutrient uptake by lettuce placed closest to the inlet (Alder et al., 2000). All lettuce from a set of nine trays were harvested at the same time when the average height of lettuce reached 12.7-15.24 cm tall as measured from the media level to the top of the tallest leaf (United States Department of Agriculture, 2013). Lettuce for the CC method was cut 12.7 mm above the meristem (25.4 mm above the tray) to allow for lettuce to grow continuously, while lettuce for the OD method were cut at the base of the plant. For the CC method, the first harvest was carried out five weeks after seeds were sown and the sequential harvests were made every week for a total of six harvests.

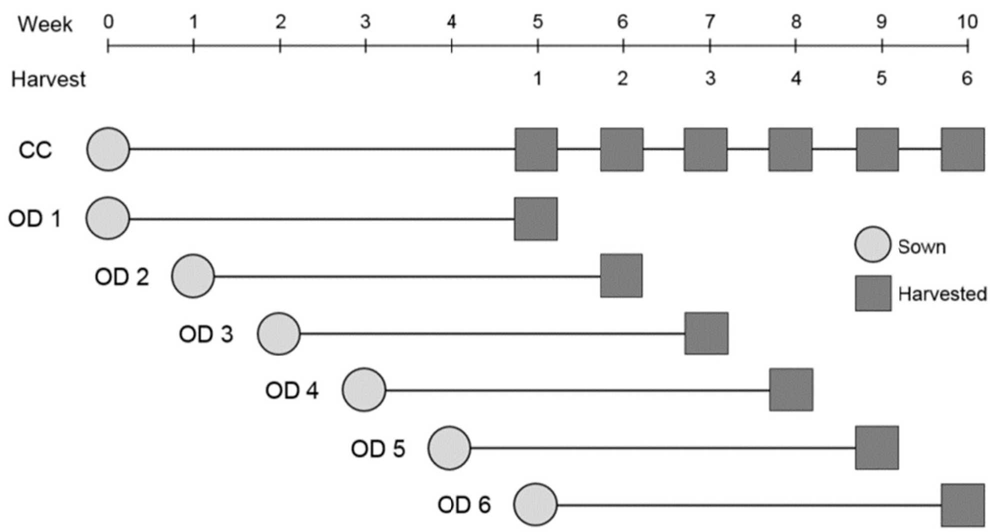


Figure 1. A timeline of the two harvest methods, Cut-and-Come-Again (CC) and Once-and-Done (OD)

*Note*. For the CC method, lettuce was first harvested five weeks after sowing and subsequently harvested every week for the next five weeks. For the OD method, lettuce was harvested once every five weeks. Seeds were sown every week for six weeks in the trays for the OD method so that the harvests for the OD method could coincide with the CC harvests. Six separate harvests for the OD method are labeled (OD 1-6). Circles and squares indicate sowing and harvesting time, respectively. The numbers above the line on top of the diagram shows the number of weeks after the first seeds were sown and the numbers below the line are harvest numbers.

*2.5 Stand Establishment, Average Yield, Productivity*

Stand establishment (SE) described the ability of the seedlings to survive after germination and was determined as the percentage of two-week old seedlings in a tray. SE of trays for the CC method was determined once from the initial trays. SE for the OD method was the average of six sets of individual SE. The average yield and productivity of ‘Red Sails’ lettuce harvested by Cut-and-Come-Again (CC) and Once-and-Done (OD) methods were determined. The average yield was determined by the average harvest fresh weight of the aerial part of the lettuce per tray. Lettuce productivity was determined by the average yield per week.

### *2.6 Quality Analysis*

The deleterious effect of multiple harvests on the lettuce quality ratings of leaf color, texture, decay, and visual quality were determined according to Kader, Lipton, and Morris (1973) and the description of market quality rating scale was provided in Table 1.

Table 1. Market quality rating scale of lettuce from Kadar et al. (1973)

| Score[z] | Visual quality description |
|---|---|
| 5 | Excellent, essentially free from defects |
| 4 | Good, minor defects, not objectionable |
| 3 | Fair, slight to moderate objectionable defects, lower limit of sales appeal |
| 2 | Poor, excessive defects, limit of salability |
| 1 | Extremely poor, not usable/salable |
| Score | Color description |
| 5 | Dark green leaves/heavy redness of leaves |
| 4 | Green leaves/slight to moderate redness of leaves |
| 3 | Dull green leaves/no redness in leaves |
| 2 | Light leaves, premature or dying |
| 1 | Yellow/dead leaves, not usable/salable |
| Score | Leaf texture description |
| 3 | Crispy, ruffed (crunchy – romaine, iceberg; smooth, soft – butterhead) |
| 2 | Wilted, poor texture, not usable/salable |
| 1 | Dead, not usable/salable |
| Score | Decay description |
| 5 | None |
| 4 | Slight, slightly objectionable, may impair salability |
| 3 | Moderate, objectionable, definitely impairs salability |
| 2 | Severe, salvageable, but normally not salable |
| 1 | Extreme, not usable |

*Note*. [z] Ratings are based on presumed consumer acceptance and a rating of one is least desirable.

### *2.7 Statistical Analysis*

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 among treatment means were determined using Tukey’s significance test at $P \leq 0.05$.

## 3. Results

### *3.1 Nutrient Removal*

The concentrations of TAN, $NO_2^{-1}$, $NO_3^{-1}$, $PO_4^{-3}$ and total suspended solids consist of particulates ≥ 1.2 µm (TSS) in the influent and effluent of all channels was measured. There was no difference in nutrient or TSS removal by lettuce grown for CC or OD method (Table 2). The control contained only vermiculite substrate and did not remove nutrient or reduce TSS (Table 2).

Table 2. Level of total ammonia nitrogen (TAN), nitrite ($NO_2^{-1}$), nitrate ($NO_3^{-1}$), and phosphate ($PO_4^{-3}$) in effluent of a cold water flow-through aquaponic system

| Date | Week | TAN | | | $NO_2^{-1}$ | | | $NO_3^{-1}$ | | | $PO_4^{-3}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control[z] | CC | OD | Control | CC | OD | Control | CC | OD | Control | CC | OD |
| | | mg $L^{-1}$ | | | | | | | | | | | |
| 30-May | 2 | 0.39Aa[y] | 0.39Aa | 0.39Aa | 3.90Aa | 4.06Aa | 4.00Aa | 0.28Ca | 0.28Aa | 0.28BCa | 0.15Ba | 0.15Ba | 0.15Ba |
| 13-Jun | 4 | 0.20Da | 0.20Da | 0.20Ea | 1.33Ba | 1.53Ba | 1.85Ba | 0.32Aa | 0.30Aa | 0.31Aa | 0.18Aa | 0.18Aa | 0.18Aa |
| 27-Jun | 6 | 0.38ABa | 0.37Ba | 0.37Ba | 4.00Aa | 4.00Aa | 4.00Aa | 0.30Ba | 0.29Aa | 0.29Ba | 0.17Aa | 0.17Aa | 0.18Aa |
| 11-Jul | 8 | 0.35Ba | 0.36Ba | 0.35Ca | 4.00Aa | 4.00Aa | 4.00Aa | 0.29BCa | 0.29Aa | 0.30Ba | 0.15Ba | 0.15Ba | 0.15Ba |
| 25-Jul | 10 | 0.31Ca | 0.31Ca | 0.31Da | 3.67Aa | 3.33Aa | 3.67Aa | 0.26Da | 0.27Aa | 0.26Ca | 0.15Ba | 0.15Ba | 0.15Ba |

*Note*. [z] Control, vermiculite only; CC, Cut-and-Come-Again; OD, Once-and-Done.

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

Different upper case letters down the column indicate significant difference among sampling dates within a treatment by Tukey's significance test at $P \leq 0.05$.

Different lower case letters across the row indicate significant difference among treatments by Tukey's significance test at $P \leq 0.05$.

### 3.2 SE, Average Yield and Productivity

The average SE of lettuce for the CC and OD harvest methods were 81.6 and 83.3%, respectively. A total of six harvests were made for each harvest method during the ten week growing period. The total yield or harvest biomass of the six harvests combined by the CC and OD harvest methods were 6.7 and 22.6 kg, respectively.

The average yield (harvest biomass per tray) between CC and OD methods at each harvest and among harvests for each method were compared (Table 3). The average yields of the first two harvests were lower than the rest of harvests for each harvest method and were not significantly different between the two harvest methods. However, the average yield from the CC method gradually declined from the third harvest and the average yield of the 5$^{th}$ and 6$^{th}$ harvest by the CC method were nearly 9 and more than 13 times lower than those by the OD method at the same harvest, respectively. On the contrary, the average yield of lettuce harvested by the OD method generally increased or showed no change (Table 3).

Because the growing period of the lettuce in the two harvest methods was different, productivity (average yield per week) at each harvest was also compared (Table 3). The productivity at the first harvest of the CC method was not different from the OD method, but after the first harvest the productivity of the CC method significantly increased (Table 3). The first four harvests of CC had productivity that was generally equivalent to or higher than that of the OD method. However, the productivity of the CC method declined significantly at 5$^{th}$ and 6$^{th}$ harvests, while the productivity of the newly sown lettuce in the OD method increased or stayed at the same level (Table 3).

Table 3. Lettuce yield and productivity from two harvest methods, Cut-and-Come-Again (CC) and Once-and-Done (OD) at each harvest

| Harvest | Average yield (g/tray) | | | Productivity (yield/number of growing weeks) | | |
|---|---|---|---|---|---|---|
| | Treatment[z] | | Significance | Treatment | | Significance |
| | CC | OD | | CC | OD | |
| 1 | 180.91 ABa[y] | 221.02 Da | NS | 36.18 ABa | 44.20 Da | NS |
| 2 | 225.78 Aa | 232.97 Da | NS | 225.78 Aa | 46.59 Db | *** |
| 3 | 130.99 Bb | 459.87 BCa | *** | 130.99 Ba | 91.97 BCb | * |
| 4 | 144.59 ABb | 614.90 Aa | *** | 144.59 ABa | 122.98 Aa | NS |
| 5 | 46.36 Cb | 406.59 Ca | *** | 46.36 Cb | 81.32 Ca | ** |
| 6 | 42.70 Cb | 572.42 ABa | *** | 42.70 Cb | 114.48 ABa | *** |
| Significance | *** | *** | | *** | *** | |

*Note*. [z] CC, Cut-and-Come-Again; OD, Once-and-Done.

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

Different upper case letter down the column indicate significant difference among harvests within a treatment by Tukey's significance test at $P \leq 0.05$.

Different lower case letter across the row indicate significant difference between treatments by Tukey's significance test at $P \leq 0.05$.

NS, *, **, ***: Non-significant, significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

### *3.3 Quality Analysis*

The lettuce harvested by the OD method showed no decay in visual ratings (Figure 2). However, the lettuce harvested by the CC method showed decay and consequently visual ratings began to decline from the third harvest. In some cases, not only dead leaves, but redness along the ribs and leaf margins were visible. There was no difference in ratings of leaf color, and leaf texture among the lettuce harvested by CC or OD method (data not shown).

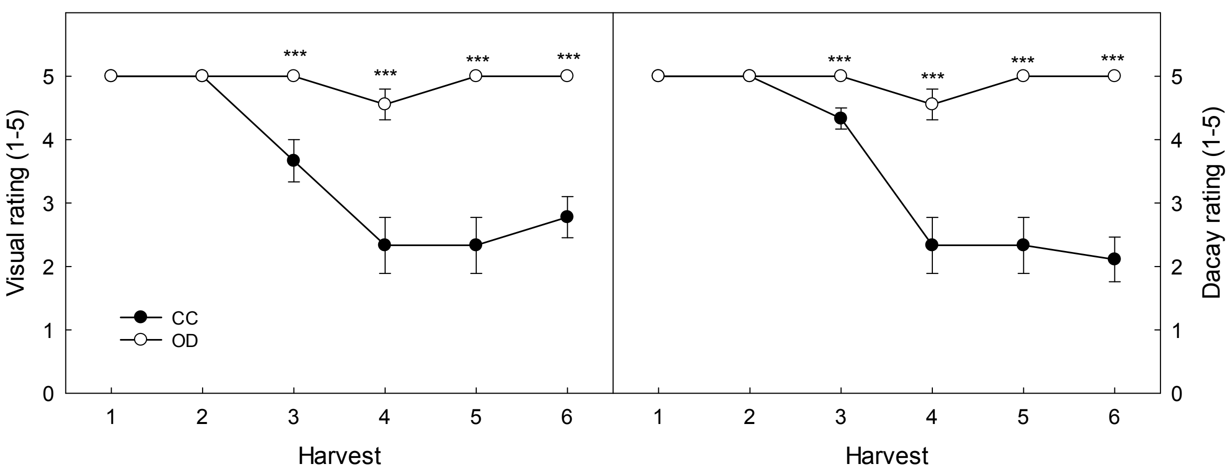


Figure 2. Visual (left) and decay (right) ratings of lettuce in Cut-and-Come-Again (CC) and Once-and-Done (OD) harvest methods at each harvest

*Note*. Vertical bars are standard errors of the means with three replications (n = 3). ***: Significant at $P \leq 0.001$.

## 4. Discussion

### *4.1 Nutrient Removal*

There appeared to be no significant nutrient removal in any channel for control, CC and OD treatments during the ten weeks of the growing period in our study. Because the aquaculture effluent continuously flowed with a low level of nutrient in the FTS, it was difficult to determine the exact amount of nutrient removed by the lettuce. Even though low level of nutrient was provided through FTS, constant supply of those nutrients might have helped lettuce to grow without any nutrient deficiency symptoms. However, nutrient removal was observed in the previous report in an FTS (Buzby et al., 2016b). This discrepancy could be nutrient compensation effect of nutrients generated from the solids accumulated at the bottom of the channels. The solids might have decayed and released nutrient to compensate for the amount of nutrient removed by lettuce, resulting in no apparent removal. In the experiment performed by Buzby et al. (2016b), the accumulated solids were removed prior to water analysis.

### *4.2 SE, Average Yield, and Productivity*

The feasibility of lettuce production in a cold water FTS has been demonstrated (Buzby et al., 2016b). However, an efficient harvest method to increase lettuce yield has not been evaluated. In our study, the harvest method designed to harvest multiple times from a single planting (CC) was compared to a method of a single harvest from a single planting (OD). The average SE was over 80% in both harvest methods. Total yield of lettuce obtained from all six harvests by the CC method was 6.7 kg compared to 22.6 kg by the OD method. The reason for this large discrepancy was because in the CC treatment lettuce was harvested repeatedly from the same plants sown once using nine trays, while total of 54 trays were used for the OD method (nine trays each harvest for six harvests). However, when the yield per tray was taken into consideration for the 10 week of growing period, 744.4 g of lettuce per tray were harvested by the CC method (6.7 kg/9 trays), while 418.5 g of lettuce per tray (22.6 kg/54 trays) was obtained by the OD method. Additionally, it would have taken 30 weeks to harvest, if lettuce were harvested every 5 weeks in the OD method. Thus, the CC method not only increased harvest biomass per tray and production efficiency, but also reduced the cost of resources such as labor for seeding, number of seeds and trays.

The average yields of lettuce measured from the 3$^{rd}$ through 6$^{th}$ harvest using the OD method were higher than the first two harvests. The first two harvests were made on June 20$^{th}$ and 27$^{th}$. Nutrient analyses of the aquaponic water were initiated on May 30$^{th}$, and repeated every two weeks until July 25$^{th}$ (Table 2). According to the nutrient analysis, significantly lower concentrations of TAN (0.2 mg $L^{-1}$) were detected in all treatments on June 13$^{th}$, which was one week prior to the first harvest, compared to the concentration of TAN (0.31-0.39 mg $L^{-1}$) detected during all other time periods. On June 8$^{th}$ through 10$^{th}$, fish were harvested. Prior to the fish harvest, feeding was discontinued temporarily and feeding resumed after the harvest was completed. Nutrient concentrations in the aquaculture water, especially TAN, decreased during this transition period before nutrient level increased to 0.37-0.38 mg $L^{-1}$ in all treatment as observed on the second lettuce harvest (June 27$^{th}$). The fish harvest might have influenced the nutrient level in the wastewater, consequently resulting in lower average yield of the first two harvests in both harvest treatments. This low level of TAN could have affected the 3$^{rd}$ harvest as well, while lettuce harvested after 3$^{rd}$ harvest might not have been affected as much, if not at all. Well-coordinated planning for fish and lettuce production is strongly recommended.

As observed in the last four harvests (harvests 3 through 6), the average yield using the CC method gradually declined to less than 50 g per tray at the 5$^{th}$ and 6$^{th}$ harvest, while the average harvest using the OD method was over 400 g per tray through the 6$^{th}$ harvest. In the CC method, not only average yield decreased, but also visual and decay ratings declined as well (Figure 2), resulting in only the first two harvests being marketable using the CC method which was comparable to the OD method.

Initially, it took five weeks until lettuce reached the marketable size of 12.7-15.24 cm. The productivity at the second harvest by the CC method was particularly high because it only took one more week to regenerate and reach harvestable size. In the CC method, when lettuce was harvested, the meristem remained intact to allow continued growth after the harvest. Because of this accelerated regeneration, the productivity of CC method was significantly higher than that of the OD method at the 2$^{nd}$ and 3$^{rd}$ harvest, and comparable at the 4$^{th}$ harvest even with the declining harvestable yield. However, the productivity of CC treatment significantly declined following the fourth harvest due to a damage from repeated harvests of the same lettuce as evidenced by the decay and visual ratings (Figures 2 and 3). On the other hand, the productivity of OD method was significantly higher at 5$^{th}$ and 6$^{th}$ harvest compared to that of the CC method and remained relatively high from 3$^{rd}$ through the last harvest because they were grown from a new set of seeds at every harvest (Table 3).

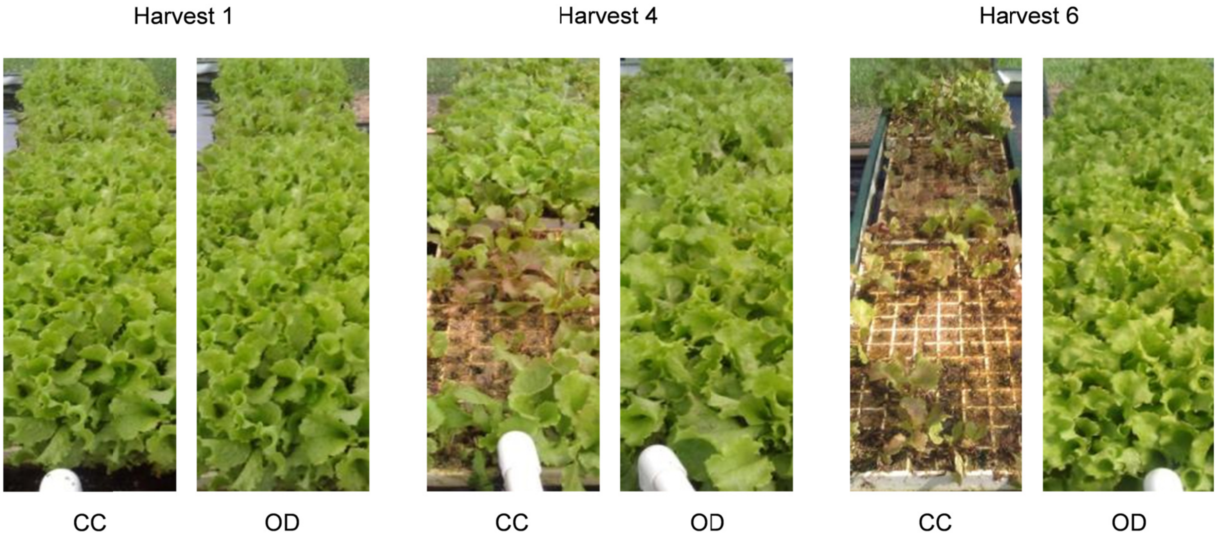


Figure 3. Representative photos of lettuce during the first, fourth, and sixth harvests by Cut-and-Come-Again (CC) and Once-and-Done (OD) harvesting methods

*4.3 Quality Analysis*

The quality of lettuce was analyzed because the repeated harvest of the same plants in the CC method would damage the lettuce and potentially decrease the marketability. There was no difference in ratings of leaf color and leaf texture among the lettuce harvested by CC or OD method (data not shown). However, decay and poor visual ratings due to uncharacteristic reddish leaves and rib veins were observed in the CC method after 2$^{nd}$ harvest (Figure 2), while decay and visual quality ratings were consistently better for lettuce in the OD treatment from the 3$^{rd}$ harvest until the last harvest. Multiple harvesting from the same plants might have caused the lettuce to be stressed (Figures 2 and 3), and consequently resulted in the reduction of average yield (Table 3). Increase in red pigmentation could be due to an increase of anthocyanins in response to the stress (Gazula, Kleinhenz, Streeter, & Raymond, 2005). Rib discoloration was known to be one of the physiological disorders induced by heat stress in lettuce (Jenni, 2005). High quality ratings are essential to crop production for marketability. Although productivity was comparable or greater using the CC method compared to the productivity of OD method for the first four harvests, it is recommended to resow new lettuce seeds after two harvests. While some lettuce at the 3$^{rd}$ or 4$^{th}$ harvest was salvageable, it was time consuming to carefully harvest healthy looking lettuce. Furthermore, direct contact with decaying lettuce using tools and hands while harvesting can potentially spread the damage in other production areas and therefore the lettuce must be handled carefully to prevent further damage and/or the spreading of disease. Decay can render a crop unsalable and affect profitability of the lettuce crop. More importantly, mishandling during harvest or post-harvest could cause an epidemic of plant diseases or food contamination that may result in harmful food consumption to consumers and therefore, food safety testing is also required to guarantee food quality. However, no plant disease symptoms were observed in this study.

Results may also vary based on the time of year. This experiment was conducted in the summer when lettuce could regrow within a week due to warmer temperature and high light levels, however this warm environment would have increased the likely hood of decay. If this experiment were conducted in the spring or fall the growth rate may have been slower, but the reduction in quality using the CC harvest method may also have been ameliorated allowing for more marketable harvests increasing the average yield. With the CC method, lettuce was harvested as loose leaf, because lettuce was cut above the meristem without a base that holds the leaves together. Separated leaves tend to be sensitive to water deficit stress and cannot be stored as long. Therefore, these lettuce leaves need to be stored in a refrigerated storage, sold, or eaten quickly.

*4.4 Estimated Lettuce Production Using Combination of CC and OD Methods*

It required six weeks to harvest the first two rounds of lettuce with good quality from one planting using the CC method, compared to ten weeks of growing time from two separate plantings using the OD method. The CC harvest method provided several advantages over the OD method; the same quantity and quality of lettuce can be harvested in six weeks instead of ten weeks, the seed needs to be sown only once instead of twice, and the number of trays used for lettuce production also can be reduced by half. This result suggests that lettuce production could be increased by choosing the CC harvest method instead of replanting after every harvest. Considering the comparable average yields and quality of the first two harvests between CC and OD methods as well as significantly higher productivity in CC, the OD method combined with the CC method would increase total yield and reduce cost for lettuce production (Table 4). For example, a similar amount of lettuce was harvested by the CC method (1.8 kg; average of 203.3 g/tray × 9 trays) and OD method (2.0 kg; average of 227.0 g/tray × 9 trays) for the first two harvests. However, it would take ten weeks to grow lettuce for the OD method, instead of just six weeks for the CC method. When harvest time is considered, the productivity of CC method would be 67.8 g per tray per week based on our harvest result (total of two first harvest 406.7 g/tray for a six week growing period) compared to 45.4 g per tray per week using the OD method (total of two first harvest 454.0 g/tray for a ten week growing period). When two consecutive harvests using the CC method is repeated as one OD harvest, significantly increased total lettuce yield would have been obtained (Table 4). Therefore, the CC method used in our study will increase not only the total yield of lettuce, but also reduce the cost of production by reducing the number of the trays, seeds and the amount of labor. In addition, there was also a large saving of space when the square footage used for growing lettuce was compared between the CC and OD methods. While the OD method maintained harvestable lettuce for all six harvests it required six times the space. To get the best utilization of space without reduction in yield it would be recommendable to use the CC harvest method twice as it would save space without reduction of productivity. Depending on the size of growing operation it could be desirable. Not all crops can be harvested by the CC method in FTS. Other vegetables such as Swiss chard, herbs and cilantro would also be good candidates for FTS production using the CC method combined with the OD method (Buzby, Waterland, Semmens, & Lin, 2016a; Buzby et al., 2016b; Rakocy, Bailey, Shultz, & Thoman, 2004).

Table 4. Estimated average yield per tray and growing periods using Cut-and-Come-Again (CC) and Once-and-Done (OD) methods

| Harvest Method[z] | | | |
|---|---|---|---|
| CC | | OD | |
| Estimated Average Yield (g/tray)[y] | No. of Weeks[x] | Estimated Average Yield (g/tray)[w] | No. of Weeks[v] |
| 406.8 | 6 | 454.0 | 10 |
| 813.4 | 12 | | |
| 1220.1 | 18 | 908.0 | 20 |
| 1626.8 | 24 | | |
| 2033.5 | 30 | 1362.0 | 30 |

*Note*. [z] CC, Cut-and-Come-Again; OD, Once-and-Done.

[y] The estimated yield was calculated by sum of first two harvest average yield based upon 180.9 g/tray for 1st harvest and 225.8 g/tray for 2nd harvest using the CC method in our study.

[x] Number of weeks was calculated by sum of first two harvest period using the CC method in our study.

[w] The estimated yield was calculated by sum of first two harvest average yield based upon 221.0 g/tray for 1st harvest and 233.0 g/tray for 2nd harvest using the OD method in our study.

[v] Number of weeks was calculated by sum of first two harvest period using the OD method in our study.

## 5. Conclusions

Our study showed that the CC method in an FTS had an advantage over the OD method in productivity because it allowed multiple harvests in a shorter cultivation time and had a benefit of increasing yield. Additionally, the production would be further increased by utilizing a combined harvest method of CC and OD, and the cost labor and materials could also be reduced.

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