Shallow Aggregate Ebb-and-Flow System for Greenhouse Lettuce Production

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Shallow Aggregate Ebb-and-Flow System for Greenhouse Lettuce Production

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science in Horticulture

by

Joseph R. Chidiac
University of Arkansas
Bachelor of Science in Biological Engineering, 2011

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This thesis is approved for recommendation to the Graduate Council

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ABSTRACT

A novel soilless technique for the production of lettuce was developed and evaluated for its viability for ornamental greenhouse growers adapting their ebb-and-flood irrigation benching systems to diversify into growing lettuce, without having to purchase the conventional nutrient film technique (NFT) or deep flow technique (DFT) hydroponic systems. The experimental design was a three by four factorial, with three treatments for aggregate depth (19, 38, and 57 mm) and four treatments for irrigation frequency (once every 1, 2, 4, or 8 hours), for a total of twelve treatment combinations. Each treatment was replicated three times in a randomized complete block treatment design. Prior to each replication, seeds of ‘Rex’ lettuce were planted in phenolic foam sheets and grown until the emergence of four true leaves on each seedling was noted. Sixteen seedlings were then transplanted into each shallow-aggregate ebb-and-flood (SAEF) system. After 42 days, shoot diameters and chlorophyll content were measured and shoots were harvested, weighed fresh, and then dried to acquire their dry weights. Aggregate depth and irrigation frequency were demonstrated to significantly affect lettuce shoot diameter, chlorophyll content, as well as fresh and dry weight. The treatment with 38 mm of aggregate and irrigated every 2 hours resulted in significantly higher average fresh and dry shoot weights than all other treatments. The treatment with 57 mm of aggregate and irrigated every 8 hours resulted in significantly lower average fresh and dry shoot weights than all other treatments as well as the lowest average shoot diameters. Chlorophyll content was significantly higher in the 57 mm treatment irrigated every 8 hours than in all other treatments, and the 19 mm treatments irrigated every 1 and 2 hours produced the lowest average chlorophyll content. There were no significant differences in shoot fresh weight, dry weight, chlorophyll, or diameter among the plants grown...
in optimal SAEF, DFT, or NFT treatments. Therefore, the SAEF system can be considered a viable alternative to produce uniformly marketable lettuce shoots reliably.
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DEDICATION

This thesis is dedicated to my late grandfather Clarence Bernard Norgaard, a great agriculturist, and my late grandmother Evelyn Beatrice Norgaard, a great horticulturist, both of whom had a profound influence on me and helped shape my personality, future, and career. I would also like to dedicate this thesis to my parents Roland and Lisa, both avid gardeners, as well as my wife Katy, who has supported and encouraged me throughout the course of this work.
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Literature Review

Greenhouse Crop Production

Controlled environments (CEs), such as greenhouses, are increasingly being used to grow fresh produce such as tomatoes, peppers, cucurbits, melons, berries, and salad greens (Parry et al., 2004). The greenhouse food crop industry in the United States was estimated to have grown 3 billion USD worth of produce in 2012 and is projected to grow over 4 billion USD by the year 2020 (Rabobank, 2013). In 2012, 1.5 billion USD of this total revenue was attributed to greenhouse grown salad greens, including lettuce, and over 1 billion USD to tomatoes (USDA, 2014).

With over 8000 total hectares of commercial greenhouses in the U.S. (USDA, 2009), growers and consumers are taking advantage of the produce quality, crop consistency, and resource usage efficiency that CEs can provide. Greenhouse production reduces the economic barriers for entry into farming, such as the purchase of agricultural lands and heavy farming equipment. Moreover, small operations may be economically viable in niche markets due to year round production of high value crops. Greenhouses can be tailored to meet the needs of practically any grower and even allow them to cultivate crops on non-arable land, such as deserts, brownfields, and urban rooftops. Introducing crops into CEs, such as greenhouses, can have numerous additional advantages over conventional field production, including giving growers the ability to monitor the plants’ water usage (Giacomelli, 1998; Jones and Tardieu, 1998) and irrigate with maximal efficiency using capillary mat, ebb-and-flow, or drip irrigation systems, for example (Beerling et al., 2014; Stanghellini, 2014). Greenhouses also offer climactic control (Durazzo et al., 2013) and physical protection against weather conditions and pests that have been known to damage outdoor crops and reduce yield and quality (Parry et al.,...
2004; Rosenzweig et al., 2001). Pests and diseases, however, present a challenge even in greenhouse systems. In certain regions, greenhouse crop cultivation also may help to avert heavy metal toxicity in produce due to contaminated agricultural soils (Chang et al., 2014).

While shading has been found to reduce greenhouse temperature and crop transpiration (Kitta et al., 2012), it is also an effective means of reducing light intensity in cases where it exceeds the light saturation point so as to reduce potential damage to the crop. Shading may also be used to limit the Daily Light Integral (DLI) as well as the photoperiod. Conversely, supplemental lighting applied to a greenhouse crop may serve to extend the photoperiod or increase the intensity of Photosynthetically Active Radiation (PAR). Various types of light sources have been tested in the greenhouse setting, including High Intensity Discharge (HID) lamps and Light Emitting Diodes (LEDs). Both HID lamps, such as High Pressure Sodium (HPS) bulbs, and LEDs have shown potential in growing and supplementing crops, and the next generation lighting technologies promise to increase energy efficiency, uniformity, and photosynthetic efficacy (de Villiers et al., 2012) in greenhouse crop cultivation. Since all of these technological advances are easier to apply to greenhouse crop production than field crop production, they give greenhouses additional advantages over field production.

Integrated pest management (IPM) is generally considered to be the strategic combination of control strategies including bio-control agents (BCAs), physical protections, and agricultural control chemicals to minimize potential ecological effects. While IPM is generally considered a success within the field of CE agriculture, the effectiveness of the strategies employed rests on available technology as well as crop species and cultivars. The main advantage to IPM that greenhouses provide is that physical controls, such as screens, climate control, and resistant cultivars, enhance the efficacy of biological and chemical controls (Poncet
et al., 2012). For example, predatory insects confined to the intended crop by the greenhouse structure more effectively control the population of their prey insects than if they were applied in an open field. In addition, applications of pesticides and fungicides in a greenhouse can be more effective and long-lasting than in the field due to the reduction of new pathogen and pest infiltration and prolonging of residual activity. Although field and greenhouse applications of BCAs have not been shown to achieve the success rates that laboratory trials do (Fleckleton, 2000), a BCA is considered effective if it can suppress a pest or pathogen enough to provide sufficient crop protection to ensure that yield and quality are minimally affected. Real-time greenhouse temperature and humidity mapping, using sensors, helps determine the effect of abiotic factors on biotic interactions between crops, pests, and beneficial organisms (Fatnassi et al., 2006). New strategies must continuously be developed to manage the emergence of pest and pathogen resistance, as well as the removal of various chemical agents (Wezel et al., 2009).

Despite the advantages of greenhouse cultivation, there remain concerns that need to be addressed. Consumers are increasingly demanding produce that has been grown with ecological considerations (i.e. with a small carbon footprint and little to no use of agricultural chemicals such as pesticides and fungicides) as well as high quality food for the lowest price possible. This, in turn, means that greenhouse crop growers must continue to reduce their use of resources, reduce their waste levels, and increase productivity through better greenhouse design and management practices that focus on the optimization of efficiency and the early detection of sources of plant stresses that may reduce yield and quality (Stanghellini and Montero, 2012). The development of innovative greenhouse glazing materials will further improve energy conservation, diffuse lighting, allow spectral selectivity, and minimize pest pressure. Moreover, the use of renewable energy sources such as solar, wind, and geothermal power will decrease
energy costs and increase operational efficiency and overall sustainability of greenhouse crop cultivation.

Lettuce Production

*Lactuca sativa* L., or cultivated lettuce, is a diverse species with numerous cultivars and is derived from *L. serriola*, its wild counterpart, through cultivation. *L. sativa* is an annual angiosperm that belongs to the Asteraceae family. *L. sativa* is a dioecious plant, with each individual displaying either male or female flowers exclusively and flower stalks reaching one meter in height. Individuals can continue flowering for up to four weeks, commonly opening flowers in the morning and closing them in the evening. A single female plant can produce dozens of viable seeds. The first evidence of the cultivation of lettuce comes from ancient Egypt, approximately 7000 B.C. (Large, 1972).

Lettuce is currently cultivated around the world wherever temperate climates or microclimates and cool seasons are experienced or can be simulated. The global production of lettuce in 2013 totaled almost 25 million tons, with China, the United States, and India producing 13.5, 3.6, and 1.1 million tons, respectively (FAO, 2014). Lettuce is produced extensively in the United States both in the field and under greenhouse structures either in soil, soilless media, or hydroponic systems. The lettuce produced in the U.S., on over 130,000 hectares, was valued at a total of $2 billion (USDA, 2014), and the vast majority of production took place in California and Arizona and was subsequently distributed across the country (Boriss and Bunke, 2005).

Commercially, lettuce is produced from seed that is propagated by companies dedicated to breeding, maintaining, and propagating select cultivars. Lettuce can also be rapidly regenerated and propagated asexually by tissue culture (Teng et al., 1992). Optimal germination of seed occurs around 25°C, and germination rate decreases steadily as the temperature is decreased.
Temperatures over 27°C inhibit seed germination as well as plant growth (Smith et al., 2011). Optimal vegetative growth for most cultivars has been observed between 16°C and 18°C, and most tolerate temperatures as low as 7°C (Smith et al., 2011). The ideal pH range for lettuce production was found to be between 6.0 - 6.7 in field production and 5.6 - 6.0 for hydroponic production (Morgan et al., 2012). Bolting, and subsequent flowering, is promoted by temperatures above 20°C, as well as long days, and can often be mitigated by shading plants from full sunlight or cooling the root zone by chilling the fertilizer solution or irrigation water.

Heads of lettuce are generally harvested by hand; in the field, the shoot is cut at the base and the roots are left in the soil, while hydroponically grown lettuce heads may be cut but are often pulled from the cultivation system, packaged, and sold with the roots intact. Living lettuce that still has an intact root ball can stay turgid and appear fresh for up to two weeks when refrigerated, as compared to one week or less for field-grown or processed lettuce (Couture et al., 1993). The limiting of oxygen and control of relative humidity is crucial for lettuce storage and transport, and nitrogen gas is often supplemented in packages of cut lettuce produced for supermarket sale.

High value cultivars of lettuce (e.g. romaine, oakleaf, bibb, butterhead types) are increasingly replacing crisp-head varieties (e.g. iceberg) at cultivation facilities (Morgan et al., 2012) due to their aesthetics, textures, and nutritional qualities. These cultivars are being incorporated into greenhouses for soilless or hydroponic production due to their high aesthetic, nutritional, and economic value (Davis et al., 1997; Morgan et al., 2012).
Hydroponic Crop Cultivation

Advantages of Hydroponic Crop Production over Field Crop Production

Hydroponics is the cultivation of plants without the use of soil, either in sterilized containers of dilute fertilizer solution or in inert soilless media that is irrigated. Most hydroponic systems involve the recirculation of fertilizer solution. Hydroponic cultivation allows growers to control the fertilizer regimen and root zone pH (Savvas, 2003), which can be monitored and controlled automatically in real-time (Domingues et al., 2012; Jung et al., 2014) with the use of sensors and injectors. Moreover, hydroponic systems can provide numerous potential advantages over field production, including the ability to separately regulate the shoot and root temperatures, which is not possible under field conditions. The fertilizer solution is often heated or cooled to a different temperature than that of the ambient or greenhouse air, and this helps provide crops with optimal conditions for growth (Thompson et al., 1998). It also enables growers to meet increasingly strict regulations on water and fertilizer usage and runoff (Beerling et al., 2014) as well as contribute to sustainable food production practices and food security (Schnitzler, 2013).

Many other diverse benefits of hydroponics have been reported. For example, the nutritional quality of lettuce and soybean has been improved using hydroponic cultivation when compared to conventional production methods (Bito et al., 2013; Palmero et al., 2012). These production systems also help growers avoid food contamination by human pathogens, such as Escherichia coli and Salmonella enterica (Franz et al., 2007) and even allow the roots to be protected from pathogens during cultivation by using organic additives (Chinta et al., 2014) or carbon dioxide (Kobayashi et al., 2013). Furthermore, bacterial inoculation of hydroponic fertilizer solutions can degrade excess ferulic acid, released from plant roots, which inhibits root
growth (Caspersen et al., 2000). Many of these advantages contribute to higher yields per area in addition to the fact that hydroponic crop cultivation does not require any arable land.

Customizing fertilizer regimens to match crops and available fertilizers is facilitated in hydroponic crop production systems compared to field production. Hydroponics has been evaluated as a means of removing and fixing phosphorus from aquaculture effluent (Adler et al., 2000) and is often paired with aquaculture, making use of the plant nutrients found in fish waste. Aquaponics is the cultivation method linking the production of fish to the production of crops. In addition, hydroponic cultivation systems have allowed growers to utilize brackish water, a mixture of fresh and salt water, to grow crops (Soares et al., 2008).

In effect, hydroponics serves as a technological tool that supports greenhouse crop cultivation. Hydroponics facilitates the application of integrated crop management strategies (Savvas, 2003), which generally include soil conservation, biological control, and minimal use of non-renewable energy sources.

**Nutrient Film Technique**

The nutrient film technique (NFT) has been used as a method of hydroponic cultivation since the early twentieth century and has become the most common hydroponic method for the production of salad greens and herbs (Morgan et al., 2012). It makes use of long troughs or tubes sloped at a 2-3% decline. Hydroponic fertilizer solution is fed into the high side of the troughs through an irrigation manifold that is pressurized by a pump in the reservoir. The solution moves down the troughs as a shallow liquid film (2-3 mm deep) and is collected by a gutter at the low end that transports it back to the reservoir (Smith, 1999). The troughs used in NFT hydroponic systems are usually made of food grade PVC (polyvinyl chloride) and can range from less than
1.5 m to over 20 m in length (commonly 2 - 4 m in length); they can be circular or rectangular cylinders.

Seedlings in their germination cubes or plugs are placed into holes on the top side of the NFT troughs and rest on the bottom surface, where their roots lay flat and are wetted by the recirculated shallow film of nutrient solution. Despite the diffusion of oxygen being maximal into a shallow film of liquid, the fertilizer solution will equilibrate with the temperature of ambient air, which may be too hot to allow sufficient diffusion of oxygen from the air. Therefore, the fertilizer solution is commonly chilled in hot climates or seasons, and a venturi device is usually used when cultivating plants in an NFT system. A venturi supplies additional oxygenation to the solution in the reservoir (Smith, 1999); it functions by using negative pressure caused by the flow of solution through a pipe to inject air bubbles from an adjoining smaller tube in contact with ambient air. When using an NFT system, a grower must check and adjust the reservoir water level, the electrical conductivity (E.C.), and the pH of the fertilizer solution daily to within the desired ranges. This can be done manually or automatically with the use of chemical dosing machines. This hydroponic method is also compatible with aquaponic crop production in that aquaculture effluent can be used as the recirculating fertilizer solution.

The limitations of this system include pump failures, which may result in rapid wilting damage to the crop due to the absence of a humidity buffer. Warm fertilizer solution cannot dissolve as much oxygen, and gradients in temperature, oxygen, and fertilizer can occur down the troughs, especially when troughs are relatively long. Clogs in the feed line micro-tubing may also result in isolated trough dry-outs due to algal accumulation constricting nutrient solution flow in the narrowest components. When using NFT systems for crop production, it is vital to
maintain an alternative source of power and it is recommended to minimize the exposure of the nutrient solution to light so as to inhibit algal growth.

Deep Flow Technique

The Deep Flow Technique (DFT), also known as a floating raft system, is another common hydroponic method for growing salad greens and herbs. Aquaponic crop cultivation commonly involves DFT systems. This method relies on 10 cm to 40 cm deep ponds or runs filled with fertilizer solution and actively aerated by an air compressor pumping through air-stones placed along the bottom of the reservoir (Smith, 1999). Fertilizer solution may or may not be recirculated by a pump. The reservoirs can be adapted to any size and may even take the form of an entire greenhouse floor or section that has been designed to function as a watertight container. Rafts, usually made of extruded polystyrene foam or LDPE (low density polyethylene), are floated on top of the fertilizer solution in the reservoir, and plants are fastened to these with shoots above and roots descending into the fertilizer solution.

When using a DFT system, the grower must monitor and maintain the reservoir water level, the E.C. and pH of the fertilizer solution and must pay special attention to dissolved oxygen levels throughout the reservoir. It has been shown that the application of micro-bubbles to the solution of DFT systems can enhance the growth of lettuce (Park and Kurata, 2008), and that root zone cooling can alleviate heat stress induced by high ambient air temperatures (Thompson et al., 1998).

Large quantities of water and nutrients are required to operate DFT systems, relative to NFT. Dissolved oxygen levels within the reservoir can vary by location and change with temperature. This effect is exacerbated as plants develop and larger root systems that remove more and more oxygen from the solution. It should be noted, however, that maintaining a stable
fertilizer solution temperature is more easily feasible in DFT system as compared to NFT systems. Aquaponic crop cultivation most commonly involves DFT systems.

**Aggregate Beds**

Aggregate Beds (ABs) are tracks, troughs, or channels filled with a soilless aggregate substrate for the cultivation of aquatic or semi-aquatic plant crops, such as watercress and water spinach, which require flooding (Morgan et al., 2012). The soilless aggregate substrates employed include calcined clay gravel or expanded clay beads, as these do not float excessively or degrade rapidly. The aggregate is generally more than 8 cm deep and is either constantly or periodically flooded and can be sub-irrigated or top-irrigated. Aggregate particle size will affect water retention and air-filled pores (Lipiec et al., 2007), as will the bulk density of the aggregate. While some fertility may come from the substrate itself, the rest must be provided to the crop by the addition of organic or inorganic fertilizers in the case of biologically active or sterile systems, respectively.

**Aeroponics**

Aeroponics is a method of cultivating plants without using a root substrate and without immersing roots in fertilizer solution directly. In an aeroponic system, plants are fastened to boards with the shoots pointing outwards and the roots pointing inwards into a dark, air filled chamber in which the roots are sprayed or misted with the fertilizer solution that is recollected and recirculated. These systems can have a horizontally flat conformation but they may also comprise an A-frame set-up or be pyramidal in shape. A grower will need to check and adjust the water level, E.C. and pH of the fertilizer solution periodically. The advantage of aeroponic over hydroponic cultivation is that oxygenation of the roots is guaranteed by the root zone air
chamber. However, if the pump fails or if a spray nozzle clogs, roots can dry out quickly and the crop may be irreversibly damaged. In addition, attention must be paid to controlling the temperature of the fertilizer solution in aeroponic systems, as a fine mist is subject to warming to ambient air temperatures faster than a film or pool of solution.

**Ebb-and-Flow Irrigation**

Although it is not conventionally used as a food crop production system, ebb-and-flow benching is a common and widely employed method of irrigation for potted ornamental crops, such as poinsettia and pansy. Similar in format to aggregate beds, the main difference is that the aggregate or potting substrate used with ebb-and-flow irrigation is only flooded momentarily but generally kept much dryer. Ebb-and-flow benching involves large, watertight trays that are 15 – 30 cm deep and made of food grade styrene plastics or aluminum. The trays are grooved at the bottom to increase rigidity and direct the flow of water. Each tray must be level throughout for proper function, and each has two drain fixtures: one supplies the flow of fertilizer solution to the surface of the tray and drains once the irrigation is over, and the other acts as a safety mechanism to ensure the fertilizer solution does not exceed the desired depth in the trays. This method generally involves flooding the trays to a depth of 10 to 20 cm for up to 10 minutes to allow the potting substrate to absorb fertilizer solution before being drained (Bartok, 1989). The nutrient solution is typically recirculated to reservoirs to be used again, and the pH and electrical conductivity of the solution must be monitored and adjusted, since they affect the physiology of the crop (Poole and Conover, 1992; Wortman, 2015). The trays can be flooded from a reservoir containing nutrient solution to fertilize, but they can also be flooded from a reservoir with pure water, to flush out salts, or with a cleaning agent to disinfect the system.
Ebb-and-flow irrigation makes use of the concept of capillary action, as sub-irrigating potted plants reduces the leaching of fertilizer out of the growing substrate and minimizes the quantity of water and fertilizer used (Dole et al., 1994; Poole and Conover, 1992). It has been shown to use 88% less water than an overhead irrigation system (Neal and Henley, 1992). Although sub-irrigation does cause a salt-buildup in the top-most layer of the growing substrate, this appears to have no detrimental effect on plant growth.

Dutch tray systems are ebb-and-flow trays with wheels on the bottom that allow them to be easily moved across greenhouse benches. Flood floors, on the other hand, are built into the greenhouse structure or a section thereof. Both flood floors and ebb-and-flow irrigation benching have demonstrated high water usage efficiencies in greenhouses, especially under partial saturation conditions (Gent et al., 2012; Gent and McAvoy, 2011), which have the added benefit of suppressing Pythium and Phytophthora and reducing the occurrence of root rot and plant death when compared with top-irrigation practices (Elmer et al., 2011; Stanghellini et al., 2000).

The effect of the potting media on water uptake and distribution during irrigation has been simulated for ebb-and-flow irrigation (Anlauf, Rehrmann, and Schacht, 2012), and the depth, duration, and frequency of flooding affect the moisture levels of the growing substrate. The frequency and duration of flooding are controlled with electronic timers, and the depth is determined by the safety drain adjusted to the desired height. Ebb-and-flow irrigation benching has been used to grow potted herbs, but not to grow lettuce. However, due to its versatility, ebb-and-flow benching can potentially be used for novel applications, such greenhouse lettuce production.
Shallow Aggregate Ebb-and-Flow System

The objective of our research is to develop a new hydroponic cultivation system that integrates elements from NFT, aggregate beds, and ebb-and-flow irrigation benching and to optimize it to deliver comparable performance to NFT and DFT systems in the production of greenhouse lettuce. Greenhouse growers who currently employ ebb-and-flow benching commonly do so to irrigate potted ornamental plants. These growers have been showing increasing interest in diversifying into fast cycling, edible crops such as lettuce. Our novel system will enable them to convert their irrigation systems for growing lettuce hydroponically without purchasing NFT or DFT systems.

We have named our novel system the Shallow Aggregate Ebb-and-Flow (SAEF) system. The ebb-and-flow irrigation trays are filled with a shallow layer of aggregate and flooded to a shallow depth of 2-3 mm based on what is commonly used for NFT (Smith, 1999). We chose expanded clay aggregate due to its physical properties, including bulk density, air pore size, hydrophilicity, and potential for indefinite reuse. The particle and pore size helps determine the water retention properties of the aggregate (Lipiec et al., 2007) The aggregate’s moisture retention allows for pulse irrigation instead of continuous recirculation as is the case in NFT and DFT systems. It also serves to block light from the root zone and thereby contribute to inhibiting algae growth in the fertilizer solution.

The performance of this system and the growth and development of lettuce depend on the substrate used, the depth of the substrate, and the frequency and duration of irrigation. Therefore, we sought to evaluate different aggregate depth and irrigation frequency treatments, using expanded clay aggregate, to optimize the SAEF system before performing a side by side comparison with the NFT and DFT systems.
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Additional index words: Lactuca sativa, salad greens, controlled environment agriculture, hydroponics, soilless, hydroton, vegetable, pulse irrigation, subirrigation.

Abstract

A novel soilless technique for the production of lettuce was developed and evaluated for its viability for ornamental greenhouse growers adapting their ebb-and-flood irrigation benching systems to diversify into growing lettuce, without having to purchase the conventional nutrient film technique (NFT) or deep flow technique (DFT) hydroponic systems. The experimental design was a three by four factorial, with three treatments for aggregate depth (19, 38, and 57 mm) and four treatments for irrigation frequency (once every 1, 2, 4, or 8 hours), for a total of twelve treatment combinations. Each treatment was replicated three times in a randomized complete block treatment design. Prior to each replication, seeds of ‘Rex’ lettuce were planted in phenolic foam sheets and grown until the emergence of four true leaves on each seedling was noted. Sixteen seedlings were then transplanted into each shallow-aggregate ebb-and-flood (SAEF) system. After 42 days, shoot diameters and chlorophyll content were measured and shoots were harvested, weighed fresh, and then dried to acquire their dry weights. Aggregate
depth and irrigation frequency were demonstrated to significantly affect lettuce shoot diameter, chlorophyll content, as well as fresh and dry weight. The treatment with 38 mm of aggregate and irrigated every 2 hours resulted in significantly higher average fresh and dry shoot weights than all other treatments. The treatment with 57 mm of aggregate and irrigated every 8 hours resulted in significantly lower average fresh and dry shoot weights than all other treatments as well as the lowest average shoot diameters. Chlorophyll content was significantly higher in the 57 mm treatment irrigated every 8 hours than in all other treatments, and the 19 mm treatments irrigated every 1 and 2 hours produced the lowest average chlorophyll content. There were no significant differences in shoot fresh weight, dry weight, chlorophyll, or diameter among the plants grown in optimal SAEF, DFT, or NFT treatments. Therefore, the SAEF system can be considered a viable alternative to produce uniformly marketable lettuce shoots reliably.

Controlled environments, such as greenhouses, are being increasingly used in the US to grow crops such as tomatoes, peppers, cucurbits, berries, and salad greens including lettuce (Parry et al., 2004). The greenhouse food crops industry in the United States produced 3 billion USD worth of produce in 2012 and is projected to exceed 4 billion USD by the year 2020 (Rabobank, 2013). High value cultivars of lettuce (e.g. romaine, oakleaf, bibb, butterhead types) are increasingly replacing crisp-head varieties (e.g. iceberg) at cultivation facilities (Morgan et al., 2012) due to their aesthetics, flavors, textures, and nutritional qualities. These cultivars are being incorporated into greenhouses for soilless or hydroponic production due partly to their sensitivity to extreme environmental conditions, such as drought, flood, high winds, and abnormally cold or hot weather (Davis et al., 1997; Morgan et al., 2012). Over 1.5 billion USD
could be attributed to greenhouse and field grown lettuce and salad greens produced in the USA in 2012 (NASS/USDA, 2014).

Growing crops in a greenhouse environment with hydroponic systems can have numerous advantages over conventional field production, including giving growers the ability to better monitor the plants’ water usage (Giacomelli, 1998; Jones and Tardieu, 1998) and irrigate more efficiently (Beerling et al., 2014; Stanghellini, 2014). Greenhouses also offer better climactic control than field crop production systems (Durazzo et al., 2013) as well as increased protection against pathogens and pests that have been known to damage field crops (Parry et al., 2004; Rosenzweig et al., 2001) and reduce yield and quality. This is due to growers’ improved ability to limit the proliferation of pests and pathogens and to control the environment in favor of conditions that inhibit pest and pathogen health and spread. Growers and consumers are taking advantage of the produce quality, crop consistency, and operational efficiency that greenhouses offer due in part to limiting plant stress. Moreover, greenhouse production reduces the economic barriers for entry into farming due mainly to the fact that it is scale neutral and takes advantage of niche markets and year round production.

Hydroponic cultivation methods offer the advantages of improving control over the fertilizer regimen and solution pH (Savvas, 2003), which can even be controlled automatically in real-time (Domingues et al., 2012; Jung et al., 2014). Moreover, the ability to separately regulate the shoot and root temperatures in hydroponic systems helps provide crops with optimal conditions for growth (Thompson et al., 1998). Hydroponic cultivation has been shown to improve the nutritional quality of certain crops (Bito et al., 2013; Palmero et al., 2011) as well as reduce the accumulation of heavy metals from contaminated field soils (Chang et al., 2014). Hydroponic cultivation systems also help growers avoid food contamination by *E. coli* and *S.*
*E. coli* (Franz et al., 2007) and even allow the roots to be free from pathogens during cultivation (Chinta et al., 2014; Kobayashi et al., 2013). All of these factors are contributing to the expansion of the greenhouse industry worldwide.

Lettuce is by far the most common salad green grown, and the greenhouse salad greens industry commonly employs the Nutrient Film Technique (NFT) or Deep Flow Technique (DFT) for the hydroponic production of lettuce. The NFT production system uses tubes, troughs, or gullies made of food grade PVC and sloped at a 2 - 3 % decline. Plants are inserted from the top of the tubes, and their roots lay flat and are wetted by a thin film (2 - 3 mm) of continuously recirculated fertilizer solution (Morgan et al., 2012). This technique requires a pump to continuously circulate fertilizer solution; therefore, rapid damage or death of the crop can result if the pump fails. The temperature of the fertilizer solution is difficult to maintain in a thin film and dissolved oxygen levels in the root zone decrease as the temperature of the fertilizer increases. Therefore, the fertilizer solution in NFT systems is often cooled, and is usually aerated with a venturi device. The water level, electrical conductivity (E.C.) and pH of the fertilizer solution in the reservoir must be closely monitored and adjusted within the desired ranges.

In contrast, the DFT system comprises large tanks or raceways of fertilizer solution on top of which plants are floated on foam or plastic boards with their roots extending below and hanging freely in the 15 cm to 30 cm deep fertilizer solution (Morgan et al., 2012). These systems may or may not have pumps for circulation yet usually employ bubblers for oxygenation of the fertilizer solution. This technique requires large volumes of solution compared to NFT, and therefore large amounts of fertilizer, and it is crucial that it be actively aerated by an air compressor to ensure proper root oxygenation (Park and Kurata, 2008). These systems can also
be expensive to build, and present the challenge of maintaining uniformly oxygenated fertilizer solution throughout the reservoir.

Aggregate beds are a type of hydroponic system used mainly for growing flooded crops such as watercress and water spinach. They comprise long channels, ditches, or runs filled with aggregate (e.g. expanded clay beads or calcined clay gravel) to a depth of 8 cm or more. The aggregate is then either constantly or periodically flooded and can be sub-irrigated or top-irrigated (Morgan et al., 2012).

Many greenhouse growers of ornamental crops have expressed interest in diversifying into food crops to increase profits and alleviate the price pressures and low margins on their traditional crops, such as poinsettia, mums, and bedding plants. However, the cost of buying NFT or DFT systems often proves to be prohibitive, especially since the greenhouses would have to be emptied during the installation, and production would cease temporarily. Many of these growers use ebb-and-flow irrigation benches, which involve trays or flood floors that are flooded to a depth of 10 to 20 cm for up to 10 minutes to sub-irrigate potted plants; this functions to reduce leaching and the quantity of water and fertilizer used (Bartok, 1989; Schmal et al., 2011). The fertilizer solution is typically recirculated to reservoirs to be used again. Dutch tray systems are ebb-and-flow trays on rolling benches that allow them to be moved around the greenhouse. Therefore, a technique to cultivate lettuce using this type of irrigation benching would solve their problem.

We propose to develop an alternative system to NFT and DFT to enable growers to use their ebb-and-flow benching for lettuce cultivation. This novel system, referred to as Shallow Aggregate Ebb-and-Flow (SAEF), combines aspects of ebb-and-flow benching and aggregate bed systems. It involves ebb-and-flow trays filled with aggregate 19-57 mm deep, sub-irrigated
periodically 2-3 mm deep, and drained immediately. The advantages of this method would include added savings in water, mineral nutrients, and energy. This is due to the fact that large volumes of nutrient solution will not be necessary and that pumps will not be running continuously. In addition to providing root support for larger plants, the aggregate medium employed would serve as a wet/dry wick during every irrigation cycle. This would, in effect, increase root oxygenation and decrease root disease incidence (Elmer et al., 2011). With the proposed system, these growers could make use of their current irrigation equipment and only have to purchase the reusable aggregate medium. They would also be able to easily convert back to ebb-and-flow irrigation for potted plants whenever necessary, as no irreversible changes would be made to the benching system.

Two major variables that would affect the efficacy of this system would be the depth of the aggregate, which affects the seedling cube placement, and the irrigation frequency. Both variables affect the availability of water, nutrients, and oxygen to the roots and could potentially affect the physical support received by the plant. Therefore, the first objective of this study was to determine the optimal aggregate depth and irrigation frequency for lettuce production in SAEF systems. The second objective was to determine the overall viability and competitiveness of the SAEF by side by side comparison with NFT and DFT systems.

Materials and Methods

Effects of Aggregate Depth and Irrigation Frequency

The experiments were conducted in a glass-glazed A-frame greenhouse located at the University of Arkansas Agricultural Research Station in Fayetteville, AR (36.0764° N, 94.1608° W, 427 m ASL) under naturally occurring ambient light. The greenhouse had heating and
cooling set-points of 15 °C and 20 °C, respectively. Temperature, relative humidity (RH) and daily light integral (DLI) were monitored using a Watchdog™ 2475 weather station (Spectrum Technologies Inc., Bridgend, U.K.).

Seed of ‘Rex’ lettuce (Johnny’s Selected Seeds, Fairfield, ME) were sown into 2.5 cm x 2.5 cm x 3.7 cm phenol-formaldehyde polymer foam sheets (162 Horticubes, Smithers-Oasis, Kent, OH). One seed was sown per cell, and the foam sheets were placed in 108 cm x 25 cm x 5 cm grooved propagation trays (AmHydro, Arcada, CA) that were sub-irrigated for 2 minutes every 6 hours with water until seedlings had two expanded cotyledons, at which point a fertilizer solution (Table 1) was applied at an electrical conductivity (E.C.) of 0.6 dS m⁻¹ and a pH of 5.9. When the seedlings developed two expanded true leaves, the E.C. of the fertilizer solution was increased to 1.0 dS m⁻¹. This propagation method was developed at the University of Arkansas based on personal communications with local hydroponic lettuce producers and technicians from AmHydro and Smithers-Oasis.

After seedlings developed four expanded leaves, they were transferred to the SAEF systems. These SAEF systems consisted of 90 cm x 90 cm x 15 cm plastic ebb-and-flow trays (Botanicare, Chandler, AZ) filled with 5-15 mm diameter expanded round clay aggregate (Hydroton® Plant!, Binley, Coventry, U.K.). Each ebb-and-flow tray was a self-contained unit, with its own fertilizer solution reservoir and pump. All tanks were filled with fertilizer solution maintained at an E.C. of 1.4 dS m⁻¹ and a pH of 5.9 for the duration of the 42-day cultivation period. The fertilizer recipe was developed by the Evans lab at the University of Arkansas’ Department of Horticulture (Table 1).

One of each of three trays was filled with aggregate to a depth of 19 mm, 38 mm, or 57 mm. In the trays filled to 19 mm and 38 mm, the cubes were placed so they were in contact with
the tray surface and covered by the aggregate by 50% and 100% of the cube height, respectively. The seedlings were planted on 18 cm centers. In the tray filled to 57 mm, the cubes were 100% covered by the aggregate, but the bottoms of the cubes were elevated 19 mm off the ebb-and-flow tray surface, making this treatment similar to dry hydroponics in that the seedling cubes are not wetted during irrigation (Smith, 1999). During irrigation, the trays were flooded to a depth of 2-3 mm, based on NFT, and drained after 30 seconds. The duration of the irrigation was determined by the time required for the pump to wet the entire surface of the tray and related to the size of the pumps and trays. This resulted in a wetting of the aggregate-tray interface with fertilizer solution. For each aggregate depth and cube placement, irrigation was conducted every 1, 2, 4, and 8 hours, which resulted in a 3 x 4 factorial experimental design.

According to our personal communications with hydroponic lettuce farmers, 42 days after transplant was determined to be the average time required for lettuce to grow to an average marketable weight of approximately 200 g, depending on cultivar. After 42 days in the SAEF systems, the plants’ relative chlorophyll content was determined using a SPAD-501 portable leaf chlorophyll meter (Minolta Corp., Tokyo, Japan) by taking a measurement on the youngest fully expanded leaf of each head of lettuce (Rodriguez and Miller, 2000). Chlorophyll content was measured to assess greenness, which growers and consumers attribute to quality. Two perpendicular diameters were measured on each head and averaged to determine shoot diameter per sub-sample. The shoots were then harvested and their fresh mass was determined. Dry shoot mass was determined after drying lettuce shoots at 60 °C until completely dry.

The experimental unit was a tray consisting of one SAEF system and containing 16 plants or subsamples, with a set depth of aggregate and irrigation frequency. The optimization experiment had a 3 by 4 factorial treatment structure (Edmondson, 1994) and each treatment was
replicated 3 times with complete random blocking (Fernandez, 2007) across time and space. Due to equipment limitations, each block of time was divided into two runs consisting of 6 out of the 12 total treatments each (Table 2). A 30% light reduction shading was applied to the greenhouse at the end of April each year and was removed in September.

An analysis of variance (ANOVA) was conducted to determine if significant differences occurred among the treatments for any of the dependent variables. Where significant differences occurred, a least significant difference (LSD) mean separation test ($\alpha=0.05$) was conducted to determine specific differences between treatments (Schaarschmidt and Vaas, 2009).

**Comparison of SAEF to NFT and DFT**

Experimental protocols and statistical analysis for the comparison experiment were as previously described, except where indicated. After seedlings developed four expanded leaves, 90 seedlings were transplanted into a SAEF system comprised of two 120 cm x 240 cm x 15 cm reinforced plastic ebb-and-flow trays (Botanicare, Chandler, AZ) filled to 38 mm ± 5 mm with expanded round clay aggregate (Hydroton® Plant!, Binley, Coventry, U.K.) and irrigated for 2 minutes every 2 hours using a single pump and reservoir. The seedling cubes were all in contact with the ebb-and-flow tray surface. Additionally, 90 seedlings each were transplanted into an NFT and a DFT system. The NFT system comprised twelve 1.5 m long troughs (AmHydro, Arcata, CA), and the DFT system comprised two 90 cm x 180 cm x 15 cm grow trays (Botanicare, Chandler, AZ) on which 5 cm thick polystyrene foam boards (Dow, Midland, MI) were floated. Treatments in this experiment were the three types of cultivation systems and the experimental unit for this study was one SAEF, NFT, or DFT system, each containing 90 plants or subsamples and each replicated three times, for a total of 9 experimental units (Table 3). Fertilizer solutions for all systems were maintained at an E.C. of 1.4 dS m$^{-1}$ and a pH of 5.9 for
the duration of the 30-day cultivation cycle. We adjusted our duration for the cultivation cycle from 42 days to 30 days based on our observation that, at 42 days after transplanting, our average lettuce shoot weights were exceeding the average marketable shoot weight.

The occurrence of tip-burn and root discoloration was recorded for each lettuce plant on harvest day, and these values were reported by percentage of incidence. These data were collected to determine whether any of the treatments produced unmarketable heads due to tip-burn, or if they resulted in root discoloration which hydroponic farmers consider undesirable.

**Results**

*Effects of Aggregate Depth and Irrigation Frequency*

There was no significant interaction between aggregate depth and irrigation frequency (Table 4), therefore, the data were pooled across aggregate depth and irrigation frequency. Aggregate depth significantly affected lettuce shoot fresh mass, chlorophyll, and mean shoot diameter (Table 4), and the p-value for shoot dry mass was 0.095. Irrigation frequency significantly affected lettuce shoot fresh mass, shoot dry mass, chlorophyll, and mean shoot diameter (Table 4). Block significantly affected lettuce shoot fresh mass, shoot dry mass, chlorophyll, and mean shoot diameter (Table 4).

The treatment with 38 mm of aggregate resulted in significantly higher average fresh shoot mass than the treatment with 57 mm, while the treatment with 19 mm did not result in significantly different fresh shoot mass than the treatments with 38 mm and 57 mm of aggregate (Fig. 1). While aggregate depth did not significantly affect average dry shoot mass, the numerical trend was similar to that of average fresh shoot mass (Fig. 2). The treatment with 57 mm of aggregate resulted in significantly higher average shoot chlorophyll than the treatment with 19
mm, and neither were significantly different than the treatment with 38 mm for the average shoot chlorophyll variable (Fig. 3). The treatments with 19 and 38 mm of aggregate both resulted in significantly higher average shoot diameters than the treatment with 57 mm of aggregate (Fig. 4).

The treatments irrigated every 1 and 2 hours resulted in significantly higher average fresh mass than the treatment irrigated every 8 hours, and the treatment irrigated every 4 hours did not result in significantly different fresh shoot mass than the rest of the treatments (Fig. 5). The treatment irrigated every 2 hours resulted in a significantly higher average dry shoot mass than the treatments irrigated every 4 and 8 hours, but not significantly different than the treatment irrigated every 1 hour (Fig. 6). The treatment irrigated every 8 hours resulted in a significantly higher average shoot chlorophyll than the treatments irrigated every 4, 2, or 1 hours (Fig. 7). The treatments with irrigation every 1, 2, and 4 hours resulted in significantly greater average shoot diameters than the treatment irrigated every 8 hours (Fig. 8).

**Comparison of SAEF to NFT and DFT**

Block significantly affected lettuce shoot dry mass and diameter (Table 5), and the p-values for shoot fresh mass and chlorophyll were 0.1692 and 0.165, respectively. Treatment had no significant effect on lettuce fresh shoot mass, dry shoot mass, chlorophyll, or mean shoot diameter (Table 6).

Root discoloration and tip burn were observed in the DFT and NFT systems but not in the SAEF system. All incidents of both root discoloration and tip burn were very mild, barely visible damage that did not affect marketability. The NFT system had a 6.7% average rate of occurrence for both root discoloration and tip burn, and the DFT system had a 1.8% average rate of occurrence for both variables.
Discussion

An aggregate depth of 38mm resulted in the highest average fresh shoot mass (marketable weight) as well as average dry shoot mass (biomass production) while resulting in average shoot chlorophyll and shoot diameters that were not significantly different from the highest observed treatment averages. Average chlorophyll SPAD measurements increased as the depth of aggregate increased. Darker leaves with abnormally concentrated chlorophyll have been associated with drought stress (Songsri et al., 2009), and our treatment with 57 mm deep aggregate having resulted in the highest chlorophyll values supports this conclusion. In the treatments with 57 mm of aggregate, the seedling cubes were not in contact with the tray surface and therefore did not come into direct contact with the fertilizer solution during sub-irrigation and initially relied on capillary action for moisture. Note that these results may differ with the use of other types or sizes of aggregate (Anlauf et al., 2012; Lipiec et al., 2007). While the treatment with 38 mm of aggregate did not result in significantly higher average shoot fresh mass than the treatment with 19 mm of aggregate, some algal growth was observed in the treatment with 19 mm, indicating that 38 mm of aggregate may more effectively block unwanted light from the root zone. Therefore, 38 mm was chosen as the optimal depth of expanded clay aggregate for this system.

The treatments irrigated every 1 hour and 2 hours resulted in the highest average shoot fresh and dry masses. The treatment irrigated every 8 hours may have experienced lower aggregate moisture content between irrigations than other irrigation treatments, resulting in comparatively stunted growth and the lowest average shoot diameter (Stanhill, 1957). The highest average chlorophyll content was observed in the treatment irrigated every 8 hours, which also supports the association of abnormally high chlorophyll content with drought stress (Songsri
et al., 2009). Since there were no significant differences in our variables between the treatments irrigated every 1 hour and 2 hours, the treatment irrigated every 2 hours was chosen as optimal for this system. This also allows the greenhouse grower to irrigate twice as many zones within the same period of time, compared to irrigating every 1 hour.

Block significantly affected the average fresh shoot mass, dry shoot mass, shoot chlorophyll, and shoot diameters. This may have occurred due to varied temperature, airflow, and relative humidity conditions dependent upon the physical location in the greenhouse over the course of the experiments (Gruda, 2007). PAR intensity also fluctuated throughout the different seasons. A complete randomized block experimental design accounts for this as much as possible and was therefore used in this study (Fernandez, 2007).

The comparison experiment resulted in no significant differences between the treatments for average fresh shoot mass, dry shoot mass, chlorophyll content, or shoot diameter. The SAEF system produced numerically higher average values for all variables than the NFT and DFT systems over three replications (Table 6).

While cases of root browning and tip burn were not observed in the SAEF systems in either the optimization nor the comparison experiment, a few cases were observed in the DFT and NFT systems, and they were all mild and did not affect marketability. The aggregate in the SAEF system may have alleviated the occasional lack of access to moisture, oxygen, or darkness that the roots experience in DFT and NFT systems; however, this effect on roots and shoot tips was shown to be insignificant in this study. The root browning was likely to be abiotic in nature, due to the fact that all systems were thoroughly sterilized with 5% hydrogen peroxide before each use.
The results of the optimization experiment indicate that the depth of the expanded clay aggregate and the frequency of sub-irrigation significantly affected the growth and development of ‘Rex’ butterhead lettuce in a SAEF production system. The lack of significant differences in lettuce growth variables between the treatments in the comparison experiment signifies that the SAEF system can produce heads of lettuce of comparable quality to the DFT and NFT systems.

Conclusion

The SAEF system was considered a viable and competitive alternative to the NFT and DFT hydroponic crop cultivation systems and can be recommended to growers currently using ebb-and-flow irrigation benching as a method for growing lettuce. Further study will elucidate whether this conclusion holds for lettuce cultivars other than that which was used in this study and for climates and greenhouses different than those available to us in this study. The SAEF system may also have the potential to grow other types of crops such as other salad greens and herbs, and further evaluations will determine this potential. It is plausible that mimicking the conventional wet and dry cycle of the root zone in field agriculture while in a sterile and inert medium is what allowed the SAEF system to be successful for the cultivation of greenhouse grown lettuce.


Smith, R. 1999. Hydroponic crop production. NZ Hydroponics International Ltd., Tauranga, NZ.


Table 1. University of Arkansas standard recipe for concentrated hydroponic fertilizer stock solution for growing salad greens.\(^z\)

<table>
<thead>
<tr>
<th>Ingredient(^y)</th>
<th>Tank A (g)</th>
<th>Tank B (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (NO(_3))(_2)·3(H(_2)O)</td>
<td>1350</td>
<td></td>
</tr>
<tr>
<td>KNO(_3)</td>
<td>450</td>
<td></td>
</tr>
<tr>
<td>10% Fe-DTPA</td>
<td>61.6</td>
<td></td>
</tr>
<tr>
<td>KH(_2)PO(_4)</td>
<td></td>
<td>330</td>
</tr>
<tr>
<td>Mg SO(_4)·7(H(_2)O)</td>
<td></td>
<td>645</td>
</tr>
<tr>
<td>K(_2)SO(_4)</td>
<td></td>
<td>115.5</td>
</tr>
<tr>
<td>MnSO(_4)·H(_2)O</td>
<td></td>
<td>4.65</td>
</tr>
<tr>
<td>H(_3)BO(_3)</td>
<td></td>
<td>4.11</td>
</tr>
<tr>
<td>ZnSO(_4)·7(H(_2)O)</td>
<td></td>
<td>0.246</td>
</tr>
<tr>
<td>CuSO(_4)·5(H(_2)O)</td>
<td></td>
<td>0.585</td>
</tr>
<tr>
<td>(NH(_4))(_6)Mo(_7)O(_24)·4(H(_2)O)</td>
<td></td>
<td>0.1665</td>
</tr>
</tbody>
</table>

\(^z\) Amounts shown are to be added to 20 L of water in each tank, making a 100x concentrated stock solution. When adding concentrated stock solution to the hydroponic fertilizer reservoir, equal amount from Tank A and Tank B must be used. B = boron, Ca = calcium, Cu = copper, Fe = iron, H = hydrogen, Mg = magnesium, Mn = Manganese, Mo = molybdenum, N = nitrogen, K = potassium, O = oxygen, P = phosphorus, S = sulfur, and Zn = zinc.

\(^y\) Inorganic salt fertilizers listed: Ca (NO\(_3\))\(_2\)·3(H\(_2\)O) = calcium nitrate trihydrate, KNO\(_3\) = potassium nitrate, 10% Fe-DTPA = 10% iron chelate, KH\(_2\)PO\(_4\) = potassium monophosphate, Mg SO\(_4\)·7(H\(_2\)O) = magnesium sulfate heptahydrate, K\(_2\)SO\(_4\) = potassium sulfate, MnSO\(_4\)·H\(_2\)O = manganese sulfate hydrate, H\(_3\)BO\(_3\) = boric acid, ZnSO\(_4\)·7(H\(_2\)O) = zinc sulfate heptahydrate, CuSO\(_4\)·5(H\(_2\)O) = copper sulfate pentahydrate, (NH\(_4\))\(_6\)Mo\(_7\)O\(_24\)·4(H\(_2\)O) = ammonium molybdate tetrahydrate.
Table 2. Dates for start and end of each block and run during the optimization experiment. 

<table>
<thead>
<tr>
<th>Block</th>
<th>Run</th>
<th>Start Date</th>
<th>End Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1/29/2015</td>
<td>3/12/2015</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3/26/2015</td>
<td>5/5/2015</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>10/2/2015</td>
<td>11/13/2015</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>11/15/2015</td>
<td>12/26/2015</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>12/31/2015</td>
<td>2/11/2016</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2/14/2016</td>
<td>3/27/2016</td>
</tr>
</tbody>
</table>

Start date refers to the day the lettuce seedlings were transplanted into the cultivation systems, and end date refers to the day plants were harvested. Treatments were randomized within each block, with half of the treatments occurring within the first run and half within the second run of each block.

Table 3. Dates for start and end of each block during the comparison experiment. 

<table>
<thead>
<tr>
<th>Block</th>
<th>Start Date</th>
<th>End Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3/15/2016</td>
<td>4/14/2016</td>
</tr>
<tr>
<td>2</td>
<td>4/16/2016</td>
<td>5/16/2016</td>
</tr>
<tr>
<td>3</td>
<td>5/18/2016</td>
<td>6/17/2016</td>
</tr>
</tbody>
</table>

Start date refers to the day the lettuce seedlings were transplanted into the cultivation systems, and end date refers to the day plants were harvested.
Table 4. Analysis of variance (ANOVA) table for optimization experiment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Fresh Wt. Pr &gt; F</th>
<th>Dry Wt. Pr &gt; F</th>
<th>Chlorophyll Pr &gt; F</th>
<th>Diameter Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>0.0045</td>
<td>0.0149</td>
<td>0.0002</td>
<td>0.0021</td>
</tr>
<tr>
<td>Depth</td>
<td>0.0494</td>
<td>0.0950</td>
<td>0.0180</td>
<td>0.0025</td>
</tr>
<tr>
<td>Frequency</td>
<td>0.0022</td>
<td>0.0135</td>
<td>0.0006</td>
<td>0.0004</td>
</tr>
<tr>
<td>Depth.Frequency</td>
<td>0.7543</td>
<td>0.8699</td>
<td>0.3477</td>
<td>0.7205</td>
</tr>
</tbody>
</table>

A P-value less than 0.05 indicates a significant effect.

The listed sources of error were tested for significant effects on the recorded variables.

The block represents the physical position in the greenhouse as well as the time period in which each replication was run.

The depth was one of three treatments: either 19 mm, 38 mm, or 57 mm of expanded clay aggregate used in the ebb-and-flow tray.

The frequency was the interval between separate irrigations and was one of three treatments: irrigation every 1 hr, 2 hrs, 4 hrs, or 8 hrs.

The interaction between depth and frequency treatments was evaluated for significant effects on the recorded variables.

Table 5. Analysis of variance (ANOVA) table for comparison experiment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Fresh Wt. Pr &gt; F</th>
<th>Dry Wt. Pr &gt; F</th>
<th>Chlorophyll Pr &gt; F</th>
<th>Diameter Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>0.1692</td>
<td>0.0327</td>
<td>0.1650</td>
<td>0.0199</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.1740</td>
<td>0.2184</td>
<td>0.1996</td>
<td>0.0278</td>
</tr>
</tbody>
</table>

A P-value less than 0.05 indicates a significant effect.

The listed sources of error were tested for significant effects on the recorded variables.

The block represents the physical position in the greenhouse as well as the time period in which each replication was run.

The treatments were three cultivation systems: nutrient film technique (NFT), deep flow technique (DFT), and shallow aggregate ebb-and-flow (SAEF).
Table 6. Average shoot fresh mass, dry mass, chlorophyll, and diameter of ‘Rex’ lettuce grown in deep flow technique (DFT), nutrient film technique (NFT), and shallow aggregate ebb-and-flow (SAEF) cultivation systems in a greenhouse.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh Wt. (g)</th>
<th>Dry Wt. (g)</th>
<th>Chlorophyll (SPAD)</th>
<th>Diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFT</td>
<td>195.17</td>
<td>8.17</td>
<td>29.35</td>
<td>18.99</td>
</tr>
<tr>
<td>NFT</td>
<td>182.32</td>
<td>8.66</td>
<td>32.01</td>
<td>18.81</td>
</tr>
<tr>
<td>SAEF</td>
<td>214.47</td>
<td>9.01</td>
<td>32.31</td>
<td>20.72</td>
</tr>
</tbody>
</table>

*Measurements were taken 30 days after transplanting lettuce seedlings with 4 true leaves into the production systems.*
Figure 1. Mean fresh shoot mass in grams of ‘Rex’ butterhead lettuce grown in shallow aggregate ebb-and-flow (SAEF) systems for 42 days with means pooled for aggregate depth.

Means were pooled for each aggregate depth (19 mm, 38 mm, and 57 mm). Means are for three replications. Analysis of variance was performed, and mean separation was done using least significant differences. Bars indicate least significant difference = 59.19. 1 mm = 0.0294 inch.
Figure 2. Mean dry shoot mass in grams of ‘Rex’ butterhead lettuce grown in shallow aggregate ebb-and-flow (SAEF) systems for 42 days with means pooled for aggregate depth.

Means were pooled for each aggregate depth (19 mm, 38 mm, and 57 mm). Means are for three replications. Analysis of variance was performed, and mean separation was done using least significant differences. Bars indicate least significant difference = 2.06. No significant differences were found between these means. 1 mm = 0.0294 inch.
Figure 3. Mean shoot chlorophyll in SPAD units of ‘Rex’ butterhead lettuce grown in shallow aggregate ebb-and-flow (SAEF) systems for 42 days with means pooled for aggregate depth.

Means were pooled for each aggregate depth (19 mm, 38 mm, and 57 mm). Means are for three replications. Analysis of variance was performed, and mean separation was done using least significant differences. Bars indicate least significant difference = 2.9. 1 mm = 0.0294 inch.
Figure 4. Mean shoot diameter in centimeters of ‘Rex’ butterhead lettuce grown in shallow aggregate ebb-and-flow (SAEF) systems for 42 days with means pooled for aggregate depth.

Means were pooled for each aggregate depth (19 mm, 38 mm, and 57 mm). Means are for three replications. Analysis of variance was performed, and mean separation was done using least significant differences. Bars indicate least significant difference = 1.86. 1 mm = 0.0294 inch.
Figure 5. Mean fresh shoot mass in grams of ‘Rex’ butterhead lettuce grown in shallow aggregate ebb-and-flow (SAEF) systems for 42 days with means pooled for irrigation frequency.

Means were pooled for each irrigation frequency (every 1, 2, 4, or 8 hours). Means are for three replications. Analysis of variance was performed, and mean separation was done using least significant differences. Bars indicate least significant difference = 58.62. 1 mm = 0.0294 inch.
Figure 6. Mean dry shoot mass in grams of ‘Rex’ butterhead lettuce grown in shallow aggregate ebb-and-flow (SAEF) systems for 42 days with means pooled for irrigation frequency.

Means were pooled for each irrigation frequency (every 1, 2, 4, or 8 hours). Means are for three replications. Analysis of variance was performed, and mean separation was done using least significant differences. Bars indicate least significant difference = 2.14. 1 mm = 0.0294 inch.
Figure 7. Mean shoot chlorophyll in SPAD units of ‘Rex’ butterhead lettuce grown in shallow aggregate ebb-and-flow (SAEF) systems for 42 days with means pooled for irrigation frequency.

Means were pooled for each irrigation frequency (every 1, 2, 4, or 8 hours). Means are for three replications. Analysis of variance was performed, and mean separation was done using least significant differences. Bars indicate least significant difference = 2.87. 1 mm = 0.0294 inch.
Figure 8. Mean shoot diameter in centimeters of ‘Rex’ butterhead lettuce grown in shallow aggregate ebb-and-flow (SAEF) systems for 42 days with means pooled for irrigation frequency.

Means were pooled for each irrigation frequency (every 1, 2, 4, or 8 hours). Means are for three replications. Analysis of variance was performed, and mean separation was done using least significant differences. Bars indicate least significant difference = 1.86. 1 mm = 0.0294 inch.