TIMING AND RATES OF TWO HYDROGEN PEROXIDE PRODUCTS TO CONTROL ALGAE AND EVALUATION OF WATER TEMPERATURE ON LETTUCE QUALITY FOR HYDROPONIC PRODUCTION

By

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Abstract: Hydroponic systems are an ideal environment for algal growth. Algae poses several threats to hydroponic crops and needs to be controlled. Use of hydrogen peroxide (H_2O_2) as an algaecide has been on the rise in recent years. Hydrogen peroxide also serves as a signaling molecule for processes involved in plant growth and development. Thus, the objective of our study was to find the optimum rate and application timing of two H_2O_2 products 'Zerotol' and 'PERpose Plus' to control algae in an Ebb and Flow hydroponic system without inhibiting growth of pepper and tomato plants. Among various factors which affect hydroponic production, temperature of the nutrient solution is considered one of the most important determining factors of crop yield and quality. Optimum water temperature in a hydroponics system positively influences the production of vegetables. Hence, the effect of three different water temperature (18.3°C, 21.1°C, and ambient) on growth and quality of seventeen cultivars of lettuce was studied in an NFT hydroponic system. Results indicated that weekly application of 70 mL of either Zerotol or PERpose Plus produced the best results in terms of controlling algae and improving the growth of pepper and tomato plants. For lettuce, hydroponics growers can maintain the water temperature at 21.1°C for greater growth and yield, while 18.3°C water temperature can be maintained to produce lettuce with greater °Brix. Our results also indicated that all the cultivars in Romaine type performed better in terms of growth and quality compared to all other lettuce types.

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CHAPTER I

LITERATURE REVIEW

Hydroponics

Soil has long been the most popular growing medium for plants and provides nutrients, water, and anchorage that are important for successful plant growth (Dholwani et al., 2018). However, growing plants in soil does have some serious limitations like presence of pest, diseases and nematodes, unfavorable soil compaction, and unsuitable soil reactions. In addition, field production is labor intensive and also requires large space and large volumes of water (Sardare and Admane, 2013). Conventional practice of growing plants in soil is facing various challenges these days as the fertility of land has been decreasing because of urbanization, haphazard use of chemicals, and climate change (Jensen, 1997). Under these circumstances, soilless culture was introduced successfully as an alternative and has been gaining popularity all over the world (Butler and Oebker, 2006). Hydroponics allows for intensive crop production in areas where the soil is unfit for agricultural use (Jensen, 1999).

The word ‘hydroponics’ is derived from two Greek words ‘hydro’ which means water and ‘ponos’ which means labor. Hydroponics in its simplest form is the science of providing the essential mineral nutrients to the plant from a culture solution (Berry, 1996). In hydroponic production, plants can be grown with or without the use of an inert medium like rock wool, coco coir, saw dust, peat moss, and vermiculite, but the common denominator is that plants get all of the required nutrients from an enriched water nutrient solution (Singh, 2017).

Hydroponics as a growing technique has an old and honorable history starting long before W. F. Gericke first coined and published the term hydroponics in the 1937 (Jones, 1982). The hanging gardens of Babylon and the floating gardens of China were the earliest examples of hydroponics system. Julius von Sachs and Wilhem Knop introduced water as a growing medium for plants for their research which was investigating the effects of chemical compounds on plant growth and the use of water as a growth media allowed for easier isolation of compounds (Morgan and O'Hare, 1978). In the United States, interest began to develop in the possible use of complete nutrient solutions in 1925 when research workers were having difficulties with frequent replacement of greenhouse soil year to year. This resulted in the replacement of a natural soil system with nutrient solution as an alternative growing medium (Jensen, 1997).

Hydroponics offers advantages compared to soil-based systems due to greater control of irrigation, nutrient management as well as modification of water temperature (Landers, 2017). Hydroponics is gaining in popularity because of less pest and disease incidence, absence of weeds, faster growth, and year-round production of crops (Resh, 1978). It is also a relatively clean and easy method of production. In addition, hydroponics also helps in water conservation as the water used in hydroponics is filtered, replenished, and recycled. It has been found that good-quality vegetables can be grown effectively in hydroponics using 85% to 90% less water than the traditional field production (Sharma et al., 2019). Many growers have found the benefits of hydroponics to be multiple, with greater efficiency of space, quicker yield, and better consistency of products all year-round

(Hughes, 2016). Plants grown in hydroponic systems are also found to have better qualities and increased content of antioxidants compared to the crops grown in a conventional soil-based system. Sgherri et al. (2010) reported increased contents of vitamin C, vitamin E, lipoic acid, total phenols, and rosmarinic acid in hydroponically grown basil (*Ocimum basilicum* L.) in comparison with that grown in soil. Hydroponically grown crops are also reported to have greater yield than soil-grown crops. For example, 10% higher fruit yield per plant was found in hydroponically grown raspberries (*Rubus idaeus* L.) compared to the soil-grown raspberries (Treftz and Omaye, 2015). Similar results were found in hydroponic lettuce (*Lactuca sativa* L.) with 11 ± 1.7 times higher yields compared to conventionally produced lettuce (Barbosa et al., 2015). Furthermore, environmental advantage of hydroponics is also worth mentioning, particularly in environmentally protected areas, or regions with limited water resources. Rouphael et al. (2005) reported greater water use in soil (256 $L \cdot m^{-2}$) during the growing cycle compared with the water requirements of plants grown respectively in coco fiber (221 $L \cdot m^{-2}$), perlite (200 $L \cdot m^{-2}$), and pumice (185 $L \cdot m^{-2}$). Hydroponic systems make use of recycled nutrient solution which enables a considerable reduction of fertilizer application and a drastic restriction or even a complete elimination of nutrient leaching from greenhouses to the environment (Savvas, 2002).

Nutrient Film Technique (NFT)

There are many systems of hydroponics such as nutrient film technique (NFT), drip system, Ebb and Flow, water culture, wick system, and the list continues as different designs emerge and are renamed (Mohammed and Sookoo, 2016). Among various hydroponics systems, NFT and its modifications are the most popular and commonly used systems

(Jensen and Collins, 1985). It is a modification of the circulating type of hydroponics in which plants are grown without using a substrate, by maintaining a channel of liquid media around the roots (Resh, 1978). In this system, plant roots are suspended in channels where a thin film of nutrient solution passes through thus keeping the roots moist but not water-logged (Mohammed and Sookoo, 2016).

An NFT system consists of a trough on a slope of 0.3% to 2% with the roots of plants floating at the bottom of this trough. Nutrient solution is recycled through this trough as the nutrient solution is applied at the elevated end so that the solution flows down through the trough at exactly the rate required to keep the roots completely wet (Singh, 2017). The width and length of the channels can vary according to the crops. For smaller crops like lettuce and chrysanthemums (*Dendranthema grandiflora* L.), troughs of 4 to 8 cm wide are sufficient, whereas for crops like tomato (*Lycopersicum esculentum* L.) and sweet pepper (*Capsicum annuum* L.), wider trough of about 15 cm is said to be optimum (Os et al., 2019).

An NFT system is among the most versatile hydroponics system being both energy-efficient as well as environmentally friendly. An NFT system seemed to be an ideal system of growing plants when it first appeared, as there was no expense of substrate because of optimal control over the watering of the plant roots (Os et al., 2019). A simple means of watering and great control over the rooting environment makes an NFT system a popular and easy technique of growing plants (Graves, 1983). Furthermore, NFT is a more convenient system for growing crops like basil, lettuce, and Swiss chard (*Beta vulgaris* L.) which requires frequent cutting, as the channels can be placed at required heights above the ground which makes it more comfortable for greenhouse employees during transplanting and harvesting (Singh, 2017). However, NFT systems also has disadvantages. Since roots are growing in confined channels, this system is not suitable for plants with large root system as roots can clog the channels. Sometimes, entire crops can wilt and die if the pump stops working even for a few hours, especially in hot weather. In addition, there is also a greater risk of infection from plant to plant as all the plants are growing in a same nutrient solution (Jones Jr., 2005).

Ebb and flow system

The Ebb and Flow table is a versatile system which can be used with or without a variety of growing mediums. This system works on a very simple principle. This system works by temporarily flooding the grow tray with nutrient solution through a water pump until a certain level is reached. The solution stays there for a certain period of time to provide nutrients and moisture to the plants and then the solution is drained back into the reservoir (Sharma et al., 2019). The table is slightly sloped so that the nutrient solution runoff goes to a drain and then returns to the tank below. The flow can be either made constantly on or a timer can be installed for keeping the flow on and off according to the need of the plants. In this system, the nutrient solution is recirculated to the reservoirs and used again, and the pH and electrical conductivity (EC) of the solution should be monitored and adjusted according to the crops requirement as both affect the physiology of a crop (Wortman, 2015). Different kinds of crops have been successfully grown in Ebb and Flow systems, however, the problem of root rot, algae, and mold is very common (Nielsen et al., 2006).

Algae

Algae is an informal term for a group of organisms carrying out oxygenic photosynthesis but are not higher plants. However, there is not a definition of algae which is generally accepted. The phylogenetic definition of algae does not agree that all algae have oxygenic photosynthetic capacity as mentioned in the definition above because there have been multiple losses of the capacity for photosynthesis (Raven and Giordano, 2014). According to another definition, algae are thallophytes that have chlorophyll as their primary photosynthetic pigment and lack a sterile covering of cells around their reproductive cells. Likewise, the colorless Prototheca under Chlorophyta are all devoid of any chlorophyll (Lee, 2008). The size range of the algae spans seven orders of magnitude. Many algae like diatoms, Euglenophyta, and Dinoflagellates consist of only one cell while there are some which have millions of cells like giant kelp and brown algae (Andersen and Lewin, 2021).

Algae reproduce very quickly and can be found in almost every water source with good sunlight and a few inorganic nutrients to grow. Observations on algae that occur in hydroponic culture suggests that optimum growing conditions for hydroponic plants, which include water, nutrients, and light are also optimum for the growth of algae (Coosemans, 1995; Morgan, 2014). Algae not only creates an unsightly mess in hydroponics but can also create several other problems like reduction of dissolved oxygen, depletion of nutrients, pH swings, and production of harmful toxins (Morgan, 2014). It is not surprising that algae poses serious problems from a technical viewpoint (obstructions), from a cultural viewpoint (nutritional competition), as well as from an economic viewpoint (Coosemans, 1995). Algae can cause problems with the water supply system by clogging drippers or pipes (Ravina et al., 1997). Some algae species are found to produce toxins that can inhibit or even stop the growth of crops (Borowitzca, 1995). The harmful effects of algae are fairly well-known. However, some of the algae species are also found to be beneficial to the plants. Several reports suggest that certain types of algae release growth regulators like auxins, cytokinins, gibberellins, abscisic acid, and ethylene (Van Staden, 1999). Some researchers have also found that certain algae species can help in prevention of root disease by producing anti-fungal and anti-bacterial compounds (Morgan, 2014).

In any hydroponics system, complete control over all algae growth can be difficult. However, there are several options that can be used to control algae in a hydroponic system if there is excessive growth. One of the popular practices is to prevent or at least control algae growth using a black plastic sheet to cover the water tank in hydroponic systems (Schwarz and Gross, 2004). Controlling algae by basic cleaning or using plastic films to cover physical structures have been found to be effective, but time for meticulous application and high use of labor makes it impractical (Caixeta et al., 2018). Alternatively, sanitization with chlorine bleach and application of algaecides are also among the popular methods for controlling the algae growth in a hydroponic system (Morgan, 2014). Woo et al. (2004) used NaOCl to control algae in hydroponic lettuce production and reported 95% inhibition in algae occurrence. However, lettuce yield decreased with increasing NaOCl concentration. Further, application of chemical methods have not been actively explored for controlling algal bloom, partly because of concerns on environmental acceptability of the chemicals, and the potential side effects on other organisms (Anderson, 1997; Anderson, 2009).

Hydrogen peroxide (H_2O_2)

Several options exist for controlling algae growth in a hydroponic system with application of hydrogen peroxide being one of them. Hydrogen peroxide is a chemical compound with the formula H_2O_2. It is defined as a Reactive Oxygen Species (ROS) generated from molecular oxygen (O_2) with relatively high stability and a long half-life (Hossain et al., 2015). It is a well-known agent for disinfection and water treatment with a strong oxidizing capability (Bauza et. al, 2014), which is devoid of any harmful byproducts since it decomposes into water and oxygen (Barroin and Feuillade, 1986). However, the toxicity of H_2O_2 on algae is found to be affected by several factors such as light intensity, temperature, and algae species (Drábková et al., 2007). Many studies have been conducted to investigate the effects of hydrogen peroxide in water sources with positive results that hydrogen peroxide has been found effective in destroying algae species like cyanobacteria as well as the cyanotoxins produced by the algae (Svrcek and Smith, 2004). Zhou et al. (2017) reported that combining hydrogen peroxide with sunlight gave better results for eradicating cyanobacterial blooms. It was found that the cyanobacterial stress and injury due to H_2O_2 were dose dependent, and the control effectiveness and degradation of H_2O_2 were better and faster under full light than under shading. Hydrogen peroxide decomposes forming molecular oxygen and water that then reacts with organic matter in a redox reaction to oxidize it. In other words, algae and hydrogen peroxide cannot coexist (Caixeta et al., 2018). The impacts of H_2O_2 on algae mainly include inhibiting the photosynthetic activity and genes expression and affecting the membrane integrity (Qian et al., 2010).

In plant cells, H_2O_2 is produced predominately during photosynthesis, photorespiration, and also in respiration (Ismail et al., 2015). Application of H_2O_2 as a plant growth promoter has increased recently in small as well as large scale farming. Hydrogen peroxide is said to be the most stable ROS, thus playing an important role as a signaling molecule in various physiological processes, including photosynthesis, respiration, translocation, and transpiration which ultimately leads to improved crop yield and productivity (Slesak et al., 2007). Khan et al. (2016) reported increased activity of Rubisco and PS II which ultimately increased net photosynthesis with H_2O_2 treatment in mustard (*Brassica juncea* L.) plants. Similar results were found by Nazir et al. (2002) who reported improved gas exchange parameters, chlorophyll content, net photosynthesis, along with improved scavenging of ROS and recovery of photosynthetic efficiency with H_2O_2 treatment.

On the other hand, H_2O_2 at higher levels has also been recognized as a toxic molecule that can cause damage at different levels of cell organization and thus losses in cell viability (Gechev et al., 2005). Xia et al. (2014) reported that a low concentration of H_2O_2 promoted stomatal opening, whereas a high concentration of H_2O_2 promoted stomatal closure in tomato (*Solanum lycopersicum* L.) 'Ailsa Craig'. Similarly, Khan et al. (2016) reported decreased net photosynthesis and plant dry weight with greater concentration of H_2O_2 application. The mutual relationship between positive and negative effects of H_2O_2 in any biological system depends mostly on the H_2O_2 dose and concentration, on physiological conditions, and on the specificities of processes affected by H_2O_2 (Wojtyla et al., 2016).

Pepper

Pepper belongs to the family Solanaceae and is native to northern South America and southern North America. Its fruit is characterized by high levels of antioxidants such as ascorbic acid, vitamin C, carotenoids, and phenolic compounds (Chaki et al., 2015). The species includes a wide variety of shapes, sizes, and tastes such as jalapenos, bell peppers, cayenne peppers and New Mexico chile. Bell pepper is the second most important crop of the Solanaceae family after tomato and contains sufficient amounts of all antioxidants like vitamin A, C, E, along with wide range of vitamin B (Khan et al., 2018). In hydroponics, peppers require 21 to 28°C for germination and 18 to 23°C during propagation (Resh, 1978).

Very limited number of studies have been conducted on the effects of hydrogen peroxide on growth and quality of pepper plants. The addition of hydrogen peroxide (H_2O_2) in hydroponics systems was found to affect the size of the pepper fruits (Hernandez et al., 2012). Hydrogen peroxide has also been effectively used to protect peppers against pepper golden mosaic geminivirus (PepGMV) infections. In another study, hydrogen peroxide at 6, 14, and 18 mM induced tolerance to PepGMV either by absence of symptoms as well as by attenuating and/or delaying infection (Mejía-Teniente et al., 2019). Effect of hydrogen peroxide on germination of pepper seeds was studied by Nandi et al. (2016) who reported that the treatment of pepper seeds with hydrogen peroxide, regardless of concentration, significantly reduced seed infestation with seed borne pathogens and improved health of the seeds.

Tomato

Tomato belongs to the nightshade family Solanaceae and is cultivated extensively for its edible fruits ("Tomato", 2019). It is a rich source of energy, carotenoids, flavonoids, phenolics, mineral nutrients, vitamin C, and dietary fibers which are beneficial and serve as protective ingredients for human health (Beecher, 1998). Tomatoes have been grown successfully as a hydroponic crop. Among open and closed hydroponic systems, better performance of various cultivars of tomato was found in closed system with greater marketable yield. Because of fruit cracking, yield was reduced in open system (Maboko et al., 2011). There are several advantages of growing tomatoes hydroponically in comparison to the soil-based cultivation. Akhtar-Jehan et al. (1994) recorded greater number, size and, weight of tomato fruits as well as increased number of leaves in hydroponically grown tomatoes compared to the soil- grown plants. The temperature requirement of tomatoes varies according to the growth stage. However, as a warm season crop, tomatoes can do well in temperature ranging from 18.3℃ to 26.1℃ during the day and 18.3℃ to 22.2℃ during the night (Resh, 1978).

Evaluating the effects of hydrogen peroxide on tomato plants with respect to its growth and quality surprisingly resulted in few studies. According to a research carried out by Orabi et al. (2015), a lower level of treatment with H_2O_2 (0.5 mM) can have a significant positive effect on plant growth, as endogenous growth regulators, antioxidant enzyme activity, fruit yield, and quality of two tomato cultivars 'Streentb' and 'Floridat'. The effect of postharvest application of hydrogen peroxide on quality and decay of tomato fruits during storage was studied by Al-Saikhan and Shalaby (2019) who reported that hydrogen peroxide treatments reduced weight loss and disease incidence percentage of fruits compared with control and was useful to keep quality of tomato fruits under storage conditions.

Lettuce

Lettuce belongs to the family Asteraceae. Originally, lettuce was cultivated from its wild parent thousands of years ago in ancient Egypt. Its consumption had spread by the late 1900s to be enjoyed by the people worldwide (Harlan, 1986). Growing lettuce in a hydroponic system does not require a high skill level, because of that it is one of the most popular and common hydroponic crops and occupies the largest proportion of area under hydroponics (Jones Jr., 2005; Kaiser and Ernst, 2012). Owing to its high demand and popularity, lettuce needs to be produced all year-round and growing lettuce in hydroponics system can help the growers to meet this demand. Lettuce is the most cultivated vegetable in NFT hydroponic systems due to its ease of adaptation to hydroponic systems and has shown high productivity and reduction in crop production cycles in comparison to soil cultivation (Ohse et al., 2001). Lettuce is considered a cool season crop with an optimal temperature ranging from 15°C to 20°C (Qin et al., 2002). It is susceptible to a number of physiological problems at greater than optimal temperatures, including tip burn, loose heads, rib discoloration, and bolting (Landers, 2017). Lettuce types that are popular in hydroponics production system are Summer Crisp, Butter head, Romaine, Leaf, and Crisp head (Ryder, 1999). The effect of cooling of nutrient solution was studied on Butterhead lettuce by Ilahi et al. (2017), who reported significant differences in size and leaf number with root-zone cooling and an increase in incidence of tip burn on those plants which were under the non-chilled condition. In another study, Crisp head lettuce plants (‘Marbello' and 'Bastion') grown in a solution heated to either 15°C or 20°C were found to have significantly greater shoot fresh weights than those grown in the unheated solution at 10°C (Economakis and Said, 2002). He et al. (2001) reported increased values for photosynthesis, leaf relative water content, and chlorophyll of hydroponics lettuce by maintaining a constant 20°C water temperature compared to a fluctuating ambient temperature (23 to 40°C). In hydroponic systems, temperature of the nutrient solution affects the oxygen content and, in lettuce, high temperature can cause root death and accelerate the bolting process. In this case, it is recommended that the temperature does not exceed 20°C (Magalhaes, 2006).

Water temperature management

The development of hydroponic techniques in agriculture has permitted easy regulation of the root environments with respect to humidity, nutrient solution, and temperature (Sakamoto and Suzuki, 2015). Among various factors which affect hydroponic production, temperature of the nutrient solution is considered one of the most important determining factors of crop yield and quality (Al-Rawahy et al., 2018). Heating the nutrient solution can help to increase hydroponic production in cooler seasons, whereas cooling nutrient solution may help crop production in hotter months, especially in the regions where high temperatures occur much of the year (Landers, 2017). Just like air temperature, maintaining root temperature has also been recognized as an important factor for the production of hydroponic crops.

Temperature that is above the optimum in the root zone can affect the integrity of the cell membrane of the roots. This disruption of the cell membranes can further affect the function of the roots resulting in less nutrient uptake, which affects crop cycles and yields (Al-Rawahy et al., 2018). Warmer water has the added side effect of being a breeding ground for bacteria and fungus that are harmful to plants (Nxawe et al., 2010). In addition, water temperature is also directly related to how much dissolved oxygen a hydroponic system can support. At lower temperature, sufficient dissolved oxygen is available to the plants to meet their requirement. Whereas, at higher temperature, dissolved oxygen levels decrease and evaporation of the solution can occur, resulting in a higher concentration of nutrients in the solution (Bartok, 2018). As temperatures rise, less and less oxygen can stay in the solution resulting in oxygen deficiency in the root zone which could further lead to poor root and plant performance and an increase in the incidence of diseases and pests (Al-Rawahy et al., 2018). Lee and Takakura (1995) reported greater growth of 'Okame' spinach (*Spinacia oleracea* L.) when nutrient solution temperatures were chilled to between 18°C and 26°C. Cooling of nutrient solution has also been found to affect the photosynthetic activity of plants. According to a study conducted on lettuce, photosynthetic rates of the plants were increased by 50% with root-zone cooling than with plants that were exposed to hot ambient temperatures (Jie and Kong, 1998). Further**,** the temperature of the nutrient solution has also been found to affect the production of metabolites in various crops. Increasing the root-zone temperature in African snake tomato (*Trichosanthes cucumerina* L.) resulted in an increased amount of phenols, ascorbic acid, and chlorophyll content in the leaves (Adebooye et al., 2009). In contrast, decreasing the root-zone temperature of cucumber seedlings promoted soluble sugar production (Yan et al., 2013).

On the other hand, if the water is too cold it will cause plants to start to shut down and not intake as many nutrients (Nxawe et al., 2010). Low temperature can also affect dissolved nutrients and physiological properties (Bartok, 2018). Research has shown that leaf numbers, leaf length, and total fresh and dry weight can be affected. Lettuce grown in a solution heated to either 15°C or 20°C had significantly greater shoot fresh weights, increased water content, and more leaves than those grown in the unheated nutrient solution (10°C) (Economakis and Said, 2002). Similarly, Thompson et al., (1998) observed significant differences in plant dry weight of 'Ostinata' Butterhead lettuce when exposed to various water temperatures. They found that 24°C pond water temperature maintained market quality lettuce with the greatest dry weight compared to 31°C and 17°C water temperature in 'Ostinata' Butterhead lettuce. Root-zone heating or cooling by monitoring the temperature of nutrient solution can be easily implemented in a hydroponic system and can be more efficient due to the high heat capacity of water compared to air (Morgan et al. 1980).

CHAPTER II

TIMING AND RATES OF TWO HYDROGEN PEROXIDE (H_2O_2) PRODUCTS TO CONTROL ALGAE IN EBB AND FLOW HYDROPONIC SYSTEMS

Abstract

Algae is not desirable in hydroponics and creates problems of reduced yield, decreased dissolved oxygen, and affects the physiology of plants and thus, needs to be controlled. An experiment was conducted in Ebb and Flow hydroponic systems to investigate the application timing and rates of two hydrogen peroxide products (Zerotol and PERpose Plus). Treatments included 35 mL weekly, 35 mL biweekly, 70 mL weekly, 70 mL biweekly, and a control using a 40 gallon reservoir of water. Pepper 'Early Jalapeno' and 'Lunchbox Red' and tomato 'Geronimo' and 'Little Sicily' were used. The study was conducted in a split-plot design with two replications over time. Plant height, flower number, net CO_2 assimilation, fresh weight, and dry weight were recorded. Algae data including dry weight, algae cell counts, and chl *a* were also measured. Results indicated that with increasing rate and timing of either product, algae counts, dry weight, and chl *a*

values decreased. However, weekly and biweekly application of 70 mL of both products were not different for algae quantification. In pepper, plant height, shoot fresh and dry weight, and root fresh and dry weight were found to be significantly greater with Zerotol 35 mL biweekly, Zerotol 70 weekly, PERpose Plus 35 mL biweekly, and PERpose Plus 70 mL weekly compared to the control. 'Lunchbox Red' was significantly greater than 'Early Jalapeno' in all growth parameters, except SPAD. 'Lunchbox Red' had the greatest flower number with weekly application of 70 mL PERpose Plus. In tomato, greatest flower number and SPAD were observed in 'Geronimo' with a weekly application of 70 mL PERpose Plus and 70 mL Zerotol, respectively. Greater shoot and root fresh and dry weight for both tomato cultivars were recorded with 35 mL biweekly or 70 mL weekly application with either product. The results from both plants as well as algae analysis suggests that weekly application of 70 mL of either Zerotol or PERpose Plus produced the best results in terms of controlling algae and improving the growth of pepper and tomato plants.

Introduction

Hydroponics is the method of growing plants under soilless conditions with nutrients, water, and an inert medium (Savvas, 2003). Systems can be circulating, in which the nutrient solution is recirculated and nutrients levels are manipulated, or non-circulating, in which the nutrient solution is not recirculated and flow through the system only once (Singh, 2017). Although there are different types of hydroponic systems, every system must deliver water, nutrients, and oxygen to achieve success for plant production (Aires, 2018). Algae needs water, nutrients, and light to grow and since a hydroponic system provides it all, algae growth is often seen in hydroponics (Morgan, 2017), especially those with recirculating nutrient solution (Schwarz and Gross, 2004). Algae enters the system through irrigation water and is commonly present in the physical structures that make up the hydroponic system. Under uncontrolled conditions, algae can cause organic loading and clogging of pipes (Supraja et al., 2020). Algae poses several threats to hydroponic crops by competing for the nutrients and releasing harmful toxins which might inhibit or even stop crop growth (Borowitzka, 1995; Schwarz and Gross, 2004). It is generally accepted that algae growth can affect the water quality parameters such as pH, dissolved oxygen, and nutrients in the water, and may compete with the target vegetables (Abdel-Raouf et al., 2012). Thus, it is critical that algal levels are kept to a minimum in hydroponic systems (Tesoriero et al., 2010).

It is a common practice in hydroponics to clean the system manually by basic cleaning or using black plastic sheets to reduce algal growth (Vanninnen and Koskula, 1998), but time for application and high use of labor adds to costs (Caixeta et al., 2018). Alternatively, chemical algaecides are also used occasionally (Nonomura et al., 2001). The interest in environmental-friendly chemicals has been on the rise in recent years. One such chemical is hydrogen peroxide (H_2O_2), which decomposes rapidly into harmless products water and oxygen (Randhawa, 2013). Many studies have reported application of H_2O_2 resulted in effective and selective inhibition of algal growth with low or no effects on other phytoplankton groups in natural waters (Barrington and Ghadouani, 2008). In plants, H_2O_2 plays an important physiological role as a Reactive Oxygen Species (ROS), which is produced under both biotic and abiotic conditions and plays a key role against O_2 derived cell toxicity (Almeida et al., 2005). Exogenous application of H_2O_2 was found to increase plant growth, physiological activities, and biochemical properties of wax apple (*Syzygium samarangense* L. var. Jambu Madu) fruits (Khandaker et al., 2012). Similarly, Orabi et al.

(2015) reported that a low level of H_2O_2 can have a significant positive effect on plant growth, endogenous growth regulators, antioxidant enzyme activity, fruit yield, and quality of tomato (*Solanum lycopersicum* L.). On the other hand, high concentration of H_2O_2 can promote stomatal closure in plants reducing net photosynthesis and growth (Xia et al., 2014), and produce carcinogenic effects and induce cell death (Nurnaeimah et al., 2020). Although H_2O_2 based products have been used widely as aquatic herbicide and algaecide in waste-water treatment, its application in hydroponic crop production is still limited. The signaling role of H_2O_2 in plants is well established, particularly with reference to plant processes like stress acclimation, cell wall cross-linking, stomatal behavior, phytoalexin production, regulation of the cell cycle, and photosynthesis. However, if not applied within the optimum range, H_2O_2 can result in decreased net photosynthesis and plant dry mass (Khan et al., 2016). So, the toxicity or danger associated with H_2O_2 on one hand and signaling cascades on other makes it a versatile chemical whose rate and concentration need to be tightly controlled in plants during the application (Petrov and Breusegem, 2012). The goal of this study was to find the optimum amount and timing of application of two hydrogen peroxide products to control algae and improve the growth and quality of pepper and tomato plants using an Ebb and Flow hydroponic system.

Materials and Methods

Plant materials and growth conditions:

The experiment was conducted at the Department of Horticulture and Landscape Architecture Research Greenhouses in Stillwater, OK. Seeds of tomato (*Lycopersicum* *esculentum* L.) 'Geronimo' and 'Little Sicily' and pepper (*Capsicum annuum* L.) 'Early Jalapeno' and 'Lunchbox Red' were obtained from Johnny's Selected Seeds (Winslow, MN). Pepper seeds were sown in oasis cubes (Harris Seeds, Rochester, NY) of size 1.5 cm^3 on 22 April, 2020 and kept on the mist bench. Tomatoes were planted 3 weeks later on 13 May, 2020 in oasis cubes of the same size. Nutrient was applied once when the seedlings were three weeks old. Nutrient solution was made by mixing 4.9 g Jack's 5-12-26 and 3.2 g of calcium nitrate diluted in 10 L of water and watered over the seedlings. Both peppers and tomatoes were transplanted to an Ebb and Flow table (Gro Master, Maple Park (Virgil), IL) on 17 June, 2020. A Styrofoam sheet containing 5 cm diameter slots spaced 28 cm apart was placed on each table to support the plants. A 5 cm net pot was placed on each slot of the Styrofoam and one plant was placed into each net pot. Each Ebb and Flow bench was supplied with a 40-gallon capacity tank. Tanks were filled with tap water and 147.41 g Jack's 5-12-26 (J.R. Peters, Allentown, PA) along with 97.52 g of calcium nitrate (American Plant Products, OKC, OK) was added initially according to recommended rates. The pH and EC of the solution were checked every day to maintain the pH between 5.5 to 6.5 and the EC at 1.5 to 2.5 $mS \cdot cm^{-1}$.

Treatments and data collection:

Both Zerotol (Biosafe Systems, East Hartford, CT) and PERpose Plus (Bioworks, Victor, NY) had four different combinations of rate and timing, 35 mL weekly and twice weekly (biweekly), 70 mL weekly and biweekly, and a control without any hydrogen peroxide application. Each plant on the table was scanned using a chlorophyll meter (SPAD-502, Konica Minolta, Japan) 63 d after transplanting. SPAD readings were taken from each plant from top, middle, and bottom leaf and then averaged to determine the chlorophyll concentration. Data on photosynthesis rate was taken using a Li-cor 6400 (LI-COR, NE). The Li-cor with 6400-02B LED light source chamber was used by keeping the reference CO_2 at 400 ppm. The block temperature was set at 28°C and the light level was set at 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The third leaf from the top was selected per plant and was used as non-destructive sample for the Li-cor measurement. Plants were harvested 70 d after transplanting and data were collected on number of flowers (fully opened), plant height, root and shoot fresh weight and dry weight (oven dried for 2 d at 53.9°C). Dissolved oxygen level of the solution was measured every day with a dissolved oxygen (D.O.) meter (Hanna Instruments, Woonsocket, RI).

Quantification of algae:

After harvesting plants, 300 mL of solution was collected from five tables (control, Zerotol and PERpose Plus 35 mL weekly, Zerotol and PERpose Plus 70 mL biweekly) and sent to EnviroScience Lab (Stow, OH) for quantitative algae analysis. Total suspended solids method was used to measure the dry weight of algae. A 100 mL solution was collected per table and thoroughly mixed by shaking each bottle before vacuum filtering it through a filter paper (Whatman GF/A Glass Microfiber, Cytiva, Marlborough, MA) of known weight. The suspended algae in the filter paper was then oven dried for 24 h at 53.9°C. The dry weight of algae along with the filter paper was measured and the dry weight of algae ($mg \cdot L^{-1}$) was calculated by using the following formula as reported by Joy (1994).

[weight of filter + dried residue (mg) - weight of filter (mg)]x 1000 / volume used

A hemocytometer was used to count the number of algae cells. A 100 µl of water sample was collected from each table and 100 µl of trypan blue dye was added to make the solution for the slide before 1 µl homogenous solution was added to the hemocytometer slide. The slide was examined under a compound microscope at 40X and the average number of viable algae cells was counted. The average cell count was multiplied by 10,000X the dilution factor (2) in order to calculate the algae concentration (viable cells/mL) according to LeGresley and McDermott (2010). Water sample from each treatment was collected to measure the chlorophyll (chl)-a of algae with a spectrophotometer (GENESYS 30, The Lab Depot, Dawsonville, GA) to measure the absorbance of the samples at various wavelengths (750 nm, 665 nm, 647 nm, and 630 nm) as reported by Kumar and Saramma (2013).

Experimental design and statistics:

Eight plants per cultivar per species were randomly planted in tables and treatments were arranged in a split-plot design with two replications over time. The experiment was replicated by planting another set of pepper seeds on 28 August, 2020 and tomatoes on 18 September, 2020 adopting the similar methods listed above. Statistical analysis was performed using SAS/STAT software (Version 9.4; SAS Institute, Cary, NC). Tests of significance were reported at the 0.05, 0.001, and 0.0001 level. The data were analyzed using generalized linear mixed models methods. Tukey multiple comparison methods were used to separate the means.

Results

Quantification of algae

Dry weight of algae, chlorophyll *a*, and algal cell counts were significantly affected by the rate and application timing of H_2O_2 products (Table 2.1). As H_2O_2 rate increased, dry weight, algal cells, and chl *a* were found to decrease. Lowest algae dry weight was recorded with 70 mL biweekly application of either product; however, this was not different from 70 mL weekly treatment, and 35 mL biweekly treatment. A similar trend was observed in algal cell counts as well, with the lowest cell counts in 70 mL biweekly treatment of either product. Lowest chl *a* was recorded in samples collected from 70 mL biweekly treatment of either product, which was similar to all other treatments except control and 35 mL weekly treatment for either product. Algae species were still found in treatments that were sent for analysis with *Chlamydomonas* spp., *Gleocystis vesiculosa*, and *Scenedesmus acutus* being recorded in the greatest numbers collectively from all treatments (Table 2.2). *Chlamydomonas* spp. was found in all treatments, while *Gloeocystis vesiculosa* was found in all treatments except Zerotol 70 mL biweekly treatment and *Scenedesmus* spp. was found in all treatments except PERpose Plus 70 mL biweekly treatment. Average cells/mL of *Scenedesmus acutus* decreased 99.9%, 99.8%, and 94.8% in Zerotol 35 mL weekly, PERpose Plus 35 mL weekly, and Zerotol 70 mL biweekly treatment, respectively compared to the control. Average cells/mL of *Pennate Diatom spp.* decreased 99.8%, 98%, 66.3%, and 52.1% in Zerotol 70 mL biweekly, Zerotol 35 mL weekly, PERpose Plus 70 mL biweekly, and PERpose Plus 35 mL weekly treatment, respectively compared to the control. While, average cells/mL of *Centric Diatom spp.* reduced 17.5%, 62.1%, and 99.2% compared to the control in Zerotol 35 mL weekly, PERpose Plus 35 mL weekly, and Zerotol 70 mL biweekly treatment, respectively. Average cells/mL of *Leptolyngbya spp.* was recorded to be 2,016 in the control treatment but was not found in any other treatments.

H_2O_2 and cultivar main effects and their interaction on plant growth parameters for pepper

In pepper, there was a significant cultivar × H_2O_2 interaction for flower number and carbon dioxide assimilation (Table 2.3). Greatest CO_2 assimilation was observed in 'Early Jalapeno' with Zerotol 35 mL biweekly treatment which was not different than Zerotol 35 mL weekly and Zerotol 70 mL weekly treatments. Within cultivar, application of Zerotol 35 mL biweekly and 70 mL weekly resulted in greatest value for CO_2 assimilation in 'Lunchbox Red'. Between cultivars for CO_2 assimilation, 'Early Jalapeno' was found to have greater CO_2 assimilation in comparison with 'Lunchbox Red'. Greatest flower number was recorded in 'Lunchbox Red' with PERpose Plus 70 mL weekly treatment (Table 2.4). SPAD, plant height, and fresh and dry root and shoot weight showed significant main effects for both cultivar and H_2O_2 (Table 2.3). Within H_2O_2 treatments, Zerotol 35 mL weekly treatment had the greatest SPAD reading and was found to be similar to all other treatments except the control and Zerotol 70 mL biweekly treatments (Table 2.5). For plant height, greatest value was recorded with Zerotol 70 mL weekly treatment but was not different than all other treatments except control, Zerotol 35 mL weekly, and Zerotol 70 mL biweekly treatments (Table 2.5). Greatest root fresh weight was recorded with 70 mL weekly treatment of either products and Zerotol 35 mL biweekly treatment and were different than the control treatment only. Whereas, biweekly application of 35 mL Zerotol produced plants with the greatest root dry weights but was similar to all other treatments except the control and 70 mL biweekly treatments of either product. Weekly application of 70 mL PERpose Plus resulted in the greatest shoot fresh weight. However, 70 mL weekly application of Zerotol and 35 mL biweekly application of either product also resulted in plants with greater shoot fresh weights. Whereas, greatest shoot dry weight was recorded with Zerotol 35 mL biweekly and PERpose Plus 70 mL weekly treatments but were similar to all other treatments except control and 70 mL biweekly treatments of either product (Table 2.5). ‘Lunchbox Red’ performed better in terms of all the growth parameters except SPAD which was greater in ‘Early Jalapeno’ (Table 2.6).

H_2O_2 and cultivar main effects and their interaction on plant growth parameters for tomato

In tomato, there was a significant cultivar × H_2O_2 interaction for all growth parameters except for net CO_2 assimilation. Whereas, CO_2 assimilation was found to be affected by H_2O_2 product only (Table 2.7). Zerotol application at the rate of 35 mL biweekly and 70 mL weekly resulted in the greatest CO_2 assimilation compared to any other treatments (Table 2.8). Greatest flower number was observed in ‘Geronimo’ with a weekly application of 70 mL PERpose Plus, which was not different than Zerotol 35 mL biweekly and PERpose Plus 35 mL weekly treatments as well as Zerotol 35 mL weekly and 35 mL biweekly treatment of either product in ‘Little Sicily’. Greatest SPAD value was recorded in ‘Geronimo’ within Zerotol 70 mL weekly treatment which was also similar to PERpose Plus 70 mL weekly treatment and 35 mL biweekly treatment of either product. In ‘Lunchbox Red’, both weekly and biweekly application of 35 mL Zerotol resulted in the greatest SPAD and were similar to all other treatments except the control and 70 mL biweekly treatments of either products (Table 2.9). In ‘Little Sicily’, Zerotol 35 mL biweekly application resulted in the greatest plant height and was similar to all the treatments except Zerotol 70 mL biweekly treatment. While, ‘Geronimo’ had the greatest plant height with PERpose Plus 70 mL biweekly treatment and was similar to all the treatments except Zerotol 35 mL biweekly and PERpose Plus 70 mL biweekly treatments. When compared across the cultivars, ‘Little Sicily’ had greater plant height than ‘Geronimo’ (Table 2.9). Greater fresh and dry root weight were observed in ‘Little Sicily’ in comparison to ‘Geronimo’. In ‘Little Sicily’, greatest root fresh was observed with biweekly application of 35 mL Zerotol and was similar to all other treatments except control and 70 mL biweekly treatments of either products. Whereas, the greatest root dry weight was observed in PERpose Plus 35 mL biweekly treatment which was similar to 35 mL weekly and biweekly treatments of Zerotol as well as 35 mL and 70 mL weekly treatments of PERpose Plus. In ‘Geronimo’, 70 mL weekly application of either products resulted in the greatest fresh weight of roots and were similar to all the treatments except the control, Zerotol 35 mL weekly, and PERpose Plus 70 mL biweekly treatments. However, greater root dry weight was recorded in PERpose Plus 70 mL weekly treatment compared to any other treatments (Table 2.9). ‘Little Sicily’ had the greatest shoot fresh weight with PERpose Plus 70 mL weekly treatment, which was similar to all other treatments except the control and Zerotol 70 mL biweekly treatments. Whereas, the shoot dry weight was found to be greatest with PERpose Plus 70 mL biweekly treatment but was only different from the control treatment. In ‘Geronimo’, PERpose Plus 35 mL biweekly and 70 mL weekly treatments resulted in greatest shoot fresh weight but was not different than PERpose Plus 35 mL weekly, Zerotol 35 mL biweekly, and Zerotol 70 mL weekly treatments. For shoot dry weight, PERpose Plus 70 mL weekly had the greatest value but was similar to PERpose Plus 35 mL biweekly treatment (Table 2.9).

Dissolved oxygen (DO)

Dissolved oxygen (DO) levels varied from 5.2 to 6.6, 6.2 to 7.5, 6.2 to 7.9, and 6.8 to 8.2 $mg \cdot L^{-1}$ for PERpose plus treatments of 35 mL weekly, 35 mL biweekly, 70 mL weekly, and 70 mL biweekly, respectively (Figure 2.1). Average DO levels were 6.0, 6.6, 7.0, and 7.7 $mg \cdot L^{-1}$ for PERpose plus treatments of 35 mL weekly, 35 mL biweekly, 70 mL weekly, and 70 mL biweekly, respectively. Dissolved oxygen (DO) levels varied from 5.4 to 6.4, 6.0 to 7.6, 6.1 to 7.8, and 6.1 to 8.1 $mg \cdot L^{-1}$ for Zerotol treatments of 35 mL weekly, 35 mL biweekly, 70 mL weekly, and 70 mL biweekly, respectively (Figure 2.1). Average DO levels were 5.9, 6.6, 6.9, and 7.6 $mg \cdot L^{-1}$ for Zerotol treatments of 35 mL weekly, 35 mL biweekly, 70 mL weekly, and 70 mL biweekly, respectively. Whereas, the control levels varied from 5.1 to 5.7 $mg \cdot L^{-1}$ and averaged 5.5 $mg \cdot L^{-1}$. Spikes in DO levels were observed on days in which H_2O_2 was added to the tanks, then levels decreased within a couple days until the next application. The trend showed that with increasing dose and application of H_2O_2, there was an increase in DO level.

Discussion

In the present study, dry weight of algae, chl *a*, and algal cell counts were found to decrease with increasing rate and application timing of H_2O_2 products. Hydrogen peroxide is a strong oxidant that has been used in industrial application and water treatment processes (Ismail et al., 2015). When catalyzed in water, H_2O_2 can generate a wide variety of free radicals and other reactive species that are capable of transforming or decomposing organic chemicals (Petri et al., 2003). Hydrogen peroxide based products have been used as aquatic herbicides and microbiocide to manage algae, cyanobacteria, fungi, and microorganisms in water (Randhawa et al., 2012). However, the efficiency of H_2O_2 can be influenced by abiotic factors such as light intensity and nutrient availability as well as biotic factors like phytoplankton and bacterioplankton composition (Santos et al., 2021). According to a study conducted by Vanninen and Koskula (1998), a single application of 1 dL of 125 ppm hydrogen peroxide or daily applications over 3 weeks of 1 dL of 100 ppm peroxide reduced algal growth by 40-60% which was similar to our findings. However, our results showed that both Zerotol and PERpose Plus reduced algae by as much as 95% even at lower rates. Exposure of *Planktothrix agardhii* to H_2O_2 resulted in the degeneration of cells and filaments. Swollen cell walls and some degree of cytoplasmatic alteration were observed after 24 h of treatment with 0.83 $mg{\cdot}L^{-1}$ H_2O_2. Whereas, after 48 h under the highest concentration (3.33 $mg{\cdot}L^{-1}$) resulted in cell wall and plasmatic membrane disruption, disorganized and degraded thylakoids, and changes in the cytoplasmatic inclusions (Bauzá et al., 2014). The same study also reported that the chl *a* decreased 40%, 86%, 86%, 78%, and 79% respectively with application of 0.17, 0.33, 0.83, 1.67, and 3.33 $mg{\cdot}L^{-1}$ H_2O_2 which corresponds to our finding that chl *a* decreases with increasing rate of H_2O_2. Santos et al. (2021) evaluated the effects of a single application of H_2O_2 (10 $mg{\cdot}L^{-1}$) over 120 h in mesocosms introduced in a reservoir and reported that H_2O_2 efficiently decreased the biomass of cyanobacteria, green algae, and diatoms over 72 h along with the chlorophyll concentration, leading to an increase in transparency. However, after 120 h subsequent dominance of green algae was observed. In some cases, suppression of one algae species might benefit the growth of another species which could be the reason why some of the treatments in our experiment had new species that were not recorded in the control treatment. Sinha et al. (2018) observed that a reduction in cyanobacteria biomass was followed by an increase in the abundance of eukaryotic diatom *Synedra* sp. and green algae

Cladophora sp., suggesting that these species benefited from the collapse of cyanobacteria and utilized the available nutrients. In another study conducted by Wang et al. (2019), where a *Microcystis aeruginosa* bloom was suppressed with the application of H_2O_2, growth of *Chlamydomonas* spp. was found to be promoted.

Our results indicated that either 70 mL weekly or 35 mL biweekly application of either H_2O_2 product resulted in improved growth performance of both pepper and tomato plants. This may be due to the positive role of H_2O_2 in plant growth and development. H_2O_2 is said to be the most stable reactive oxygen species (ROS), and therefore plays a crucial role as a signaling molecule in various physiological processes, including photosynthesis, respiration, translocation, and transpiration (Slesak et al., 2007). Growth (root and shoot length; fresh and dry weight) and photosynthetic performance of cowpea (*Vigna unguiculata* L.) was found to increase by foliar application of H_2O_2 at 0.5 to 1.0 mM solution (Hasan et al., 2016). Similarly, root dipping treatment of H_2O_2 to tomato plants under copper stress and stress-free conditions improved growth, including shoot and root length, shoot and root fresh weight, shoot and root dry weight, and leaf area (Nazir et al., 2019). Foliar application of diluted H_2O_2 (at 20 mM) has been shown to increase the dry weight of leaves, increase fruit set, biomass and quality of fruit as well as decreasing bud drop in wax apple ('Jambu Madu') crops (Khandaker et al., 2012). Uchida et al. (2002) reported that H_2O_2 application induces the activity of sucrose phosphate synthase (SPS), which is an enzyme important in the formation of sucrose from triose phosphates during and after photosynthesis and positively regulate the metabolic and antioxidant enzyme activities in favor of plant growth and development. Furthermore, application of H_2O_2 has been found to increase the shoot and root length in two wheat (*Triticum aestivum* L.)

cultivars by increasing the activity of starch hydrolyzing enzymes which resulted in better root carbohydrate. As the root length increases, absorption of water and nutrients usually increases leading to increased growth parameters and prevention against the harmful effects of stress (Latef et al., 2019). On the other hand, since H_2O_2 functions by decomposing into an unstable free radical oxygen molecule which can destroy biotic cell tissue, and has the potential to indiscriminately damage healthy living root tissue, consequently reducing the fresh weight as well (Lau and Mattson, 2021). Lower root and shoot weight at 70 mL biweekly treatments in our study may be the result of this phytotoxicity.

Khan et al. (2018) studied the effects of root treatment of two varieties (S-22 and PKM-1) of tomato on three different concentrations of H_2O_2 (0.01, 0.1, and 0.5 mM). The 0.1 mM application of H_2O_2 for 4 h proved best and resulted in improved growth and photosynthetic attributes, enhanced activity of various antioxidant enzymes, and greater accumulation of proline. However, the effects were more prominent in 'S-22' compared to 'PKM-1'. The induced activity of antioxidant enzymes and proline content by H_2O_2 was recorded to be more in 'S-22' than 'PKM-1', which helped in protecting photosynthesis and maintaining high plant fresh and dry mass than in PKM-1. They concluded that both the concentration of H_2O_2 as well as cultivars influenced the effects of H_2O_2 on growth of tomato which supports our findings.

Physiological effects of exogenous application of H_2O_2 and Ca were studied on sweet and hot pepper (*Capsicum spp.* L.) under heat stress and a significant change in biochemical, physiological, and fatty acid contents in both cultivars was reported, however, sweet peppers absorbed greater amounts of Ca in comparison with the hot peppers (Motamedi et al., 2019). Calcium is an essential macronutrient in plants and provides structural stability to cell walls and membranes and acts as a secondary messenger in cellular signaling (White and Broadley, 2003). This could be a reason for greater height and shoot and root weights in cultivar Lunchbox Red compared to Early Jalapeno.

H_2O_2 treatment also improved photosynthesis as a result of improved gas exchange parameters, chlorophyll content, and net photosynthesis along with improved scavenging of ROS and recovery of photosynthetic efficiency in tomato (Nazir et al., 2019). In addition, H_2O_2 treatment also improved chlorophyll content in mung bean (*Vigna radiata* L.) and tomato by modulating endogenous plant hormones (Fariduddin et al., 2014; Orabi et al., 2015). Root dipping treatment with H_2O_2 significantly increased photosynthesis of tomato plants because of increase in the activity of Rubisco and PS II resulting in increased stomatal conductance and intercellular CO_2 concentration (Khan et al., 2018). In a similar study conducted by Nurnaeimah et al. (2020), plant height, leaf area, chlorophyll content, net photosynthetic rate, stomatal conductance, and quantum yield of mistletoe fig (*Ficus deltoidea* L.) increased after treatment with 16 and 30 mM H_2O_2. They concluded that the application of H_2O_2 increased stomatal opening, CO_2 concentration, and accumulated photosynthetic pigments, which ultimately resulted in the increased photosynthetic rate. However, increasing the concentration of H_2O_2 to 60 mM led to a decrease in growth parameters and increased the accumulation of arsenic, iron, and sodium content in the leaves of mistletoe fig. These results can be supported by the fact that H_2O_2 at low concentration acts as a regulator of some major processes, such as assimilation, photosynthesis, respiration, stomatal conductance, cell cycle, growth and development, and plant response to biotic and abiotic pressure, however, it can increase the oxidative damage, and ultimately cause the cell death above a certain threshold (Latef et al., 2019). The study of Xia et al. (2014) also reported that a low concentration of H_2O_2 promotes stomatal opening, whereas a high concentration of H_2O_2 promotes stomatal closure in tomatoes. This might be a reason why a decrease in growth parameters of pepper and tomato was observed in our study with 70 mL biweekly application of either H_2O_2 products. In addition, H_2O_2 has also been reported to promote reproductive growth in fruits by inhibiting the growth of rudimentary leaves as well as by promoting the expression of the flower related gene, LcLFY and reducing bud drop (Ismail et al., 2015).

Dissolved oxygen (DO)

An increase in DO levels with increased rate and application timing of H_2O_2 was observed. In hydroponic nutrient solutions, decomposition of H_2O_2 gives rise to byproducts H_2O and O_2, and this released O_2 can increase the dissolved oxygen concentration in the root zone (Lau and Mattson, 2021). Hydrogen peroxide, when added into nutrient solution, breaks down readily releasing a molecule of water and a reactive O-molecule which can bind with another O-molecule. This O-molecule can also react with organic compounds which often results in degradation of the compound (Fredrickson, 2015). In addition, reduced amount of algae in water can result in greater dissolved oxygen level as the result of increased permeability of light in water (Lauguico et al., 2020). Plant growth of hydroponic crops has been shown to increase when irrigated with additional oxygen in the solution (Soffer et al., 1990). Butcher (2016) reported that the treatment with high frequency application of H_2O_2 alone produced the greatest level of dissolved oxygen (DO), whereas combination of high frequency application (every 3 d) of hydrogen peroxide, vortex oxygenation, and air-pump injection treatment yielded the greatest production of chlorophyll in peppermint geranium (*Pelargonium tomentosum* L.). The increased production of chlorophyll was likely due to healthy root growth and respiration within a nutrient solution where high levels of dissolved oxygen and turbulence were facilitated. According to Chérif et al. (1997), tomato plant roots were susceptible to *Pythium* infection when the oxygen in root zone dropped below 2.8 mg·L^{-1}. Poor root and plant performance as well as an increase in the incidence of disease were reported when the root zone was oxygen deficient (Chérif et al., 1997). Marfà et al. (2005) reported greater yield of pepper plants grown in perlite with nutrient solution enriched with oxygen (16 mg·L^{-1}) compared to unenriched solution (6 mg·L^{-1}). In contrast to our result, Ouyang et al. (2021) reported a significant increase in growth, photosynthesis, yield, and quality of tomato when the dissolved oxygen of irrigation water was increased (9 mg·L^{-1}) compared to the control (4 mg·L^{-1}). This could be because they used aeration as a method of increasing dissolved oxygen, whereas the high level of hydrogen peroxide in our study might have caused phytotoxicity and resulted in poor plant growth.

Conclusion

The main objective of this study was to find the optimum rate and application timing of H_2O_2 that reduced the algae growth in an Ebb and Flow hydroponic system while not inhibiting the growth of pepper and tomato plants. From the results, we can conclude that with increasing rate and timing of either product, algae counts, dry weight, and chl *a* values decreased. However, weekly and biweekly application of 70 mL of the products were not significantly different from each other for controlling algae growth so weekly would be recommended. Surprisingly, Zerotol 35 mL biweekly, Zerotol 70 weekly, PERpose Plus 35 mL biweekly, and PERpose Plus 70 mL weekly resulted in better growth in both tomato and pepper. Our results also indicated that 70 mL biweekly application of either product caused phytotoxicity and resulted in decreased growth. Results from both plants as well as algae analysis suggested that weekly application of 70 mL of either Zerotol or PERpose Plus produced the best results in terms of controlling algae and improving the growth of pepper and tomato plants. Future research should investigate other plants using these two products and rates, effects on fruiting, and overall nutrition of fruit.

Tables and figures

Table 2.1: Least square means for rate and application timing of two hydrogen peroxide products on algae samples from Ebb and Flow hydroponic systems in Stillwater, OK.

Chemical	Rate (ml)	Timing of application	Dry weight ($mg \cdot L^{-1}$)	Algal cells (10^5)	Chl *a* ($\mu g \cdot L^{-1}$)
Control	0	None	1082.50a[z]	37.51a	2556.75a
Zerotol	35	Weekly	361.30bc	27.91ab	1949.30a
Zerotol	35	Biweekly	113.20cd	11.77bc	1409.85ab
Zerotol	70	Weekly	105.65d	8.79cd	1262.85ab
Zerotol	70	Biweekly	60.05d	3.53d	626.65b
PERpose Plus	35	Weekly	444.70ab	27.76ab	1929.55a
PERpose Plus	35	Biweekly	154.75cd	13.94abc	1297.85ab
PERpose Plus	70	Weekly	149.50cd	11.46cd	1323.30ab
PERpose Plus	70	Biweekly	67.30d	3.84d	593.35b

[z]Means (n = 20) within a column followed by same lowercase letter are not significantly different by pairwise comparison in mixed model ($P \leq 0.05$).

Table 2.2: Different rates and application timing of two hydrogen peroxide products on taxonomic counts of algae present in Ebb and Flow hydroponic systems in OSU research greenhouses, Stillwater, OK.

Chemical	Rate (ml)	Time of application	Scientific Name	Average cells/mL[z]	Average natural units/mL[z]
Control	0		*Scenedesmus acutus*	183,477	144,989
			Scenedesmus ecornis	127,991	94,733
			Pennate Diatom spp.	22,452	22,452
			Gloeocystis vesiculosa	16,410	1,675
			Centric Diatom spp.	12,810	12,810
			Chlamydomonas spp.	2,066	2,066
			Leptolyngbya spp.	2,016	2,016
Zerotol	35	Weekly	*Gloeocystis vesiculosa*	331,969	331,969
			Chlamydomonas spp.	163,278	163,278
			Centric Diatom spp.	10,567	8,884
			Pennate Diatom spp.	443	443
			Scenedesmus acutus	266	89
Zerotol	70	Biweekly	*Chlamydomonas spp.*	1,568	1,568

			Scenedesmus acutus	9,676	4,718
			Chlorella minutissima	309	309
			Centric Diatom spp.	103	103
			Chlorella ellipsoidea	103	103
			Monoraphidium pusillum	82	82
			Tetrastrum komarekii	41	10
			Pennate Diatom spp.	34	34
PERpose Plus	35	Weekly	*Gloeocystis vesiculosa*	57,388	46,309
			Chlamydomonas spp.	602,261	602,261
			Pennate Diatom spp.	10,744	10,744
			Centric Diatom spp.	4,855	4,855
			Scenedesmus acutus	413	207
PERpose Plus	70	Biweekly	*Pennate Diatom spp.*	7,556	7,556
			Gloeocystis vesiculosa	3,142	399
			Aphanocapsa nubilum	1,771	59
			Chlamydomonas spp.	1,391	1391
			Oocystis parva	266	59
			Chroococcus dispersus	142	14

	Cyclotella spp.	30	30

[z]Derived from a 100 mL solution.

Table 2.3: Tests of effects for pepper cultivars (Early Jalapeno and Lunchbox Red) and rate and application timing of Zerotol and PERpose Plus (H_2O_2) in Ebb and Flow hydroponic systems at OSU research greenhouses in Stillwater, OK.

	Cultivar	H_2O_2	Cultivar × H_2O_2
No. of flowers	***[z]	***	*
SPAD	***	***	NS
Height	***	***	NS
CO_2 assimilation	***	***	*
Root fresh wt.	***	***	NS
Shoot fresh wt.	***	***	NS
Root dry wt.	***	***	NS
Shoot dry wt.	***	***	NS

[z]Indicates significant at or non-significant (NS) at *P ≤ 0.05, **P ≤ 0.001, or ***P ≤ 0.0001.

Table 2.4: Least squares means for cultivars (Early Jalapeno and Lunchbox Red) and different rate and application timing of two hydrogen peroxide products for flower number and net CO_2 assimilation of peppers grown in ebb and flow hydroponic systems at OSU research greenhouses in Stillwater, OK.

Cultivars	Chemical	Rate (ml)	Timing of application	No. of flowers	CO_2 assimilation ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
Early Jalapeno	Control	0	None	2.1h[z]	21.9cdefgh
	Zerotol	35	Weekly	4.9g	24.4abc
	Zerotol	35	Biweekly	5.9g	26.8a
	Zerotol	70	Weekly	4.7g	26.0ab
	Zerotol	70	Biweekly	3.6gh	20.7efghi
	PERpose Plus	35	Weekly	4.9g	22.7cdefg
	PERpose Plus	35	Biweekly	4.8g	22.8cdefg
	PERpose Plus	70	Weekly	5.3g	23.3bcdef
	PERpose Plus	70	Biweekly	4.0gh	20.8efghi
Lunchbox Red	Control	0	None	11.5f	19.3hi
	Zerotol	35	Weekly	15.7fe	20.6fghi

Zerotol	35	Biweekly	23.2bcd	23.6bcde
Zerotol	70	Weekly	26.7b	23.8bcd
Zerotol	70	Biweekly	18.8cde	20.2ghi
PERpose Plus	35	Weekly	26.3b	20.7efghi
PERpose Plus	35	Biweekly	24.0bc	22.0cdefgh
PERpose Plus	70	Weekly	33.9a	22.5cdefg
PERpose Plus	70	Biweekly	17.8de	21.0defghi

[z]Means (n = 16) within a column followed by same lowercase letter are not significantly different by pairwise comparison in mixed model ($P \leq 0.05$).

Table 2.5: Least square means for rate and application timing of two hydrogen peroxide products on growth of pepper ('Early Jalapeno' and 'Lunchbox Red') grown in Ebb and Flow hydroponic systems at OSU research greenhouses in Stillwater, OK.

Chemical	Rate (ml)	Time of application	SPAD	Height (cm)	Shoot wt. (g)	Root wt. (g)	Shoot dry wt. (g)	Root dry wt. (g)
Control	0	None	44.9c[z]	31.1cd	36.4ef	23.7c	8.0cd	2.6d
Zerotol	35	Weekly	49.4a	33.2bcd	46.3de	37.5ab	9.9abc	3.1ab
Zerotol	35	Biweekly	47.8abc	37.9ab	68.7abc	42.3a	13.4a	3.6a
Zerotol	70	Weekly	47.9abc	39.4a	71.4ab	43.4a	12.6ab	3.0abc
Zerotol	70	Biweekly	45.3bc	30.5d	38.8ef	31.5b	9.2bcd	2.6bcd
PERpose Plus	35	Weekly	46.6abc	37.0abc	55.1bcd	37.9ab	10.1abc	3.1ab
PERpose Plus	35	Biweekly	48.7ab	34.2abcd	62.3abcd	41.1ab	11.4ab	3.1ab
PERpose Plus	70	Weekly	47.5abc	36.4abcd	78.9a	45.4a	13.1a	3.3ab
PERpose Plus	70	Biweekly	46.8abc	34.4abcd	50.1cde	35.3ab	8.0cd	2.3cd

[z]Means (n = 16) within a column followed by same lowercase letter are not significantly different by pairwise comparison in mixed model ($P \leq 0.05$).

Table 2.6: Least square means for cultivars (Early Jalapeno and Lunchbox Red) on growth of peppers grown in ebb and flow hydroponic systems at OSU research greenhouses in Stillwater, OK.

Cultivar	SPAD	Height (cm)	Shoot fresh wt. (g)	Root fresh wt. (g)	Shoot dry wt. (g)	Root dry wt. (g)
Early Jalapeno	50.4a[z]	29.0b	28.0b	24.1b	4.7b	2.4b
Lunchbox Red	43.4b	40.7a	96.5a	51.6a	21.6a	3.5a

[z]Means (n = 16) within a column followed by same lowercase letter are not significantly different by pairwise comparison in mixed model ($P \leq 0.05$).

Table 2.7: Tests of effects for tomato cultivars (Little Sicily and Geronimo) and rate and application timing of two hydrogen peroxide products in Ebb and Flow hydroponic systems at OSU research greenhouses in Stillwater, OK.

	Cultivar	H_2O_2	Cultivar × H_2O_2
No. of flowers	*[z]	***	**
SPAD	***	***	*
Height	***	***	**
CO_2 assimilation	NS	***	NS
Root wt.	***	***	***
Shoot wt.	***	***	*
Root dry wt.	***	***	***
Shoot dry wt.	***	***	*

[z]Indicates significant at or non-significant (NS) at *P ≤ 0.05, **P ≤ 0.001, or ***P ≤ 0.0001.

Table 2.8: Least square means for rate and application timing of two hydrogen peroxide products on net CO_2 assimilation of tomato ('Little Sicily' and 'Geronimo') grown in Ebb and Flow hydroponic systems at OSU research greenhouses in Stillwater, OK.

Chemical	Rate (ml)	Time of application	CO_2 assimilation ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
Control	0	None	20.7de[z]
Zerotol	35	Weekly	23.4b
Zerotol	35	Biweekly	25.9a
Zerotol	70	Weekly	25.5a
Zerotol	70	Biweekly	20.2e
PERpose Plus	35	Weekly	22.7bc
PERpose Plus	35	Biweekly	23.1b
PERpose Plus	70	Weekly	23.1b
PERpose Plus	70	Biweekly	21.6cd

[z]Means (n = 16) within a column followed by same lowercase letter are not significantly different by pairwise comparison in mixed model ($P \leq 0.05$).

Table 2.9: Least square means for cultivars (Little Sicily and Geronimo) and different rate and application timing of two hydrogen peroxide products for growth of tomatoes grown in ebb and flow hydroponic system at OSU research greenhouses in Stillwater, OK.

Cultivars	Chemical	Rate (ml)	Timing of application	No. of flowers	SPAD	Height (cm)	Shoot fresh wt. (g)	Root fresh wt. (g)	Shoot dry wt. (g)	Root dry wt. (g)
Little Sicily	Control	0	None	17.6efg[z]	47.2f	97.0ab	276.3def	70.1efg	55.8bcdef	6.6cdefg
	Zerotol	35	Weekly	25.6abc	55.5bcd	101.6ab	527.0abc	110.6abcd	105.2ab	7.7abcde
	Zerotol	35	Biweekly	26.5abc	56.1bcd	102.2a	500.6abcd	142.8a	97.6ab	9.0abc
	Zerotol	70	Weekly	22.9bcde	55.2bcde	101.0ab	453.3abcd	111.4abcd	85.6abc	7.3bcde
	Zerotol	70	Biweekly	15.3g	50.3ef	72.5c	357.9bcdef	89.6cdef	69.0abcd	5.6efgh
	PERpose Plus	35	Weekly	23.7bcd	51.5def	100.0ab	528.3abc	103.5abcd	84.5abc	7.7abcde
	PERpose Plus	35	Biweekly	25.9abc	52.0def	95.3ab	635.4ab	133.3ab	91.9ab	10.5a
	PERpose Plus	70	Weekly	24.0bc	55.2bcde	97.2ab	680.6a	124.2abc	88.0ab	9.4ab
	PERpose Plus	70	Biweekly	21.9defg	50.5ef	90.6abc	506.7abcd	93.4bcde	119.6a	6.9bcdef
Geronimo	Control	0	None	21.7cdef	51.4def	45.8def	214.7f	52.9g	27.9g	4.9ghi
	Zerotol	35	Weekly	22.6cde	56.6bcd	46.0def	223.9f	67.1efg	44.3def	5.3fgh

Zerotol	35	Biweekly	25.3abc	58.2abc	42.6efg	391.8bcd	82.9def	39.6efg	5.9efgh
Zerotol	70	Weekly	24.6bc	62.9a	45.2def	366.1bcde	103.8abcd	40.7def	5.7efgh
Zerotol	70	Biweekly	15.1g	56.5cde	46.3def	251.7ef	90.6cde	37.5fg	5.8efgh
PERpose Plus	35	Weekly	29.2ab	55.1bcde	50.6de	284.5def	82.8def	33.9fg	5.9efgh
PERpose Plus	35	Biweekly	24.1bc	58.8ab	48.7de	415.8abcd	90.5cde	48.2def	6.2defgh
PERpose Plus	70	Weekly	31.3a	59.7ab	52.0d	442.3abcd	112.8abcd	51.0cdef	8.0abcd
PERpose Plus	70	Biweekly	22.0cde	52.7bcd	39.5fg	211.5f	67.3efg	33.7fg	3.9i

[z]Means (n = 16) within a column followed by same lowercase letter are not significantly different by pairwise comparison in mixed model ($P \leq 0.05$).

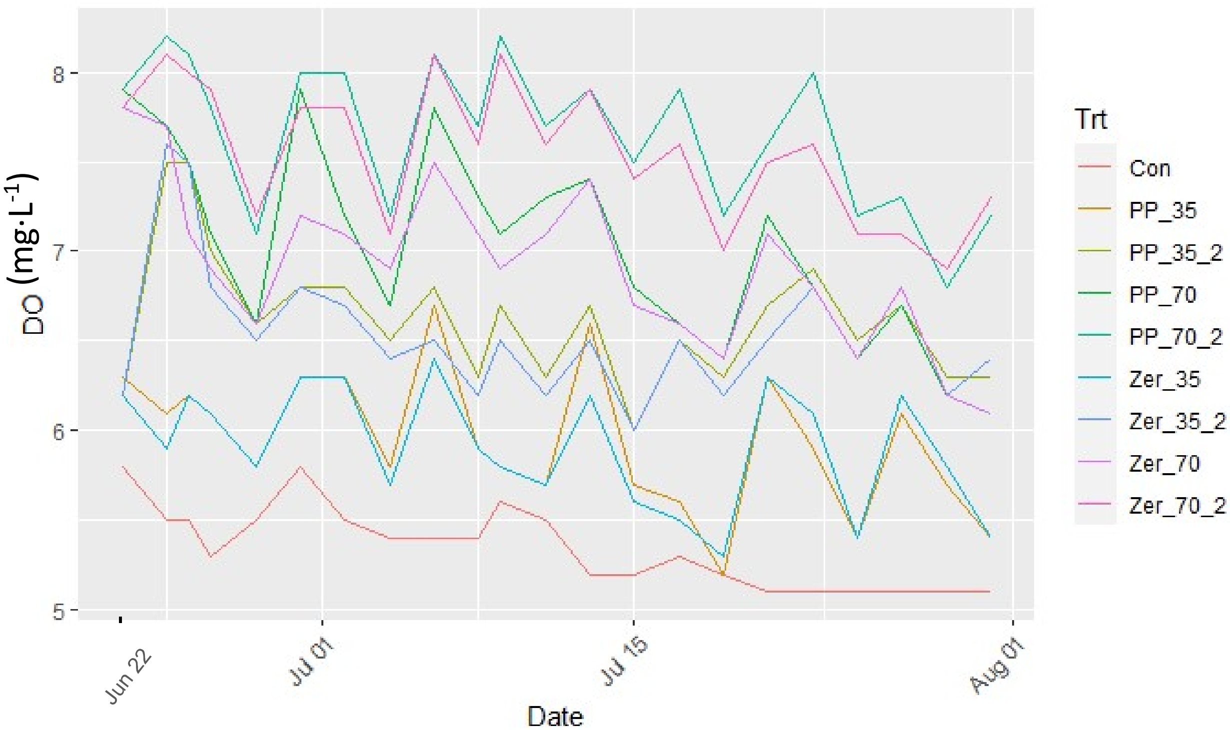

Figure 2.1: Effects of rate and application timing of two H_2O_2 products Zerotol (Zer) and PERpose Plus (PP) (35 mL weekly and biweekly, 70 mL weekly and biweekly, and control) on dissolved oxygen (DO) levels of nutrient solution in Ebb and flow hydroponic systems at OSU research greenhouses in Stillwater, OK.

CHAPTER III

WATER TEMPERATURE ON LETTUCE GROWTH AND QUALITY IN NFT HYDROPONIC SYSTEMS

Abstract

Nutrient solution temperature in a hydroponic system has been found to affect the quality as well as the yield of lettuce, thus it is important to maintain the water temperature within an appropriate range. Nutrient-film technique (NFT) trials were conducted to investigate the effects of different water temperature (18.3°C, 21.1°C, and ambient) on growth and quality of seventeen cultivars from five different types (Loose leaf, Romaine, Butterhead, Salanova, and Batavian) of lettuce. The average daily water temperature for ambient treatment was recorded to be 20 to 26.5°C. The study was conducted in a split-plot design with three replications over time. Ten plants per cultivar were planted per treatment. Data was collected on °Brix, SPAD, CO_2 assimilation, plant height, width, shoot and root fresh weight, and shoot and root dry weight. Results indicated that water temperature affected both root and shoot fresh as well as dry weight, plant width, and °Brix for lettuce. Lettuce grown at 21.1°C were significantly greater in terms of growth parameters compared to other treatments, however, °Brix was found to be greater at 18.3°C. All the growth and quality parameters of lettuce were found to be affected by cultivars, with 'Coastal Star' showing the best results in both growth and quality parameters. All the cultivars of

Romaine type showed better growth and quality, while Salanova lettuce did not perform well in all treatments compared to other lettuce types. For CO_2 assimilation, interaction between water temperature and cultivars was significant, with ‘Parris Island’ having the greatest rate at ambient water temperature. These results suggested that maintaining water temperature at 21.1°C produced lettuce with greater growth and yield, but lower °Brix than the lettuce grown at 18.3°C.

Introduction

Hydroponics is a technique of growing plants by placing the roots in a nutrient solution with or without mechanical root support (Jones, 2005). It is also referred as “controlled environment agriculture” or CEA since raising plants hydroponically requires control of various environmental factors such as water temperature, light intensity and duration, humidity, and pH of the solution and mineral nutrients (Pandey et al., 2009). Hydroponics offers advantages compared to soil-based systems due to more efficient use of fertilizers and water, greater control of the growing climate, and pest control (Bradley and Marulanda, 2000). An important characteristic of hydroponic cultivation is the need and ability to control the temperature of the nutrient solution or of the root system using heaters or a cooling spiral. It has been reported that relatively small changes in root-zone temperature can have a significant impact on root development, depending on phenological stage of crops and duration of temperature (Rodrigues, 2002).

Water temperature can affect many physiological processes during plant growth and development. Studies have shown that when the temperature is above or below an optimum level, it may influence plant metabolic activities including the accumulation of different metabolites such as phenolic compounds, nutrient uptake, chlorophyll pigment formation, photosynthesis, and finally the growth and development of the plant (Nxawe et al., 2010). Calatayud et al. (2008) suggested that, in most plant species, nutrient uptake by roots is decreased at low temperatures. In addition, temperature of the nutrient solution also affects the amount of oxygen dissolved in the solution. Oxygen solubility has been found to be reduced at high solution temperature (Falah et al., 2010). At prolonged high temperature, oxygen deficiency in the root zone can lead to poor root and plant performance and an increase in the incidence of diseases and pests (Chun and Takakura, 1994).

Lettuce (*Lactuca sativa* L.) is a herbaceous annual belonging to the family Compositae. It is an important cool season leaf/salad vegetable having high content of vitamin A and C and is consumed widely (Masarirambi et al., 2018). It is a cool season hydroponic crop that often suffer from growth and physiological disorders when the nutrient solution temperature is above or below optimum (Landers, 2017). Maintaining optimum root-zone temperature is important as it is the plant temperature, not air temperature, that is the regulating factor in plant growth (Nelson 2012). The effect of cooling of nutrient solution was studied on Butterhead lettuce by Ilahi et al. (2017) who reported significant increases in canopy diameter, leaf number, and yield with root-zone cooling at 19℃ when compared to the non-chilled condition. On the other hand, heating the nutrient solution to 15℃ and 20℃ increased shoot growth for 'Marbello' and 'Bastion' lettuces, respectively (Economakis and Said, 2002). In another experiment, In a related experiment, Boxall (1971) reported that heating the soil to 18°C, thereby heating the root zone, decreased the length of the production cycle of butterhead lettuce by 14 to 17 days in the field. However, the response in lettuce growth to temperature is cultivar dependent (Al-harbi, 2001).

The importance of optimal air temperature has been widely recognized, and it is equally important to optimize the water or root temperature in hydroponic crops as it is an important parameter to maximize crop growth and minimize possible damages (Thompson et al., 1998). While the effects of the root-zone has not been widely studied in hydroponic lettuce, there is evidence that by optimizing water temperature, market quality lettuce can be grown all year round even in warmer climates. The objectives of our research were to identify the optimum water temperature needed for the highest quality and yield of five different types of lettuce grown in NFT hydroponic systems.

Materials and methods

Plant materials and growth conditions:

Seventeen cultivars of lettuce, including five different types, were obtained from Johnny's Selected Seeds (Winslow, MN) on 1 March, 2020. Cultivars included Black Seeded Simpson, Waldman's Dark Green, and Panisse (Loose leaf type), Parris Island, Jericho, and Coastal Star (Romaine type), Nancy, Optima, and Buttercrunch (Butterhead type), Butter, Sweet Crisp Red, Sweet Crisp Green, Oakleaf Red, and Oakleaf Green (Salanova type), Nevada, Cherokee, and Sierra (Batavian type). Seeds were sown in oasis cubes (Harris Seeds, Rochester, NY) of size 1.5 cm^3 on 6 March, 2020 and kept under the mist bench at the Department of Horticulture and Landscape Architecture Research Greenhouses in Stillwater, OK. Nutrients were applied once when the seedlings were 2 weeks old. Nutrient solution was made by mixing 4.9 g Jack's 5-12-26 and 3.2 g calcium nitrate diluted in 10 L of water and applied over the seedlings. Seedlings were transplanted from the mist bench to the Hydrocycle Pro NFT table (Growers Supply, Dyersville, IA) on 29 March, 2020. Each NFT table had 10 channels measuring 10 cm wide x 5 cm deep x

900 cm long and each channel lids had 18 of the 2.5 cm site holes, spaced 20 cm on center. One lettuce plant per slot was planted and 10 plants per cultivar per table were transplanted in a randomized arrangement. The tables had a slope of 2.8% approximately between the irrigation and drainage end and the water flowing along the slope was collected in the tank and recirculated by pump to the irrigation pipe. Nutrient solution was made using Jack's 5-12-26 (J.R. Peters, Allentown, PA) and calcium nitrate (American Plant Products, Oklahoma City, OK). Tanks were filled to 40-gallon capacity and 147.41 g of Jack's along with 97.52 g of calcium nitrate was added initially according to the recommended rates. pH of the nutrient solution was maintained at 5.5 to 6.5 and the EC level was maintained between 1.5 to 2.5 $mS \cdot cm^{-1}$ throughout the growing period. No supplemental lighting was used in the greenhouse and DLI levels ranged from 10 to 15 $mol \cdot m^{-2} \cdot d^{-1}$. The greenhouse temperature was set at 21°C/18°C (day/night) regime. Water temperature in the NFT nutrient tanks were set at 18.3°C, 21.1°C, and a control at ambient temperature. Water chillers (Active Aqua, Hydrofarm, Petaluma, CA) were used to control water temperature. Ten healthy plants of each cultivar were selected and planted randomly per table.

Data Collection:

Data on photosynthesis rate and °Brix value were collected after 4 weeks of plants being on the NFT tables. For the °Brix value, a mature leaf from the middle of a stem was selected from each plant. The juice from the leaf was squeezed using a sap squeezer and measured by a refractometer (Cole-Parmer, Vernon Hills, IL) in sunlight. One °Brix is equivalent to 1 g sugar in 100 mL of water. Photosynthesis rate was measured using a Li-cor 6400 (LI-COR, NE). The Li-cor with 6400-02B LED light source chamber was used by keeping the reference CO_2 at 400 ppm. The block temperature was set at 28°C and the light level was set at 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. One of the top young leaves was selected per plant and was used as a non-destructive sample for the photosynthesis rate.

SPAD, height (cm), and width (cm) of each plant were measured at the time of harvesting, which was 35 d after transplanting. Each plant was scanned using a chlorophyll meter (SPAD-502, Konica Minolta, Japan) for the measurement of leaf chlorophyll concentration. From each plant, three mature leaves at top, middle, and bottom were selected in a non-destructive manner. Plants were harvested after 35 d of being on the table and data was collected on shoot and root fresh weight then oven dried for 2 d at 53.9°C for dry root and shoot weight. A data logger (HOBO, Onset, Bourne, MA) was used to record the average daily water temperature of the solution in all three tanks throughout the growing period.

Experimental design and statistical analysis:

The study was conducted in a split-plot design with three replications. Factors were water temperature (three levels) and cultivars of lettuce (seventeen levels). The experiment was replicated by starting the second set of seeds on 7 June 2020 and the third on 17 September, 2020 and similar growth conditions as described above were followed. Statistical analysis was performed using SAS/STAT software (Version 9.4; SAS Institute, Cary, NC). Tests of significance were reported at the 0.05, 0.001, and 0.0001 level. The data were analyzed using generalized linear mixed models methods. Tukey multiple comparison methods were used to separate the means.

Results and discussion

Water temperature

Daily water temperature in the ambient treatment ranged from 18.8 to 25.5, 23.3 to 30.4, and 16.7 to 24.7 °C during the first, second, and third replications, respectively (Figure 3.1, 3.2, 3.3). Average temperature for the ambient treatment was 23.6, 26.5, and 20 °C during the first, second, and third replications, respectively. Daily water temperature in the 18.3°C chilled treatment ranged from 17.9 to 18.8, 17.7 to 19.0, and 17.6 to 18.5 °C during the first, second, and third replications, respectively. Average temperature for the 18.3°C chilled treatment was 18.3, 18.4, and 18.3 °C during the first, second, and third replications, respectively. Daily water temperature in the 21.1°C chilled treatment ranged from 20.5 to 21.3, 20.5 to 21.3, and 20.5 to 22 °C during the first, second, and third replications, respectively. Average temperature for the 21.1°C chilled treatment was 21.1, 21.1, and 20.9°C during the first, second, and third replications, respectively. Temperature drops seen in both the first and third rep was the result of heaters malfunctioning.

Temperature and cultivar effect and their interaction on plant growth parameters

There was a significant cultivar × temperature interaction for carbon dioxide assimilation (Table 3.1). Greatest net CO_2 assimilation was recorded in Romaine type 'Parris Island' within the ambient water temperature (Table 3.2). Temperature is said to be one of the major environmental factors affecting the physiological processes in plants, especially photosynthesis and plant growth (Zobayed et al., 2005). Similar to our findings, root zone cooling has been found to affect the net photosynthetic rate (P_N) and CO_2 assimilation of hydroponic lettuce and several other species (Martin et al., 1989; Udomprasert et al., 1993;

Sun et al., 2016). Sun et al. (2016) reported increased P_N, stomatal conductance (G_S), intercellular CO_2 concentration (C_i), and transpiration rate (T_r) in 'Romaine' lettuce by root zone cooling at 25°C compared to a 30°C control treatment, although the chlorophyll content in lettuce leaves was not affected by root zone cooling, suggesting the increase of photosynthesis was caused by improved stomatal conductance, which enabled sufficient CO_2 for photosynthesis. Zhang et al. (2008) also suggested that changes in photosynthesis induced by root temperatures were mainly attributed to corresponding changes of *G*s since there was a similarity in the trends for P_N and G_s, however the responsiveness to root-zone temperature depends on the plant species and cultivars. He et al. (2013) were able to prevent photoinhibitory damage in temperate lettuce plants grown in the tropics under hot ambient temperature by exposing the roots to a lower temperature of 20°C. They concluded that the reduction in photosynthesis in lettuce plants with their roots exposed to hot ambient temperature was mainly due to the reduction in water uptake.

Plant width showed significant main effects for both cultivar and temperature (Table 3.1). There was no difference in plant width of lettuce grown at water temperatures of 18.3°C and 21.1°C, but both were greater than the ambient temperature treatment (Table 3.3). 'Waldman's Dark Green' had the greatest width, but was not different than 'Nancy', 'Optima', 'Jericho', 'Parris Island', 'Coastal Star', and 'Sweet Crisp Green' (Table 3.4). Within plant types for plant width, 'Waldman's Dark Green' of Loose Leaf type was greater than 'B.S. Simpson' and 'Panisse' and Salanova type 'Sweet Crisp Green' was greater than 'Butter' (Table 3.4). For root and shoot fresh and dry weight, there was significant cultivar and water temperature main effects (Table 3.1). Greatest root and shoot fresh and dry weights were observed when the water was at 21.1°C. 'Jericho' had the greatest root weight but was not different than all other cultivars except ‘Oakleaf Green’, ‘Oakleaf Red’, and ‘Butter’ (Table 3.4). ‘Optima’ had the greatest root dry weight and was similar ‘Oakleaf Green’, ‘Oakleaf Red’, ‘Sweet Crisp Green’, and ‘Butter’ (Table 3.4). For shoot fresh weight, ‘Coastal Star’ and ‘Sweet Crisp Green’ were greatest but not different than all other cultivars except ‘Panisse’, ‘Oakleaf Green’, ‘Oakleaf Red’, and ‘Butter’. Within Loose leaf type, ‘Black Seeded Simpson’ and ‘Waldman’s Dark Green’ had greater fresh and dry shoot weight compared to ‘Panisse’.

Water temperature is an important growth factor that may influence plant development in hydroponic systems. At optimum temperatures, water can nourish growth, while at lower or high levels, plant growth can be negatively affected (He et al., 2002). Similar to our results, root-zone cooling at 20℃ increased the biomass of aeroponically grown lettuce from that in plants with ambient conditions (24℃ to 38℃) in a tropical greenhouse (He at al., 2013). Since the roots of plants are more sensitive to heat stress than the above-ground parts, high root-zone temperature can easily damage the root, which can restrict the length and diameter of stem (Lam et al., 2020). High root-zone temperature (27.5°C) resulted in water and nutrient loss in tomatoes (*Solanum lycopersicum* L.) which ultimately led to reduction of plant growth (Díaz-Pérez et al., 2007). In another study, high temperature in the root-zone resulted in reduced leaf, stem, and fresh and dry weights of coriander (*Coriandrum sativum* L.), as a result of water deficits in the plants (Nguyen et al., 2019). According to Al-Rawahy et al. (2019), temperature of the nutrient solution has a direct relation to the amount of oxygen consumed by plants, and an inverse relation to the oxygen dissolved. Nutrient solution cooling to 22°C and 25°C provided positive effects on the availability of dissolved oxygen levels in the nutrient solutions as well as on all growth parameters (plant height, leaf number, chlorophyll content, leaf area) and production parameters (number of fruits and yield) in cucumber (*Cucumis sativus* L.).

On the other hand, when water temperature in hydroponic system is too low, it can affect nutrient uptakes of plants. A study on red leaf lettuce showed that the exposure of lettuce roots to low temperature (10°C) significantly reduced leaf area, stem diameter, and fresh weight of tops and roots compared to 20°C (Sakamoto & Suzuki, 2015). Calatayud et al. (2008) revealed that, in most plant species, nutrient uptake by roots is found to decrease at low temperatures, which ultimately affects plant growth and yield. Cometti et al. (2013) conducted research with the objective of evaluating the effect of cooling of nutrient solution on growth and development of lettuce 'Vitória de Santo Antão' in hydroponics and reported greater fresh weight of leaves and stem, greater volume of roots, dry weight of leaves and roots, and greater percentage of water in the plants with cooling of the nutrient solution (maximum 26°C) compared to the fluctuating ambient water temperature which ranged from 24°C to 29.9°C. Further, controlling the temperature to a maximum of 26°C resulted in greater amount of oxygen (9.3 $mg \cdot L^{-1}$) in the solution compared to the ambient temperature (6.2 $mg \cdot L^{-1}$). In another study, using 24°C root zone temperature, lettuce crop growth was maximized and damages minimized at various air temperatures ranging from 17°C to 31°C. Head size, leaf color, leaf thickness and root structure were found to be best in 24°C water compared to 17°C and 31°C regardless of the air temperature. 31°C water temperature resulted in the presence of root diseases in lettuce which ultimately reduced the head size, whereas poorly formed roots in 17°C water also contributed to the reduced head size of lettuce (Thompson et al., 1998). Lam et al. (2020) studied the response of Korean mint plants (*Agastache rugose* L.) to various root zone temperature and reported an increase in nine plant growth parameters, namely, shoot and root fresh weights, stem and root lengths, leaf length and leaf width, leaf area, and shoot and root dry weights at 28°C compared to 10°C and 36°C. They concluded that too high as well as too low temperature led to water stress, ion imbalance, and growth inhibitors and promoter imbalance in the plant. These results support our findings in which increased root fresh weight, dry weight, and shoot dry weight at 21.1℃ compared to lower (18.3℃) or higher ambient temperature was found (Table 3.3).

In the present study, plant height was found to be affected by cultivars (Table 3.1). 'Black Seeded Simpson' had the greatest plant height and was found to be significantly similar to 'Waldman's Dark Green', 'Jericho', and 'Coastal Star' (Table 3.4). Within types for plant height, 'B.S. Simpson' and 'Waldman's Dark Green' were greater than 'Panisse' while Salanova type 'Sweet Crisp Green' was greater than 'Oakleaf Green', 'Oakleaf Red', and 'Butter' (Table 3.4). Lettuce growth response is known to be cultivar dependent (Al-harbi, 2001). An experiment conducted by Holmes et al. (2019) evaluated the effects of four types (Romaine, Butterhead, Bibb, Summer Crisp) and 18 lettuce cultivars, on growth and bolting, some of which were common in our experiment. Similar to our findings, large differences in growth characteristics including the size index and head fresh weight between the cultivars of various types as well as within types was reported and these differences were attributed to genetic diversity. Another study was conducted to study the growth characteristics of nine commonly grown lettuce cultivars of four different types (Leaf, Butterhead, Romaine, and Crisphead subtype Batavia), in which Romaine and leaf lettuce showed greater plant growth rates during the initiation period of plant growth than 'Butterhead' and 'Batavia' lettuce (Lee et al., 2015). They reported that the interaction between genotype and environment affects lettuce plant development and plays a critical role in determining the marketable yields of lettuce.

Temperature and cultivar effect and their interaction on SPAD readings and °Brix of lettuce

There was a significant main effect of cultivar on SPAD readings (Table 3.1). Greatest mean for SPAD was observed in 'Butter', which was not different than 'Sweet Crisp Green', 'Sweet Crisp Red', 'Oakleaf Red', 'Parris Island', and 'Coastal Star'. SPAD readings and total chlorophyll content of leaves have been found to be significantly correlated (Jiang et al., 2017). Wang et al. (2005) concluded that leaves with high chlorophyll concentration not only show increasing greenness visually but also indicate the sound growth of plants physiologically. In a study conducted by Gazula et al. (2005), differences in chlorophyll concentration among three different cultivars of lettuce 'Impuls', 'Valeria', and 'Lotto' were found and were attributed to differences in gene numbers. Irrespective of the water temperature, there was a difference in chlorophyll concentration which supports the fact that genetic factors affecting the plant growth also influences the chlorophyll concentration in various horticultural crops (Reay, 1999; Dela et al., 2003; Gazula et al., 2005).

Significant main effects of cultivar and temperature occurred for °Brix content (Table 3.1). For water temperature, °Brix was greatest in water temperature of 18.3℃ (Table 3.3). Among cultivars for °Brix, 'Sierra' had the greatest value but was not different than all other cultivars except 'Jericho' and 'Black Seeded Simpson' (Table 3.4). Root-zone temperature can affect the production of various plant metabolites in many plants, including leafy vegetables (Chadirin et al., 2011; Sakamoto and Suzuki, 2015). Sakamoto and Suzuki (2015) conducted a study on red leaf lettuce 'Red Wave' and found that there was a significant increase in production of anthocyanin and total phenol content in the leaves of red lettuce when grown in 10℃ water than the ones that were grown in 20℃ and 30℃. There was also a significant difference in the total soluble solids among various temperature with the greatest °Brix found in lettuce grown in 10℃. Similarly, low temperature treatment of roots was found to enhance °Brix in spinach (*Spinacia oleracea* L. *cv.* Orai*).* It is suggested that low temperature treatment induces water stress and the plants exposed to osmotic stress such as salt or water stress induce osmoregulation by the accumulation of osmolites like sugar, minerals, and amino acids in order to maintain the turgor pressure in leaves (Hasegawa et al., 2000; Chadirin et al., 2011). Similarly, Guinn and Hunter (1968) observed rapid increases in sugar contents of all plant parts, including leaves, epicotyls, hypocotyls, and roots of young cotton plants (*Gossypium hirsutum* L.) at lower root temperature of 10℃. Not only temperature, but lettuce cultivars also affect °Brix. According to the sensory panel and analytical evaluations of five different lettuce cultivars, it was demonstrated that genetic constitution had a greater impact on sensory qualities and total phenolic content. Flavor, total phenolic content, and degree of bitterness varied greatly among lettuce cultivars of different types and pigmentation but, with few exceptions, did not vary within cultivars across the growing season (Bunning et al., 2010).

Conclusion

In the present experiment, lettuce grown at 21.1℃ chilled water were greater in terms of shoot fresh and dry weight as well as root fresh and dry weight compared to 18.3℃ and ambient water treatments, whereas greater ºBrix was recorded at 18.3℃ water temperature. This shows that ºBrix of lettuce increases with decreasing water temperature. All the cultivars in Romaine type showed better growth and quality, while Salanova lettuce did not perform well in all treatments compared to other lettuce types. For CO_2 assimilation, interaction between water temperature and cultivars was found to be significant, with 'Parris Island' having the greatest rate at ambient water temperature. While this study shows some possible advantages of chilling the nutrient solution in a NFT hydroponic system, further research could be done investigating full effects on nutritional quality. Future research should investigate other cultivars suitable for hydroponics using these water temperatures or should evaluate other temperatures for these lettuce types and cultivars.

Tables and figures

Table 3.1: Tests of effects for water temperature (18.3℃, 21.1℃, ambient) and seventeen lettuce cultivars grown in NFT hydroponics systems at OSU research greenhouses in Stillwater, OK.

	Cultivar	Temperature	Cultivar × Temperature
CO_2 assimilation	***[z]	NS	*
SPAD	***	NS	NS
Height	***	NS	NS
Width	***	**	NS
°Brix	*	***	NS
Root fresh wt.	***	***	NS
Shoot fresh wt.	***	**	NS
Root dry wt.	***	***	NS
Shoot dry wt.	***	**	NS

[z]Indicates significant at or non-significant (NS) at *P ≤ 0.05, **P ≤ 0.001, or ***P ≤ 0.0001.

Table 3.2: Least square means for lettuce cultivar and water temperature (18.3°C, 21.1°C, ambient) for net CO_2 assimilation in NFT hydroponic systems at OSU research greenhouses in Stillwater, OK.

Types	Cultivars	Water temperature (°C)	CO_2 assimilation ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
Loose leaf	B.S. Simpson	18.3	18.9abcdefgh[z]
		21.1	19.8abcdefg
		Ambient[y]	19.7abcdefgh
	Waldman's Dark Green	18.3	18.7abcdefgh
		21.1	18.8abcdefgh
		Ambient	18.1bcdefgh
	Panisse	18.3	16.5gh
		21.1	16.2h
		Ambient	15.3h
Butterhead	Buttercrunch	18.3	20.3abcde
		21.1	19.0abcdefgh
		Ambient	18.1abcdefgh
	Optima	18.3	18.5abcdefgh
		21.1	18.8abcdefgh
		Ambient	20.6abc
	Nancy	18.3	18.8abcdefgh
		21.1	19.3abcdefgh
		Ambient	18.9abcdefgh
Romaine	Jericho	18.3	18.2abcdefgh
		21.1	20.6abc
		Ambient	19.8abcdefgh
	Coastal Star	18.3	19.3abcdefgh
		21.1	19.1abcdefgh

		Ambient	19.3abcdefgh
	Parris Island	18.3	20.2abcdef
		21.1	18.3abcdefgh
		Ambient	21.5a
Batavian	Cherokee	18.3	19.7abcdefgh
		21.1	21.3ab
		Ambient	19.8abcdefgh
	Sierra	18.3	19.4abcdefgh
		21.1	20.4abcde
		Ambient	20.5abcd
	Nevada	18.3	19.8abcdefgh
		21.1	19.7abcdefgh
		Ambient	18.9abcdefgh
Salanova	Oakleaf Red	18.3	17.6bcdefgh
		21.1	17.1efgh
		Ambient	16.8fgh
	Oakleaf Green	18.3	16.5gh
		21.1	17.7bcdefgh
		Ambient	16.2h
	Sweet Crisp Green	18.3	19.4abcdefgh
		21.1	18.5abcdefgh
		Ambient	17.1defgh
	Sweet Crisp Red	18.3	18.2abcdefgh
		21.1	17.7bcdefgh
		Ambient	17.6bcdefgh
	Butter	18.3	17.4cdefgh
		21.1	18.9abcdefgh
		Ambient	17.2cdefgh

[z]Means (n = 30) within a column followed by same lowercase letter are not significantly different by pairwise comparison in mixed model ($P \leq 0.05$).

[y]Ambient treatment indicates control water temperature which ranged from 20°C to 26.5°C.

Table 3.3: Least square means for water temperature on growth and °Brix of five types of lettuce grown in NFT hydroponic systems at the OSU research greenhouses, Stillwater, OK.

Temperature (°C)	°Brix	Width (cm)	Shoot fresh wt. (g)	Root fresh wt. (g)	Shoot dry wt. (g)	Root dry wt. (g)
18.3	5.7a[z]	22.5a	86.8ab	22.7b	5.2b	0.8b
21.1	4.5b	23.3a	96.8a	26.8a	5.9a	1.1a
Ambient	4.3c	20.7b	84.1b	22.5b	5.0b	0.9b

[z]Means (n = 30) within a column followed by same lowercase letter are not significantly different by pairwise comparison in mixed model ($P \leq 0.05$).

Table 3.4: Least square means for five types and seventeen cultivars on growth and quality of lettuce grown in NFT hydroponic systems at OSU research greenhouses in Stillwater, OK.

Types	Cultivars	°Brix	SPAD	Height (cm)	Width (cm)	Shoot fresh wt. (g)	Root fresh wt. (g)	Shoot dry wt. (g)	Root dry wt. (g)
Loose leaf	B.S. Simpson	4.5b[z]	17.5gh	25.2a	20.5bcd	109.3a	27.1a	6.7abc	1.0abc
	Waldman's Dark Green	4.8ab	22.3gf	21.9ab	27.5a	118.3a	27.3a	7.5ab	1.1ab
	Panisse	4.9ab	15.5h	13.2def	21.9bcd	65.1bcd	21.8abcd	4.7cdefg	0.9abcd
Butterhead	Buttercrunch	4.9ab	30.5bc	12.7def	22.1bcd	82.8abc	21.9abcd	4.9cdefg	0.9abcd
	Nancy	5.1ab	22.8efg	14.7cdef	25.6ab	104.8a	24.8ab	5.9abcde	1.0abcd
	Optima	5.1ab	28.9bcd	15.2bcde	24.4ab	92.2abc	26.6ab	6.2abcd	1.2a
Romaine	Jericho	4.5b	24.3def	20.4abc	25.2ab	122.9a	28.9a	7.5abc	1.1abc
	Parris Island	5.4a	31.4abc	17.3bcd	22.7abcd	91.2abc	24.7ab	5.6abcdef	1.1abc
	Coastal Star	4.7ab	31.7abc	21.7ab	25.4ab	123.0a	27.9a	7.4abc	1.1ab
Batavian	Cherokee	5.0ab	25.7def	14.7cdef	21.7bcd	97.6ab	26.8ab	5.0cdef	1.0abcd
	Nevada	4.6ab	23.1defg	14.9bcdef	18.9bcd	80.8abcd	21.5abcd	4.5cdefg	0.9abcd
	Sierra	5.3a	24.5def	14.5cdef	21.5bcd	85.2abc	24.5abc	4.4defg	1.0abcd

Salanova	Oakleaf Green	4.9ab	27.2cde	7.2f	18.7cd	51.3d	18.9d	3.5g	0.8d
	Oakleaf Red	4.7ab	32.6ab	9.2ef	19.8bcd	63.7cd	20.9bcd	4.0fg	0.8d
	Sweet Crisp Green	4.7ab	33.5ab	16.3bcd	23.4abc	123.0a	22.9abcd	7.7a	0.9bcd
	Sweet Crisp Red	4.9ab	31.9ab	13.5cdef	20.6bcd	89.7abc	22.5abcd	5.4bcdef	0.9abcd
	Butter	5.0ab	35.2a	9.1ef	17.9d	61.8cd	19.1cd	4.2efg	0.8cd

^{z}Means (n = 30) within a column followed by same lowercase letter are not significantly different by pairwise comparison in mixed model ($P \leq 0.05$).

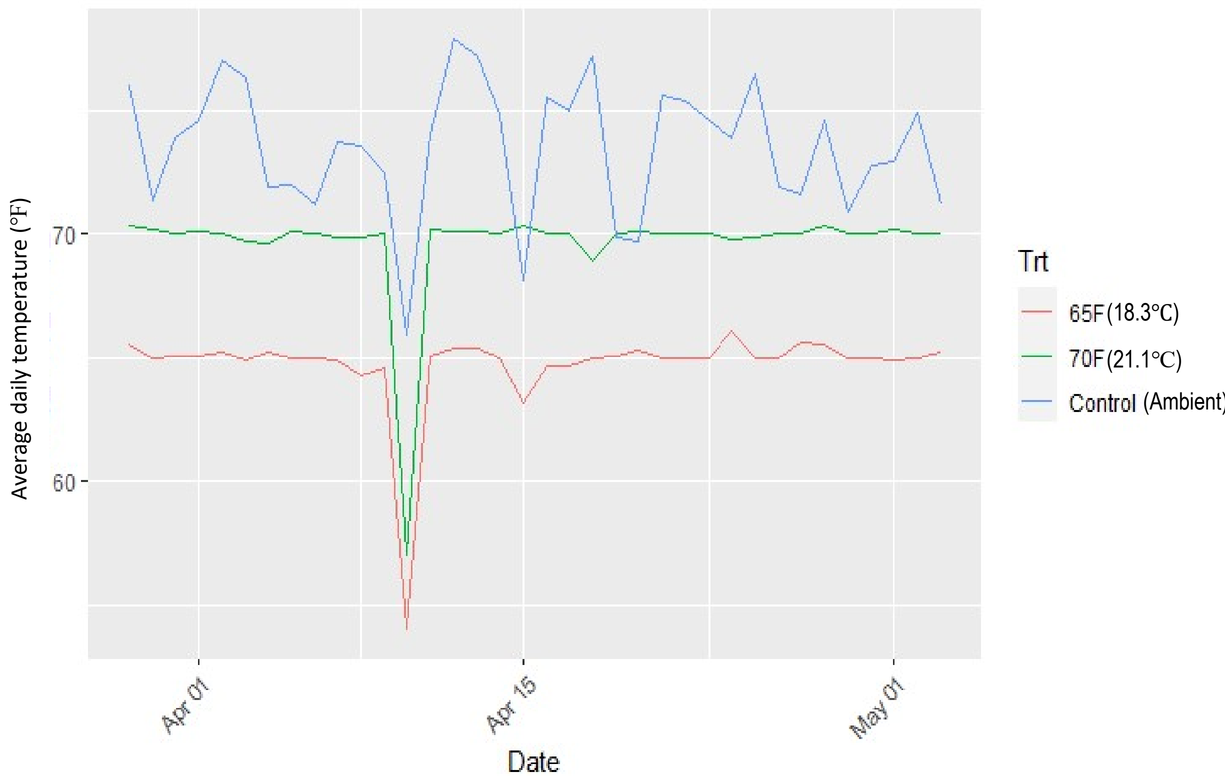

Figure 3.1: Average daily water temperature for three treatments during the first replication in Stillwater, OK greenhouses in 2020.

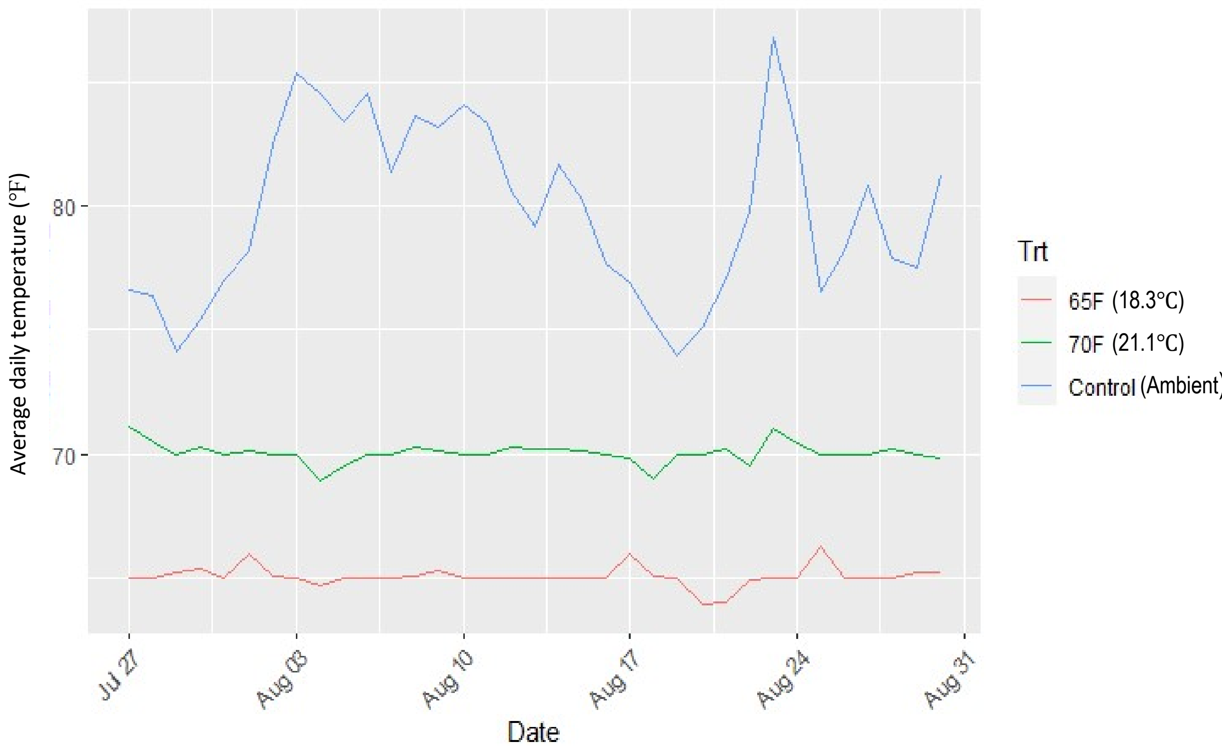

Figure 3.2: Average daily water temperature for three treatments during the second replication in Stillwater, OK greenhouses in 2020.

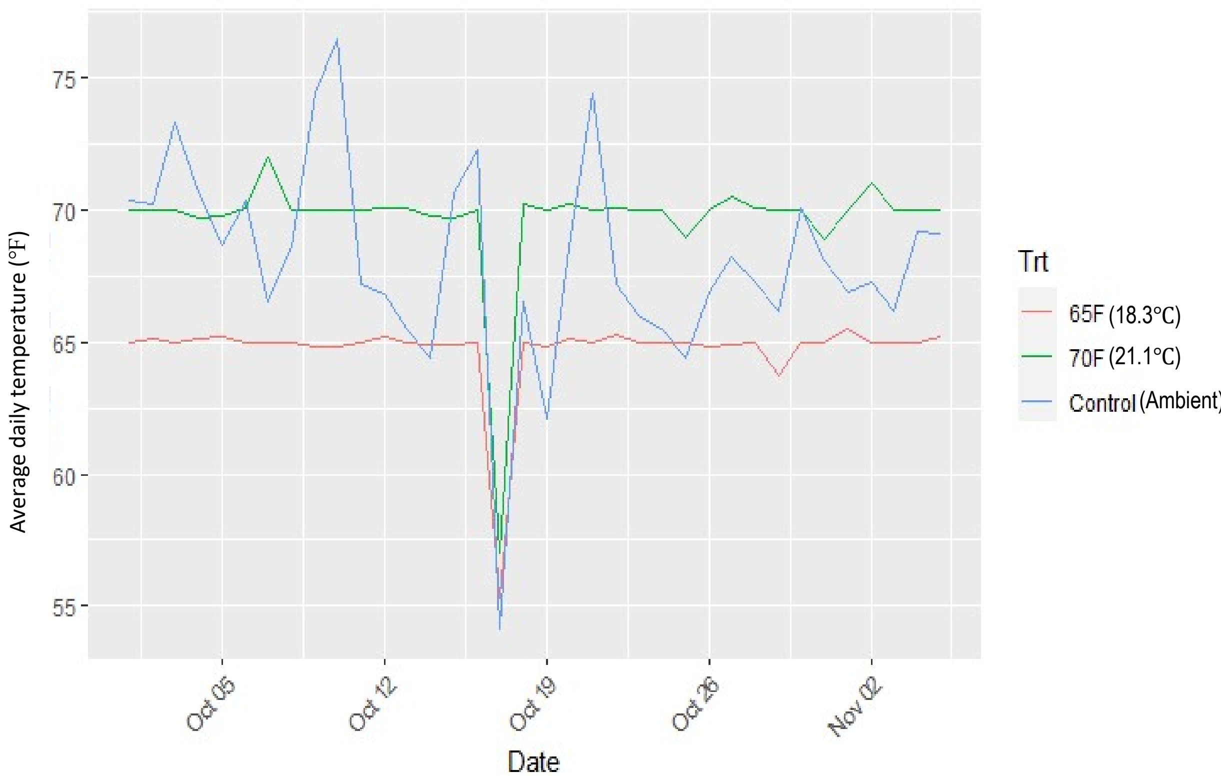

Figure 3.3: Average daily water temperature for three treatments during the third replication in Stillwater, OK greenhouses in 2020.

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