# THE EFFECT OF WATER TEMPERATURE ON BASIL GROWTH IN HYDROPONICS AND EVALUATION OF HYDROGEN PEROXIDE PRODUCTS ON ALAGE AND PLANT GROWTH

By

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EVALUATION OF HYDROGEN PEROXIDE

PRODUCTS ON ALAGE AND PLANT GROWTH

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Abstract: Root zone temperature is an important factor in growing plants, whether in the field or hydroponically. In this research, three temperatures (23°C, 27.5°C, and 31°C) were evaluated to measure their effect on plant growth on 17 cultivars of basil in an Nutrient Film Technique hydroponic system. Algae is a common problem in agricultural water usage, especially in hydroponic systems. Hydrogen peroxide ($H_2O_2$) has been commonly used in other agricultural systems to prevent or lessen the buildup of algae. In this research, three hydrogen peroxide products (Zerotol, PERpose Plus, and 3% hydrogen peroxide) were evaluated based on their ability to limit algae growth while not causing phytotoxic effects on the lettuce and basil crops in the hydroponic system. Results of this research found that warmer nutrient solutions, such as 27.5°C and 31°C, produced a greater shoot fresh weight and leaf number in most varieties of basil. For all cultivars of basil except purple basil and ‘Large Leaf Italian’, growing with a 27.5°C or 31°C nutrient temperature will result in a greater yield.  For control of algae, there were no significant differences between hydrogen peroxide products and the control, though concentrations of 60 mL of hydrogen peroxide products such as Zerotol and PERpose Plus can visually lessen the appearance of algae. Lower concentrations of hydrogen peroxide treatments had less phytotoxic effects on lettuce, while basil was not influenced by the presence of greater concentrations of hydrogen peroxide, except in terms of shoot dry weight.

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# CHAPTER I

# LITERATURE REVIEW

## HYDROPONICS

Soilless agriculture has become more popular in recent years (Bhargaw and Chauhan, 2020; El-Kazzaz and El-Kazzaz, 2017). Unlike traditional soil-based agriculture, soilless agriculture can be used year-round in many climates that were previously unsuitable for in-ground agriculture. Not only that, but soilless agriculture reduces time between plantings, as there is no need for fallowing or crop rotation, allows for more control over nutrient levels and the growing environment, provides for better working conditions due to the elevated systems, and faster growth/increased yield due to easier oxygen and water access for the roots of the crop (Shrestha and Dunn, 2016). Since the need for soil is eliminated, increased pollution, pesticide use, urbanization, and desertification may be avoided with what appears to be an environmentally friendly agricultural system (Sambo et al., 2019). Food insecurity is also a growing concern (Bhargaw and Chauhan, 2020). With the use of soilless agriculture, space, soil quality, and climate can all be negated, allowing for fresh fruits and vegetables even in food deserts (Treftz and Omaye, 2015).

There are several different methods for soilless agriculture, including aquaponics, aeroponics, and hydroponics. While the term "hydroponics" was first mentioned by Dr. W. A. Gericke in 1937, the practice of growing plants in water or a nutrient solution was used in civilizations such as the Aztecs, Egyptians, and Babylonians (Jones 1982, 2005; Resh, 1978). Hydroponics comes from the Greek words *hydro*, meaning water, and *ponos*, meaning labor, based off of Gericke's experiments using a nutrient solution in place of soil. Hydroponics can include inert mediums such as vermiculite, perlite, gravel, or mineral wool can be solely based on a water-nutrient solution (Sardare and Admane, 2013; Sharma et al., 2018). Some of the main concerns with using soilless media and hydroponic systems can include pathogens traveling quickly through the closed system, expensive setup and operational costs, need for skilled maintenance to operate the system, and wastewater pollution (Hughes, 2016; Shrestha and Dunn, 2016). However, hydroponic systems often have a greater yield, mitigating some of the costs of production (Papadopoulos, 2008).

## EBB AND FLOW HYDROPONICS

Though less popular commercially in recent years, the Ebb and Flow hydroponics system is often a common method for hobbyists and home growers. Consisting of a watertight grow bed, nutrient solution tank, pump, and pipe system, Ebb and Flow hydroponics, also called "flood-and-drain" hydroponics, is a closed recirculating system. This means that it can recirculate water and nutrients within the system.

During World War II, the Ebb and Flow system was used to produce lettuce (*Lactuca sativa* L.) and tomatoes (*Solanum lycopersicum* L.) by the U.S. Army, eventually leading to commercial usage of Ebb and Flow systems (Bhargaw and Chauhan, 2020; Jones, 2014). However, the Ebb and Flow system had a few disadvantages. Firstly, there was an increased susceptibility to root diseases. Once a diseased plant was introduced into the system, the likelihood that the disease would wipe out the entire system increased dramatically (El-Kazzaz and El-Kazzaz, 2017). Secondly, the systems were highly difficult to clean. Every inch of the table would need to be sterilized as frequently as any disease was introduced. This became especially arduous when the use of an inert substrate was included, as the substrate would have to be entirely replaced or sterilized (El-Kazzaz and El-Kazzaz, 2017). Due to these reasons, commercial use of this system has declined, and hobby growers instead have taken to the system as Ebb and Flow hydroponics are relatively easy to assemble and maintain compared to more technical tables (Jones, 2014).

## NUTRIENT FILM TECHNIQUE HYDROPONICS

Nutrient Film Technique, commonly abbreviated to NFT, was developed in the 1970s and debuted at the Hydroponics Worldwide: State of the Art in Soilless Crop Production conference when it was presented by Allen Cooper (Jones, 2014). The technique was named due to the design's emphasis of shallow liquid flowing past the plant's roots, though others also called it the nutrient flow technique, as the liquid flowed in a recirculating system and is currently used mainly for low growing crops and herbs (Dholwani et al., 2018; Mohammed and Sookoo, 2016; Resh, 1978).

The first NFT “table” was a vinyl-lined trench dug into the center of a greenhouse with polyethylene troughs slated down to it as the reservoir. A pump and pipeline would transport the nutrient solution into the top of the troughs (Resh, 1978) In the 1970s, Dr. Schippers developed the “nutrient flow technique” as a method of taking an NFT table and turning it vertical. He developed techniques such as the NFT “tower,” a system with a vertical pipe, where the nutrient solution dripped downward to the plants, and the “cascade” NFT system, where the nutrient solution enters the highest vertical sloped trough and then is pulled down into the next one and so on, a system successful with lettuce and other low growing crops (Resh, 1978).

Even as new designs are created, the NFT method does not solve many of the previous issues with hydroponics. There are, however, many advantages to the NFT method, nonetheless. Unlike other methods, the NFT method is relatively easy and cheap to construct. Also, the closed recirculating system allows the nutrient solution to be cycled through many times before needing to be replaced by measuring the electrical conductivity and pH of the solution (Jones, 2014; Wortman, 2015).

## BASIL

Basil (*Ocimum basilicum* L.) is commonly used as a culinary herb, medicinal herb, and as an agent to control insects (Raimondi, 2006). Basil is one of the most popular herbs to grow hydroponically, as its yield is said to be better in soilless media (Currey, 2020; Saha et al., 2016). Found mainly in tropical and subtropical regions, basil is a high-light, warm temperature crop (Currey, 2020; Raimondi, 2006). Optimal air temperatures tend to be around 26°C (Currey, 2020). Terrosi et al. (2020) found that heating sweet basil seedlings grown in soil using a basal heating system, such as a heat pump or condensing boiler, warmed the soil significantly more than air heating systems, subsequently leading to a higher biomass yield. Contrarily, Doty et al. (2020) suspected that increased root zone temperatures (RZT) may have decreased plant growth in basil cultivars 'Cinnamon', 'Mrs. Burns Lemon', 'Genovese', and 'Sweet Thai' because of nutrient solution temperature increasing to extremes due to lack of water volume in the NFT system.

There are several types of basil, including sweet basil and large leaf basil, some of the more popular basils for cooking and hobby gardening. Basil is popular in gardens and planters due to its fragrance and high phenolic content. In more recent years, basil has been grown more frequently in indoor controlled environments, as it is hard to control the phenolic content and phytochemical concentration (Dou et al., 2018). Basil can withstand a variety of different conditions, such as a wide range of temperatures, light intensities, and dissolved oxygen levels, leading many to consider basil as an ideal hydroponic plant (Solis-Toapanta et al., 2020). Another important component to basil quality is leaf area and number (Chang et al., 2005; Pennisi et al., 2020). Leaves can often have a greater nitrate content due to an increase in available nutrients while growing, which negatively effects leaf quality (Raimondi et al., 2006).

## LETTUCE

Lettuce is a cool season crop that thrives between 15 to 21°C (University of Illinois Extension, 2021). At temperatures that are greater than optimal, lettuce tends to develop several physiological disorders such as tip burn, ribbiness, rib discoloration, and bolting (Landers, 2017). Greenhouse and hydroponically produced lettuce has become more common in recent years and has been shown to produce 11 times greater yield per area than conventional lettuce production (Barbosa et al., 2015). Lettuce is relatively easy to grow in a hydroponic system, especially in an NFT system, as lettuce has very shallow roots (Barbosa et al., 2015; University of Illinois Extension, 2021). Leafy greens are generally desired year-round, leading to producers to lean more heavily on climate-controlled production as opposed to soil agriculture due to climate fluctuations (Lee et al., 2015). High temperatures can cause bolting and bitterness, affecting the quality of the crop. Other conditions that may affect the quality of a lettuce crop include light intensity, photoperiod, nutrients applied, pest management, and high humidity (Kaiser and Ernst, 2016; Landers, 2017; Lee et al., 2015; Ohse et al., 2001; Pennisi et al., 2020).

## WATER TEMPERATURE MANAGEMENT

Since the increase of greenhouse use, temperature management has become more important to agricultural processes. Air temperature is often the focus, especially in germination. Each crop's preferred temperature varies, with some, like oregano (*Origanum vulgare* L.) more sensitive and requiring specific temperatures, and others, like basil, with a broader spectrum of acceptable temperatures (Putievsky, 2015). Often, increased temperatures can increase the rate and percentage of germination, such as hyssop (*Hyssopus officinalis* L) and oregano at ~25°C, and sweet basil at ~27.5°C (Mijani et al., 2013). Warmer temperatures can also mean greater volatile oil content and differences within its composition (Chang et al., 2005). Some plants even show their adaptability to rising air temperatures due to temperature management, showing that some increase in temperature with many warmer climate plants may be acceptable (Al-Huqail et al., 2020). Also, with the use of hydroponic systems, there has been an increased focus on RZTs.

Root temperatures can be altered in soil as well, such as with geothermal pipelines, and have been shown to correlate an increase in rooting percentages and shoot growth in both rootstocks and crops such as tomato, cucumber (*Cucumis sativus* L.), and pepper (*Capsicum annuum* L.) (Nxawe et al., 2009; Rosik-Dulewska and Grabda, 2002). RZT has also shown to impact nutrient uptake. A greater RZT allows more nutritional uptake, while a lower RZT slows nutritional uptake (Thakulla et al., 2021; Yan et al., 2012). In tomatoes, warmer root temperatures between 25 to 30°C increased transpiration, nutritional absorption, root length, and leaf area (Moorby and Graves, 1980). In spinach (*Spinacia oleracea* L.), warming the nutrient solution sped up metabolic processes and therefore nutrient uptake and shoot growth (Nxame et al., 2009). Optimized root conditions can also substitute for poor air temperature, as seen in lettuce with a RZT of 24°C grown in 31°C air temperature that still produced a marketable product, in contrast with a RZT of 31°C, which caused wilting (Thompson et al., 1998).

## ALGAE

Algae is a photosynthetic organism that can form as single cell organisms or in colonies while producing asexually by cell division or sexually by gametes (Camberato and Lopez, 2009). There are three types of algae: microscopic, mat-forming, and chara (Lembi, 2009). Mat-forming and microscopic algae are the main problems in hydroponic systems (Raudales, 2016). Algae is often found in hydroponic systems due to favorable light and nutrient conditions and can form mats or sheets on the surface of the nutrient solution, the edges of rafts, and other surfaces, causing competition between the algae and the crop, as well as attract many pests, such as shore flies (Raudales, 2016). As algae degrades after death, oxygen is absorbed from the water, causing a lower dissolved oxygen environment (Varma and DiGiano, 1968).

Several different methods of eliminating algae have been tested with moderate degrees of success. Free chlorine in a concentration of 1 $mg \cdot L^{-1}$ has been used to remove algae from hydroponic systems while lessening the phytotoxic effects (Dannehl et al., 2015). Chlorine combined with ultraviolet (UV) light radiation was shown to disrupt the integrity of the algal cells, as well as degrade any release of algal organic matter (Chen et al., 2020). The chemical 3-(3-indolyl) butanoic acid was discovered to have some algicidal effects while attempting to control bacterial wilt pathogens and might be relatively non-phytotoxic to many crops (Nonomura et al., 2001). More classic methods of preventing algae are to cover tanks with black and white mulching plastic to prevent sunlight from reaching the tank or the addition of barley straw, which produces algicidal enzymes as the straw degrades (Camberato and Lopez, 2009; Lembi, n.d.).

Algae can be harvested to create biofuels and biomaterials for the food industry, as well as absorb high nitrogen levels of aquaponic water (Addy et al., 2017; Baron et al., 2019; Hellebust and Ahmad, 1989). Microalgae can be used to absorb nutrients in hydroponic wastewater and lessen potential pollution regarding mine waste run off by absorbing heavy metals and organic pollutants (Supraja et al., 2020; Kováčik et al., 2018). It has even been suggested that co-cultivation of microalgae and certain crops could be beneficial (Huo et al., 2020). Tomato plants growing in an eco-hydroponic system with microalgae had increased biomass due to aeration from algal photosynthesis (Zhang et al., 2017). Similarly, Supraja et al. (2020) found that adding adequate microalgae inoculum facilitates positive interaction between plants and algae, improving overall system performance. In aquaponic systems, algae have been found to help balance the pH value, add oxygen, and control ammonia, but the microorganism can compete with vegetable plants for total nitrogen and growth space (Addy et al., 2017).

## HYDROGEN PEROXIDE

Hydrogen peroxide has been used in everything from antiseptic to algicide products and is also a signaling molecule in plants. Hydrogen peroxide is a form of reactive oxygen species (ROS) that are generated because of oxidative stress and is involved in growth, cell cycle, apoptosis, hormone signaling, development, and responses to biotic and abiotic stress (Mejia-Teniente, 2019; Ślesak et al., 2007). Produced in all cellular compartments as a result of energy transfer, electron leakage, and oxidase and peroxidase reactions, hydrogen peroxide has a low reactivity to other ROS species and easily crosses across biological membranes (Gadjev et al., 2008). In addition, hydrogen peroxide can act as a signaling molecule and regulator of expression of some genes in cells (Quan et al., 2008). One of the more well-known signals is hydrogen peroxide signaling abiotic stress. However, hydrogen peroxide could enhance stress resistance by protecting organelle structure (Nui and Liao, 2016). Exogenous hydrogen peroxide can even be used as a foliar spray to induce drought, salinity, extreme temperatures, and heavy metal tolerance (Wojtyla et al., 2016). There is a downside, as high levels of ROS can cause extensive damage to proteins, lipids, and DNA, leading to oxidative stress, metabolic dysfunction, and redox imbalance (Hossain et al., 2015). Many plants have developed an extensive system to combat high levels of ROS and to utilize low levels as a signaling system, as previously discussed (Hossain et al., 2015). Low doses can also build up stress tolerance, while high doses can cause programed cell death (Gechev et al., 2005).

In addition to functioning as a signaling molecule, exogenous hydrogen peroxide can be used as an environmentally safe way to break down microalgae as it is a natural photochemical product formed in waters under sunlight and quickly broken down into oxygen and water (Qian et al., 2010; Tesoriero et al., 2010). Hydrogen peroxide can decrease metabolic processes, destroy pigment synthesis and membrane integrity, inhibit photosynthetic activity and genes expression, alter circadian rhythms, and induce apoptotic-like cell death while limiting growth (Draäbkova et al., 2007; Zhou et al., 2017). However, the efficacy of hydrogen peroxide is dependent on culture density and how protected the pigments are inside of the chloroplasts (Barroin and Feuillade, 1986). Hydrogen peroxide can also be combined with UV radiation to help eliminate algae in filtration to prevent membrane fouling, using the UV to rupture the membrane of the algae before the hydrogen peroxide degraded any pigments (Sarathy and Mohseni, 2010; Wan et al., 2019). There is a slight concern of secondary oxidation byproducts in water filtration due to microcystins produced from cyanobacteria and secondary pollution (Bauzá et al., 2014; Zong et al., 2013).

More recently, hydrogen peroxide has been used in hydroponic systems to help prevent disease and to positively effect growth and development. Hydrogen peroxide has been shown to cause oxidative stress and eventual death on *Penicillium expansum*, a widespread fungal pathogen (Qin et al., 2011). Foliar sprays of hydrogen peroxide have been shown to induce resistance of peppers to the *Pepper golden mosaic geminivirus* in a similar manner to how it induces higher stress tolerances (Mejia-Teniente, 2019). Foliar sprays can also be used to enhance fruit growth and development, as well as increase the quality of fruit, such as in wax apple trees (*Syzygium samarangense* L.) (Ismail et al., 2015). In mistletoe fig (*Ficus deltoidei* L.), exogenously applied hydrogen peroxide increased height and chlorophyll content, as well as mineral uptake (Nurnaeimah et al., 2020). A 50 µM solution of hydrogen peroxide applied to mustard (*Brassica juncea* L.) in 200 $mg \cdot kg^{-1}$ Ni-rich soil counteracted the toxic effects of the nickel and induce defensive responses as well as increasing photosynthesis rate (Khan et al., 2016). Addition of hydrogen peroxide to the nutrient solution of the system can help oxygenate the roots of peppers and improve the crop (Hernandez et al., 2012).

Lastly, hydrogen peroxide has been used to help reduce algae concentration in hydroponic systems (Thakulla et al., 2022). Using 125 ppm of hydrogen peroxide on cucumber seedlings in rockwool cubes reduced the concentration of green algae on the cubes, therefore reducing the shore fly population, but it also had temporary phytotoxic effects on the seedlings that were dependent on temperature and light intensity (Vänninen and Koskula, 1998). Similar results were seen in lettuce seedlings, as hydrogen peroxide tended to effect germination rates due to oxidative stress (Caixeta et al., 2018). After observing many different pesticide products and their effect on corn salad (*Valerianella locusta* L.), Coosemans (1995) found that specific application, such as time and dose, is not universal and is dependent on the crop.

CHAPTER II

# EFFECTS OF ELEVATED WATER TEMPERATURE ON GROWTH OF BASIL (*OCIMUM BASILICUM* L.) IN NUTRIENT FILM TECHNIQUE HYDROPONICS


## ABSTRACT

Hydroponic systems have become increasingly popular for growers in recent years for year-round local production. While optimum air temperature has been considered, optimal root zone temperatures have not been examined as thoroughly. Optimal air temperature for basil has been reported to be between 25°C and 30°C. The goal of this research was to determine the optimal water temperature for growing different types of basil hydroponically. Research was conducted at the Department of Horticulture and Landscape Architecture greenhouses in Stillwater, OK. Seventeen cultivars were selected from six main types of basil and transplanted into Nutrient Film Technique hydroponic systems, and three water temperature treatments were applied: 23°C, 27.5°C, and 31°C. Height, width, average leaf area, leaf number, SPAD, shoot fresh weight, shoot dry weight, and root dry weight were evaluated. In general, the 27.5°C and 31°C treatments were not greater than each other in terms of leaf number and root dry weight but were greater than the 23°C treatment. The 31°C treatment had the greatest height, while width, average leaf area, shoot fresh weight, and shoot dry weight

were not different in the 27.5°C and 31°C treatments but were significantly greater than the 23°C treatment. The 23°C treatment had the greatest SPAD and was significantly different from the 31°C treatment. Cultivar differences were significant in average leaf area and SPAD, with 'Spicy Bush' having the smallest leaf area, and purple basil having the greatest chlorophyll content. For all cultivars except purple basil and 'Large Leaf Italian', a 27.5°C water temperature would be recommended for greater plant growth.

## INTRODUCTION

Hydroponic systems have been utilized since ancient times, such as in the Babylonian hanging gardens and the Aztecs' chinampas (Resh, 1978). The term "hydroponics" itself was mentioned in 1937 by Dr. Gericke as he experimented with growing plants in a nutrient solution (Jones, 1982). Hydroponic systems have become increasingly popular in recent years due to the changing climate and decreased abundance of agricultural water as hydroponics predominantly is a closed recirculating system that recycles water (Sambo et al., 2019). In terms of agricultural research, hydroponic systems provide more environmental control than traditional soil-based research (Shrestha and Dunn, 2016). These systems allow for the control of everything from air temperature to specific soilless media used, though there is concern about ease of pathogen travel and expensive equipment and maintenance. In recent research, water temperature has been observed to impact plant root and shoot growth (Nxawe et al., 2009; Rosik-Dulewska and Grabda, 2002). The Nutrient Film Technique (NFT) system is ideal for herbs and other short crops, as it provides a thin layer, or film, of constantly recirculating nutrient solution (Dholwani et al., 2018; Mohammed and Sookoo, 2016; Resh, 1978).

As hydroponics allows for more absolute control over environmental variables in experimental research, temperature is an essential component of production (Nxawe et al., 2009). Air temperature has long been considered while growing crops, as extreme cold and heat can cause a multitude of issues. Temperature influences most plant processes such as photosynthesis, transpiration, respiration, germination, and flowering (Van Der Zanden, 2008). Generally, increasing temperature increases the rate of photosynthesis, transpiration, and respiration to a point (Van Der Zanden, 2008). Rice (*Oryza sativa* L.) has been shown to increase photosynthesis and yield in high temperatures, but, conversely, cotton (*Gossypium hirsutum* L.) decreased in photosynthesis and therefore boll filling (Mondal et al., 2016). For many leafy and vegetable crops, lower temperatures are desired to reduce energy usage and increase sugar storage, creating a sweeter fruit or leafy green, while adverse temperatures can create bitterness in greens (VanDerZanden, 2008). However, air temperature is only one factor in examining how temperature effects crops overall throughout their growth cycle, and, as the cost of fossil fuels and winter heating climbs, heating the root zone or hydroponic water may be an adequate solution (Kawasaki et al., 2014).

Root zone temperature (RTZ) is often a little thought about concept as compared to air temperature in relation to plant growth; however, RZTs have been shown to correlate to an increase in rooting percentages and shoot growth in both rootstocks and crops such as tomato (*Solanum lycopersicum* L.), cucumber (*Cucumis sativus* L.), and pepper (*Capsicum annuum* L.) (Nxawe et al., 2009; Rosik-Dulewska and Grabda, 2002).

A greater RZT allows more and quicker nutritional uptake, while a lower RZT can slow nutritional uptake (Yan et al., 2012). Optimized root conditions can also substitute for poor air temperature, as seen in lettuce with a RZT of 24°C grown in 31°C air temperature which still produced a marketable product, in contrast with a higher RZT of 31°C, which caused wilting (Thompson et al., 1998). In soil environments, time of year and air temperature can have a significant impact on RZT. Tomatillos (*Physalis ixocarpa* Brot. ex Hornem) grown in the spring had an increase in dry shoot weight as the RZT increased; however, in the summer, dry shoot weight decreased with increased RZT during the establishment phase of growth (Diaz-Perez et al., 2005). The roots themselves are also highly sensitive to temperature change, especially root depth and root width, as roots adapt to their environment (Luo et al., 2020). High temperature may significantly accelerate the root meristem cell division, thus the development of lateral root primordium, creating more branches but possibly thinner roots depending on species (Luo et al., 2020). This concept is not only applicable in soil media, but also in hydroponic nutrient solutions as well.

In previous research, using chilled water temperatures to mimic cold air temperatures in order to convince cool season crops to grow has been the norm (Thompson et al., 1998). However, other research has looked at heating water to encourage greater nutrient uptake, increased transpiration, nutritional absorption, root length, and leaf area (Moorby and Graves, 1980). Plant such as tomatoes and spinach (*Spinacia oleracea* L.) often thrive with warmer root temperatures as a result of increased metabolism that allows for more nutrient uptake and shoot growth (Wang et al., 2022). In a study using condensation irrigation in pots with soil, pots treated with the elevated temperatures secondary to condensation irrigation stimulated root growth in green basils, though specific cultivars were not reported (Arabnejad et al., 2021).

Unlike some hydroponic crops that prefer cooler temperatures such as most lettuce (*Lactuca sativa* L.), basil (*Ocimum basilicum* L.) is commonly found in tropical and subtropical regions, preferring high light and an optimal temperature around 26°C (Currey, 2020; Raimondi, 2006; Thakulla et al., 2021). Basil is a highly popular crop due to its usefulness in a variety of fields, such as a culinary herb, a medicinal plant, and an agent to control pests (Raimondi, 2006). Nevertheless, controlling the phenolic content and phytochemical concentration is difficult, and basil has been cultivated more frequently in controlled environments in recent years (Dou et al., 2018). Unfortunately, many herbs may exhibit low yield in hydroponic systems due to unknown reasons (Bulgari et al., 2016). Thus, the objective of this research was to ascertain if various cultivars of basil would produce better growth under different RZTs that more accurately match their preferred tropical and subtropical climate.

## MATERIALS AND METHODS

### *Plant Materials and Growth Conditions*

The experiment was conducted at the Oklahoma State University Department of Horticulture and Landscape Architecture research greenhouses in Stillwater, Oklahoma. Average air temperatures for each run were 23.27°C, 27.97°C, and 30.11°C. Average humidity was 45.86%, 70.44%, and 66.28% each run. Daily light integral (DLI) averaged 26.6, 19.2, and 29.9 $mol \cdot m^{-2} \cdot d^{-1}$. Seeds of 17 cultivars of basil, ‘Sweet Thai’, ‘Cardinal’, ‘Red Rubin’, ‘Prospera’, ‘Cinnamon’, ‘Nufar’, ‘Italian Large Leaf’, ‘Karpoor Tulsi’, ‘Lemon’, ‘Amethyst’, ‘Dark Opal’, ‘Spicy Bush’, ‘Lime’, ‘Rutgers Devotion’, ‘Genovese’, and ‘Elide’ were obtained from Johnny’s Selected Seeds (Winslow, MN) and planted in Horticubes Grow Cubes (Smithers Oasis, Kent, Ohio) on 1 April 2021, and repeated on 7 May 2021 and 1 July 2021, and placed under misters for 4 weeks. Seedlings in cubes were transplanted to a Nutrient Film Technique (NFT) hydroponic table (Botanicare, Vancouver, Washington) on 22 April 2021, and was repeated on 4 June 2021 and 29 July 2021. The 40-gal 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) and were added initially according to the recommended rates. The pH and electrical conductivity (E.C.) of the solution were checked every other day to maintain the pH between 5.5 to 6.5 and the E.C. at 1.5 to 2.5 $mS \cdot cm^{-1}$ as recommended by Singh and Dunn (2016).

***Treatments and Data Collection***

The NFT tanks were heated or cooled to three temperatures: 23°C, 27.5°C, and 31°C. Two different heaters, Orlushy Submersible Aquarium Heater at 300 watts (NOVA Pet Appliance CO., Guangdong, China) and the Aqua Heat Titanium Reservoir and Aquarium Heater at 200 watts (Sunlight Supply Inc., Vancouver, Washington), were used in tandem to maintain temperatures within a single tank due to heat retainment issues. For the 23°C table, an Active Aqua Water Chiller (Hydrofarm, Petaluma, California) was used to maintain a cooler temperature. Temperature readings were taken every 2 h using HOBO thermocouple data loggers (Onset, Cape Cod, MA). Dissolved oxygen (D.O.) was measured daily using a D.O. meter (Milwaukee Instruments, Rocky Mount, North Carolina).

Each plant on the table was scanned using a chlorophyll meter (SPAD-502, Konica Minolta, Japan) 31 d after transplanting. SPAD readings were taken from each plant from the middle of the top and bottom leaf and were averaged to determine the chlorophyll concentration. Plants were harvested 30 d after transplanting. Fresh shoot weight and leaf area were assessed on the day of harvest. Leaf area was measured using a Li-Cor L1-3100C Area Meter (LI-COR Biosciences, Lincoln, NE), selecting the third leaf from the top. Each leaf was measured twice and averaged. Shoots and roots were dried at 59°C for 2 d. Samples of five cultivars, 'Genovese', 'Dark Opal', 'Kapoor Tulsi', 'Lemon', and 'Prospera', three of each from each treatment per repetition, were chosen to be analyzed for total nutrients, phosphorus, magnesium, sulfur, calcium, potassium, boron, zinc, copper, and manganese and were then sent to the Soil, Water and Forage Analytical Laboratory at Oklahoma State University for analysis of leaf mineral element concentrations using a nutrient analyzer (TruSpec Carbon and Nitrogen Analyzer; LECO Corp., St. Joseph, MI, USA).

***Experimental design and statistical analysis***

The study was conducted using a split-plot in a randomized complete block design with three replications. The whole main plot was water temperature (three levels), and cultivar of basil (17 levels) was the subplot factor. Statistical analysis was performed using SAS/STAT software (Version 9.4; SAS Institute, Cary, NC). Tests of significance were reported at the 0.05, 0.01, and 0.001 level. The data were analyzed using generalized linear mixed models methods. Tukey multiple comparison methods were used to separate the means.

## RESULTS

***Temperature and cultivar effect and their interaction on plant growth parameters***

There was a significant cultivar × temperature interaction for number of leaves and root dry weight (Table 2.1). The cultivar 'Cinnamon' in the 31°C heated treatment had the greatest number of leaves but was not different from the 27.5°C treatment (Table 2.2). For 'Lime', 'Sweet Thai', 'Kapoor Tulsi', 'Nufar', and 'Prospera' either 27.5°C or 31°C treatments also resulted in greater leaf numbers. For 'Red Rubin', 'Mrs. Burns Lemon', and 'Large Leaf Italian', greater number of leaves were recorded with the 31°C treatment, while 'Cardinal' had greater numbers with the 27.5°C treatment (Table 2.2). Within the purple type, 'Amethyst' has the least number of leaves compared to 'Red Rubin' and 'Dark Opal'. 'Mrs. Burns Lemon' had a significantly greater number of leaves in the 23°C treatment than 'Lime', though the 27.5°C and 31°C treatments were not significantly different. 'Sweet Thai' and 'Cinnamon' had the greatest number of leaves in the Asian basils but were only significantly greater than 'Cardinal'. In the large leaf basils, 'Nufar' had significantly greater number of leaves overall than 'Italian Large Leaf'. The number of leaves for classic basils was not significantly different from one another but were significantly lower than citrus basils and some Asian basils, such as 'Sweet Thai' and 'Cinnamon' (Table 2.2).

‘Nufar’ in the 31°C heated treatment had the greatest root dry weight and was not different than the 27.5°C, but both were greater than the 23°C treatment. ‘Dark Opal’ and ‘Mrs. Burns Lemon’ were greater at 31°C than 23°C but were not different from 27.5°C. For ‘Amethyst’, root dry weight under 27.5°C was greater than 23°C, but was not different than the 31°C treatment. Purple, citrus, and fine leaf basils had the lowest root dry weight as compared to the other types. Large leaf basils had greater root dry weights than other types. Asian and classic basils tended to have greater root dry weights at warmer temperatures but were not different from other types at the lowest temperature (Table 2.2).

Plant height, average leaf area, shoot fresh weight, shoot dry weight, and SPAD showed significant main effects for cultivar (Table 2.1). ‘Kapoor Tulsi’ was the tallest cultivar but was significantly not different from any other cultivar except ‘Spicy Bush’ (Table 2.3). Average leaf area was greatest for ‘Nufar’, but not significantly different than any of the other cultivars except ‘Spicy Bush’ (Table 2.3). In regard to shoot fresh weight and dry weight, ‘Cinnamon’ had the greatest weight, but was only significantly different from ‘Amethyst’ (Table 2.3). Greatest value for SPAD was observed in ‘Amethyst,’ which was not significantly different from ‘Red Rubin’, ‘Dark Opal’, ‘Cinnamon’, ‘Sweet Thai’, ‘Kapoor Tulsi’, and ‘Rutgers Devotion’ (Table 2.3). In general, purple and Asian varieties had greater values, while fine leaf and large leaf varieties were lower (Table 2.3).

Root zone temperature main effects were observed for parameters including plant height, width, average leaf area, shoot fresh weight, shoot dry weight, and SPAD (Table 2.1). The 31°C treatment produced the tallest plants, while there was no difference between the other two temperature treatments (Table 2.4). The basil plant widths under 31°C and the 27.5°C treatments were not different from each other but were significantly greater than that under 23°C treatments (Table 2.4). The 31°C had the greatest average leaf area but was not different than 27.5°C (Table 2.4). The 27.5°C and 31°C shoot fresh and dry weights were not significantly different from each other but were greater than the 23°C treatment (Table 2.4). The greatest SPAD value was found in 23°C treatment but was not significantly different than the 27.5°C treatment (Table 2.4).

For nutrient content, cultivar effect was significant for magnesium, sulfur, and iron content, while temperature treatment effect was significant for total nutrient content, copper, and manganese (Table 2.5). Total nutrient content and copper content were greatest in the 27.5°C treatment, and this treatment was significantly greater than the 23°C and 31°C treatments. Similarly, manganese content was found to be the greatest in the 27.5°C treatment, which was significantly greater than the other two treatments (Table 2.6). 'Dark Opal' had the greatest iron content but was only significantly different from 'Mrs. Burns Lemon' (Table 2.7). 'Mrs. Burns Lemon' had reduced amounts of sulfur compared to all other cultivars. 'Kapoor Tulsi' had the greatest magnesium content, which was significantly greater than all other cultivars (Table 2.7)

***Water temperature and dissolved oxygen***

Daily water temperature in the 23°C chilled control treatment ranged from 19.0 to 23.0 and 22.8 to 25.4°C during the first and second replications, respectively (Figure 2.1). Average temperature for the 23°C chilled control treatment was 21.6 and 23.8°C during the first and second replications, respectively. Data for the third repetition of 23°C was lost due to a data logger malfunction. Daily water temperature in the 27.5°C heated treatment ranged from 22.0 to 30.3, 24.9 to 31, and 23.2 to 30.4℃ during the first, second, and third replications, respectively. Average temperature for the 27.5°C heated treatment was 26.4, 28.5, and 27.9℃ during the first, second, and third replications, respectively. Daily water temperature in the 31℃ heated treatment ranged from 21.7 to 33.1, 24.9 to 33.8, and 29.8 to 34.1℃ during the first, second, and third replications, respectively. Average temperature for the 31℃ heated treatment was 29.1, 31.2, and 31.9℃ during the first, second, and third replications, respectively. In terms of daily dissolved oxygen within each NFT tank, the 23°C treatment was significantly greater than the 31°C treatment at a mean of 6.47 $mg \cdot L^{-1}$ throughout the growing season (Figure 2.2).

## DISCUSSION

### *Effects of cultivar and water temperature on plant growth*

Air temperatures in this research averaged 23.27°C, 27.97°C, and 30.11°C in each repetition, respectively. Generally, 27.5ºC is considered the ideal air temperature for growing basil (Currey, 2020). Warmer temperatures allow for increased nutrient uptake, and therefore, increased leaf number (Moorby and Graves, 1980). Similarly in this study, we hypothesized that warmer RZTs would cause increased basil plant growth. Root growth did increase in this research, with greater temperatures increasing root dry mass for most cultivars. Root mass was greater under greater temperatures for most cultivars, with the exception of purple basils and some large leaf basils, in this research. While root growth can also show an increase under optimal air conditions, root zone heating allows for the same root growth under suboptimal air temperatures (Kawasaki et al., 2014). Root weight and architecture are largely dependent on species, as different species have different optimal temperatures (Luo et al., 2020).

In this study, root dry weights increased as the temperature increased for most basil cultivars. These results are similar to what Kawasaki et al. (2014) saw in heating the root zone of tomatoes. On the seventh and the 14th day of heating the root zone, root growth and dry weight were greater than the ambient control under suboptimal air conditions. Unlike in this study, when exposed to low or high RZTs, Korean mint (*Agastache rugosa* L.) exhibited significantly lower fresh and dry root weight due to temperature stress (Lam et al., 2020). In a study with cucumber cultivar 'Super N3', Haghighi et al. (2015) found that 25°C was ideal for increasing dry root weight, as lower and higher temperatures caused stress on the plants. Al-Rawahy et al. (2019) found that cooled RZTs of 22ºC and 25ºC produced significantly more leaves than the 28ºC treatment or the 33ºC control in cucumber 'Reema F1', once again stressing the importance of optimal temperature to produce better yield is dependent on crop and cultivar. Sakamoto and Suzuki (2015) found that root zone heating to 33ºC significantly reduced leaf number in carrots (*Daucus carota* L. cv Tokinashigosun), while, in lettuce, cooling root temperatures did not significantly change leaf number (Sun et al., 2016). However, in soybeans (*Glycine max* L.), increasing RZT increased leaf number (Dashti et al., 2016). Crop and cultivar can strongly impact how RZTs can affect plant growth.

Cultivar itself can also play a significant role in root biomass, magnifying the effect of elevated water temperature (Lazarević et al., 2019). Cultivar differences were seen in height, average leaf area, shoot fresh weight, shoot dry weight, and SPAD in this research. Walters and Currey (2015) found that citrus basils had significantly greater height when compared to sweet, purple, Asian, large leaf, and fine leaf basil, which was contrary to what was found in this study. Like this research, 'Spicy Bush' has been found to be one of the shortest cultivars, often being approximately 11 to 15 cm tall, (Svecov and Neugebauerova, 2010; Walters and Currey, 2015). Asian basils, such as 'Kapoor Tulsi', are generally among the taller cultivars, though usually not significantly different from other cultivars, unlike in the present study (Simon et al., 1999; Walters and Currey, 2015). Upadhyay (2017) reported that 'Kapoor Tulsi' is usually 30 to 60 cm tall, which matches the findings of our study, leading to the conclusion that 'Kapoor Tulsi' is a taller cultivar than other cultivars of basil.

In the present study, 'Spicy Bush', a fine leaf basil, had the smallest leaf area, while 'Nufar', a large leaf basil, had the largest average leaf area. Marotti et al. (1996) and Walters and Currey (2015) found similarly that average leaf area was found to be greatest in large leaf varieties, while fine leaf, bush, and compact varieties had the smallest leaf size. This was due to genetic differences between the plants.

Basil shoot fresh weight and dry weight are often gauged by basil color. In this study, 'Amethyst' had the lowest shoot fresh and shoot dry weight, with 'Cinnamon', a green Asian basil, having the greatest shoot weight. In general, purple basils have been found to have less fresh and dry shoot weight in comparison to green basils (Abbas, 2014; Marotti et al., 1996; Walters and Currey, 2015; Yaldiz et al., 2015). However, Svecov and Neugebauerova (2010) found that 'Lime' had the lowest fresh weight, and 'Spicy Bush' had the lowest dry weight yield.  This was contrary to what we found but is likely due to the differences between hydroponic and field studies in regard to basil.

Amongst basil cultivars, there are significant differences in chlorophyll content due to genetic variability causing differences in pigmentation (Lazarević et al., 2020). SPAD readings for the green varieties (Citrus, Asian, Fine Leaf, Large Leaf, and Classic) from this study correspond to similar studies, the average reading being between 23 and 31 (Lazarević et al., 2020; Singh et al., 2019; Teliban et al., 2020; Yang and Kim, 2020). The purple basil had greater SPAD values as compared to most of the other green varieties; however, these numbers match up with the values found in previous studies of red basil, which are generally between 32 and 40 (Saha et al., 2016; Teliban et al., 2020; Yang and Kim, 2020). SPAD readings and total chlorophyll content of leaves have been found to be significantly correlated (Jiang et al., 2017).

Water temperature was found to have significant effects on plant growth and development. At ideal temperatures (27.5°C), water temperature improved growth. White jute (*Corchorus capsularis* L.), an annual herb native to tropical Asia, when grown at high temperatures, showed to increase stem length but reduced leaf area, because high temperature can increase photosynthetic rate which make leaves less important (Luo et al., 2020). But if the temperature strays too high or too low, then damage can occur to the plant and its metabolism (He et al., 2002). Low temperature can affect the imbalance between growth inhibitors and promoters in plants such as abscisic acid, cytokinin, and gibberellins, which are mainly synthesized in the root apical meristems (Lam et al., 2020). Korean mint was thought to suffer water stress at the 10°C treatment due to the possibility that water uptake was inhibited from root to shoot during the treatment's duration (Lam et al., 2020). Spinach (*Spinacia olearacea* L.) was found to have increased leaf number and length, as well as shoot fresh weight, at higher temperatures of

24, 26, and 28°C as compared to the 10°C control (Nwaxe et al., 2009). At 25°C, Pak choy (*Brassica rapa* L.) produced the greatest growth and yield at 38°C as compared to the control of 25°C (Maludin et al., 2020). Low temperatures can also precede to low rates of cell expansion, leading to many small cells per given area, making the leaves denser and smaller, reducing single leaf area (Carotti et al., 2021). Sakamoto and Suzuki (2015) found in a study with red leaf lettuce 'Red Wave' that the exposure of lettuce roots to a low temperature of 10°C significantly reduced leaf area, stem diameter, and fresh weight of tops and roots compared to 20°C.

However, if the water temperature is higher than the optimal level, roots may be damaged, affecting stem length and diameter (Lam et al., 2020). High temperatures, such as 32°C, have been known to cause tip-burn and other physiological issues in lettuce (Carotti et al., 2021). High root-zone temperatures, such as 27.5°C, resulted in water and nutrient loss, which ultimately led to reduction of plant growth in tomatoes (Díaz-Pérez et al., 2007). In cucumbers, a high temperature of 35°C was found to lower fresh and dry shoot weight, as well as decreasing chlorophyll and antioxidant content (Haghighi et al., 2015). Higher temperatures can also be greatly beneficial to plants. Elad et al. (2017) found that heating sweet basil allowed for the repression of disease, specifically white and gray mold. Wang et al. (2016) found that, in newly unfurled cucumber leaves, warming of the root zone caused an increase in leaf area expansion and reduced the stomatal limitation of photosynthesis, while improving water supply from the roots. The present study found that warmer water temperatures led to a greater leaf count and average leaf area, which are often the most important factors in basil quality.

Water temperature can also cause variability in chlorophyll content. Higher RZTs can affect the physiological processes of the plant, such as chlorophyll content, and, consequently, plant metabolism, while any resultant heat stress can trigger significant changes in plant physiological processes, such as water uptake and leaf photosynthesis, as well as decreased SPAD values due to a decrease in chlorophyll content (Al-Rawahy et al., 2019). SPAD readings are a quick, non-destructive gauge of relative chlorophyll concentration, and Ruiz-Espinoza et al. (2008) found that SPAD readings were strongly correlated with chlorophyll content in basil. Lower SPAD values at high temperatures may be due to cessation of chlorophyll production or chlorophyll degradation under higher temperatures (Haghighi et al., 2015). However, Kalisz et al. (2016) found that lower temperatures increased the chlorophyll-*a* to chlorophyll-*b* ratio, increasing SPAD values.

Nutrient content is often an important indicator of plant nutrient uptake and quality. Differences in nutrient content amongst cultivar are common in basil, often expressing in magnesium, iron, calcium, and zinc, aligning similarly with the differences found in this study (Lazarević et al., 2020). Values for iron were similar to previous studies which averaged between 80 to 100 ppm, while this study averaged 135 ppm due to high outliers such as ‘Dark Opal’ and ‘Prospera’; however, ‘Kapoor Tulsi’ and ‘Genovese’ were within the normal average range (Lazarević et al., 2020; Saha et al., 2016). In the case of sulfur, known values and values from the current study both stayed around 0.30 ppm (Saha et al., 2016). Magnesium levels in the current study were somewhat greater than found in some literature, but this is thought to be due to differences in cultivars (Saha et al., 2016). Root zone heating has been shown to increase the uptake of total nutrients and promote their transport to the shoot of the plant (Kawasaki et al., 2014; Lam et al., 2020). In a study on RZTs and their influence on tomatoes in winter, increased temperatures allowed for greater total nutrient uptake and lowered uptake in cooler temperatures; however, at high air temperatures, root cooling allowed for better total nutrient uptake (Kawasaki et al., 2014). Lam et al. (2020) reported that, in cucumbers, a higher RZT allowed for an increase in nutrient uptake. As each species has its own optimal temperature, a study on melons (*Cucumis melo* L. cv. Arava), cucumbers, and rape (*Brassica napus* cv. Emerald) found that each species had a different optimal RZT for optimal nutrient uptake (Yan et al., 2012). Saha et al. (2016) found, in a study comparing hydroponic and aquaponic basil growth with the green basil cultivar 'Aroma II', that magnesium content was similar to the 23°C treatment, due to their average water temperature of 23.4°C. The warmer temperatures investigated in this study are suspected to be the cause of the increase in comparison with the Saha et al. (2016) study.

***Dissolved oxygen and water temperature***

In the present study, dissolved oxygen was significantly greater in the coolest temperature treatment but increased dissolved oxygen did not increase plant growth. Dissolved oxygen levels are inversely related to water temperature, with lower temperatures holding more oxygen and higher temperatures holding less oxygen (Falah et al., 2010; United States Environmental Protection Agency, 2012). As reported by Al-Rawahy et al. (2019), the temperature of a nutrient solution has a direct relationship 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. Low levels of oxygen in the water can reduce plant growth and development, as well as increase the likelihood of pathogens in hydroponic systems (Yan et al., 2012). However, according to Ruso et al. (2021), basil can persist with dissolved oxygen levels as low as 4 $mg \cdot L^{-1}$, with optimal levels at 6.5 $mg \cdot L^{-1}$. In the present study, dissolved oxygen rates were well above the minimum requirements for basil, leading towards the conclusion that elevated temperature provided more benefits than could be accounted for by increased oxygen.

## CONCLUSION

The main objective of this research was to determine the optimal RZT, and, consequently, optimal nutrient solution temperature, for different basil cultivars in NFT hydroponic systems. In this study, most types of basil grown in nutrient solution heated to 27.5°C and 31°C had overall better growth than basil grown at a 23°C nutrient solution. Purple basil and some large leaf basils, such as ‘Italian Large Leaf’ were unaffected. SPAD values were greatest in the 23°C treatment, however, but were not significantly different from the 27.5°C treatment. Total nutrient content was greatest in the 27.5°C treatment. These results suggest that heating nutrient solutions to 27.5°C will promote basil growth in most types of basil in NFT hydroponics. Further research is needed to determine the most economical and efficient way to heat the nutrient solution and what effect warmer RZT has on phenolic content.

## TABLES AND FIGURES

Table 2.1: Tests of effects for water temperature (23℃, 27.5℃, and 31℃) and 17 basil cultivars grown in Nutrient Film Technique hydroponics systems at OSU research greenhouses in Stillwater, OK.

| | Cultivar | Temperature | Cultivar × Temperature |
|---|---|---|---|
| SPAD | ***[z] | * | NS |
| Height | * | *** | NS |
| Width | NS | ** | NS |
| Number of leaves | *** | *** | *** |
| Average leaf area | ** | * | NS |
| Shoot fresh wt. | ** | *** | NS |
| Root dry wt. | *** | *** | *** |
| Shoot dry wt. | *** | *** | NS |
| Dissolved oxygen | --- | ** | --- |

[z]Indicates significant at or non-significant (NS) at $*P \leq 0.05$, $**P \leq 0.01$, or $***P \leq 0.001$.

Table 2.2: Least square means interaction between basil cultivar and water temperature (23°C, 27.5°C, and 31°C) for number of leaves and root dry weight for 17 basil cultivars in Nutrient Film Technique hydroponic systems at OSU research greenhouses in Stillwater, OK.

| Types | Cultivar | Water temperature (°C) | Number of leaves | Root dry wt. (g) |
|---|---|---|---|---|
| Purple | Amethyst | 23 | 2.77uv[z] | 0.54l[z] |
| | | 27.5 | 2.68v | 0.94efghijk |
| | | 31 | 2.81tuv | 0.28ijkl |
| | Red Rubin | 23 | 3.27pq | 0.80jkl |
| | | 27.5 | 3.27pq | 0.27jkl |
| | | 31 | 3.50klmno | 0.31ijkl |
| | Dark Opal | 23 | 3.23pqr | 0.69l |
| | | 27.5 | 3.30opq | 0.01jkl |
| | | 31 | 3.35nop | 0.32hijk |
| Citrus | Mrs. Burns Lemon | 23 | 3.70ghi | 0.25jkl |
| | | 27.5 | 3.84def | 0.88fghijk |
| | | 31 | 4.01ab | 1.27defghi |
| | Lime | 23 | 3.47klmno | 0.70kl |
| | | 27.5 | 3.98abcd | 1.11defghij |
| | | 31 | 4.01ab | 1.95abcde |
| Asian | Cinnamon | 23 | 3.75fgh | 0.16jkl |
| | | 27.5 | 3.94abcde | 1.52bcdefg |
| | | 31 | 4.07a | 2.38ab |
| | Cardinal | 23 | 3.38mnop | 0.27ijkl |
| | | 27.5 | 3.68ghij | 2.32abc |
| | | 31 | 3.41klmnop | 1.86abcde |
| | Sweet Thai | 23 | 3.82efg | 0.09jkl |
| | | 27.5 | 4.01ab | 0.36hijkl |
| | | 31 | 3.94bcde | 0.29ijkl |
| | Kapoor Tulsi | 23 | 3.51jklmn | 0.32hijkl |
| | | 27.5 | 3.86cdef | 1.64cdef |
| | | 31 | 3.92bcde | 2.16abcd |
| Fine leaf | Spicy Bush | 23 | --- | 0.84jkl |
| | | 27.5 | --- | 0.13jkl |
| | | 31 | --- | 0.38hijkl |
| Large leaf | Large Leaf Italian | 23 | 2.99rstuv | 0.78jkl |
| | | 27.5 | 2.96stuv | 2.13abcde |
| | | 31 | 3.40klmnop | 2.76a |
| | Nufar | 23 | 3.12qrs | 0.35hijkl |
| | | 27.5 | 3.57hijk | 1.67abcdefg |
| | | 31 | 3.73fgh | 2.69a |
| Classic | Prospera | 23 | 3.07rstq | 0.90jkl |
| | | 27.5 | 3.42klmnop | 2.11abcde |
| | | 31 | 3.59hijkl | 2.47ab |

| | | | |
|---|---|---|---|
| Rutgers Devotion | 23 | 3.14qrs | 0.11jkl |
| | 27.5 | 3.41klmnop | 0.73ghijkl |
| | 31 | 3.41klmnop | 1.38cdefgh |
| Elida | 23 | 3.01rstu | 0.94jkl |
| | 27.5 | 3.10qrs | 1.51bcdefg |
| | 31 | 3.40lmnop | 2.15abcd |
| Genovese | 23 | 3.10qrs | 0.13jkl |
| | 27.5 | 3.40lmnop | 1.75abcdef |
| | 31 | 3.49klomn | 1.65bcdef |

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

Table 2.3: Least square means of six types and 17 cultivars on growth and quality of basil grown in Nutrient Film Technique hydroponic systems at OSU research greenhouses in Stillwater, OK.

| Types | Cultivars | SPAD (unitless) | Height (cm) | Average leaf area ($cm^2$) | Shoot fresh wt. (g) | Shoot dry wt. (g) |
|---|---|---|---|---|---|---|
| Purple | Amethyst | 36.76a[z] | 15.54ab | 12.40ab | 14.23b | 0.85b |
| | Red Rubin | 33.09abc | 20.24ab | 12.78ab | 25.70ab | 1.81ab |
| | Dark Opal | 35.12ab | 19.77ab | 9.60ab | 21.94ab | 1.48ab |
| Citrus | Mrs. Burns Lemon | 26.99ed | 24.53ab | 12.70ab | 43.99ab | 3.51ab |
| | Lime | 30.86bcd | 23.09ab | 10.09ab | 27.94ab | 2.84ab |
| Asian | Cinnamon | 32.55abc | 29.06ab | 13.51ab | 58.30a | 5.01a |
| | Cardinal | 29.64cd | 22.28ab | 12.48ab | 41.96ab | 3.66ab |
| | Sweet Thai | 35.54ab | 20.23ab | 6.41ab | 27.65ab | 2.81ab |
| | Kapoor Tulsi | 33.24abc | 31.61a | 15.23ab | 50.57ab | 4.75ab |
| Fine leaf | Spicy Bush | 24.67e | 15.50b | 2.63b | 44.85ab | 2.84ab |
| Large leaf | Large Leaf Italian | 29.85cd | 22.33ab | 16.99ab | 36.76ab | 2.75 ab |
| | Nufar | 29.61cd | 23.20ab | 19.05a | 53.42ab | 4.47ab |
| Classic | Prospera | 30.01cd | 25.75ab | 17.74a | 54.23ab | 4.40ab |
| | Rutgers Devotion | 32.51abc | 22.81ab | 16.79ab | 37.76ab | 3.15ab |
| | Elide | 31.03bcd | 20.48ab | 14.52ab | 34.16ab | 2.65ab |
| | Genovese | 31.47bcd | 25.85ab | 15.10ab | 36.40ab | 3.02ab |

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

Table 2.4: Least square means of three temperatures on growth and quality of basil grown in Nutrient Film Technique hydroponic systems at OSU research greenhouses in Stillwater, OK.

| Treatment (°C) | SPAD | Height (cm) | Width (cm) | Average leaf area ($cm^2$) | Shoot fresh wt. (g) | Shoot dry wt. (g) | Dissolved oxygen ($mg \cdot L^{-1}$) |
|---|---|---|---|---|---|---|---|
| 23 | 32.03a[z] | 19.18b | 12.44b | 10.57b | 27.78b | 2.23b | 6.47a |
| 27.5 | 31.7ab | 21.99b | 16.07a | 12.62ab | 40.12ab | 3.28ab | 5.88ab |
| 31 | 30.52b | 27.55a | 17.81a | 15.81a | 46.45a | 3.88a | 5.63b |

[z]Means 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: ANOVA for nutrient content in five basil cultivars grown in Nutrient Film Technique hydroponics systems at OSU research greenhouses in Stillwater, OK.

| Nutrient | Cultivar | Water Temperature | Cultivar × Water Temperature |
|---|---|---|---|
| Total Nutrients | NS[z] | * | NS |
| Phosphorus | NS | NS | NS |
| Calcium | NS | NS | NS |
| Potassium | NS | NS | NS |
| Magnesium | ** | NS | NS |
| Sulfur | ** | NS | NS |
| Boron | NS | NS | NS |
| Iron | * | NS | NS |
| Zinc | NS | NS | NS |
| Copper | NS | ** | NS |
| Manganese | NS | *** | NS |

[z]Indicates significant at or non-significant (NS) at $*P \leq 0.05$, $**P \leq 0.01$, or $***P \leq 0.001$.

Table 2.6: Least square means of water temperature on nutrient levels in five basil cultivars ('Kapoor Tulsi', 'Dark Opal', 'Mrs. Burns Lemon', 'Genovese', and 'Prospera').

| Temperature (°C) | Total nutrients (ppm) | Copper (ppm) | Manganese (ppm) |
|---|---|---|---|
| 23 | 4.19b[z] | 7.99b | 87.30b |
| 27.5 | 4.62a | 10.51a | 131.24a |
| 31 | 4.20b | 9.90a | 102.58b |

[z]Means (n = 9) within a row and nutrient followed by same lowercase letter are not significantly different by pairwise comparison in mixed model ($P \leq 0.05$).

Table 2.7: Least square means cultivar effects of nutrient content of select basil cultivars grown in a Nutrient Film Technique hydroponic system at the OSU research greenhouses in 2021.

| Cultivar | Magnesium (ppm) | Sulfur (ppm) | Iron (ppm) |
| --- | --- | --- | --- |
| Dark Opal | 0.78b[z] | 0.30ab | 189.09a |
| Genovese | 0.79b | 0.37a | 77.96ab |
| Kapoor Tulsi | 0.98a | 0.37a | 86.07ab |
| Mrs. Burns Lemon | 0.75b | 0.25b | 52.84b |
| Prospera | 0.70b | 0.36a | 136.49ab |

[z]Means (n = 9) 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: Average daily temperature of nutrient solution in three treatments (23°C, 27.5°C, and 31°C) in Nutrient Film Technique hydroponic systems over three replications (spring, summer, and fall).

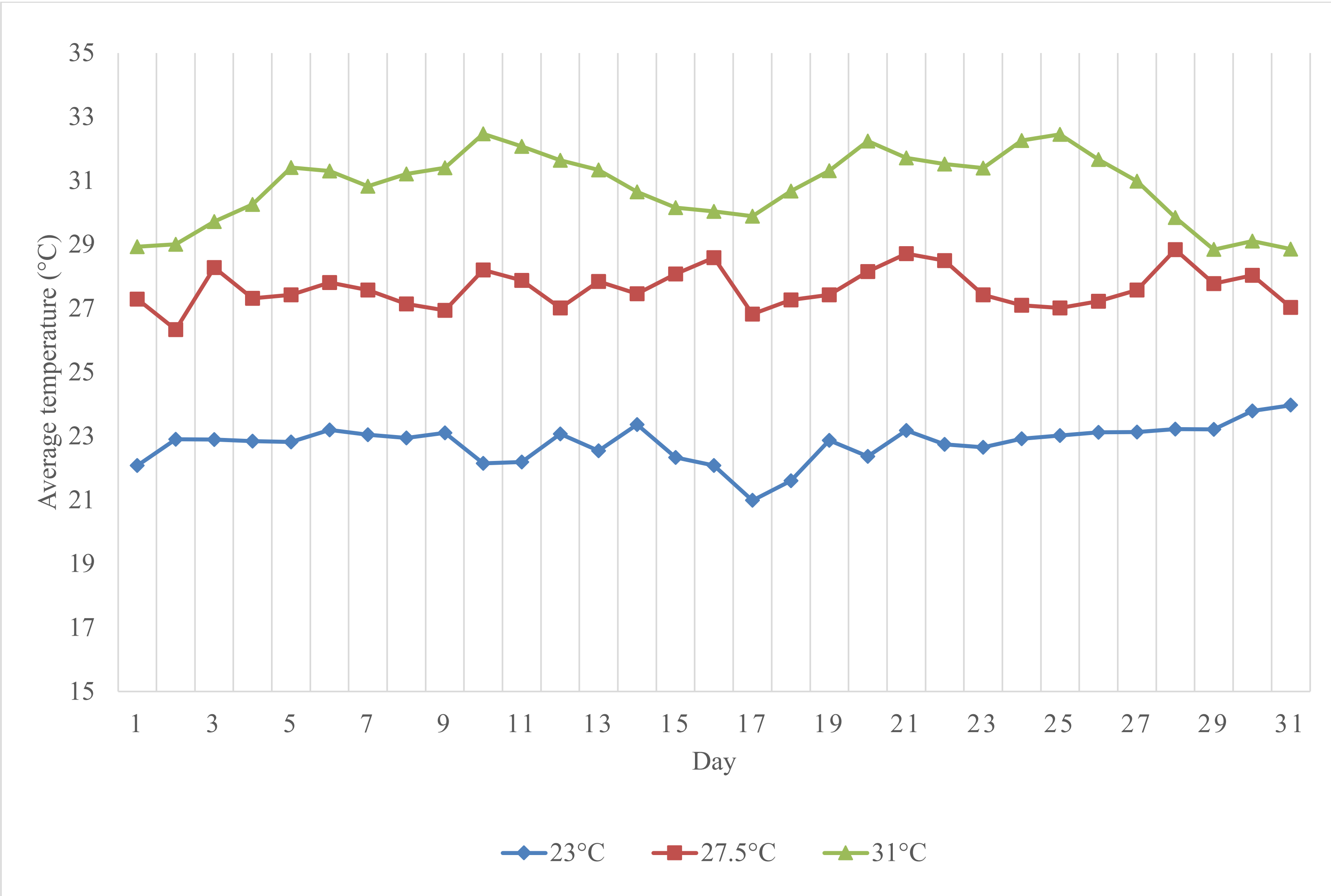
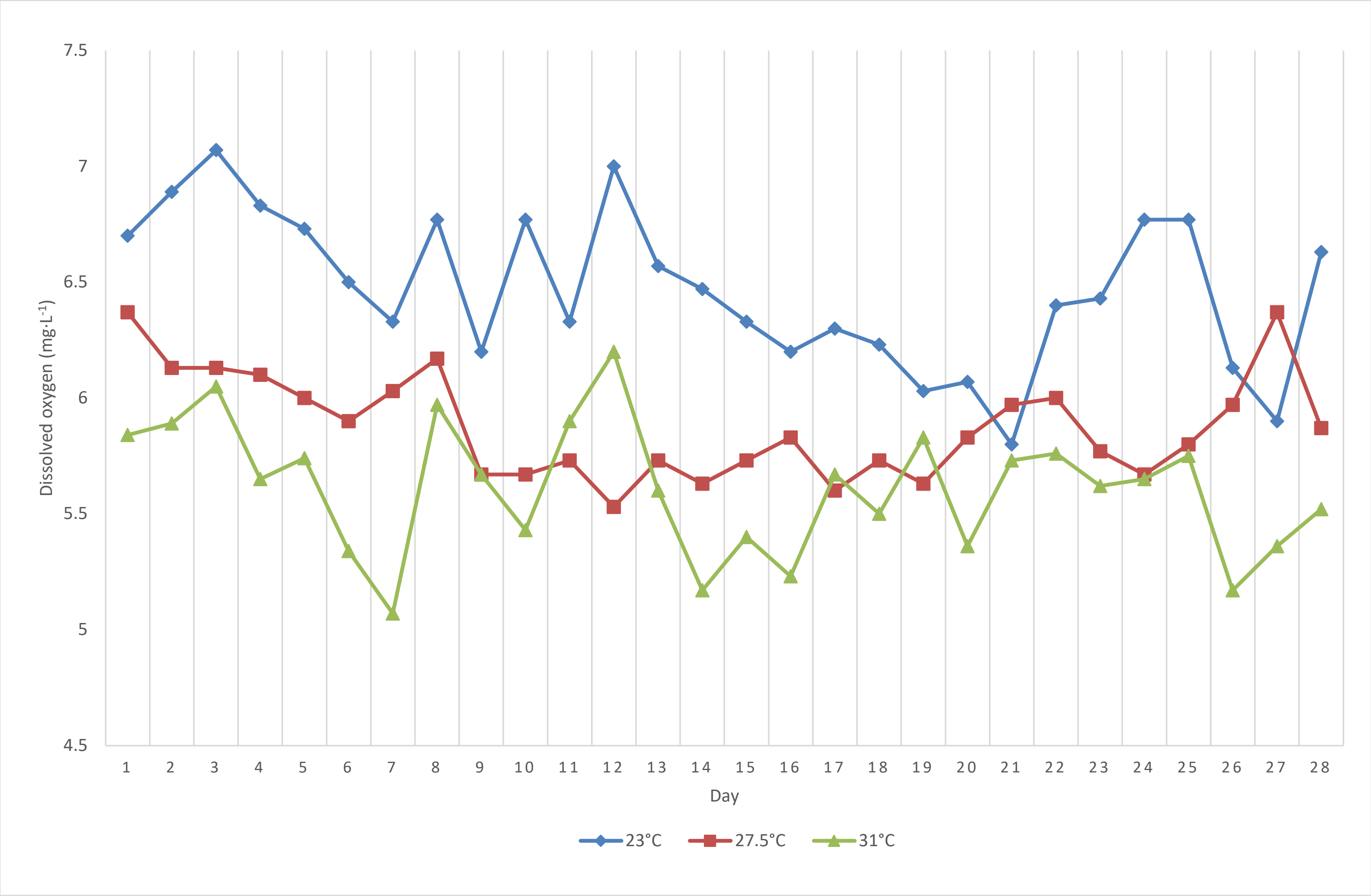


Figure 2.2: Effects of temperature difference (23°C, 27.5°C, and 31°C) on average dissolved oxygen (D.O.) levels of nutrient solution in Nutrient Film Technique hydroponic systems at OSU research greenhouses in Stillwater, OK.



CHAPTER III

# THE EFFECTS OF THREE DIFFERENT HYDROGEN PEROXIDE PRODUCTS ON PLANT GROWTH AND ALGAE IN EBB AND FLOW HYDROPONICS

## ABSTRACT

Hydrogen peroxide has been used as a sanitation agent for many years. Recently, hydrogen peroxide products have been used to remove algae from irrigation lines and sanitize hydroponic systems between uses. However, hydrogen peroxide can have phytotoxic effects on plants at high concentrations. In this study, the effect of hydrogen peroxide treatments on algae, lettuce, and basil growth was observed. The goal of this research was to determine if hydrogen peroxide treatments effected plant and algae growth in Ebb and Flow hydroponic systems. Research was conducted at the Department of Horticulture and Landscape Architecture. greenhouses in Stillwater, OK. Two cultivars of lettuce, 'Green Forest' and 'Tropicana', and two cultivars of basil, 'Aroma II' and 'Genovese', were transplanted into Ebb and Flow hydroponic systems and three different hydrogen peroxide products, PERpose Plus, Zerotol, and 3% hydrogen peroxide, were applied at different rates and combinations in two experiments. Shoot

fresh weight in lettuce was found to be significantly greater in the control and the 3% hydrogen peroxide treatments. Greater amounts of PERpose Plus and Zerotol appeared to restrict plant growth. Algae growth was not significantly restricted by any treatment in this research, though higher levels of hydrogen peroxide treatments did produce differences visually.

## INTRODUCTION

Since ancient times, the usage of hydroponic systems has been prevalent in advanced societies; however, the term “hydroponics” was not coined until 1937 by Dr. Gericke (Jones 1982, 2005; Resh, 1978). Hydroponics is the usage of a nutrient solution mixed with water to grow plants. Soilless agriculture reduces time between plantings, with no need for fallowing or crop rotation, as well as allows for more control over more variables, such as nutrient levels, temperature, and more while producing crops faster and with greater yield than soil-based systems due to easier oxygen and water access for the crop’s roots (Shrestha and Dunn, 2016). Many iterations of this system have been developed, from soilless media hydroponics such as Dutch bucket to flooding of the root zone, such as in the Ebb and Flow technique. Despite the iterations and the precautions taken, some problems have existed since the beginning. One such problem is the universal existence of algae in fresh water sources.

Algae are photosynthetic organisms that generally live in aquatic habitats and can be unicellular or multicellular (Camberato and Lopez, 2009). Most freshwater algae are unicellular and form colonies, or mats, on any surfaces in or on top of the water (Raudales, 2016). These microorganisms are capable of capturing nutrients from wastewater, atmospheric carbon dioxide ($CO_2$) or industrial flue gas, in addition to their natural photosynthetic processes that use sunlight (Supraja et al., 2020). As algae is common in most, if not all, fresh water supplies, it is no surprise that algae has made its way into the agricultural sector via irrigation lines, pumps, and hydroponic systems. While some research has suggested that co-cultivation of microalgae with crops in hydroponics can be beneficial to increase crop biomass and increase the release of growth-promoting substances, these studies have predominately used two main microalgal species: *Chlorella* sp. and *Scenedesmus* sp. (Huo et al., 2020; Supraja et al., 2020). Due to lack of study, the full range of native microalgae may not contribute to hydroponic systems in this manner (Supraja et al., 2020). On the other hand, uncontrolled growth of algae can cause several issues such as clogging lines and pumps, attracting pests such as shore flies and fungus gnats, and triggering decreased dissolved oxygen (D.O.) due to mass die-offs, as well as causing organic loading (Raudales, 2016; Supraja et al., 2020; Varma and DiGiano, 1968). In hydroponic systems, algae tends to collect around the edges of rafts and in tanks, which can lead to competition with the crop, producing a lower yield (Raudales, 2016).

Several methods exist for eliminating or preventing algae. Some of the most popular methods include covering tanks in black plastic or covering root zones in black and white plastic mulching, which can be more expensive due to high labor needs, and the application of barley straw, which can often be unreliable due to degradation rate (Camberato and Lopez, 2009; Lembi, 2009). More recently, other means of preventing or eliminating algae have been tested, such as using UV light to disrupt the integrity of algae cell membranes, as well as to degrade any organic material released by the algae, and various chemicals used to prevent or manage algae growth (Chen et al., 2020). Free chlorine and 3-(3-indolyl) butanoic acid were both discovered to have some algicidal effects, especially when used in tandem with UV, while being relatively non-phytotoxic to the crop itself (Dannehl et al., 2015; Nonomura et al., 2001). Hydrogen peroxide is often one of the more popular choices, especially early in the process of investigating the best chemical means to prevent algae (Draäbkova et al., 2007; Kay et al., 1982). Known for its abilities as a sterilizing agent, hydrogen peroxide has been shown to prevent or contain algal growth in various hydroponic environments (Caixeta et al., 2018; Vänninen and Koskula, 1998). Hydrogen peroxide has been shown to cause limited degradation on intracellular materials when used alone, as well as having oxidative properties. This allows hydrogen peroxide to be useful in curbing algal growth while maintaining a low phytotoxic effect on the crops within the system (Caixeta et al., 2018; Ou et al., 2011; Vänninen and Koskula, 1998). There is a slight downside to hydrogen peroxide which is a high phytotoxicity in seedlings.

As environmental runoff is a concern, hydrogen peroxide degrades rapidly into two byproducts: oxygen and water (Qian et al., 2010; Tesoriero et al., 2010). Both byproducts are relatively harmless in the environment and may even increase the D.O. content in the remaining water (Hernandez et al., 2012; Thakulla et al., 2021). Internally in the plant, hydrogen peroxide exists as a signaling molecule for abiotic stresses and is thought to protect organelle membranes to increase stress resistance (Mejia-Teniente, 2019; Nui and Liao, 2016; Ślesak et al., 2007). As an algicide, hydrogen peroxide can decrease metabolic processes, destroy pigment synthesis and membrane integrity, inhibit photosynthetic activity and genes expression, alter circadian rhythms, and induce apoptotic-like cell death while limiting growth; however, the efficacy of hydrogen peroxide is dependent on culture density and how protected the pigments are inside of the chloroplasts (Barroin and Feuillade, 1986; Draäbkova et al., 2007; Zhou et al., 2017). While hydrogen peroxide is often combined with UV in irrigation lines to prevent algae build up, the chemical can also cause some phytotoxic effects in seedlings (Caixeta et al., 2018; Sarathy and Mohseni, 2010; Vänninen and Koskula, 1998; Wan et al., 2019). While hardier seedlings often can recover, more delicate seedlings, such as lettuce (*Lactuca sativa* L.), are more prone to have a lower fresh weight and biomass than seedlings that were not treated with hydrogen peroxide (Caixeta et al., 2018). Therefore, specific application, such as time and dose, is not universal and is dependent on plant species (Coosemans, 1995).

Lettuce and basil (*Ocimum basilicum* L.) are common crops that are grown hydroponically; however, these two crops have different preferred climates and requirements (Currey, 2020; Saha et al., 2016; University of Illinois Extension, 2021). Nevertheless, as herbs and leafy greens are generally desired year-round, it is important to be able to feasibly produce a quality crop while reducing labor and maintenance costs, as well as reduction in crop quality, due to algae accumulation. Thus, the main objective of this research was to establish efficient rates of hydrogen peroxide products that would adequately control or prevent the algae population while not inhibiting plant growth and crop yield of lettuce and basil.

## MATERIALS AND METHODS

### *Experiment One Plant Materials and Growth Conditions*

The experiment was conducted at the Oklahoma State University Department of Horticulture and Landscape Architecture research greenhouses in Stillwater, Oklahoma. Average air temperatures for each run were 28.38°C, with greenhouse set points at 21°C/18°C as the average day/night temperature. Average humidity was 55.89%. Daily light integral (DLI) averaged to be 19.3 $mol \cdot m^{-2} \cdot d^{-1}$. Seeds of two cultivars of lettuce, 'Green Forest' and 'Tropicana', and two cultivars of basil, 'Aroma II' and 'Genovese', were obtained from Johnny's Selected Seeds (Winslow, MN) and planted in Horticubes Grow Cubes (Smithers Oasis, Kent, OH) and placed under misters for 4 weeks on 9 July 2021. Seedlings in the cubes were transplanted to an Ebb and Flow table (Gro Master, Maple Park (Virgil), IL) on 6 August 2021. A Styrofoam sheet was used as a float with 5 cm holes drilled approximately 22 cm apart. A 5 cm net pot (HydroFarm, Petaluma, California) was placed in each slot, and a single plant was placed in the net pot. The 40-gal 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) were added initially according to the recommended rates. The pH and electrical conductivity (EC) of the solution were checked every other 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*

Treatments applied included: Zerotol (Biosafe Systems, East Hartford, CT) at 45 ppm, Zerotol at 45 ppm with 50 ppm of 3% $H_2O_2$, Zerotol at 60 ppm, Zerotol at 60 ppm with 50 ppm 3% $H_2O_2$ (Greater Value 3%, Wal-Mart, Bentonville, AR), and 50 ppm 3%

$H_2O_2$, as well as a control. Zerotol was first applied 3 d after transplanting and was repeated weekly. Hydrogen peroxide was applied 4 d after the first application of Zerotol and was repeated weekly. D.O. was measured daily using a D.O. meter (Milwaukee Instruments, Rocky Mount, North Carolina).

A chlorophyll meter (SPAD-502, Konica Minolta, Japan) was used 30 d after transplanting. SPAD readings were taken from each plant from the middle of the top and bottom leaf and were averaged to determine the chlorophyll concentration. Data on photosynthesis rate was taken using a Li-Cor 6400 (LI-COR, Lincoln, 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 at 29°C, and the light level at 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The third leaf from the top was selected per plant and was used as a non-destructive sample. Plant height, width, fresh shoot weight, and leaf count were assessed 30 d after transplanting. Shoots and roots were dried at 59°C for 2 d for obtaining dry weight.

***Algae Quantification***

After harvesting plants, 300 mL of solution was collected from each table and given to EnviroScience Lab (Stowe, OH) for quantitative algae analysis. A visual scale of 1 to 3 was used to grade the algae in hydroponic tanks, with 1 being little to no algae, 2 being a moderate amount of algae on sides and bottom of the tank, and 3 being large mats of algae. 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 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·L$^{-1}$) was calculated by using the following formula as reported by Michaud (1994).

$$\frac{\left[\left(filter\ weight + dried\ residue\ (mg)\right) - \left(filter\ weight * 1000\right)\right]}{volumed\ used}$$

A hemocytometer (Hausser Scientific, Horsham, PA) 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. Then 1 µl of homogenous solution was added to the hemocytometer slide. The slide was examined under a compound microscope (Olympus, Waltham, MA) 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-*a* of algae through spectrophotometry. The spectrophotometer (GENESYS 30, The Lab Depot, Dawsonville, GA) was used to measure the absorbance of the samples at 750 nm, 665 nm, 647 nm, and 630 nm according to Kumar and Saramma (2013).

***Experimental Design and Statistics***

In both experiments, 10 plants per cultivar per species were treated as subsamples and were randomly planted in tables. Subsamples were averaged. Treatments were arranged as a split-plot in a randomized complete block design with two replications of each experiment. Treatment was the whole main plot, with four factors in the first experiment and five factors in the second experiment, and cultivar was the subplot with two factors. Statistical analysis was performed using SAS/STAT software (Version 9.4;

SAS Institute, Cary, NC). Tests of significance were reported at the 0.05, 0.01, and 0.001 level. The data were analyzed using generalized linear mixed models methods. Tukey multiple comparison methods were used to separate the means.

***Experiment Two Treatment, Data Collection, and Experimental Design***

The experiment was conducted at the Oklahoma State University Department of Horticulture and Landscape Architecture Research greenhouses in Stillwater, Oklahoma, and was carried out in the same manner as experiment one, apart from the seeds being planted on 28 March 2021, as well as on 7 May 2021, and transplanted on 26 April 2021, and repeated on 4 June 2021. Day temperature averaged 23.01°C and 27.07°C per rep, respectively, while humidity averaged 55.89% and 71.97% per repetition. Daily light intensity averaged 20.8 and 19.34 $mol \cdot m^{-2} \cdot d^{-1}$ DLI per repetition respectively. There were 10 treatments applied: Zerotol (Biosafe Systems, East Hartford, CT; 27.1% hydrogen peroxide and 2.0% peroxyacetic acid) at 15, 30, 45, and 60 ppm once weekly; PERpose Plus (Bioworks, Victor, NY; 33.0% hydrogen peroxide) at 15, 30, 45, and 60 ppm once weekly, 3% hydrogen peroxide (Greater Value 3%, Wal-Mart, Bentonville, AR) at 70 ppm weekly, and a control with two replications. The first application occurred 3 d after transplanting and was repeated every 7 d for the duration of 4 weeks. Data collection, algae quantification, and experimental design and statistics were all done in the same manner as in experiment one, except no photosynthesis data.

## RESULTS

***Interactions between cultivar and hydrogen peroxide treatments in lettuce***

In experiment one, there were significant interactions between hydrogen peroxide × cultivar for shoot fresh weight of lettuce (Table 3.1). Application of 3% hydrogen peroxide at 50 mL in 'Green Forest' resulted in the greatest amount of shoot fresh weight but was not significantly different than the control; however, both were greater than all other treatments. For 'Tropicana', the control had the greatest shoot fresh weight but was not different from any other treatment. Although not significantly different, in general, greater Zerotol and Zerotol plus 3% hydrogen peroxide treatments resulted in lower fresh weight (Table 3.2)

***Hydrogen peroxide effects on algae***

In both experiment one and two, there were no significant effects of hydrogen peroxide treatments on algae dry weight, cell number, or chlorophyll-*a* content (Table 3.3). However, the means of algae dry weight and algal cell counts were less in the presence of hydrogen peroxide products, especially greater rates of PERpose Plus and 3% hydrogen peroxide compared to Zerotol and the control, though the means were not significantly different (Table 3.3). However, visually, tanks that had been treated with greater concentrations of hydrogen peroxide, such as 60 mL of either PERpose Plus or Zerotol, appeared to have less algae than tanks treated with lower concentrations, such as 15 to 45 mL of either PERpose Plus or Zerotol, with the exception of 3% hydrogen peroxide, which appeared to cause algae matting on top of the surface of the water, and the control (Table 3.3; Figure 3.1).

There were some differences in algal species that inhabited different treatment tanks. *Microspora tumidula* was found in all treatment tanks at the greatest concentration except for the 60 mL of PERpose Plus where *Microspora* was not present (Table 3.4).

*Microspora tumidula* was found in greatest concentrations in the control, with a 96.0%, 99.2%, 94.0%, and 97.9% reduction in the 15 mL Zerotol, 60 mL Zerotol, 15 mL PERpose Plus, and 3% hydrogen peroxide treatments, respectively. Similarly, *Gloeocystis vesiculosa* was found to be dominant in all treatment tanks except for the control where *Gloeocystis* was not found and the Zerotol 60 mL treatment where *Gloeocystis* was in low concentrations. *Gloeocystis vesiculsosa* was found to be highest in the Zerotol treatment (15 mL), with a 99.2%, 66.7%, 83.6%, and 66.5% decrease in the 60 mL Zerotol, 15 mL PERpose Plus, 60 mL PERpose Plus, and 3% hydrogen peroxide treatment, respectively. *Chlamydomonas* spp. was the only algae genus to be found in every treatment tank but was found to be at lower concentrations in the PERpose Plus 60 mL and Zerotol 60 mL treatments, with a 99.9% and 96.5% reduction, respectively. The genus *Scenedesmus* was present in different species in all treatments except for Zerotol 60 mL treatment and was found in the greatest concentration in the 15 mL Zerotol treatment, with the greatest reduction in the control, 87.9%, and the 3% hydrogen peroxide treatment, 79.2%. Pennate diatoms were present in all treatments except Zerotol 15 mL and PERpose Plus 15 mL treatments, with the greatest concentration in the 60 mL Zerotol treatment, though there were 97.8%, 93.9%, and 97.4% reductions in the control, 60 mL PERpose Plus, and the 3% hydrogen peroxide treatment, respectively. Centric diatoms were similarly distributed in all treatments except the Zerotol 15 mL treatment, with the greatest reduction of 97.7% in the control. *Leptolyngbya* spp., *Microspora pachyderma*, *Sphaerocystis planktonica*, and *Tetraspora cylindrica* were found only in the control, PERpose Plus 15 mL, PERpose Plus 60 mL, and 3% hydrogen peroxide, respectively. Overall, the Zerotol treatments had less diversity than the other treatments (Table 3.4).

***Hydrogen peroxide and cultivar effects on plant growth parameters and chlorophyll content***

In experiment one, there were significant treatment effects and cultivar effects in lettuce. Treatment effects were found in plant height, number of leaves, shoot dry weight, and root dry weight in lettuce (Table 3.5). Lettuce plants were the tallest in the control, though not different from the 3% $H_2O_2$ treatment. The 45 mL of Zerotol and 50 mL of 3% $H_2O_2$ treatment plants were the shortest but were not different from the 60 mL of Zerotol and the 60 mL of Zerotol and 50 mL of 3% $H_2O_2$ (Table 3.5). The 3% $H_2O_2$ treatment had the greatest number of leaves but was not different from any other treatment except the 60 mL of Zerotol. The control had the greatest shoot dry weight but was not different from any other treatment except the 60 mL of Zerotol. Similarly, root dry weight was greatest in the control but was not significantly different from any other treatment except the 45 mL of Zerotol and 50 mL of 3% $H_2O_2$ treatment (Table 3.5). Cultivar effect was significant for parameters including SPAD, plant height, and width. There were significant cultivar effects in basil for experiment one as well. 'Aroma II' had the greatest SPAD value and was significantly different than 'Genovese' (Table 3.6).

In experiment two, there were significant treatment effects for shoot dry weight for both basil and lettuce (Table 3.7). In basil, 45 mL of Zerotol had the greatest dry shoot weight but was not different from any other treatments except the 60 mL of PERpose Plus (Table 3.8). In lettuce, 15 mL of PERpose Plus had the greatest dry shoot weight but was only different from treatments of 60 mL of Zerotol and 60 mL of

PERpose Plus. Significant cultivar effects were observed for lettuce as 'Green Forest' plants were significantly taller and had a greater SPAD value than 'Tropicana' (Table 3.9).

***Effects of hydrogen peroxide on dissolved oxygen***

In experiment one, hydrogen peroxide treatments significantly affected D.O. rates. The control had some of the lowest D.O. levels as compared to the other treatments but was only significantly different from the 60 mL of Zerotol and 50 mL of 3% hydrogen peroxide treatment on day 25 (Figure 3.2). D.O. means below 5.05 $mg \cdot L^{-1}$ were not significantly different from any other rates except the 60 mL of Zerotol and 50 mL of 3% hydrogen peroxide treatment on day 25, which had a mean of 9.95 $mg \cdot L^{-1}$ (Figure 3.2). Similar to experiment one, in experiment two, hydrogen peroxide treatments caused an increase in D.O. on treatment days (Figure 3.3). However, these treatments only caused a significant increase in D.O. on the day of application (Figure 3.4).

## DISCUSSION

***Interactions of cultivar and hydrogen peroxide treatments on lettuce***

Hydrogen peroxide and cultivars affected lettuce shoot fresh weight in this research. Individually, cultivar can have a significant impact on shoot fresh weight. Similar to this study, Lau and Mattson (2021) found that 37.5 $mg \cdot L^{-1}$ of 3% hydrogen peroxide, added in increments every 3 d to maintain concentration, produced a lettuce fresh weight that was not different from the control, but the 75 $mg \cdot L^{-1}$ produced lettuce with less fresh weight than the control and 37.5 $mg \cdot L^{-1}$ treatment due to indiscriminate damage of healthy tissue. In ‘Jessica’ and ‘Bolaria’ cucumber (*Cucumis sativus* L.) seedlings, hydrogen peroxide applications to limit algae caused a decrease in shoot weight, but the phytotoxic effects of the hydrogen peroxide treatments appeared to be dependent on temperature and amount of light effecting the speed at which hydrogen peroxide broke down (Vänninen and Koskula, 1998). Caixeta et al. (2018) found that, in lettuce seedlings, hydrogen peroxide spray treatments to limit algae, fungus gnats, and shore flies on germinating seeds did not significantly affect the fresh weight as compared to the control but did effect germination rates. In contrast, Kučerová et al. (2021) found that hydrogen peroxide increased lettuce cultivar ‘Král Máje I’ shoot weight slightly, but not significantly, from the control, which was thought to be due to plant tissue lignification. Concentration used and timing of application during the crop’s life cycle appears to have a great impact on the potential phytotoxicity of hydrogen peroxide applications. Combination of original cultivar shoot fresh weight and high levels of hydrogen peroxide stress can lower shoot fresh weight in lettuce as seen in the current study where increased rates and concentrations of hydrogen peroxide lowered fresh shoot weight significantly from the control in both ‘Green Forest’ and ‘Tropicana’.

***Hydrogen peroxide effects on algae***

Hydrogen peroxide products have long been used to disinfect water systems, especially irrigation (Arumugam et al., 2021; Raudales et al., 2014). Hydrogen peroxide is made by joining two hydrogen ions to two oxygen ions, producing relatively weak bonds and an unstable molecule that even weak UV radiation can break down that does not last as long in environmental conditions, making many consider it an environmentally friendly sanitation option (Barta and Henderson, 2000; Fisher et al., 2009; Southard, 2005; Wang et al., 2018; Yang et al., 2018). Due to its strong oxidizing abilities, hydrogen peroxide produces hydroxyl radicals under light exposure, which destroys proteins, lipids, and DNA, severely damaging unicellular organisms (Southard, 2005; Yang et al., 2018). In algae specifically, hydrogen peroxide can decrease metabolic processes, destroy pigment synthesis and membrane integrity, inhibit photosynthetic activity and gene expression, alter circadian rhythms, and induce apoptotic-like cell death while limiting growth (Draäbkova et al., 2007; Zhou et al., 2017). Hydrogen peroxide products often combine hydrogen peroxide with peracetic acid to provide stability and high reactivity both on inorganic compounds and organic compounds (Popescu et al., 2019). Rates and timing of application have been found to be largely dependent on crop, algal species and density, water chemistry and environment, and specific system (Breithaupt, 2007; Schwarz and Krienitz, 2005; Thakulla et al, 2021). Rates as low as 12.3 $mg \cdot L^{-1}$ hydrogen peroxide combined with 8 $mg \cdot L^{-1}$ peracetic acid for control of algae to 185 $mg \cdot L^{-1}$ hydrogen peroxide plus 120 $mg\ L^{-1}$ peracetic acid with 1 minute contact time are recommended to control some pathogens (Raudales et al., 2014). Hydrogen peroxide can cause antioxidant defense systems to activate in algae, allowing the microorganisms to survive oxidative stresses until a certain threshold (Liu et al., 2017). Thakulla et al. (2021) found that concentrations of 70 mL of Zerotol or PERpose Plus applied biweekly to 40-gallon tanks significantly decreased algae concentrations.

In this research, rates of 15 to 70 mL of different hydrogen peroxide products (Zerotol, PERpose Plus, and 3% hydrogen peroxide) were used; however, there were no significant effects on algae growth and density. Weenink et al. (2021) found that high populations of green heterotrophic algae may rapidly degrade hydrogen peroxide applications, protecting the other populations of algae. Water composition, especially metal components, and UV exposure can impact the rates of hydrogen peroxide decomposition and that elevated pH can influence rapid decomposition rate of hydrogen peroxide and therefore its algicidal properties (Draäbkova et al., 2006; Huang et al., 2021; Raffellini et al., 2008).

Sampling and analytical methods used may have also caused the discrepancies found between the visual grading and quantitative algae data. Marker and Bolas (1982) found that no method can be precise due to variation in collection method, including biomass dry weight, counting algae cells, and chlorophyll-*a* extraction. Biomass dry weight is only able to measure all organic and inorganic mass found within the sample and attributes the entirety of that mass is algae (Francoeur et al., 2013). This leads to other material, such as root particles or insect eggs, being included in total dry weight. Similarly, using a hemocytometer to count individual algae cells can be subjective and impractical (Marker and Bolas, 1982). Counting individual algae cells or colonies can be difficult due to obscuration from other particles and clustering of cells (Douglas, 1958). Misidentification of non-algae particles is also common, leading to higher cell counts, and different species of algae can cause increased or decreased cell counts due to filamentation and clumping (Marker and Bolas, 1982; Peniuk et al., 2016). Proper dilution is required as well, which adds more uncertainty in quantification (Douglas, 1958; Peniuk et al., 2016). Measurements of chlorophyll-*a* can also be imprecise, due to different species of algae containing different concentrations of chlorophyll and their dependance on nutrient content and light exposure (Francoeur et al., 2013). Furthermore, solvent choice for extraction is important and can be highly variable (Francoeur et al., 2013; Marker and Bolas, 1982). Simon and Helliwell (1998) found that mechanical disruption of algae cells was necessary to optimize pigment extraction, and that methanol was a more efficient solvent than acetone as long as due care was taken with the process. Similarly, Schumann et al. (2005) found that mechanical homogenization improved extraction up to 20%, but chlorophyll-*a* extraction efficiency was strongly species-specific and influenced by the growth conditions.

Thakulla et al. (2021) reported similar algae species as those found in this study, with *Chlamydomonas* spp. found in all treatments and *Gleocystis vesiculosa* found in greater concentrations in most treatments. *Chlamydomonas* spp. has been found in hydroponic systems frequently (Nonomura et al., 2001; Schwarz and Krientiz, 2005; Schwarz et al., 2005). *Scenedesmus* spp. have been similarly prevalent, though Nonomura et al. (2001) reported that *Scenedesmus* species were rare in samples in Japan (Schwarz and Krientiz, 2005; Schwarz et al., 2005; Supraja et al., 2020). *Microspora tumidula* was not found to be common in reported literature, though it was one of the most common species of algae found in this research.

***Effects of hydrogen peroxide and cultivar on lettuce and basil***

In this research, greater amounts of hydrogen peroxide products tended to decrease plant growth, especially in plant height, number of leaves, and root dry weight in lettuce, and shoot dry weight in lettuce and basil. Symptoms associated with hydrogen peroxide toxicity include leaf scorching, reduced plant growth, and plant mortality (Raudales et al., 2014). Similar to our findings, Lau and Mattson (2021) found that greater levels of hydrogen peroxide stunted root and shoot growth significantly more than the control and lower concentrations of hydrogen peroxide in lettuce. Thakulla et al.

(2021) found that lower amounts of hydrogen peroxide products applied weekly can help increase height, shoot dry weight and root dry weight, but greater concentrations applied weekly or biweekly decreased significantly in biomass and height in tomatoes. Deng et al. (2012) reported similar findings in sweet potato (*Ipomoea batatas* L.) seedlings with concentrations of less than or equal to 2.5 mM of exogenously applied hydrogen peroxide reported to have positive effects on seedling growth and root formation, while treatments that exceeded 5 mM of hydrogen peroxide had the opposite effect. Similarly, in our study, low concentrations of 3% hydrogen peroxide and low doses of stronger peroxide products were less phytotoxic than greater concentrations of hydrogen peroxide.

In this study, cultivar effects were significant for SPAD, plant height, and width in lettuce while only for SPAD and plant width in basil. Thakulla et al. (2021) found that romaine lettuce had significantly greater SPAD values as compared to leafy lettuce, similar to our research. SPAD values tend differ between lettuce cultivars due to differences in chlorophyll content (Eshkabilov et al., 2021; Suwor et al., 2020). Basil cultivars act similarly, maintaining different levels of chlorophyll content, and therefore different SPAD values (Lazarević et al., 2020; Saha et al., 2016; Yang and Kim, 2020).

Afton (2018) found that plant height was also significantly associated with cultivar, showing that romaine lettuce, such as ‘Green Forest’, was taller than leaf lettuce, such as ‘Tropicana’. Conversely, Thakulla et al. (2021) found that loose leaf lettuce cultivars were taller than romaine cultivars but not significantly so for any cultivars. Overall, Suwor et al. (2020) found that different lettuce cultivars are significantly different in height and width. Unlike in this study and the Afton (2018) study, Maynard (2013) found that ‘Green Forest’ had a greater width than ‘Tropicana’ in field studies.

Similarly, Dudai et al. (2020) found that basil widths vary among cultivars, ranging from 31 to 55 cm in field studies.

***Effects of hydrogen peroxide on dissolved oxygen***

Increases in D.O. were observed in relation with hydrogen peroxide treatments. Hydrogen peroxide decomposes into oxygen and water at different rates depending on environmental factors (Hinchee et al., 1991; Popescu et al., 2019). Tusseau-Vuillemin et al. (2002) found that hydrogen peroxide could be used as a precursor to D.O. in place of aeration due to the increased transfer rate of oxygen to solution. Without the presence of active catalysts such as metals or UV light, hydrogen peroxide degrades slowly in water and will only contribute slightly to the dissolve oxygen content (Taylor and Ross, 1988). Presence of carbons can activate hydroxyl radicals that lead to either the degradation of hydrogen peroxide or oxidation of organic compounds in the water (Oliveira et al., 2004). Similar to our study, Lau and Matton (2021) found that D.O. was greatest after application of hydrogen peroxide and greater concentrations led to greater D.O. content. However, the United States Environmental Protection Agency reported that, under aquatic aerobic non-sterile conditions, hydrogen peroxide had a half-life of 1.1 to 5.3 hours, which could be accelerated by the presence of metals in the water or UV radiation such as sunlight (Breithaupt, 2007). Soffer et al. (1991) found that chrysanthemums (*Chrysanthemum* x *morifolium* L. 'Bright Golden Anne') and weeping fig (*Ficus benjamina* L.) both grew faster in oxygen-saturated water. According to Ruso et al. (2021), basil can persist with D.O. levels as low as 4 $mg \cdot L^{-1}$, with optimal levels at 6.5 $mg \cdot L^{-1}$. However, lettuce only needs a D.O. content of at least 1.6 $mg \cdot L^{-1}$ (Sikawa and Yakupitiyage, 2010). Thus, increased D.O. did not equate to increased plant growth in this experiment due to the minimum requirements of each plant being met and the phytotoxic effects of greater hydrogen peroxide concentrations.

## CONCLUSION

In this study, applications of hydrogen peroxide did not have significant effects on algae growth quantitatively but did visually. There were, however, significant impacts on plant growth. Higher levels of hydrogen peroxide reduced plant growth, especially in lettuce, while lower concentrations of hydrogen peroxide were not toxic to the plants or the algae. Basil growth was relatively unaffected by hydrogen peroxide except at the greatest concentration of PERpose Plus. Further research is needed to identify what rates of hydrogen peroxide products more accurately could successfully limit algae growth while remaining nontoxic to plant growth. Combination treatments may be the key to limiting algae while not effecting plant growth. Lower rates of hydrogen peroxide combined with UV light treatments may be effective in hydroponic systems, as it has been shown to be effective in irrigation systems.

## TABLES AND FIGURES

Table 3.1: Test of effects for hydrogen peroxide treatments and two basil cultivars (‘Genovese’ and ‘Aroma II’) and lettuce (‘Green Forest and Tropicana) grown in an Ebb and Flow hydroponic system at OSU research greenhouses in Stillwater, OK for experiment one.

| Type | | Cultivar | $H_2O_2$ | Cultivar × $H_2O_2$ |
|---|---|---|---|---|
| Basil | SPAD | *[z] | NS | NS |
| | Height | NS | NS | NS |
| | Width | * | NS | NS |
| | Number of leaves | NS | NS | NS |
| | Shoot fresh wt. | NS | NS | NS |
| | Shoot dry wt. | NS | NS | NS |
| | Root dry wt. | NS | NS | NS |
| Lettuce | SPAD | *** | NS | NS |
| | Height | * | ** | NS |
| | Width | * | NS | NS |
| | Number of leaves | NS | * | NS |
| | Shoot fresh wt. | * | *** | * |
| | Shoot dry wt. | NS | ** | NS |

| | | | |
|---|---|---|---|
| Root dry wt. | NS | * | NS |

[z]Indicates significant at or non-significant (NS) at $*P \leq 0.05$, $**P \leq 0.01$, or $***P \leq 0.001$.

Table 3.2: Least square means interaction between lettuce cultivars and hydrogen peroxide treatment for two cultivars ('Green Forest' and 'Tropicana') in NFT hydroponic systems at OSU research greenhouses in Stillwater, OK for experiment one.

| Cultivar | Chemical | Rate (mL) | Shoot fresh wt. (g) |
|---|---|---|---|
| Green Forest | Control | 0 | 271.12a[z] |
| | 3% $H_2O_2$ | 50 | 298.80a |
| | Zerotol | 45 | 159.26bc |
| | Zerotol | 60 | 114.60bc |
| | Zerotol and 3% $H_2O_2$ | 45 and 50 | 108.40c |
| | Zerotol and 3% $H_2O_2$ | 60 and 50 | 154.79bc |
| Tropicana | Control | 0 | 223.18ab |
| | 3% $H_2O_2$ | 50 | 209.43abc |
| | Zerotol | 45 | 148.85bc |
| | Zerotol | 60 | 135.15bc |
| | Zerotol and 3% $H_2O_2$ | 45 and 50 | 148.09bc |
| | Zerotol and 3% $H_2O_2$ | 60 and 50 | 134.40bc |

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

Table 3.3: Least square means of different rates and application timing of three hydrogen peroxide products on algae samples from Ebb and Flow hydroponic systems in Stillwater, OK.

| Experiment | Chemical | Rate (ml) | Dry weight ($mg \cdot L^{-1}$) | Algal cells (10^5) | Chl *a* ($\mu g \cdot L^{-1}$) | Visual scale [y] |
|---|---|---|---|---|---|---|
| 1 | Control | 0 | 0.66a[z] | 13.66a | 740.53a | 3 |
| | 3% $H_2O_2$ | 50 | 0.61a | 13.38a | 801.59a | 3 |
| | Zerotol | 45 | 0.71a | 12.98a | 708.35a | 2 |
| | Zerotol | 60 | 0.86a | 13.26a | 755.07a | 1 |
| | Zerotol and 3% $H_2O_2$ | 45 and 50 | 1.00a | 13.28a | 856.90a | 2 |
| | Zerotol and 3% $H_2O_2$ | 60 and 50 | 0.64a | 12.57a | 875.28a | 2 |
| 2 | Control | 0 | 0.47a[z] | 6.50a | 945.57a | 3 |
| | Zerotol | 15 | 0.37a | 6.42a | 616.90a | 2 |
| | Zerotol | 30 | 0.37a | 6.75a | 637.19a | 2 |
| | Zerotol | 45 | 0.43a | 5.97a | 716.51a | 2 |
| | Zerotol | 60 | 0.23a | 6.24a | 509.46a | 1 |

| | | | | | |
|---|---|---|---|---|---|
| PERpose Plus | 15 | 0.21a | 6.15a | 573.41a | 2 |
| PERpose Plus | 30 | 0.15a | 6.01a | 597.24a | 2 |
| PERpose Plus | 45 | 0.21a | 5.93a | 607.17a | 2 |
| PERpose Plus | 60 | 0.14a | 5.76a | 690.83a | 1 |
| 3% $H_2O_2$ | 70 | 0.26a | 5.42a | 1209.09a | 3 |

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

[y] Visual scale: 1 = little to no algae, 2 = some algae collected on sides and bottom, 3 = thick algae matt.

Table 3.4: Effects of different rates of three hydrogen peroxide products on taxonomic counts of algae present in Ebb and Flow hydroponic systems in OSU research greenhouses, Stillwater, OK.

| Chemical | Rate (ml) | Scientific Name | Average cells/mL[z] | Average natural units/mL[z] |
|---|---|---|---|---|
| Control | 0 | *Microspora tumidula* | 478,071 | 976 |
| | | *Leptolyngbya spp* | 48,771 | 2,342 |
| | | *Scenedesmus acuminatus* | 92 | 92 |
| | | *Scenedesmus acutus* | 46 | 11 |
| | | Pennate Diatom spp. Live | 11 | 11 |
| | | Centric Diatom spp. Live | 11 | 11 |
| | | *Chlamydomonas spp.* | 11 | 11 |
| Zerotol | 15 | *Gloeocystis vesiculosa* | 29,494 | 1,756 |
| | | *Microspora tumidula* | 18,956 | 568 |
| | | *Chlamydomonas spp.* | 12,913 | 12,913 |
| | | *Scenedesmus acutus* | 930 | 258 |
| | | *Scenedesmus quadricauda* | 207 | 52 |
| Zerotol | 60 | Pennate Diatom spp. Live | 4,527 | 4,527 |
| | | *Microspora tumidula* | 3,587 | 244 |

| | | | | |
|---|---|---|---|---|
| | | *Chlamydomonas spp.* | 451 | 451 |
| | | Centric Diatom spp. Live | 394 | 394 |
| | | *Gloeocystis vesiculosa* | 225 | 19 |
| PERpose Plus | 15 | *Microspora tumidula* | 28,620 | 942 |
| | | *Gloeocystis vesiculosa* | 9,818 | 355 |
| | | *Microspora pachyderma* | 826 | 8 |
| | | *Chlamydomonas spp.* | 496 | 496 |
| | | *Scenedesmus acutus* | 314 | 99 |
| | | *Scenedesmus quadricauda* | 83 | 25 |
| | | Centric Diatom spp. Live | 83 | 83 |
| PERpose Plus | 60 | *Gloeocystis vesiculosa* | 4,846 | 1,183 |
| | | *Sphaerocystis planktonica* | 1,165 | 949 |
| | | *Scenedesmus acutus* | 301 | 103 |
| | | Pennate Diatom spp. Live | 272 | 272 |
| | | *Scenedesmus acuminatus* | 213 | 188 |
| | | Centric Diatom spp. Live | 150 | 150 |
| | | *Chlamydomonas spp.* | 9 | 9 |

| | | | | |
|---|---|---|---|---|
| 3% $H_2O_2$ | 70 | *Microspora tumidula* | 10,035 | 1,476 |
| | | *Gloeocystis vesiculosa* | 9,888 | 325 |
| | | *Chlamydomonas spp.* | 6,316 | 6,316 |
| | | *Tetraspora cylindrica* | 1,476 | 30 |
| | | Centric Diatom spp. Live | 472 | 472 |
| | | *Scenedesmus acuminatus* | 236 | 148 |
| | | Pennate Diatom spp. Live | 118 | 118 |

[z]Derived from a 300 mL solution.

Table 3.5: Least square means of rates of two hydrogen peroxide products on height, leaf number, shoot dry weight, and root dry weight of lettuce (‘Tropicana’ and ‘Green Forest’) grown in Ebb and Flow hydroponic systems at OSU research greenhouses in Stillwater, OK in experiment one.

| Chemical | Rate (ml) | Height (cm) | Number of leaves | Shoot dry wt. (g) | Root dry wt. (g) |
|---|---|---|---|---|---|
| Control | 0 | 28.34a | 2.56ab | 10.04a | 1.27a |
| 3% $H_2O_2$ | 50 | 26.45ab | 2.73a | 9.81a | 1.10ab |
| Zerotol | 45 | 22.80b | 2.55ab | 6.50ab | 1.07ab |
| Zerotol | 60 | 29.40bc | 2.47b | 5.59b | 0.95ab |
| Zerotol and 3% $H_2O_2$ | 45 and 50 | 17.19c | 2.53ab | 6.54ab | 0.80b |
| Zerotol and 3% $H_2O_2$ | 60 and 50 | 22.35bc | 2.57a | 6.67ab | 0.93ab |

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

Table 3.6: Least square means of cultivars ('Genovese' and 'Aroma II') on growth of basil and cultivars ('Green Forest' and 'Tropicana') on growth of lettuce grown in Ebb and Flow hydroponic systems at OSU research greenhouses in Stillwater, OK in experiment one.

| Type | Cultivar | SPAD (unitless) | Height (cm) | Width (cm) |
|---|---|---|---|---|
| Basil | Aroma II | 33.99a[z] | 35.53a | 17.35a |
| | Genovese | 32.38b | 33.53a | 15.34a |
| Lettuce | Green Forest | 40.57a | 23.96a | 27.59b |
| | Tropicana | 35.24b | 21.86b | 28.83a |

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

Table 3.7: Test of effects for hydrogen peroxide treatments and two basil and two lettuce cultivars grown in an Ebb and Flow hydroponic system at OSU research greenhouses in Stillwater, OK for experiment 2.

| Type | | Cultivar | $H_2O_2$ | Cultivar × $H_2O_2$ |
|---|---|---|---|---|
| Basil | SPAD | NS[z] | NS | NS |
| | Height | NS | NS | NS |
| | Width | NS | NS | NS |
| | Number of leaves | NS | NS | NS |
| | Shoot fresh wt. | NS | NS | NS |
| | Shoot dry wt. | NS | * | NS |
| | Root dry wt. | NS | NS | NS |
| Lettuce | SPAD | ***[z] | NS | NS |
| | Height | * | NS | NS |
| | Number of leaves | NS | NS | NS |
| | Shoot fresh wt. | NS | NS | NS |
| | Shoot dry wt. | NS | *** | NS |
| | Root dry wt. | NS | NS | NS |

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

Table 3.8: Least square means of rates of two hydrogen peroxide products on growth of basil (‘Genovese’ and ‘Aroma II’) and lettuce (‘Green Forest’ and ‘Tropicana’) grown in Ebb and Flow hydroponic systems at OSU research greenhouses in Stillwater, OK for experiment two.

| Type | Chemical | Rate (mL) | Shoot dry wt. (g) |
|---|---|---|---|
| Basil | Control | 0 | 7.98ab[z] |
| | Zerotol | 15 | 7.82ab |
| | Zerotol | 30 | 7.78ab |
| | Zerotol | 45 | 8.35a |
| | Zerotol | 60 | 7.27ab |
| | PERpose Plus | 15 | 7.99ab |
| | PERpose Plus | 30 | 7.29ab |
| | PERpose Plus | 45 | 8.16ab |
| | PERpose Plus | 60 | 6.58b |
| | 3% $H_2O_2$ | 70 | 7.79ab |
| Lettuce | Control | 0 | 14.01abc |
| | Zerotol | 15 | 15.16ab |

| | | |
|---|---|---|
| Zerotol | 30 | 13.04abc |
| Zerotol | 45 | 14.89ab |
| Zerotol | 60 | 11.17bc |
| PERpose Plus | 15 | 15.24a |
| PERpose Plus | 30 | 15.07ab |
| PERpose Plus | 45 | 13.83abc |
| PERpose Plus | 60 | 10.51c |
| 3% $H_2O_2$ | 70 | 14.38abc |

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

Table 3.9: Least square means of cultivars (‘Green Forest’ and ‘Tropicana’) on growth of lettuce grown in Ebb and Flow hydroponic systems at OSU research greenhouses in Stillwater, OK for experiment two.

| Type | Cultivar | SPAD (unitless) | Height (cm) |
|---|---|---|---|
| Lettuce | Green Forest | 40.47a | 20.12a |
| | Tropicana | 33.38b | 14.62b |

[z]Means 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: Visual scale of algae in tanks of Ebb and Flow hydroponic systems at OSU research greenhouses in Stillwater, OK.

A = little to no algae, B = some algae collected on sides and bottom, C = thick algae matt.

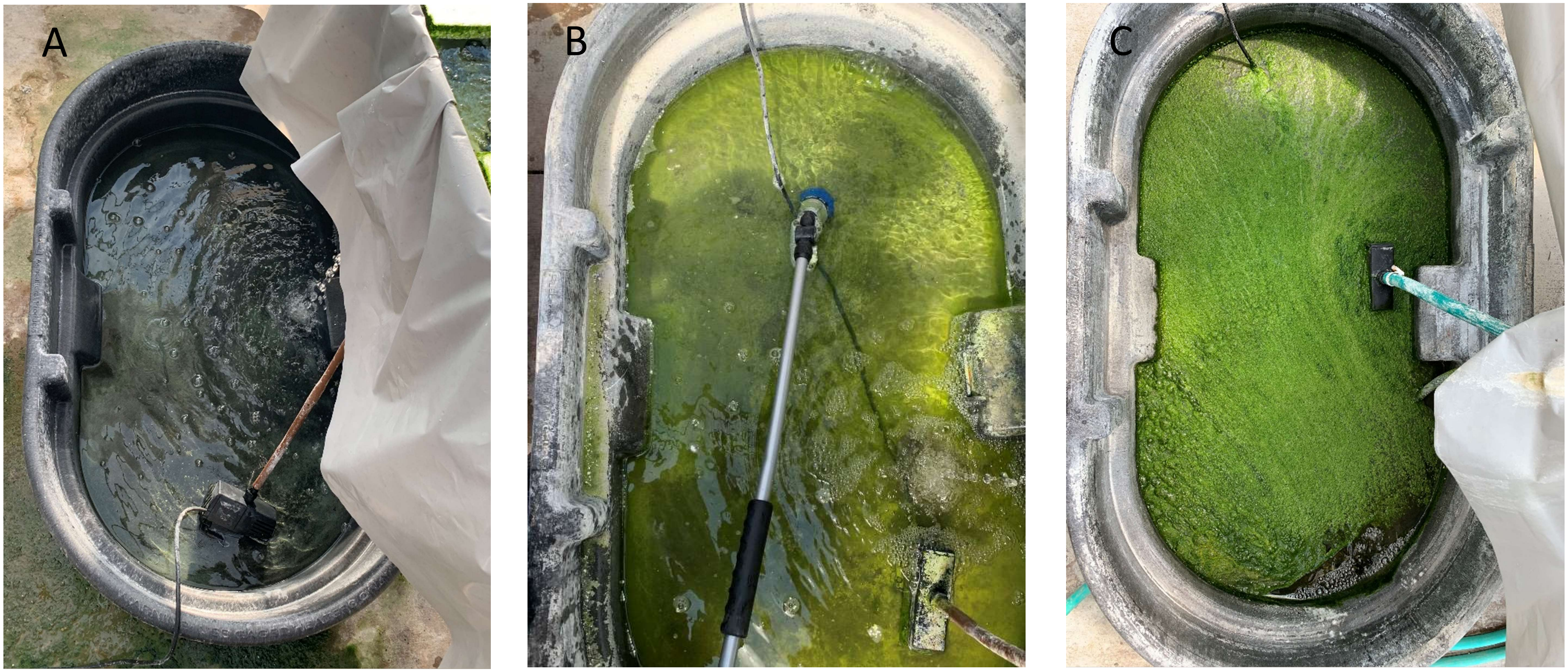


Figure 3.2: Effects of rate of two $H_2O_2$ products, Zerotol (Z) and 3% $H_2O_2$ (3%) (45 mL Z, 60 mL Z, 45mL Z and 50 mL 3%, and 60 mL Z and 50 mL 3%) on dissolved oxygen (D.O.) levels of nutrient solution in Ebb and Flow hydroponic systems at OSU research greenhouses in Stillwater, OK. Treatments were applied weekly starting on day three. Stars show significant differences for each day.

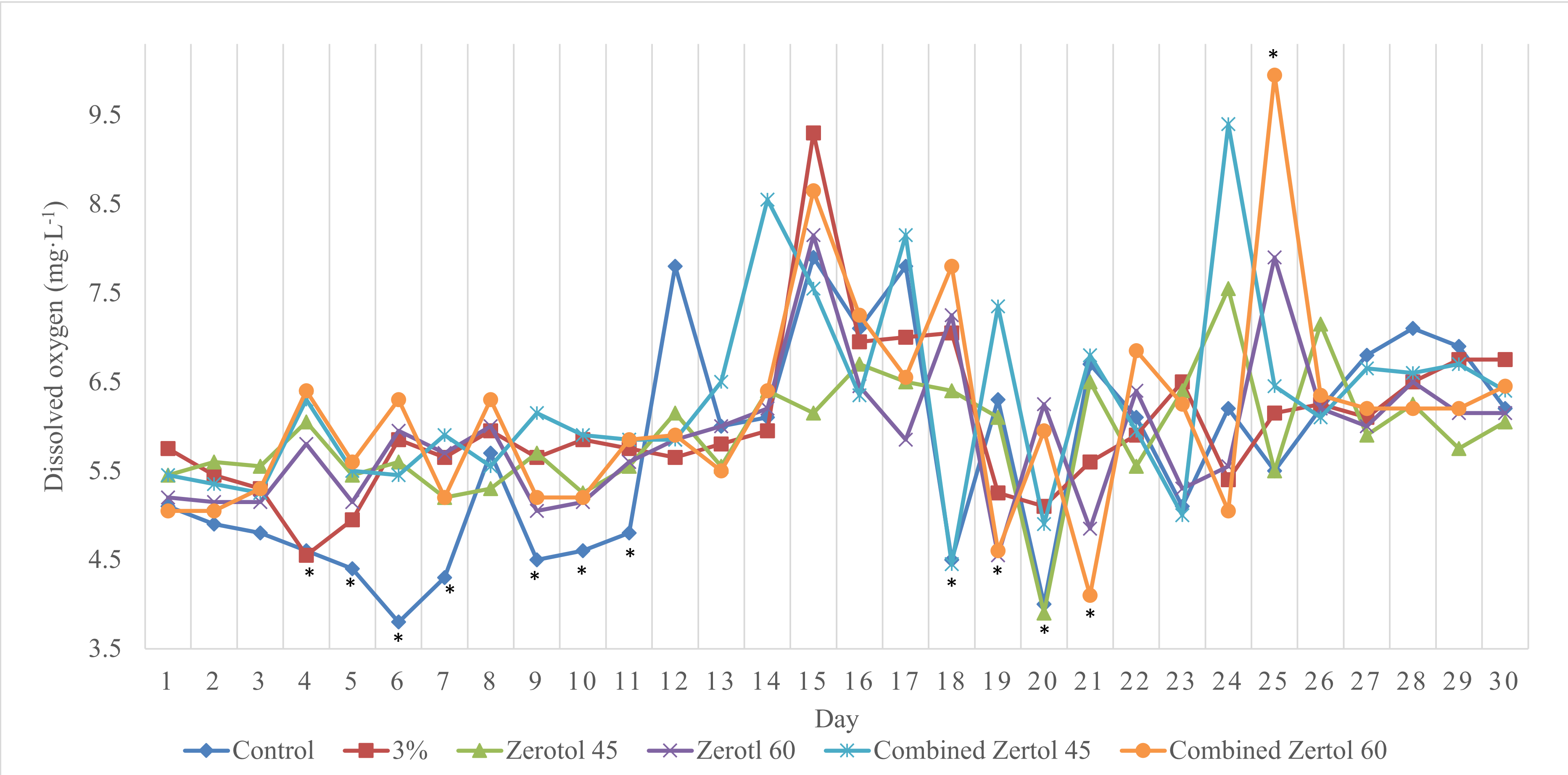


Figure 3.3: Effects of rate of three $H_2O_2$ products, Zerotol (Z), PERpose Plus (PP), and 3% $H_2O_2$ (15, 30, 45, and 60 weekly; 15, 30, 45, and 60 mL weekly; 70 mL weekly; and control) applied every seven days on dissolved oxygen (D.O.) levels of nutrient solution in Ebb and Flow hydroponic systems at OSU research greenhouses in Stillwater, OK.

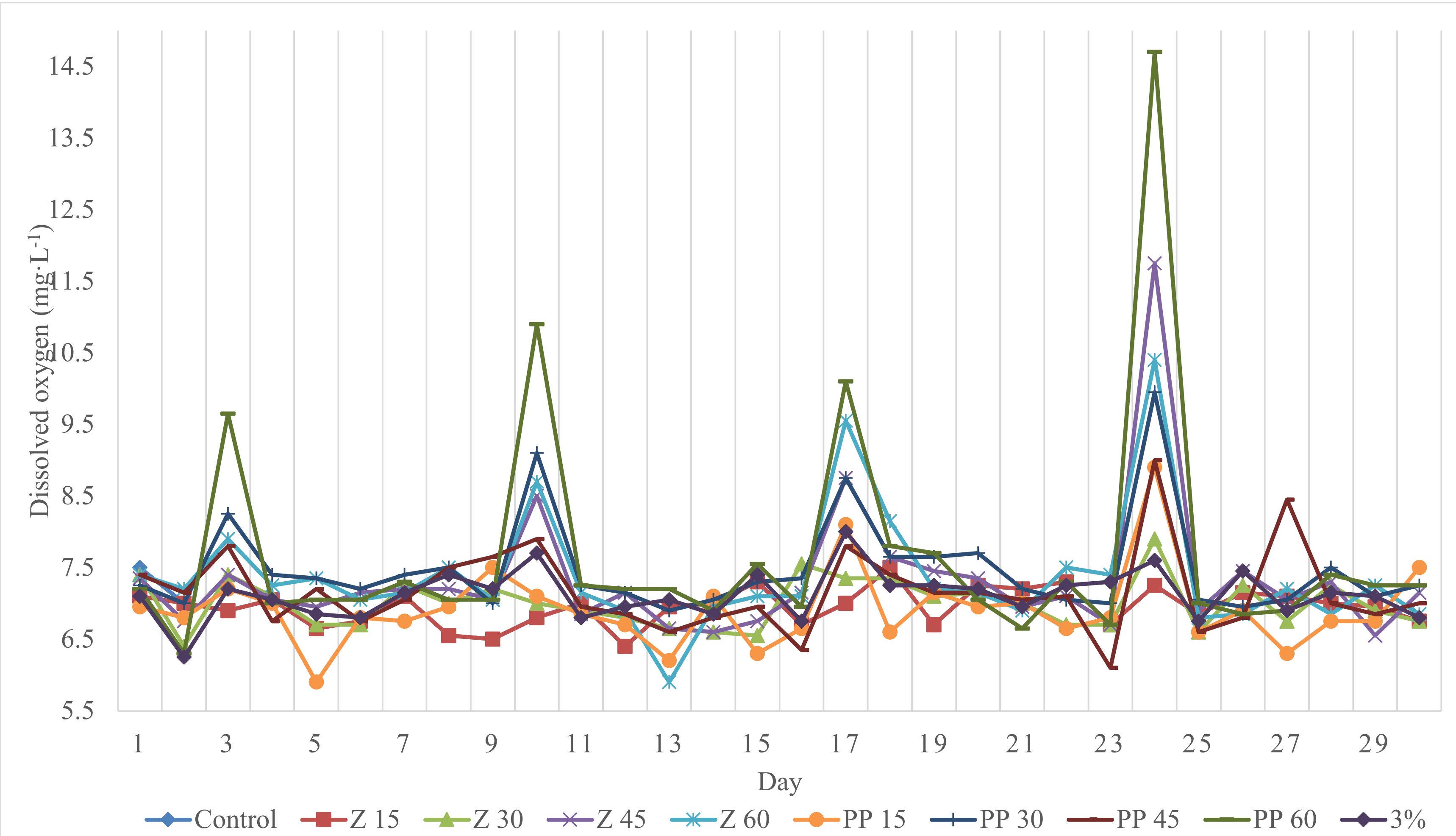
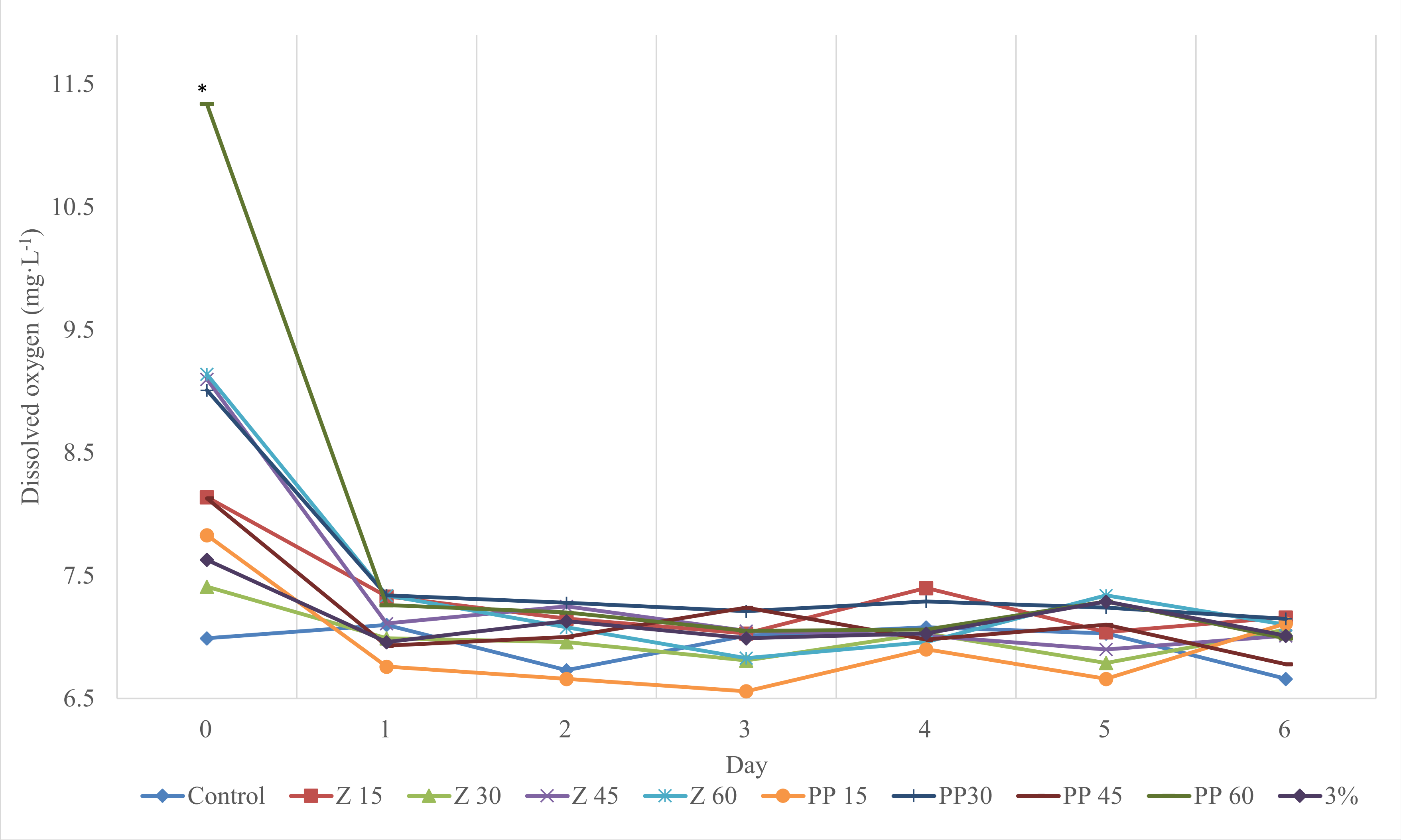


Figure 3.4: Persistent effects of three hydrogen peroxide products (Zerotol, PERpose Plus, and Greater Value 3% $H_2O_2$) on dissolved oxygen (D.O.) for six days after application. (* indicates significant difference on any given day.)



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