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Limited irradiance, artificial fluorescent irradiance, and nitrate-N:ammonium-N impact on biomass and essential oil production of Ocimum basilicum L.

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Limited irradiance, artificial fluorescent irradiance, and nitrate-N:ammonium-N impact on biomass and essential oil production of *Ocimum basilicum* L.

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ABSTRACT

In the United States, there is an increasing trend in consumer participation in herb gardening. One of the more popular herbs available, basil (*Ocimum basilicum* L.) is a culinary herb with a wide variety of uses and cultivars. With an increased need to support the growing demand for herbs, it is important to understand how various growing practices impact basil production. In particular, this research investigates several aspects of growing basil, including how limited irradiance, artificial fluorescent irradiance, and nitrate-N:ammonium-N fertilizer influences growth and essential oil production.

In our limited irradiance study, 'Genovese' basil plants were grown in a greenhouse under shade structures that provided 0%, 30%, 50%, or 70% shade. Basil fresh weight and shoot indices increased as percent shade decreased. Essential oil peak areas were predicted to be the greatest for eucalyptol at 60% shade, linalool at 30% shade, and eugenol at 40% shade.

Irradiance intensity is typically suboptimal for growing plants indoors. Fluorescent lighting systems are recommended for supplemental lighting in a residential setting. In our study with basil, it was found that plants grown under fluorescent grow lamps tended to have higher shoot indices and higher fresh weight compared with plants grown under cool white fluorescent lamps; yet no differences were found in essential oil content among lighting systems tested.

Nitrate and ammonium forms of N are commonly used to fertilize plants. Different nitrate-N:ammonium-N treatments were tested with basil plants, which included 0:100, 25:75, 50:50, and 75:25 and a non-fertilized control to compare plant growth and essential oil content. Basil plants grown with a nitrate-N:ammonium-N of

75:25 tended to have the highest fresh weights compared to other fertility treatments, whereas the 50:50 treatment tended to have the highest shoot indices. The 75:25 nitrate-N:ammonium-N treatment tended to have greater linalool, eucalyptol, and eugenol compared to other ratio treatments. Based on our results, basil appears to have greater fresh weight and essential oil production when grown with a 75:25 ratio of nitrate-N:ammonium-N.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

One of the more popular herbs, basil (*Ocimum basilicum* L.) is enjoyed by many cultures worldwide for cooking, as a source of essential oils, and as a fragrant ornamental (Simon et al, 1999). Basil can be started by seed or transplants for growing in the garden or can be purchased fresh or dried for cooking. There are more than 40 cultivars with different flavors and growth habits available (Darrah, 1974; Darrah, 1980).

Basil aroma is derived from many essential oils, including linalool, methylchavicol, and 1,8-cineole (Simon et al., 1999). Basil cultivars can be chemically categorized based upon their essential oil profiles (Grayer et al., 2004; Liber et al., 2011; Vieira and Simon, 2006), with the greatest concentration typically found in leaves compared to stem or flower tissue (Chalchat and Özcan, 2008). Sweet basil, including 'Genovese' and 'Italian Large Leaf', has the greatest quality aroma (Simon et al., 1999).

In the United States, there is an increasing trend in consumers that are growing their own vegetables and herbs. General gardening activity has increased 17% from 36 to 42 million households from 2008 to 2013 (National Gardening Association, 2014). Herb gardening in particular increased from 14 to 20 million households from 2008 to 2013 (National Gardening Association, 2014). Sales of herb gardening related products increased from $391 to $522 million from 2008 to 2013 (National Gardening Association, 2014). In 2009, 323 grower operations were reported to produce herbs in greenhouses; however, total production and sales were not reported to the public to avoid disclosing data on individual operations (United States Department of Agriculture, 2010).

The North American population in 2012 was 355 million, with 85% residing in urban areas. The North American population is projected to reach 446 million by 2050 (United Nations, 2013). Nationally, the population spends 87% of the day indoors and six percent of the time in an enclosed vehicle (Klepsis, et al 2001). While this shift in living environment poses challenges for citizens, nine million urban citizens in the United States still participated in gardening in 2013 (National Gardening Association, 2014).

Plants that are grown in an indoor environment have unique irradiance challenges compared to plants that are grown in an outdoor field or garden setting. Depending on the indoor environment, irradiance may be impacted by a variety of obstacles. Within homes, irradiance quality and quantity varies depending on the direction windows face, the type and cleanliness of the glass, and obstruction from objects, such as trees or buildings. In greenhouses, crop performance is often impacted by the amount of irradiance that is present. Research has been conducted to understand how the type and composition of greenhouse glazing materials impact irradiance (Kittas et al., 1999; Nijskens et al., 1985; Papadakis et al., 2000). The cleanliness of greenhouse glass is important, and dirty surfaces reduce irradiance (Jones, 1966). When irradiance is limited in greenhouses, supplemental lighting such as metal halide, high pressure sodium, or light emitting diodes (LED's) may be used to achieve the desired quantity and quality of irradiance (Hao and Papadopoulos, 1999; Heo et al., 2006; Pinho et al., 2013).

Additionally, artificial irradiance can be used for indoor home gardening needs. Fluorescent lamps are commonly suggested for use with indoor consumer horticulture as they are readily available and affordable. There are a variety of lamps available, such as tubular fluorescents (T5, T8, or T12 sizes) or smaller compact fluorescents. Different

spectral qualities are available, such as cool (4100 K), warm (3000 K), or full spectrum/daylight lamps (6400 K). There are also fluorescent options available that are marketed specifically as plant 'grow' lights. University extension publications typically suggest using a cool white fluorescent lamp, or a mixture of a cool and a warm fluorescent lamp/ incandescent lamp for indoor consumer horticulture (Anderson, 2004; Lerner, 2001; Rothenberger, 2012; Trinklein, 2002; Vandre, 2011).

In addition to adequate irradiance, a fertilization program is important for growth and overall plant health. Nitrogen (N) is critical for protein manufacture and growth and development of plants (Bar-Tal et al., 2001a, 2001b; Bloom, 1997; Dorais et al., 2001). In particular, more N is consumed by plants than all other essential elements combined (Epstein and Bloom, 2005). Excessive or scarce conditions of N can have adverse effects on plant health. Rates of N greater than optimal can cause toxicity in plants (Glass et al., 1997; Pilbeam and Kirkby, 1992; Ullrich, 1992; Zhu et al., 2000). Plant deformities may be caused by nutrient deficiencies, low pH, or changes in plant metabolism (Ganmore-Neumann and Kafkafi, 1983; Gerendás et al., 1997; Kandlbinder et al., 1997; Wiesler, 1997). Nitrogen is available in different forms for plant uptake, including nitrate-N and ammonium-N. Crops can respond differently in their growth and development when fertilized with either form, or a combination thereof, such as tomatoes (Gill and Reisenauer, 1993; Heeb et al., 2005), lettuce (Boroujerdnia and Ansari, 2007), cucumber (Heuer, 1991), and peppers (Marti and Mills, 1991). Adding low levels of ammonium-N to a nitrate-N based system has shown benefits to plant growth (Gill and Reisenauer, 1993; Pilbeam and Kirkby, 1992).

The purpose of this research was to explore how limited irradiance, artificial fluorescent irradiance, and nitrate-N:ammonium-N fertility impact the growth and essential oil production of basil. With the increasing trend in herb gardening, more commercial growers will be cultivating basil to meet the market demand and will have varying light and fertility programs. Additionally, as more consumers are growing herbs, including challenging urban locations, it is important to give proper guidance to succeed with limited irradiance. Overall, basil is a valuable crop and it is important to understand how growing practices impact its performance.

Thesis Organization

This thesis contains five chapters related to basil research. The first chapter gives introductory information pertinent to the foundation of this research and why it was conducted. The second chapter is a manuscript on how an increased shade percentage decreases 'Genovese' basil growth and impacts essential oil production. The third chapter is a manuscript on the characterization of indoor lighting options for growth of 'Genovese' basil. The fourth chapter is a manuscript on the influence of nitrate-N:ammonium-N on 'Genovese' basil growth and essential oil content. The fifth chapter is a conclusion of the research conducted as a whole, its implications, and discussion, followed by acknowledgements.

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CHAPTER 2. INCREASED SHADE PERCENTAGE DECREASES 'GENOVESE' BASIL GROWTH AND IMPACTS ESSENTIAL OIL CONTENT

A paper to be submitted to HortScience

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Abstract. A trend in the herb gardening market is increasing the demand for herb plants for home gardening use. One of the more popular herbs available, basil (*Ocimum basilicum* L.) is a culinary herb with a variety of uses and cultivars available. The objective of this study was to quantify the relationship between limited irradiance and the growth and essential oil production of 'Genovese' basil. Basil plants were grown in a greenhouse under shade structures that provided 0%, 30%, 50%, or 70% shade. Basil fresh weight and shoot indices increased as percent shade decreased. Essential oil peak areas were predicted to be the greatest for eucalyptol at 60% shade, linalool at 30% shade, and eugenol at 40% shade.

Additional index words. Ocimum basilicum L., limited irradiance, gas chromatography mass spectrometry

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Plants grown in an indoor environment have unique irradiance challenges compared to plants that are grown in a field or garden setting. Depending on the indoor environment, irradiance may be impacted by a variety of obstacles. Within homes, irradiance quality and quantity varies depending on the direction windows face, the type and cleanliness of the glass, and obstruction from objects, such as trees or buildings. In greenhouses, crop performance is impacted by the amount of irradiance. Research has been conducted to understand how the type and composition of glazing materials impact irradiance (Kittas et al.,1999; Nijskens et al., 1985; Papadakis et al., 2000). The cleanliness of greenhouse glass is important and dirty surfaces reduce the amount of irradiance (Jones, 1966). When irradiance is limited, supplemental lighting may be used to achieve the desired quantity and quality of irradiance (Hao and Papadopoulos, 1999; Heo et al., 2006; Pinho et al., 2013). When excess irradiance is present, typically in summer months, structures such as solar screens and shade cloth can reduce heat and/or shade plants (Miguel et al., 1994; Nijskens et al., 1985; Willits, 2001). Additionally, the geographical location of a home or greenhouse can have a large impact in the amount of irradiance present, as some regions have greater averages of cloud cover compared to others. The time of the year can also influence the percentage of cloud cover (Warren et al., 1986).

Recently in the United States, more people are growing their own herbs. Participation in herb gardening increased from 14 million households in 2008 to 20 million households in 2013 (National Gardening Association, 2014). Product sales of herb related items increased from $391 million in 2008 to $522 million in 2013 (National

Gardening Association, 2014). In 2009, 323 greenhouse operations produced herbs, but no sales data were reported (United States Department of Agriculture, 2010).

The North American population in 2012 was 355 million, with 85% residing in urban areas. The total population is projected to reach 446 million by 2050 (United Nations, 2013). Nationally, the population spends 87% of the day indoors and 6% of the time in an enclosed vehicle (Klepsis, et al. 2001). While this shift in living environment poses challenges for citizens, nine million urban citizens in the United States still participated in gardening in 2013 (National Gardening Association, 2014). One of the primary challenges for urban gardeners includes limited outdoor space or adequate natural light to grow plants within their home.

With an increasing demand for herbs in the market, more growers will be cultivating herbs to meet market demand. It is important for the successful production of these herbs for both gardening and culinary uses. The objective of this research was to determine the effect of limited irradiance on the growth and essential oil production of ‘Genovese’ basil. Our goal was to help growers understand the impact of limited irradiance on the production and flavor of basil plants, as well as provide expectations when growing basil in other areas that may have limited irradiance, such as a residential setting.

Materials and Methods

Basil plants were grown under 0%, 30%, 50%, or 70% shade for the duration of the experiment. The treatments were achieved by constructing box frames covered with 30%, 50%, or 70% polyethylene shade cloth (FarmTek, Dyersville, IA). Box frames were made with 5 cm × 10 cm dimensioned lumber and fastened together with nails and

glue. The box frames were 76 cm × 92 cm × 61 cm. Box frames were coated with Thompson's WaterSeal (The Thompson Company, Cleveland, OH) to protect wood from moisture to prevent warping. Shade cloth of the varying indices was attached with staples to cover the top and sides of the box frames. The 0% treatment used a box frame with no shade cloth to remove bias.

The experimental design included three replicate shade structures per treatment and six subsample basil plants in a randomized complete block design. This experiment was performed three times consecutively during February to August 2012, and the results herein are the combined data.

Photosynthetic Photon Flux (PPF) was measured using a SQ-110 quantum sensor (Apogee Instruments Inc., Logan, UT) connected to a universal single-ended voltage amplifier (EME Systems, Berkeley, CA) and logged using a HOBO data logger (Onset Computer Corporation, Bourne, MA). Voltage was converted to PPF for data analysis by using an Apogee voltage conversion formula. Data were recorded every 30 min during daylight hours of 6:30 AM to 8:30 PM for two weeks from 16 May to 31 May 2012 during the second trial.

'Genovese' basil seeds (Mountain Valley Seed Co., Salt Lake City, UT) were planted in 15 cm diameter plastic pots filled with a commercial substrate (Fafard 3B, Fafard, Agawam, MA). Three seeds were planted per pot to ensure germination and were thinned to the single largest seedling two weeks after germination. Pots were watered as needed based on soil dryness. Beginning two weeks after seedlings emerged, plants were fertilized weekly at 200 $mg \cdot L^{-1}$ N utilizing a 24N–6.62P–4.8K fertilizer. Plants were grown in a greenhouse under natural irradiance and supplemented with metal halide

lights for a 14 h day length. Day and night temperatures ranged from 21 to 27 °C and 18 to 24 °C, respectively.

Shoot indices, leaf pairs, and fresh weights were obtained at 10 weeks after sowing. Shoot indices were calculated by taking the average of two perpendicular plant canopy widths and one height. Fresh weights were obtained by harvesting the plant at the soil surface and weighing. Once weighed, all leaves were removed from the plant and placed into brown paper bags while the stems and petioles were discarded. The bags were placed into a SPX Blue M Electric drying oven (TPS, New Columbia, PA) set at 100 °C and were removed once leaves became brittle, in approximately 5 hours. The leaves were allowed to cool to 20 °C then transferred to a plastic bag, sealed, and stored in darkness at 20 °C until sampling for volatile compounds.

Basil subsamples were pooled into a single replicate and ground with a coffee grinder (KRUPS, Millville, NJ) for 20 s. One g of ground basil from each treatment replicate was placed into separate 60 mL vials with15 mL of methanol, sealed, and then placed into an Ultrasonik 104X sonicator (Ney Dental International, Yacaipa, CA) for 30 min to facilitate liquid extraction of the basil tissue. Vials were then stored in darkness at 20 °C for 24 h, and then mixed using a Thermolyne Maxi Mix vortex mixer (Sybron International, Milwaukee, WI) for 10 s to create a homogenous mixture. A 5 mL sample was withdrawn and passed through a 0.45 µm nylon syringe filter into a 12 × 32 mm vial. The vial was sealed with a crimp top and then loaded into the autosampler of a gas chromatograph mass spectrometer (GC/MS).

An autosampler-equipped Agilent 7890A GC system with a 5975C inert XL EI/CI MSD with Triple-Axis Detector fitted with a DB-5 fused silica capillary column

(30 m × 0.530 mm with one µm film) (Agilent Technologies, Santa Clara, CA) and ChemStation integrator was used to analyze samples in a full scan mode. Hydrogen was the carrier gas and the oven temperature was held at 20 °C for two min and then programmed to increase at a rate of 10 °C·min^{-1} to 200 °C, with a final hold at 200 °C for five min.

Ten blank methanol samples were placed into the autosampler, followed by analytical grade essential oil standards [including linalool, eugenol, eucalyptol, estragole, and methyl-cinnamate (Sigma-Aldrich, St. Louis, MO)], five methanol blanks, and then the treatment samples separated by two methanol blanks between treatments. Essential oil peak areas were determined using integrated data in Agilent ChemStation software. The peak area retention times of the essential oil standards were used to identify essential oil peak areas in basil sample GC/MS analysis.

Statistical analysis was conducted using JMP software (SAS Institute Inc., 2013). Peak areas from GC/MS basil sample analyses were used to create regression models to predict the response of basil plants to increasing shade percentages.

Results

Basil fresh weight and shoot indices tended to be highest under 0% shade and decreased as shade increased (Fig. 1). Basil leaf pairs tended to be greatest at 30% shade, both in our trial data and model (Fig. 1).

Our essential oil data resulted in 70%, 50%, and 30% tending to have the greatest eucalyptol, eugenol, and linalool peak areas, respectively (Fig. 2). Our model predicted the highest peak areas for eucalyptol at 60% shade, eugenol at 40% shade, and linalool at

30% shade (Fig. 2). Methyl-cinnamate and estragole were also analyzed via GC/MS but were not detected in our samples (data not shown).

Discussion

Basil plants grown under 0% shade were larger compared to plants grown under other shade treatments. This result was expected and similar to a study with 'Genovese' basil, where dry mass was greatest with natural irradiance and decreased as irradiance decreased with the use of shade cloth (Chang, 2008). Observationally, leaves of plants in the 50% or 70% shade tended to be thinner and less rigid in texture compared to leaves in 0% or 30% shade, which suggests that the leaves are anatomically different when grown under higher light conditions versus low light conditions. Plant response to red:far-red light and shade avoidance is a phenomenon that results in plant morphology changes, such as leaf expansion and stem elongation (Bae, 2001).

Similar results have been found in horticultural crops grown for leaf production and other herbs. A study with lettuce (*Lactuca sativa* L.) plants determined that greater biomass could be achieved with high irradiance (1000-1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) compared to medium (700-900 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and low (200-300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) irradiance levels (Zhou et al., 2009). Zhou altered natural sunlight by either filtering it with shade cloth or supplementing it with high pressure sodium lamps (HPS) to achieve desired irradiance levels. A study by Shinohara and Suzuki (1981) found similar results with lettuce in that fresh weight and leaf numbers decreased as irradiance decreased. In another lettuce study, supplemental lighting was found to increase fresh weight and number of leaves compared to natural light alone (Fukuda et al., 2004). A study with spinach (*Spinacia oleracea* L.) observed decreased growth and leaf area per plant when grown under 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$

compared to 800 µmol·m^{-2}·s^{-1} (Proietti et al., 2004). The shoot dry weight of medicinal herb horehound (*Marrubium vulgare* L.) increased linearly as irradiance increased 50 or 100 µmol·m^{-2}·s^{-1} from HPS supplemental lighting (Bergeron et al., 1995). A study of thyme (*Thymus vulgaris* L.) and HPS supplemental lighting of 200 µmol·m^{-2}·s^{-1} found a doubled increase in dry matter production (Letchamo et al., 1995).

Eucalyptol essential oil had a linear relationship to percent shade, in that the peak areas of eucalyptol tended to increase as percent shade increased. Eugenol and linalool had a quadratic relationship to percent shade, where peak areas were predicted to be higher at 40% and 30% shade, respectively. These data contrasted from results with a similar study of 'Genovese' basil, where linalool and eugenol peak areas decreased as irradiance decreased (Chang, 2008); however, in that study plants were under limited irradiance treatments for short durations, whereas our plants were under their respective treatments for the entire duration of the 10 week experiment. Plants grown under shade treatments for a portion of the lifecycle may produce different results compared to the entire lifecycle, due to the plants acclimation of the new shaded environment.

Research conducted with lettuce grown under low (200-300 µmol·m^{-2}·s^{-1}), medium (700-900 µmol·m^{-2}·s^{-1}), or high (1000-1200 µmol·m^{-2}·s^{-1}) light conditions found that lettuce grown in high light conditions had higher quantities of antioxidants (Zhou et al., 2009). Antioxidants may be indicative of essential oil content, as basil essential oil has strong antioxidant activity (Wei and Shibamoto, 2010).

Other plant research has investigated the effect of irradiance on flavor constituents. A study with thyme found that high irradiance through use of supplemental lighting increased essential oil content (Letchamo et al., 1995). However, in horehound,

additional irradiance through supplemental lighting was not found to increase premarrubin, a volatile oil bitter principle (Bergeron et al., 1995). Volatiles and compounds responsible for flavor in fruits, such as strawberry (*Fragaria x ananassa* Duch.) and apple (*Malus pumila* Mill.), have been negatively impacted by limited irradiance (Miller et al., 1998; Watson et al., 2002).

Other bodies of research have also investigated nitrate concentrations in plants under limited irradiance. In a study done by Raimondi and others (2006), two basil cultivars were found to have elevated nitrate content when grown under 50% reduced irradiance compared to 0% reduced irradiance. Accumulation of nitrate in lettuce plant tissue was found to decrease as light intensity increased (Blom-Zandstra and Lampe, 1985). Spinach plants grown under 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had plant tissues that contained more oxalate and nitrate compared to plants grown under 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Proietti et al., 2004). High levels of nitrate in food crops are a potential threat to health due to the occurrence of methaemoglobinaemia in the blood of infants and gastric cancer (Walker, 1990). While our basil research suggested that limited irradiance may produce greater essential oil concentrations and consequently more flavor, we did not investigate nitrate in our basil plants. Further research should be conducted to understand if more flavorful basil could be grown under limited irradiance yet still contain safe nitrate levels for human consumption.

Our trials focused on percent shade treatments, but in retrospect it would have been more useful to conduct the experiment in terms of PPF rather than percent shade, as PPF is more applicable to compare among other growing environments. PPF was recorded during the second replication of this trial and shade treatments of 0%, 30%, 50%,

or 70% were found to have 1630, 885, 620, or 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Data were analyzed for this individual trial with PPF as the dependent variable, and fresh weight and shoot indices were found to increase with an increase in PPF as a quadratic relationship (Fig. 7 and Fig. 8). According to our model, maximum basil fresh weight would occur at between 1300 and 1400 PPF (Fig. 3) and maximum shoot indices between 1400 and 1500 PPF (Fig. 3).

If we were to conduct this experiment again, treatments would be based on different intensities of PPF as to create a more meaningful model to predict basil response to irradiance. This would allow the results to be more universal to predict the response of basil across a variety of environments based on the available PPF. Additionally, the shade cloth did not necessarily deliver the percent shading that was advertised. The 0% shade treatment had a PPF of 1630 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when measured. In theory, the amount of light delivered for the 30%, 50%, and 70% shade treatments should have been 1141, 815, and 489 $\mu mol \cdot m^{-2} \cdot s^{-1}$ compared to the measurements of 885, 620, or 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$ that we recorded, respectively. The actual percentages as calculated are closer to 46%, 62%, and 72% per the respective treatment counterparts of 30%, 50%, and 70%. A study by Willits (2001) found when testing various types of shade cloth that the shade ratings presented by the manufacturer appeared to be a representation of the photosynthetically active radiation (PAR) transmissivity versus solar transmissivity; however, this does not agree with our findings. Our readings may have been impacted by the box frame structures we used or could have been an inaccuracy by the manufacturer. We recommend for growers utilizing shade cloth to do an irradiance measurement of

their growing environment with and without the shade cloth of their choice to help ensure the proper intensity of desired irradiance is delivered.

Our research can be used to help illustrate to growers their expected results when growing basil with limited irradiance in terms of basil growth and essential oil production. We hope that the models in this research can be used to help predict the response of basil to increasing shade percentage. More research is needed to fully understand the impact that limited irradiance has on essential oil content in basil plants due to the varying essential oil results in available published studies.

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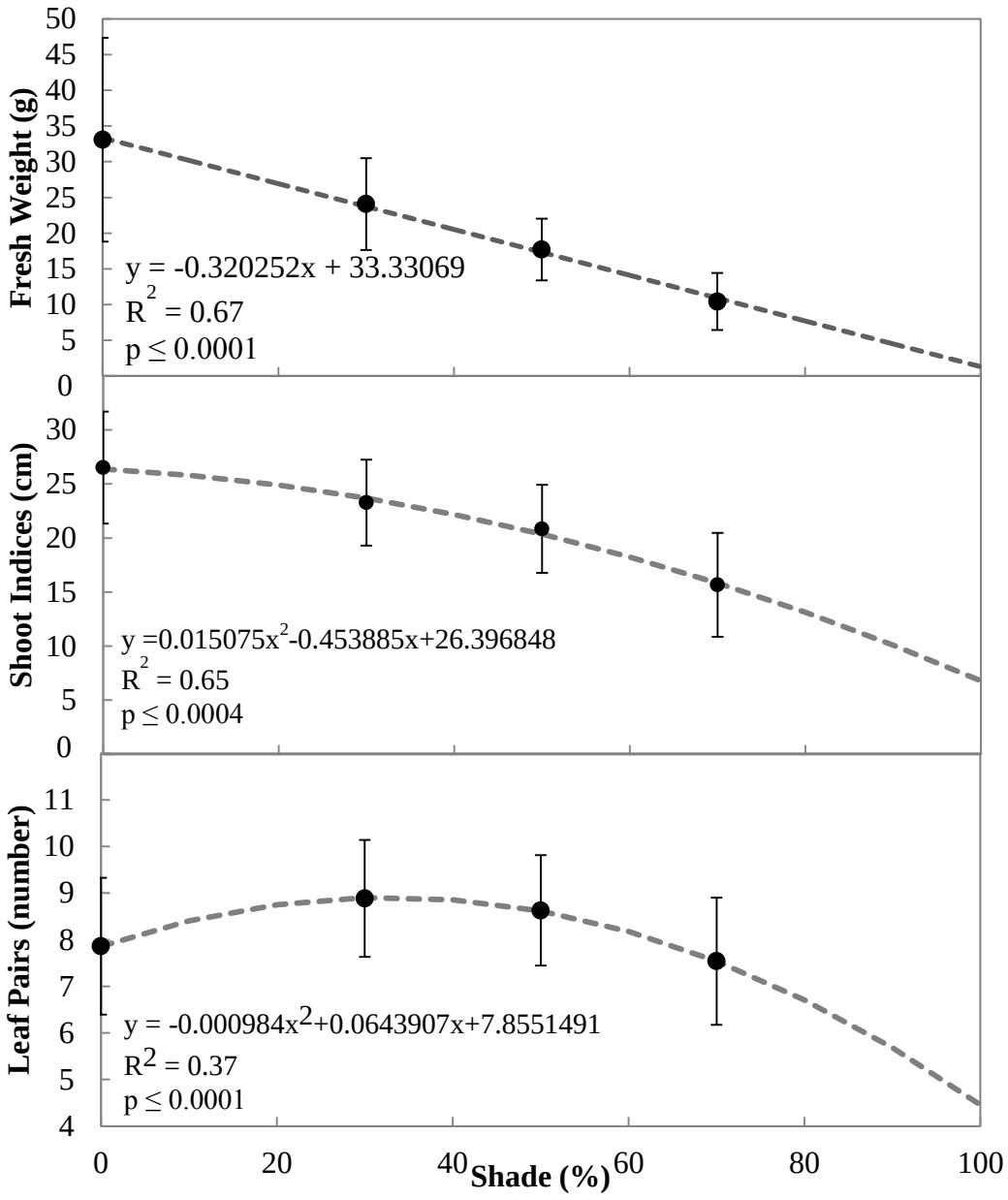

Fig. 1. Fresh weight, shoot indices, and leaf pairs of 'Genovese' basil plants grown under 0% 30%, 50%, or 70% shade. Basil plants were grown in a greenhouse under box frames covered with shade cloth at the appropriate shade indices. Data were collected at trial termination at 10 weeks after sowing. The trial contained three replicates and six subsamples per shade treatment. The trial was replicated three times and data from each trial were combined. Data are means ± standard deviation.

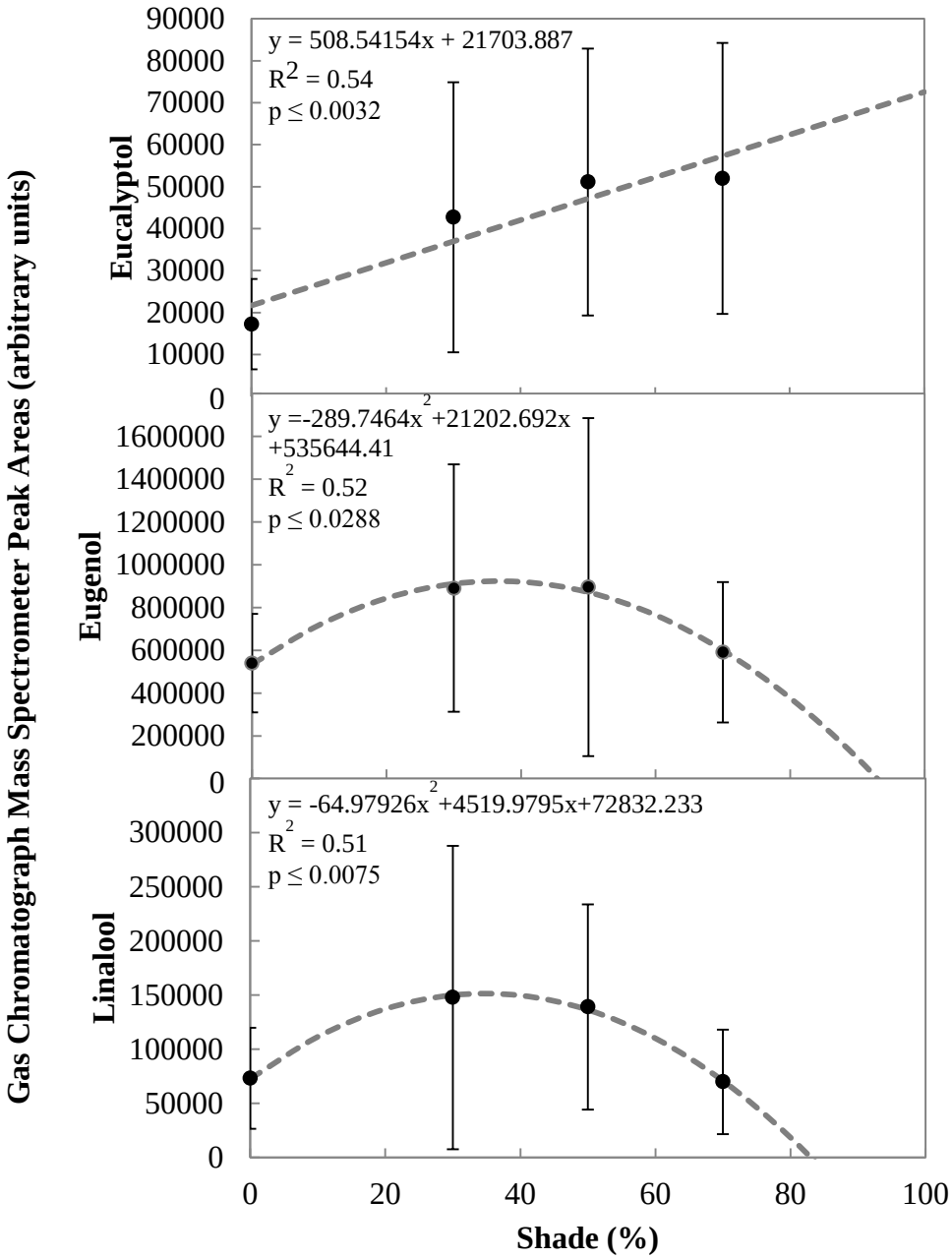

Fig. 2. Eucalyptol, eugenol, and linalool GC/MS peak areas of 'Genovese' basil plants grown under 0% 30%, 50%, or 70% shade. Basil plants were grown in a greenhouse under shade cloth at the appropriate shade indices. GC/MS peak area data were taken from dried basil tissue harvested 10 weeks after sowing. Trials contained three replicates and six subsamples per shade treatment and was repeated three times. Subsamples were pooled together for GC/MS analysis. Data are means ± standard deviation.

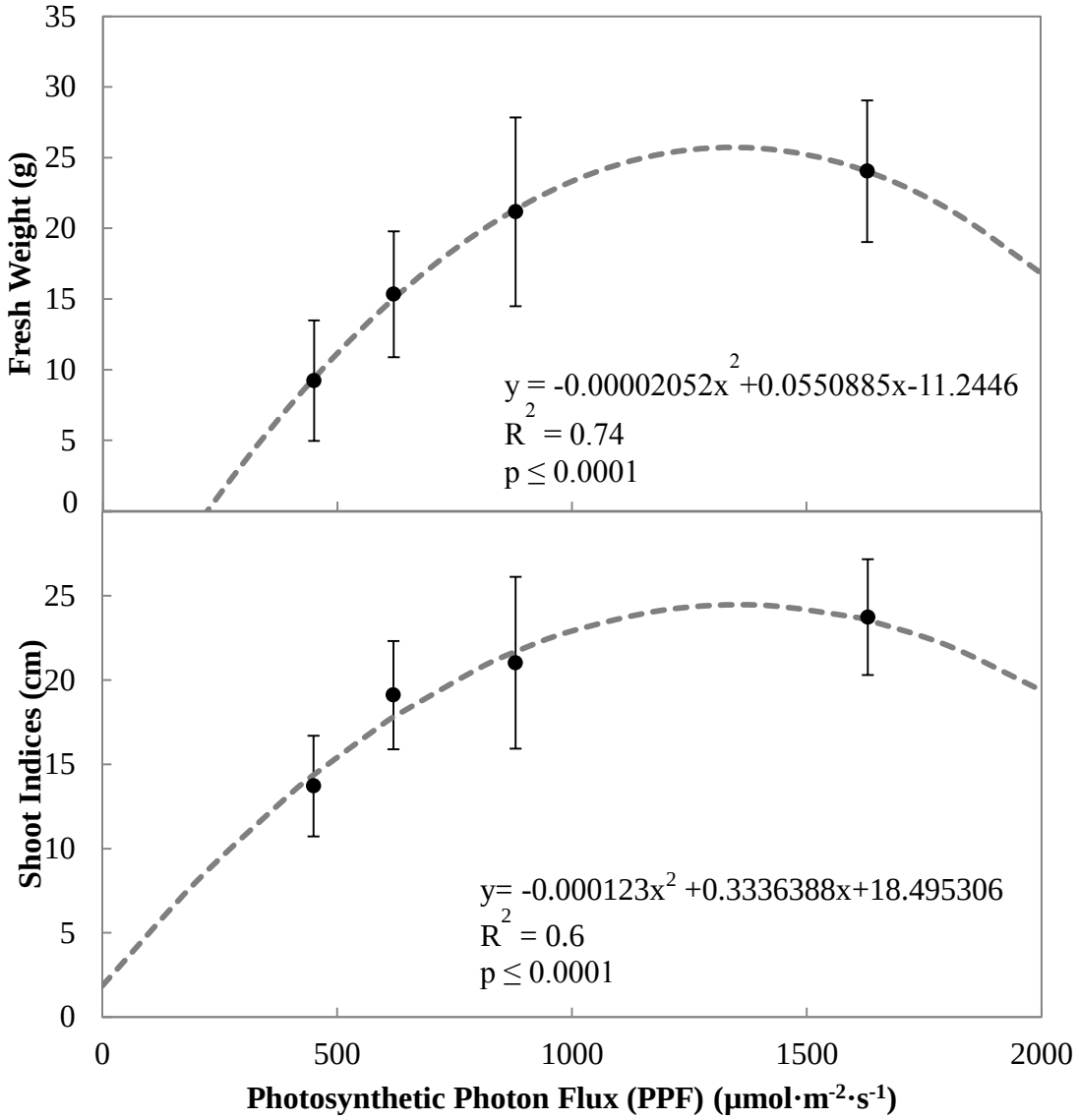

Fig. 3. Fresh weight and shoot indices of 'Genovese' basil plants grown under 1630, 885, 620, or 450 µmol·m^{-2}·s^{-1}. Basil plants were grown in a greenhouse under box frames covered with shade cloth at the respective PPF. The trial contained three replicates and six subsamples per PPF treatment and was the second trial of the series. PPF was recorded every 30 min during daylight hours of 6:30 AM to 8:30 PM for two weeks from 16 May to 31 May 2012. Data are means ± standard deviation.

CHAPTER 3: CHARACTERIZATION OF INDOOR FLUORESCENT LIGHTING OPTIONS FOR GROWTH OF 'GENOVESE' BASIL (*OCIMUM BASILICUM* L.)

A paper to be submitted to HortScience

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Abstract. Irradiance is usually suboptimal for growing plants indoors and fluorescent lighting systems are typically recommended for supplementation in a residential setting. In this study with basil plants (*Ocimum basilicum* L.), it was found that plants grown under fluorescent grow lamps had significantly greater shoot indices than cool fluorescent lamps. Fluorescent grow lamps and cool white plus incandescent lamps produced a greater number of leaf pairs compared to cool white fluorescent. Grow lamps produced greater fresh weight compared to plants grown under cool white fluorescent lamps. No differences were found in essential oil content among lighting systems tested.

Additional index words. Artificial light, fluorescent lamps, grow lights, urban gardening, essential oils

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Recently in the United States, there is an increasing trend in people that are growing their own vegetables and herbs. Gardening activity has increased 17% from 36 million households in 2008 to 42 million in 2013 (National Gardening Association, 2014). Herb gardening participation increased from 14 million households from 2008 to 20 million households in 2013. Sales of herb gardening products increased from $391 million in 2008 to $522 million in 2013 (National Gardening Association, 2014).

Basil (*Ocimum basilicum* L.) is a popular herb that is enjoyed by many cultures worldwide (Simon et al., 1999). It is used for cooking, as a source of essential oils, and as a fragrant ornamental plant. Culinary basil can be purchased fresh-cut or dried for cooking, or may be started by seed or transplant for gardening. It is a versatile herb with over 40 cultivars of different flavors and growth habits available (Darrah, 1974; Darrah, 1980).

Basil aroma is derived from many essential oils, including linalool, methylchavicol, and 1,8-cineole (Simon et al., 1999). Basil cultivars can be chemically categorized based upon their essential oil profiles (Grayer et al., 2004; Liber et al., 2011; Vieira and Simon, 2006), with the greatest concentrations typically found in the leaves of basil plants, compared to stem or flower tissue (Chalchat and Özcan, 2008). Sweet basil, including 'Genovese' and 'Italian Large Leaf', has the greatest quality aroma (Simon et al., 1999).

The act of gardening provides several benefits to the gardener. In a survey by the National Gardening Association (2014), the top reasons for gardening in the United States in 2013 included: better taste (58%), lower cost (54%), better quality (51%), and increased food safety (48%). Gardening has also shown to be personally rewarding. In a

New Zealand study, top reasons for gardening included: Connecting with nature, the ability to nurture plant growth, to challenge oneself, and enjoying the aesthetics in a relaxing (less stressful) environment (Kidd and Brascamp, 2004).

Growing plants indoors can also promote a healthier living environment. Indoor potted plants can help remove dust and other contaminants from the air, absorb harmful toxins such as formaldehyde, xylene, benzene, and ammonia, and ultimately provide cleaner air to breathe (Lohr and Pearson-Mims, 1996; Orwell et al., 2004; Orwell et al., 2006; Wolverton and Wolverton, 1993; Wood et al., 2006).

The North American population in 2012 was 355 million, with 85% residing in urban areas. The North American population is projected to reach 446 million by 2050 (United Nations, 2013), and globally reach 9.6 billion. Nationally, the population spends 87% percent of the day indoors and 6% of the time in an enclosed vehicle (Klepeis et al., 2001).

While this shift in living environment poses challenges for citizens, nine million urban citizens in the United States still participated in gardening in 2013 (National Gardening Association, 2014). One of the primary challenges for urban gardeners includes limited outdoor space or adequate natural light to grow plants within their home. When relying on natural sunlight indoors, irradiance quality and quantity varies depending on the direction windows face, the type and cleanliness of glass, and obstruction from objects, such as trees or buildings.

Artificial irradiance can serve as a supplement to natural light for indoor gardening. Fluorescent lamps are commonly suggested for use with indoor consumer horticulture as they are readily available and affordable. There are a variety of lamps

available, such as tubular fluorescents (T5, T8, or T12 sizes) or smaller compact fluorescents. Different spectral qualities are available, such as cool (4100 K), warm (3000 K), or full spectrum/daylight lamps (6400 K). There are also fluorescent options available that are marketed as plant 'grow' lights. University extension publications typically suggest using a cool white fluorescent lamp, or a mixture of a cool and a warm fluorescent lamp/ incandescent lamp for indoor consumer horticulture (Anderson, 2004; Lerner, 2001; Rothenberger, 2012; Trinklein, 2002; Vandre, 2011).

The objective of this study was to characterize common fluorescent lighting systems available to home gardeners and assess the growth and essential oil production of basil plants. It is our hope that this information can be used to give guidance to consumers when choosing lights to use for an indoor gardening environment.

Materials and Methods

Light treatments included three different light sources: fluorescent T5 lamps marketed as 'grow' lights (GL), cool white fluorescent T5 lamps (CW), and a combination of the T5 cool white fluorescent lamps plus an incandescent lamp (CW+I). The GL treatment utilized two SunBlaster™ 6400K T5 60.96 cm 24 W fluorescent lamps (Future Harvest Development, Kelowna, British Columbia, Canada). The CW treatment utilized two ELCO® 4100K T5 60.96 cm 14 W fluorescent lamps (ELCO Lighting, Vernon, CA). The CW+I treatment utilized two ELCO® T5 at 14 W and Philips incandescent lamp at 7.5 W (Koninklijke Philips Electronics N.V, Amsterdam, Netherlands).

SunLite® 58.42 cm × 29.21 cm reflectors (Gardeners Supply, Burlington, VT) were used with all treatments. The GL and CW treatments had two lamps installed under

the reflector spaced 13.97 cm apart from lamp centers. The CW+I treatment included two cool white fluorescent T5 lamps spaced 13.97 cm apart from lamp centers with the incandescent lamp placed in the center between the fluorescent lamps.

Photosynthetic Photon Flux (PPF) was measured using an Apogee Quantum Flux MQ-306 meter (Apogee Instruments Inc, Logan, UT). Three measurements were taken per treatment (left, center, and right edge of pots) and were taken 7.6 cm below the lights to represent the PPF that the plants were receiving at the canopy peak.

Pots were placed into individual light booths measuring 63.5 cm long × 43.18 cm wide × 63.5 cm tall with one light treatment per booth. Blackout cloth surrounded the sides of the booth to prevent light contamination from other sources, including other light treatments and any ambient light. Lights were suspended above each shelf with a starting height of 7.6 cm from the top of the pots. As plants developed, the 7.6 cm spacing was maintained above the canopy by adjusting lights on chains. The experimental design was a randomized complete block with three replications of the three light treatments and 10 subsample plants per light booth plot. Plants were grown at 20 °C under a 16 h photoperiod.

‘Genovese’ basil seeds (Mountain Valley Seed Co., Salt Lake City, UT) were planted in 15 cm plastic pots filled with a commercial substrate (Fafard 3B, Fafard, Agawam, MA) with three seeds per pot to ensure germination, and were thinned to the single largest plant two weeks after emergence. An 18N-7.86P-17.43K water soluble fertilizer (The Scotts Miracle-Gro Company, Marysville, OH) was added with N at 100 $mg \cdot L^{-1}$ three weeks after sowing. Pots were watered as needed based on soil dryness.

Shoot indices, leaf pairs, and fresh weights were obtained at 10 weeks after sowing. Shoot indices were calculated by taking the average of two perpendicular plant canopy widths and one height. Fresh weights were obtained by harvesting the plant at the soil surface and weighing. Once weighed, all leaves were removed from the plant and placed into brown paper bags while the stems and petioles were discarded. The bags were placed into a SPX Blue M Electric drying oven (TPS, New Columbia, PA) set at 100 °C and were removed once leaves became brittle, approximately 5 hours. The leaves were allowed to cool to 20 °C then transferred to a plastic bag, sealed, and stored in darkness at 20 °C until sampling for volatile compounds.

Basil subsamples were pooled into a single replicate and ground with a coffee grinder (KRUPS, Millville, NJ) for 20 s. One g of ground basil from each treatment replicate was placed into separate 60 mL vials with 15 mL of methanol, sealed, and then placed into an Ultrasonik 104X sonicator (Ney Dental International, Yacaipa, CA) for 30 min to facilitate liquid extraction of the basil tissue. Vials were then stored in darkness at 20 °C for 24 h, and then mixed using a Thermolyne Maxi Mix vortex mixer (Sybron International, Milwaukee, WI) for 10 s to create a homogenous mixture. A 5 mL sample was withdrawn and passed through a 0.45 µm nylon syringe filter into a 12 × 32 mm vial. The vial was sealed with a crimp top and then loaded into the autosampler of a gas chromatograph mass spectrometer (GC/MS).

An autosampler equipped 7890A GC system with a 5975C inert XL EI/CI MSD with Triple-Axis Detector fitted with a DB-5 fused silica capillary column (30 m × 0.530 mm with one µm film thickness) (Agilent© Technologies, Santa Clara, CA) and ChemStation integrator was used to analyze samples. Hydrogen was the carrier gas and

the oven temperature was held at 20 °C for two min and then was programmed to increase at 10 °C·min^{-1} to 200 °C, with a final hold at 200 °C for five min.

Ten blank methanol samples were placed into the autosampler, followed by analytical grade essential oil standards [including linalool, eugenol, eucalyptol, estragole, and methyl-cinnamate (Sigma-Aldrich, St. Louis, MO)], five methanol blanks, and then the treatment samples separated by two methanol blanks between treatments. Essential oil peak areas were determined using integrated data in Agilent ChemStation software. The peak area retention times of the essential oil standards were used to identify essential oil peak areas in basil sample GC/MS analysis. Peak areas from GC/MS basil sample analyses were used in statistical analysis to determine the effects of artificial fluorescent irradiance on basil essential oils.

This experiment was performed twice consecutively from March through June 2013 and the results herein are the combined data. Statistical analysis was conducted using JMP software (SAS Institute Inc., 2013). Analysis of variance and Tukey's HSD were used to determine means separation.

Results

The GL treatment had significantly higher PPF compared to CW+I and CW in both trials one and two individually, but was not significantly different when trial data were combined (Table 1). Basil plants grown under GL had significantly greater shoot indices than CW, but not CW+I (Table 2). Plants had a significantly greater number of leaf pairs when grown under GL and CW+I compared to CW (Table 2). Plants grown under GL treatment had significantly higher fresh weight compared to CW, but not CW+I (Table 2). Regardless of light treatment, no statistical differences in basil essential oils

were found among fluorescent lamp treatments (Table 3). Basil samples were also analyzed for methyl-cinnamate and estragole, but no significant peak areas were found during analysis (data not shown).

Discussion

Publications that discuss the testing of different types of fluorescent options are dated and predominantly from the 1960's. Helson (1964) determined that tomatoes grown under GL fluorescents were not different in height compared to CW fluorescents. Pallas (1964) tested CW fluorescents vs GL fluorescents and found no significant differences in bean plant weight, but did have significantly greater tomato plant weight with CW compared to GL. A study by Thomas and Dunn (1967) tested commercially available and experimental fluorescent lights and found that it was possible to create a GL fluorescent light that contains the desirable spectral attributes of both an I and CW fluorescent lamp for tomato growth. Our study characterizes some of the fluorescent options that are commercially available today, which have been improved compared to the fluorescent lamps that were used in the 1960's. T5 lamps, which were used in our study, were introduced into the United States market in 1995 and utilize different ballasts and phosphors compared to T8 or T12 fluorescents (Akashi, 2002). Sustainable lighting innovations impacting the ballast, phosphors, and energy usage have been recent improvements in the fluorescent lighting industry (Akashi, 2002; Hui et al., 2011). Therefore, it should be noted that the aforementioned horticultural studies are not representative of the current fluorescent lighting technologies. In more recent years, publications are comparing fluorescent lamps to light-emitting diodes (LED) or testing them in combination versus comparing various types of fluorescent lamps that are

available today (Astolfi et al., 2012; Chen et al., 2014; Heo et al., 2002; Heo et al., 2006; Huimin et al., 2013; Park et al., 2012; Shen et al., 2014). Comparison literature of currently available fluorescent options is limited and additional studies should be conducted to understand their performance.

In our study, the fluorescent GL treatment had the greatest PPF compared to other light treatments in each respective trial, which was 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for trial one and 176 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for trial two. We were concerned with the drop in PPF from trial to trial among GL, CW+I, and CW treatments, which was 75, 70, and 60 PPF, respectively. We recommend that further research be conducted to understand PPF loss over time among indoor fluorescent lighting systems.

Research by Beaman and others (2009), found that a PPF of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is optimum for greatest edible basil biomass production in a controlled-environment system utilizing CW+I lamps. Supplemental lighting studies in a greenhouse environment with other herbs and leafy crops have shown similar results in that greater irradiance promotes greater biomass production. Horehound (*Marrubium vulgare* L.) shoot dry weight increased linearly as irradiance increased 50 or 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from HPS supplemental lighting (Bergeron et al., 1995). Thyme (*Thymus vulgaris* L.) doubled in dry matter production when grown with supplemental HPS lighting of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Letchamo et al., 1995). Spinach (*Spinacia oleracea* L.) grown under 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had decreased growth and leaf area per plant when compared to 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Proietti et al., 2004). Lettuce plants were found to have greater biomass production at high light levels (1000 to 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) compared to medium (700 to 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and low (200 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) irradiance levels (Zhou et al., 2009). Zhou achieved light

levels by altering natural sunlight, either by filtering it with shade cloth for low irradiance or supplementing it with high pressure sodium lamps for high irradiance. Similar results in lettuce were found in a study by Shinohara and Suzuki (1981), where 'Butterhead' lettuce fresh weight and leaf numbers decreased as irradiance decreased.

The light treatments tested in this study did not show significant differences in essential oil content. Research with lettuce grown under low (200 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$), medium (700 to 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and high (1000 to 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) light conditions found that lettuce grown in high light conditions had higher levels of antioxidants (Zhou et al., 2009), which could be indicative of essential oil content, as essential oils are known to have strong antioxidant properties (Wei and Shibamoto, 2010). Another study found that on a fresh weight basis, β-carotene concentration increased with high light intensity in both spinach and lettuce (Oyama et al., 1999), and is known for antioxidant properties (Everett et al., 1996). Research with thyme has shown that utilizing supplemental lighting to increase irradiance resulted in increasing essential oil content (Letchamo et al., 1995). These studies suggest that with higher irradiance, differences may be seen in essential oil content of basil plants.

Lower irradiance has been correlated with higher nitrate levels in leaf tissue. Nitrate in high concentrations in food crops are a potential threat to health due to the occurrence of gastric cancer and methaemoglobinaemia in the blood of infants (Walker, 1990). A study with two basil cultivars were found to have elevated nitrate content when grown under 50% reduced irradiance compared to 0% reduced irradiance (Raimondi et al., 2006). A study with lettuce plants found that leaf tissue nitrate content decreased as light intensity increased (Blom-Zandstra and Lampe, 1985). Spinach plants had leaf

tissues that contained more oxalate and nitrate when grown under 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ compared to 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Proietti et al., 2004). In our study we did not measure the nitrate content present. We recommend that further indoor lighting research be conducted to understand if nitrate content is increased when basil plants are grown under fluorescent lamps.

We would also like to see more practical information on the labels of lamps marketed as plant grow lights to make it easier to choose the best light for a given plant species. Current labels include the amount of lumens, Kelvins, and wattage. While these metrics are helpful for some applications, they are not indicative of potential plant performance. Including PPF on the packaging materials would make it easier to determine which light system would work best for specific plant needs. Additionally, the size of lamps we used for our research was recommended to illuminate a 1020 standard sized plant tray. Our basil plants were placed underneath the lamps in the same footprint, and we observed an edge effect in which the plants on the ends of the footprint were smaller than plants located towards the center. This issue could potentially be alleviated by consistent airflow in the growing environment, a different style of reflector, or by rotating plants underneath the lamps for more even results. We recommend further research to understand what the optimum growing footprint would be with standard hardware.

Our findings suggest that for optimum basil biomass and essential oil content, a greater number of fluorescent lamps may be needed than is currently being recommended in university extension bulletins for irradiance. While the current recommendations could grow healthy basil plants, additional irradiance may be needed to improve and

maximize overall yield and flavor. Future testing could include greater numbers of lamps to test versus the current recommendations to determine if these differences would be significant and worth the additional cost to gardeners.

As more of the population becomes concentrated in urban settings, it will be important to overcome gardening barriers, such as lack of natural sunlight. Growing plants has many benefits and should be available to people in indoor environments despite these barriers. This study was an exercise to determine the growth and essential oil production of basil plants grown under fluorescent lighting systems suggested by typical university extension recommendations today. Overall, our study suggests that home gardeners should use fluorescent grow lamps or cool white fluorescent lamps in combination with incandescent lamps for indoor cultivation of basil plants. They provided irradiance for healthy growth and development of basil, as well as facilitated greater biomass compared to cool white fluorescent lamps alone.

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Table 1. Mean Photosynthetic Photon Flux (PPF) of fluorescent lamp treatments, including cool white fluorescent, cool white fluorescent plus incandescent, and fluorescent grow light. Three PPF measurements were taken per treatment (left, center, and right edge of pots) with a quantum sensor held 7.6 cm below the lamp at plant level.

	PPF ($\mu mol \cdot m^{-2} \cdot s^{-1}$)		
Fluorescent lamp treatment[z]	Trial 1	Trial 2	Combined Trials
Cool white fluorescent	187.9 b[y]	131.6 b	159.7 a
Cool white fluorescent plus incandescent	199.4 b	128.8 b	164.1 a
Fluorescent grow light	250.3 a	176.0 a	213.7 a

[z]n = Nine quantum measurements per treatment (three replicates, three subsamples).

[y] Means within columns followed by the same letter are not different at $P \leq 0.05$ according to Tukey's HSD test.

Table 2. Shoot indices, number of leaf pairs, and fresh weight of 'Genovese' basil plants grown under artificial light treatments, including cool white fluorescent, cool white fluorescent plus incandescent, and fluorescent grow light. Data were collected 10 weeks after sowing. The trial was replicated twice and the results herein are the combined data.

Fluorescent lamp treatment[z]	Shoot indices[y] (cm)	Number of leaf pairs	Fresh weight (g)
Cool white fluorescent	14.8 b[x]	6.5 b	11.0 b
Cool white fluorescent plus incandescent	15.9 ab	7.3 a	12.6 ab
Fluorescent grow light	16.5 a	7.1 a	14.7 a

[z]n = 60 plants per treatment (three replicates, 10 subsamples, two trials).

[y] Shoot indices determined by taking three plant measurements (two widths and one height) and averaging the values.

[x] Means within columns followed by the same letter are not different at $P \leq 0.05$ according to Tukey's HSD test.

Table 3. Eucalyptol, linalool, and eugenol essential oil gas chromatograph mass spectrometer (GC/MS) peak areas of 'Genovese' basil grown under artificial lighting treatments, including cool white fluorescent, cool white fluorescent plus incandescent, and fluorescent grow light. Basil leaf tissues were harvested and dried at 10 weeks after sowing. The trial was replicated twice and the results herein are the combined data. Basil subsamples were pooled into one replicate sample for GC/MS analysis.

Fluorescent lamp treatment[z]	Eucalyptol	Linalool	Eugenol
	(Arbitrary Units)		
Cool white fluorescent	1677625 a[y]	2480396 a	16203253 a
Cool white fluorescent plus incandescent	2709811 a	3660124 a	19814589 a
Fluorescent grow light	2569002 a	3784092 a	16386539 a

[z]n = 60 plants per treatment (three replicates, 10 subsamples, two trials).

[y] Means within columns followed by the same letter are not different at $P \leq 0.05$ according to Tukey's HSD test.

CHAPTER 4: INFLUENCE OF NITRATE-N:AMMONIUM-N ON 'GENOVESE' BASIL GROWTH AND ESSENTIAL OIL CONTENT

A paper to be submitted to HortScience

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Abstract. Nitrate and ammonium forms of nitrogen (N) are commonly used to fertilize plants. Many species, including basil (*Ocimum basilicum* L.), perform better when fertilized with a mixed ratio of nitrate and ammonium nitrogen sources. Different nitrate-N:ammonium-N fertilizer treatments were tested with 'Genovese' basil plants, which included 0:100, 25:75, 50:50, 75:25 or a non-fertilized control to compare plant growth and essential oil content. Basil plants grown with a nitrate-N:ammonium-N of 75:25 tended to have the greatest fresh weights compared to other fertility treatments, whereas the 50:50 treatment tended to have the greatest shoot indices. The 75:25 nitrate-N:ammonium-N had greater concentrations of linalool, eucalyptol, and eugenol compared to other ratio treatments. Based on our results, basil appears to have greater fresh weight and essential oil when grown with a 75:25 ratio of nitrate-N:ammonium-N.

Additional index words. Linalool, eugenol, eucalyptol, fertilizer, herb, flavor

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An appropriate fertilization program is important for growth and overall plant health. Nitrogen (N) is critical for protein manufacture and growth and development of plants (Bar-Tal et al., 2001a, 2001b; Bloom, 1997; Dorais et al., 2001). In particular, more N is consumed by plants than all other essential elements combined (Epstein and Bloom, 2005). Excessive or scarce conditions of N can have adverse effects on plant health. Rates of N greater than optimal can cause toxicity in plants (Glass et al., 1997; Pilbeam and Kirkby, 1992; Ullrich, 1992; Zhu et al., 2000). Plant deformities may be caused by nutrient deficiencies, low pH, or changes in plant metabolism (Ganmore-Neumann and Kafkafi, 1983; Gerendás et al., 1997; Kandlbinder et al., 1997; Wiesler, 1997).

Nitrogen is available in different forms for plant uptake, including nitrate-N and ammonium-N. Crops can respond differently in their growth and development when fertilized with either form, or a combination thereof, such as tomatoes (Gill and Reisenauer, 1993; Heeb et al., 2005), lettuce (Boroujerdnia and Ansari, 2007), cucumber (Heuer, 1991), and peppers (Marti and Mills, 1991). Adding low levels of ammonium-N to a nitrate-N based system has shown benefits to plant growth (Gill and Reisenauer, 1993; Pilbeam and Kirkby, 1992).

In the United States, there is an increasing trend in consumers that are growing their own vegetables and herbs. The number of households that have participated in gardening has increased 17% from 2008 to 2013 (National Gardening Association, 2014). A total of 42 million people participated in gardening in 2013, with 20 million of those households participating in herb gardening and spending $522 million on herb gardening products (National Gardening Association, 2014). In a 2009 United States census, there

were 323 greenhouse operations that produced herbs; however, to help keep sensitive data confidential, sales data were not reported (United States Department of Agriculture, 2010).

Basil (*Ocimum basilicum* L.) is an herb that can be used in cooking, as a source of essential oils, or as a fragrant ornamental and is enjoyed in many cultures worldwide (Simon et al., 1999). Over 40 basil cultivars are available, offering a variety of flavors and qualities (Darrah, 1974; Darrah, 1980). The distinctive aromas of basil are a result of essential oils present in plant tissues, such as 1,8-cineole, linalool, and methylchavicol (Simon et al., 1999). The leaves of basil plants contain the greatest concentrations of essential oils compared to stems or flowers (Chalchat and Özcan, 2008). Sweet basil cultivars are considered to have the greatest quality aroma and include 'Italian Large Leaf' and 'Genovese' (Simon et al., 1999). Typically, university extension bulletins recommend fertilizing basil plants sparingly and warn that over-fertilization will result in less flavorful basil (Davis, 2014; Williamson, 2014; Youger-Comaty, 1994).

With an increasing trend in herb gardening, it is important to understand proper fertilization techniques for basil plants. As a result of the trend, there will be an increase in growers producing basil for the market as well as gardeners cultivating the herb at home. The objectives of this study were to determine the influence of nitrate-N:ammonium-N fertilizer for 'Genovese' basil production and understand the impact of N form on essential oil content. A secondary objective was to determine if basil plants that did not receive fertilizer would have greater levels of essential oils compared to basil plants that were fertilized.

Materials and Methods

Four nitrate-N: ammonium-N fertilizers were tested in this study, including 0:100, 25:75, 50:50, and 75:25 at 200 $mg \cdot L^{-1}$ N formulas, respectively. A non-fertilized negative control was also included to compare against fertilized treatments. Molar solutions of individual fertilizer formulae were prepared in 1L batches and were diluted to the correct ratio and concentration (Table 1). Formula nutrients were balanced as best as possible with the given fertilizer components to achieve desired N (Table 2).

This study was performed three times consecutively from June through December 2012, and data from all three trials were combined for statistical analysis for basil plant shoot indices and fresh weight. The essential oil data from the 2nd and 3rd replications of the trial were combined for statistical analysis. The first two trials utilized 6 replications and the third trial utilized 10 replications.

'Genovese' basil seeds (Mountain Valley Seed Co., Salt Lake City, UT) were planted in 15 cm plastic pots filled with a commercial substrate (Fafard 3B) (Fafard, Agawam, MA). Three seeds were planted per pot to ensure the germination of a seedling and were thinned to the single largest seedling two weeks after germination. Plants were grown in a greenhouse under natural irradiance and supplemented with metal halide lights for a 14 h day length. Day and night temperatures ranged from 21 to 27 °C and 18 to 24 °C, respectively.

Fertilizer application began once the leaves of basil plants were above the growing media surface, about two to three weeks after seedling emergence, and fertilizer test solutions were applied twice weekly to plants in 250 mL doses and poured evenly over the soil surface. Care was taken to avoid wetting the foliage of the leaves with the

fertilizer solution. Leachate samples were collected using the NC State PourThru method and were conducted on the initial substrate and then weekly after fertilization began (Cavins et al, 2000).

Shoot indices and fresh weights were obtained at eight weeks after sowing. Shoot indices were calculated by taking the average of two perpendicular plant canopy widths and one height measured from the soil surface. Fresh weights were obtained by harvesting the plant at the soil surface and weighing. Once weighed, all leaves were removed from the plant and placed into brown paper bags while the stems and petioles were discarded. The bags were placed into a SPX Blue M Electric drying oven (TPS, New Columbia, PA) set at 100 °C and were removed once leaves became brittle, approximately 5 hours. The leaves were allowed to cool and then stored at 20 °C in plastic bags in darkness until sampling for volatile compounds.

Basil samples were ground with a coffee grinder (KRUPS, Millville, NJ) for 20 s. One g of ground basil from each treatment replicate was placed into separate 60 mL vials with 15 mL of methanol and then placed into an Ultrasonik 104X sonicator (Ney Dental International, Yacaipa, CA) for 30 min to facilitate liquid extraction of the tissue. Vials were then stored in darkness at 20 °C for 24 h, and then mixed using a Thermolyne Maxi Mix vortex mixer (Sybron International, Milwaukee, WI) for 10 s to create a homogenous mixture. A 5 mL sample was withdrawn and passed through a 0.45 µm nylon syringe filter into a 12 × 32 mm vial. The vial was sealed with a crimp top and then loaded into the autosampler of a gas chromatograph mass spectrometer (GC/MS).

An autosampler equipped 7890A GC system with a 5975C inert XL EI/CI MSD with Triple-Axis Detector fitted with a DB-5 fused silica capillary column (30 m × 0.530

mm with one µm film thickness) (Agilent© Technologies, Santa Clara, CA) and ChemStation integrator was used to analyze samples. Hydrogen was the carrier gas and the oven temperature was held at 20 °C for two min and then was programmed to increase at 10 °C·min^{-1} to 200 °C, with a final hold at 200 °C for five min.

Ten blank methanol samples were placed into the autosampler, followed by analytical grade essential oil standards [linalool, eugenol, eucalyptol, estragole, and methyl-cinnamate (Sigma-Aldrich, St. Louis, MO)], five methanol blanks, and then the treatment samples separated by two methanol blanks between treatments. Essential oil concentrations were determined using chromatograph peak areas from integrated data in Agilent ChemStation software. The peak area retention times of the essential oil standards were used to identify essential oil peak areas in basil sample GC/MS analysis.

Statistical analysis was conducted using JMP® software (SAS© Institute Inc., 2013). Peak areas from GC/MS basil sample analyses were used to create regression models to predict the response of basil plants to nitrate-N:ammonium-N fertility treatments. Tukey's HSD was used to compare the essential oil content of fertilizer treatments compared to an non-fertilized negative control.

Results

Fresh weights tended to be greater in plants grown with 75:25 nitrate-N:ammonium-N treatment. Plant fresh weights decreased linearly as percent ammonium increased and nitrate decreased (Fig. 1). Basil plant shoot indices had a quadratic relationship to increasing percentages of ammonium, with the 50:50 nitrate-N:ammonium-N treatment tending to have greater shoot indices according to our model

and trial data. Plants grown in the 0:100 nitrate-N:ammonium-N treatment tended to have the poorest shoot indices in both the trial results and our model (Fig.1).

Basil essential oils exhibited quadratic relationships with regard to increasing ammonium and decreasing nitrate. Eucalyptol, linalool, and eugenol tended to have higher GC/MS peak areas when grown with the 75:25 nitrate-N:ammonium-N treatment according to our trial data; however, the model suggests that higher essential oil content could be obtained with a higher ratio of nitrate-N (Fig. 2). Basil samples were also analyzed for estragole and methyl-cinnamate, but no significant peaks were found during GC/MS analysis (data not shown). Non-fertilized plant essential oils were also compared to plants that received fertility treatments. No significant differences were found in eucalyptol among fertilized and non-fertilized plants. However, 75:25 nitrate-N:ammonium-N treated plants had significantly higher linalool compared to non-fertilized plants. Both the 75:25 and 50:50 nitrate-N:ammonium-N treatment plants had significantly greater eugenol compared to non-fertilized plants (Table 3).

Discussion

Based on our results, 'Genovese' basil plants produced for fresh-cut basil would potentially have greater yields with a fertility program that utilizes a 75:25 nitrate-N:ammonium-N fertilizer. Basil produced for essential oils would theoretically have greater content when grown with a fertility program utilizing a 75:25 ratio of nitrate-N:ammonium-N. Overall, for both yield and essential oil content, we recommend a 75:25 ratio of nitrate-N:ammonium-N for production of basil plants.

Our results were similar to a study conducted by Beaman with 'Italian Large Leaf' basil, which found that a 50:50 or 75:25 nitrate-N:ammonium-N fertilizer produced the

greatest biomass (Beaman, 2008). These findings were from basil plants started in Oasis® LC-1 Horticubes® and then transplanted into sand. Their 0:100 nitrate-N:ammonium-N treatment resulted in plant mortality. Based on available information, chloride levels in Beaman's 0:100 nitrate-N:ammonium-N formula could have been greater than 800 ppm. Plants sensitive to chloride (Cl^-) have been described as those that have adverse effects when treated with 100 mM Cl^-, and many important vegetable and fruit plants are susceptible to Cl^- toxicity (Greenway and Munns, 1980). A study by Tarchoune et al. (2010) found that 'Genovese' basil plants treated with 50 mM NaCl did not cause plant mortality and actually enhanced the levels of antioxidants. The Cl^- in our formulas were approximately 160 ppm for both the nitrate-N:ammonium-N ratio of 50:50 and 75:25, while the 0:100 and 25:75 were approximately 190 ppm. There were a few plant mortalities in our 0:100 treatment, but it is unknown if it was Cl^- related. Additional research may be needed to understand the threshold for Cl^- toxicity in basil plants.

We also captured pH and EC data before and after basil plants were fertilized to observe any changes (Fig. 3). Combined trial data resulted in the 0:100 treatment having a pH of 4.8, the 75:25 treatment a pH of 5.6, and the non-fertilized control a pH of 5.8 at the end of the eight week trial duration. A greater ammonium concentration resulted in a more acidic pH. The change in pH between treatments may have contributed to the growth and essential oil differences we saw in basil plants, as nutrient uptake may have been impacted. The EC data trended similarly among treatments.

Additionally, while fertilizer treatment formulae were balanced to create the nitrate-N:ammonium-N ratios and keep the N rate constant, the Ca and K rate fluctuated

between treatments due to the available fertilizer components. The 0:100 treatment had the lowest Ca and K compared to other treatments (Table 2). This may have also impacted the performance of basil plants.

There are a few approaches to try and solve the variables of Cl^-, pH, and nutrient balance in future research. For Cl^-, we recommend a rate study to determine the threshold for toxicity in basil plants. In regards to pH, adding components to help buffer pH could be explored to help combat acidity and maintain pH at a more desirable level. For balanced nutrients, we recommend to investigate formulating with different fertilizer components to try and reach a more consistent nutrient profile among treatments.

Similar to our results, wheat was found to perform well in a combination nitrate and ammonium fertilizer and grew poorly in an unbuffered ammonium treatment (Gill and Reisenauer, 1993). Cucumber plants had greater shoot and root growth when treated with nitrate-based fertilizers as opposed to a combination of nitrate and ammonium (Heuer, 1991). Root crops, such as potato and taro, produced greater biomass when fertilized with a combination of nitrate and ammonium (Epstein and Bloom, 2005; Osorio et al., 2003; Serio et al., 2004). Additionally, a study by Tesi et al. (1995) observed the effects of fertilization on sweet basil and found that plants grown with ammonium sulfate had increased plant height compared to those grown with calcium nitrate; however, there were no differences in fresh weight. They also found that ammonium sulfate increased plant height and calcium nitrate promoted higher nitrate accumulation in plant leaves.

Research has also been conducted to understand the effect of N concentration on basil growth. Beaman (2008) found that N concentrations greater than 100 $mg \cdot L^{-1}$ did not produce greater biomass in ‘Italian Large Leaf’ basil plants. A study conducted by

Suh and others (1999) analyzed four all nitrate-N ionic concentrations with basil and found that sweet basil had better growth with a 0.5 ionic strength nutrient solution (9 mM NO_3^-) compared to a standard ionic strength solution (18 mM NO_3^-). A study with field basil that tested 0, 60, 120, and 180 kg·ha^{-1} N found that fields treated with up to 60 kg·ha^{-1} increased yields while the greater rates provided no significant yield increase (Zheljazkov et al., 2008). In lettuce, 120 kg·ha^{-1} N produced greater yields compared to 0, 60, 120, and 180 kg·ha^{-1} N (Boroujerdnia and Ansari, 2007).

An N concentration study by Nguyen and Niemeyer (2008) found that 'Genovese' basil had the highest phenolic content with the lowest nitrogen content tested at 0.1 mM compared to the highest treatment of 5.0 mM. Those researchers grew basil plants in sand and fertilized plants with a modified Hoagland solution with an ammonium nitrate N source and harvested plants 30 days after germination. They suggested the results may have been due to growth-differentiation balance (GDB) framework, which is described as a "physiological trade-off" that exists between plant growth and secondary metabolites (Herms and Mattson, 1992). This theory states that when environmental conditions are favorable and a nitrogen source is adequate, plant growth will be more abundant. However, when environmental conditions are harsh and a nitrogen source is scarce, growth will be minimal and secondary metabolites will be produced to help protect the plant (Herms and Mattson, 1992). Within the GDB framework, there is a carbon/nutrient balance hypothesis that states that plants in limited nutrient conditions will increase in production of carbon-based compounds, including secondary metabolites (Bryant et al., 1983). A study by Nykänen (1986) supports that field cultivated basil produces greater total essential oils with no nitrogen inputs; however, linalool and eugenol were found to

increase with added N. These results are similar to what we found, in that linalool and eugenol were positively impacted with added fertilizer. Further research could include testing nitrate-N:ammonium-N treatments and non-fertilized plants and collecting total essential oil data. Additionally, it would be interesting to explore how the flavor changes between fertilized and non-fertilized plants with the differences in linalool and eugenol essential oil content.

In our study, plants were grown in a commercial sphagnum peat based substrate whereas the majority of basil fertility studies have been performed in a sand media or hydroponic system. One of the goals of this study was to grow plants in a substrate that growers or home gardeners would use to grow potted basil plants. It is not known if substrate choice had any influence on the findings discussed, although it is likely that the higher cation exchange capacity (CEC) of the sphagnum peat moss compared to sand or water culture may have impacted nutrient availability. Future research could investigate if plants respond differently to fertilizer when grown in different substrates or a hydroponic system.

While our study focused specifically on the N source, another study conducted by Nguyen et al. (2010) found that increasing K significantly increased the phenolic concentration in ‘Genovese’ basil plants. The next phase of this research could combine the 75:25 nitrate-N:ammonium-N fertilizer and explore various rates of K to determine if greater essential oil content could be obtained in basil plants.

Overall, we recommend that a 75:25 nitrate-N:ammonium-N fertilizer be used to grow ‘Genovese’ basil plants in a sphagnum-based growing media for the greatest fresh

weight and essential oil production. We hope that this information will be useful for greenhouse growers in their cultivation of basil plants.

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Table 1. Nitrate-N: ammonium-N fertilizer treatment formulae including 0:100, 25:75, 50:50, or 75:25. One L solutions were mixed each week and fertilizer was applied twice a week to basil plants at 250 mL per application.

Ingredient (in 1 Molar Solution)[z]	Nitrate-N:Ammonium-N Fertilizer Treatment Formulas Amount in Order (mL)			
	0:100[y]	25:75	50:50	75:25
KNO_3	-	-	1.67	1.30
$Ca(NO)_3$	-	2.00	2.53	2.67
$CaCl_2$	2.67	2.33	1.80	1.70
KH_2PO_4	1.00	1.00	1.00	1.00
K_2SO_4	-	0.67	-	-
KCl	-	0.67	1.00	1.30
$NHNO_3$	-	-	0.86	7.60
NH_4NO_3	-	-	0.67	-
NH_4OH	.80	1.40	-	-
Fe EDTA	1.00	1.00	1.00	1.00
MicroMix[x]	1.00	1.00	1.00	1.00

[z] Ingredients are listed in mixing order for each fertility treatment.

[y] Fertilizer treatments were formulated to deliver 200 $mg \cdot L^{-1}$ N.

[x] Micronutrient blend consisting of 0.5 ppm boric acid, 0.02 ppm copper sulfate, 0.05 ppm zinc sulfate, 0.5 ppm manganese chloride, and 0.01 ppm molybdic acid.

Table 2. Fertilizer formula nutrient concentrations found in nitrate-N:ammonium-N treatments of 0:100, 25:75, 50:50, or 75:25. One L solutions were mixed each week and fertilizer was applied twice a week to basil plants at 250 mL per application.

	Nitrate:N-Ammonium-N Fertilizer Treatment[z] (ppm)			
Nutrient	0:100	25:75	50:50	75:25
N	201.4	212.7	200.6	200.8
P	31.0	31.0	31.0	31.0
K	39.0	139.0	143.4	143.4
Ca	106	173.7	173.7	173.7
S	-	10.7	-	-
Cl	189.1	189.1	163.1	165.4

[z]Fertilizer formulae were designed to deliver approximately 200 $mg \cdot L^{-1}$ N.

Table 3. Essential oil gas chromatograph mass spectrometer (GC/MS) peak areas of 'Genovese' basil grown with nitrate-N:ammonium-N fertilizers, including 0:100, 25:75, 50:50, or 75:25, and compared to a negative non-fertilized control. Basil leaf tissues were harvested and dried at eight weeks after sowing. The trial was performed three times in total, with essential oil data utilized and combined from the second and third trials.

Fertilizer treatment[z]	Eucalyptol	Linalool	Eugenol
	(Arbitrary units)		
Non-fertilized control	324133 a[y]	370923 b	2174921 b
75 nitrate-N:25 ammonium-N	408020 a	876120 a	4374674 a
50 nitrate-N:50 ammonium-N	280691 a	564127 b	3699243 a
25 nitrate-N:75 ammonium-N	288066 a	554710 b	3367031 ab
0-N nitrate-N:100 ammonium-N	285708 a	609470 ab	3582435 ab

[z]n = 16 plants per treatment (data from two trials, the first with six replicates and the second with 10 replicates)

[y] Means within columns followed by the same letter are not different at $P \leq 0.05$ according to Tukey's HSD test.

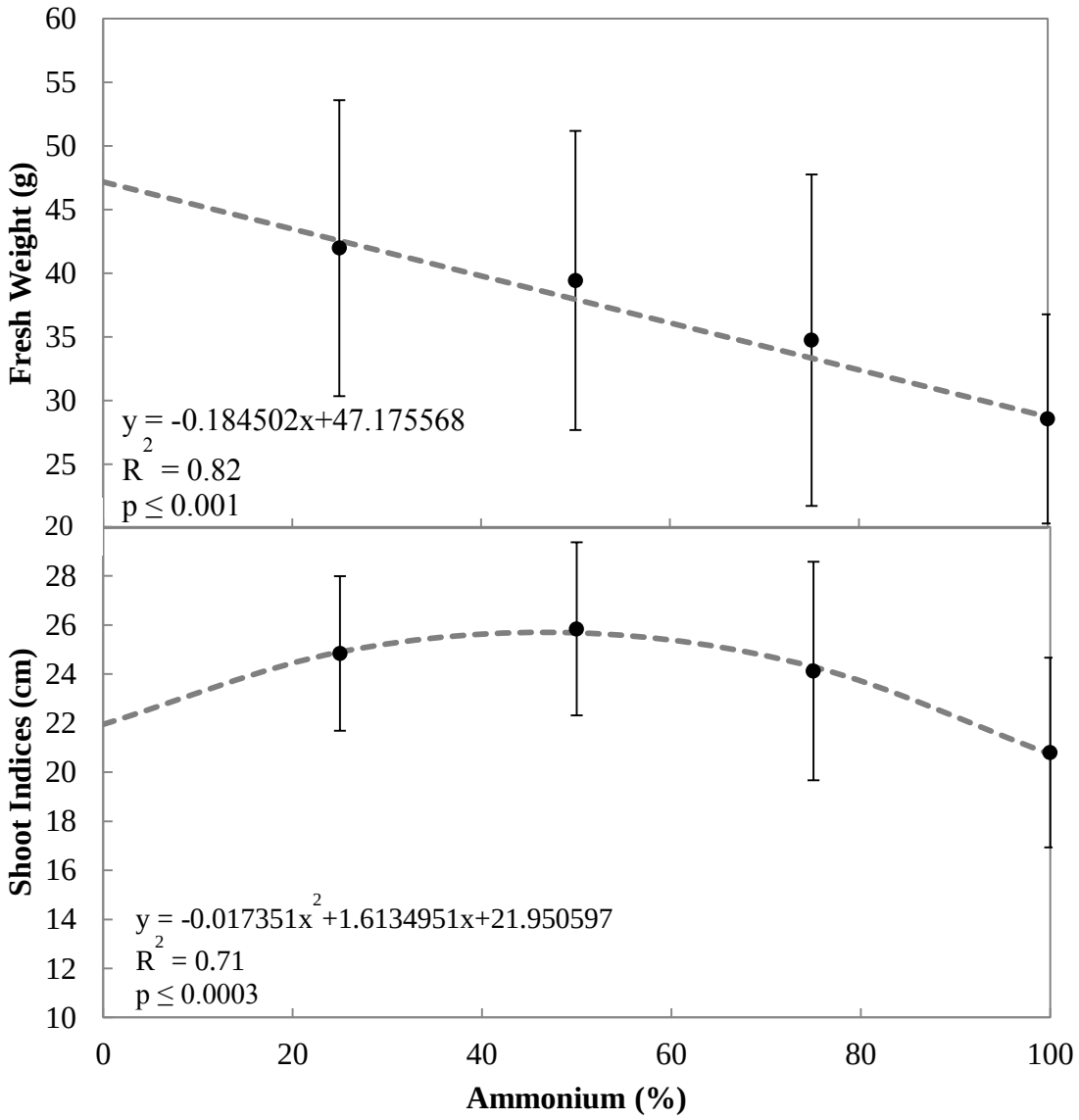

Fig. 1. Relationship of basil fresh weight and shoot indices to increasing percent ammonium versus nitrate (% ammonium + % nitrate = 100%). Basil plants were grown with nitrate-N:ammonium-N fertility treatments including 0:100, 25:75, 50:50, or 75:25. The trial was conducted three times, with six replicates in trial one and two and 10 replicates in trial three. The results herein are the combined data that were collected eight weeks after sowing. Shoot indices were calculated by taking the average of two perpendicular plant canopy widths and one height. Data are means ± standard deviation.

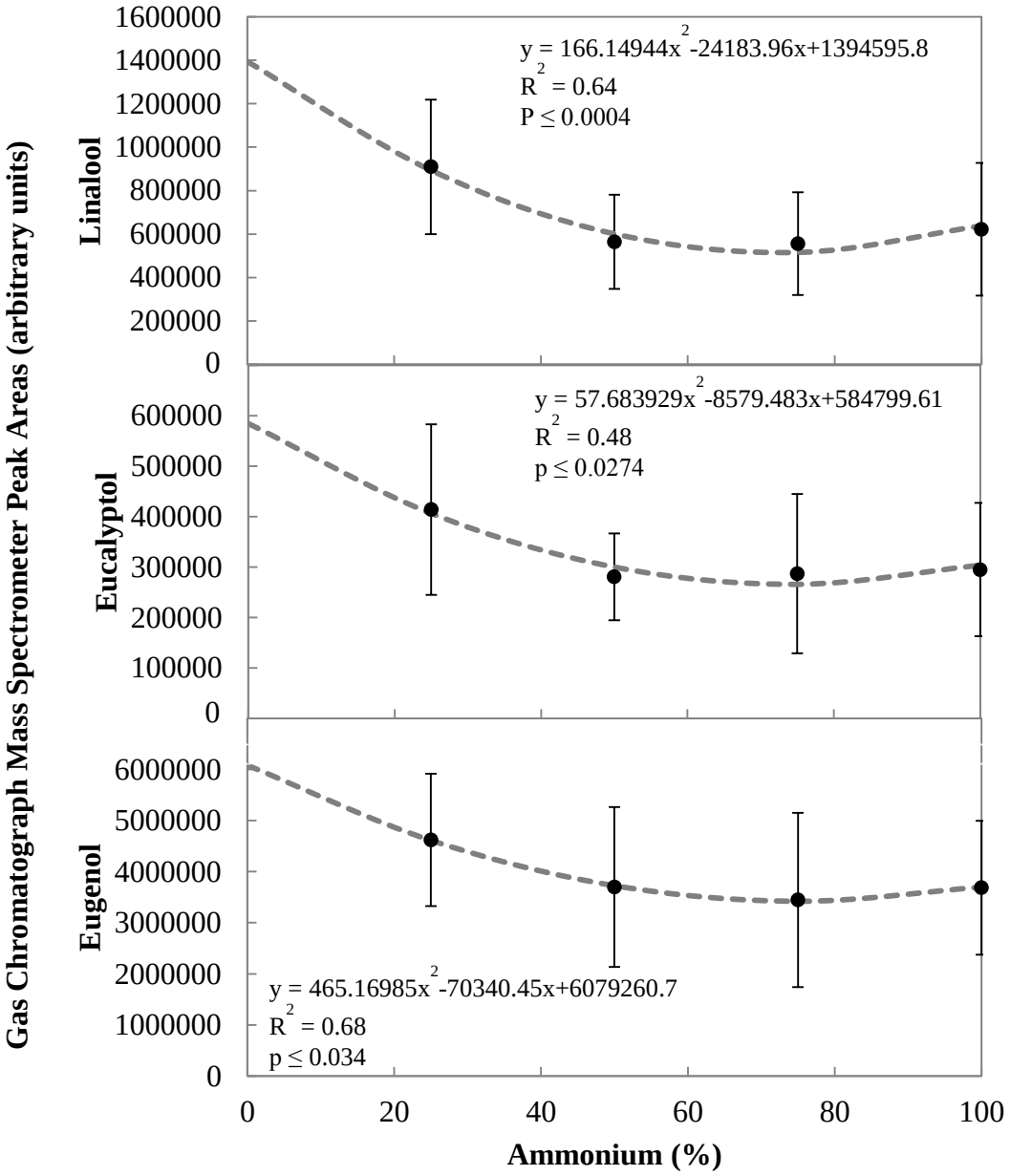

Fig. 2. Relationship of linalool, eucalyptol, and eugenol GC/MS peak areas to increasing percent ammonium versus nitrate (% ammonium + % nitrate = 100%). Basil plants were grown with nitrate-N:ammonium-N treatments of 0:100, 25:75, 50:50, or 75:25. These results are from two trials, the first with six replicates and the second with ten. Dried leaf tissue was analyzed from plants harvested at eight weeks after sowing. Data are means ± standard deviation.

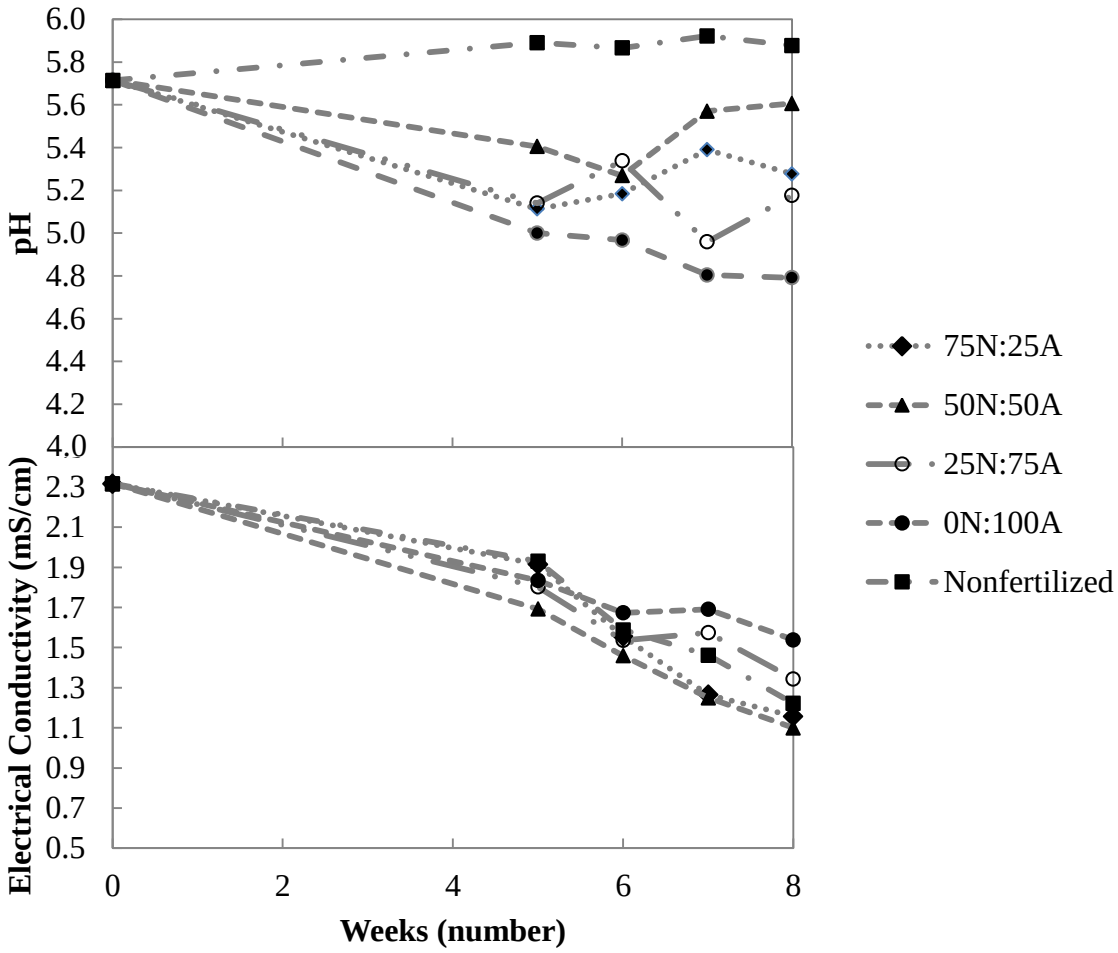

Fig. 3. The relationship of pH and Electrical Conductivity (EC) of basil fertilizer treatments over time, including nitrate-N:ammonium-N ratios of 0:100, 25:75, 50:50, 75:25, or a non-fertilized control. Initial pH/EC were recorded with non-fertilized substrate and additional pH/EC data were recorded beginning one week after fertilization began, approximately three weeks after seeding. Weekly pH/EC data were collected until trial termination at eight weeks after sowing. The trial was repeated three times, with two pH/EC subsamples pulled per treatment for the first two trials and three subsamples pulled per treatment for the third trial. The results herein are the combined data.

CHAPTER 6. GENERAL CONCLUSIONS

General Discussion

Our research provided several insights on how limited irradiance, artificial irradiance, and nitrate-N:ammonium-N fertility impacts basil growth and essential oil production. We hope that our findings can lead to recommendations for the cultivation of basil in a commercial greenhouse setting as well as for consumer gardening.

In regards to limited irradiance, basil fresh weight and shoot indices were greater under 0% shade and decreased linearly as shade increased. However, essential oil peak areas did not follow this trend. Essential oil peak areas were predicted to be the greater for eucalyptol at 60% shade, linalool at 30% shade, and eugenol at 40% shade. Our results differed from a study by Chang, who found that linalool and eugenol content decreased as irradiance decreased (Chang, 2008). However, in that study plants were under limited irradiance for short durations, whereas our plants were under their respective treatments for the entire duration of the trial. Other bodies of research have found for various crops, including thyme, apple, and strawberry, that essential oils, volatiles, and compounds responsible for flavor were increased in high irradiance growing conditions (Letchamo et al., 1995; Miller et al., 1998; Watson et al., 2002).

Our findings could potentially impact how basil crops are grown for various uses. Growers who sell fresh cut basil based on weight would benefit most from 0% shade to maximize production, whereas growers that are producing specifically for greater essential oils or flavor would benefit from growing their crop under shadecloth.

In relation to artificial irradiance, fluorescent grow lights (GL) had significantly greater PPF compared to cool fluorescent (CW) and cool fluorescent plus incandescent (CW+I) for individual trials. Basil plants grown under GL had significantly greater shoot indices than CW, but not CW+I. Plants had a significantly greater number of leaf pairs when grown under GL and CW+I compared to CW. Plants grown under GL treatment had significantly greater fresh weight compared to CW, but not CW+I. No differences were found in essential oil content among fluorescent treatments.

The GL treatment had the greatest PPF, which was 250 and 176 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for each respective trial. According to research done by Beaman and others (2009) an irradiance of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is optimum for greatest basil biomass in a controlled environmental system. Studies with other herbs and leafy crops, including thyme, horehound, lettuce, and spinach found that greater irradiance promoted greater fresh weight (Shinohara and Suzuki, 1981; Bergeron et al., 1995; Letchamo et al., 1995; Proietti et al., 2004; Zhou et al., 2009). Other research has found that growing conditions with high irradiance increased essential oils for various crops. The literature suggests that additional GL lamps would need to be used to increase the PPF output for optimal basil biomass production and increased essential oils.

In relation to the nitrate-N:ammonium-N fertilizer study, basil plant shoot indices had a quadratic relationship to increasing percentages of ammonium, with the 50:50 nitrate-N:ammonium-N treatment having the greatest shoot indices according to our model and trial data. Plants grown in the 0:100 nitrate-N:ammonium-N treatment had the poorest shoot indices in both the trial results and our model. The highest fresh weights were obtained with plants grown with 75:25 nitrate-N:ammonium-N treatment and

decreased linearly as ammonium increased and nitrate decreased. Basil essential oils had quadratic relationships with regard to increasing ammonium and decreasing nitrate ratios. Eucalyptol, linalool, and eugenol had the greatest GC/MS peak areas when grown with the 75:25 nitrate-N:ammonium-N treatment according to our trial data; however, the model suggests that higher essential oil content could be obtained with greater nitrate-N.

Our results were similar to a study conducted by Beaman with 'Italian Large Leaf' basil, which found that greater biomass could be achieved by using a nitrate-N:ammonium-N of 50:50 or 75:25 (Beaman, 2008). A sweet basil study by Tesi et al. (1995) investigated fertilizers and found that there were no fresh weight differences in plants grown with either nitrate or ammoniacal nitrogen sources. A different basil study found that plants produced greater total essential oils with no nitrogen inputs; however, eugenol and linalool were greater when additional fertilizer was provided (Nykänen, 1986). These findings are similar to ours, as our fertilized plants had greater eugenol and linalool peak areas compared to a non-fertilized control.

Recommendations for Future Research

There are several areas we recommend additional research be explored with basil plants. In relation to limited irradiance, it would be helpful to conduct a similar study to our trial that would focus on PPF treatments rather than percent shade treatments. This is especially true as the particular shade cloth that we used in our trials did not reduce irradiance to the percent as advertised. If the treatments were based upon PPF, it would be more relevant to create a model to predict the response of basil to limited irradiance, regardless of growing condition or shading material.

It is interesting that the GL treatment in the artificial irradiance trial had very low average PPF at 213.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when compared to the PPF of the shade treatments in the limited irradiance trial, which were 1630, 885, 620, or 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Granted, the growing environments were different between these two sets of trials. The results of our limited irradiance trial suggest that some shading increases essential oils in basil, and the GL treatment had lower PPF compared to the lowest PPF of the limited irradiance trial. It would be interesting to do a similar artificial light trial and increase the number of lamps to increase PPF and compare against the original two GL lamps that were the most efficacious to see the effect on fresh weight and essential oil content. In theory, the fresh weight should increase due to the additional irradiance, but the impact on essential oils is more ambiguous, especially since other research shows the opposite response of basil essential oil content to limited irradiance than we observed.

Additionally for both limited greenhouse irradiance and artificial light, we recommend for further investigations to be done on nitrate content, as elevated concentrations of nitrate in food crops are a potential threat to human health (Walker, 1990). Two basil cultivars were found to have elevated nitrate content when grown under 50% reduced irradiance compared to 0% reduced irradiance (Raimondi et al., 2006). Accumulation of nitrate was found to decrease as light intensity increased in lettuce plant tissues (Blom-Zandstra and Lampe, 1985). Spinach plants grown under 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ contained more oxalate and nitrate compared to plants grown under 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Proietti et al., 2004). It is important to determine if basil grown in limited irradiance conditions could be hazardous for human consumption.

In relation to artificial irradiance, there is a general lack of current published studies that investigate the different fluorescent options that are available today for growing plants indoors. Most comparative fluorescent research occurred in the 1960's and is not applicable to the light systems of today (Helson, 1964; Pallas, 1964; Thomas and Dunn, 1967).

In relation to the nitrate-N:ammonium-N study, it would be helpful to repeat the trial and conduct it with both peat and sand substrates to determine if the substrate influences basil's response to the various fertilizer treatments. We also suggest investigating ways to help reduce variability between the nitrate-N:ammonium-N treatments, such as buffering for pH, balancing nutrients, and reducing Cl^-. Also, it may be interesting to combine the 75:25 treatment with varying K rates. Nguyen et al. (2010) found that increasing K significantly increased the phenolic concentration in 'Genovese' basil plants. It may be possible with this combination to increase the amount of essential oils for a more aromatic and flavorful basil crop.

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