Hops Production in Virginia: Nutrition, Fungal Pathogens, and Cultivar Trials

Barslund D. Judd

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science in Life Sciences
in
Horticulture

Holly L. Scoggins
James S. Owen, Jr.
Carlyle C. Brewster

Sep. 20th, 2018
Blacksburg, VA

Keywords: *Humulus lupulus, Podosphaera macularis,* tissue testing, sufficiency range, powdery mildew, cultivar trial.

Hops Production in Virginia: Nutrition, Fungal Pathogens, and Cultivar Trials

Barslund Judd

Abstract

In the United States, hops (*Humulus lupulus* L.) are grown mainly in the Pacific Northwest (PNW). For this reason, most cultural information is based on the growing conditions of the PNW. Growing conditions in Virginia differ drastically and present unique disease and production challenges. Three studies were conducted with the intent of increasing hop cultivation knowledge for Virginia growers. For the first study, 13 cultivars of hops grown at the Virginia Tech hop yard were compared for growth, yield, and quality. Mean cone fresh weight per plant ranged from 12.00 g for Mt. Hood to 1002.87 g for Crystal in 2016 and from 97.98 g for Mt. Hood to 900.33 g for Cascade in 2017. In 2016, only Alpharoma, Cascade, Mt. Rainier, and Southern Cross had alpha acid levels, an indicator of cone quality, within the accepted range. In 2017, Alpharoma, Centennial, Mt. Rainier, and Nugget had alpha acid levels within the expected range. Three cultivars (Cascade, Crystal, and Ultra) were above the expected alpha acid range, which indicates more bittering potential for beer brewers.

In a nutrient deficiency study, hop plants were grown in hydroponic solutions, and deficiencies were induced for nitrogen (N), phosphorus (P), and potassium (K). After visual deficiency symptoms had been induced, leaf tissue samples were taken and analyzed for nutrient content. Images were taken at each deficiency stage. For N, incipient deficiency symptoms were observed at a mean of 3.18% dry weight in leaf tissue samples. Visual symptoms included a chlorotic appearance, undersized leaves, and red petioles. Incipient symptoms for P were observed at a mean of 0.307% dry weight in leaf tissue samples. Necrotic spots, leaf cupping, and undersized leaves were apparent with this deficiency. Incipient

symptoms for K were observed at a mean of 1.21% dry weight in leaf tissue samples. Symptoms included rounded leaf tips, blue veins, and marginal scorch. In the third study, a whole leaf powdery mildew (*Podosphaera macularis*) assay was developed and tested using five hop cultivars, Alpharoma, Cascade, Comet, Sorachi Ace, and Tahoma. Leaves were inoculated with powdery mildew (PM) using a settling tower. This method was used to rapidly assess the resistance of cultivars. Leaves were successfully inoculated and PM colonies were allowed to grow for two weeks. Images of the PM colony development on inoculated leaves were compared using ImageJ to determine percentage of coverage. Tahoma was the only cultivar found to produce a significantly different mean percent coverage (19.5%) compared with the resistant cultivars Cascade and Comet (<1%).

Hops Production in Virginia: Nutrition, Fungal Pathogens, and Cultivar Trials

Barslund Judd

General Audience Abstract

Hops (*Humulus lupulus)* were first grown in the United States in Massachusetts in the early 1600s. Production of this crop eventually spread throughout the Northeastern US. By the mid-1800s, commercial production spread to southern states such as Virginia. Infestation of pests, e.g., hop aphid and diseases such as downy mildew in eastern states, and prohibition on the production of alcohol caused a significant shift in hop production, which favored the Pacific Northwest (PNW). As a result, in Virginia specific knowledge of hop cultivation is now lacking. Three studies were conducted to increase our knowledge of hop cultivation for the region. For the first study, 13 cultivars grown at the Virginia Tech hop yard were compared for growth, yield, and quality. The cultivars Cascade and Alpharoma had alpha acid levels equal to or higher than expected for the 2016 and 2017 seasons. The alpha acid levels are an indicator of the hop quality from the Cascade and Alpharoma cultivars. Cascade was also a top producer of hop cones with a mean fresh weight of 989.67 g and 900.33 g in 2016 and 2017, respectively. In a nutrient deficiency study, plants were grown in Hoagland hydroponic solutions, which contain all essential nutrients needed for plant growth. This was done to provide photographic documentation to assist local growers with deficiency identification. Deficiencies were induced for nitrogen (N), phosphorus (P), and potassium (K) by removing each of the specific nutrient of interest from each treatment solution. After deficiency symptoms were induced, leaf tissue samples were taken and analyzed for nutrient content. Nutrient deficiencies were documented using photographs. For N, visual symptoms included a chlorotic appearance, undersized leaves, and red petioles. Incipient symptoms for P included necrotic spots, leaf cupping, and undersized leaves. For K, leaf cupping, blue green veins, marginal scorch, and rounded leaf tips. Disease resistance of cultivars is important information for growers and can heavily influence hop yard planning. As such, in another study, an assay for powdery mildew

(*Podosphaera macularis*) was developed that allows for rapid low cost testing of hop cultivars. The assay was tested on the following cultivars: Alpharoma, Cascade, Comet, Sorachi Ace, and Tahoma. Leaves were inoculated with powdery mildew (PM) using a settling tower. Powdery mildew colonies were allowed to grow for two weeks and were then analyzed using ImageJ software to determine percent coverage. At the end of the experiment, Tahoma had significantly greater PM coverage compared to the other cultivars, indicating that Tahoma is less resistant to the specific PM strain.

Acknowledgements

Without the support of my family, partner, friends, and advisory committee, this thesis would not have been completed. I would like to thank my parents, Gary and Margaret, for a lifetime of support. Their gardens, museum trips, and love of the outdoors inspired me to become the person I am today. Additional thanks goes out to Fred and Beverly, my grandparents, for a childhood spent in gardens and summers filled with strawberries. A special thanks for my partner Kyle Zeller, who has the patience of a saint and always knows how to cheer me up. I would like to express my gratitude to Lori Barber and Tim Pyne, two wonderful friends, who supported my professional endeavors and encouraged my return to school.

My deepest appreciation goes out to Dr. Holly Scoggins, a fellow plant nerd, for her patience and inspiration, and to Dr. Jim Owen for his advice and excited optimism. I would also like to express my appreciation to Dr. Carlyle Brewster for the many hours he spent explaining JMP to me. Without his help, I would still be trying to understand my data.

My gratitude goes out to the staff members in the department of Horticulture at Virginia Tech, including Velva Groover, for being one of the kindest people I've ever met and always having the odd piece of equipment I needed, Jeff Burr, for helping me setup experiments and taking them down, only to set them up again, John James, for his help with the cultivar trial, and Daniel Jackson, with all of his assistance on various experiments. My greatest thanks and sympathies are owed to Joel Shuman for his assistance in formatting this manuscript. My deepest gratitude goes to Grayson (Hunter) Lyons for all of his help with the cultivar trial and for being one of the hardest workers I've ever met.

Table of Contents

List of Tables

List of Figures

Chapter 1: Literature Review

Cultivar Trial

Introduction

Cultivar trials are often used to assess the overall performance of new cultivars or the performance of a cultivar for a specific region. It also gives the ability to compare cultivars side by side with those that are already in use. For hop production, it is extremely important to assess cultivars in our region because of differences in disease pressure and distribution, as well as differences in climate. Poor plant performance can make it difficult for growers to recoup expensive hop yard investments. It is important to plant varieties that perform well as hops are perennial crops and require extensive infrastructure investment.

Essential oils provide the taste and smell that is unique to each cultivar (Eyres and Dufour, 2008). They may also have other beneficial qualities and have historical usage in folk medicine (Karabin et al., 2016). Alpha and Beta acid concentrations are important for assessing the quality of hops. Brewers must know these acid concentrations when calculating hop cone additions during the brewing process (Kostelecky, 2016). Alpha acids lend bitterness to the beer while Beta acids provide flavor stabilizing benefits. Hop compounds like xanthohumol have shown promise in cancer research, while beta acids have been found to have antibacterial properties. These characteristics and compound concentrations can vary between geographic locations. This is known as terroir and includes all of the environmental factors that affect the plant. Van Holle et al. (2017) found that terroir could lead to significantly divergent flavors. Regional cultivar trials allow researchers to study these differences. These factors may help researchers to find optimal cultivars for specific regions.

Similar Studies

Variety trials are being conducted in multiple locations on the eastern part of the United States. North Carolina State University, Virginia State University, Virginia Tech, University of Maryland and the

University of Vermont are all studying hops for production in the East. Hops are grown on trellises from 16-20 feet in height. For trialing, the cultivars are randomized in multiple blocks to reduce statistical variability. In the Vermont trials, significant differences were found between production in their yard and the PNW. While many of the cultivars produced comparable acid contents to PNW industry standards, production was half of what would be expected in the PNW (Darby et al., 2017). This difference may be partially influenced by differences in management practices. The Vermont yard used organic methods to control pests. This included cultural practices which might be unfeasible in large scale production in the PNW. The majority of hop variety trials study two main factors: hop quality and hop quantity. Most hop cones are produced on sidearms (Darby and Calderwood, 2016). For this reason, it is important for plants to be properly maintained by pruning at the correct time to maintain optimal shape.

Nutrient Recommendations and Characterization of Nutrient Deficiencies in *Humulus lupulus*

Introduction

Hop (*Humulus lupulus*) production in the United States (US) has a long history with records of hop production dating back to the 1700s. Thomas Jefferson, for example, grew hops at Monticello, which he used to produce his own ale. The first commercial hop farm, located in Massachusetts, was built in 1648 and covered 18.2 hectares (Barth et al., 1994). For 150 years, Massachusetts dominated hop production. In the mid-1800s, New York displaced Massachusetts as the largest producer of hops. Eventually, the Pacific Northwest (PNW) became a favored region for hop production, and when prohibition began, production in the northeastern United States waned. In more recent years, interest in local food and beverage production has spurred the establishment of craft breweries. As local craft breweries continue to open, hop demand is expected to rise.

From 2011 and 2017, the number of breweries in Virginia increased from 40 to 226 (Virginia ABC, 2017). Increasing demand for local beer has also spiked interest in the production of local hops. Most of the hop production in the US is currently in the PNW, which has a climate and day-length favorable for cultivation (Sirrine, 2014). In 2017, 28,065 hectares were used for hop production in Idaho, Oregon, and Washington State (USDA, 2017). Overall production increased by 11% from 2015 to 2016 and another 6% between 2016 and 2017 (USDA, 2016). In 2015, approximately 14,000 plants were grown in Virginia (Siegle and Scoggins, 2017). Growers in the PNW have processing infrastructure, access to proprietary cultivars, and large hop yards. These large yards and processing facilities contribute to lower costs. Based on these lower costs and cultivar availability, it is unfeasible for Virginia growers to compete directly with the PNW. Virginia farmers also face several other challenges in hop production including shorter day length, and high pest and disease pressure that lead to reduced crop yields, and high equipment cost for market entry.

Virginia farmers have a unique opportunity to grow and market a local crop for our region. However, as demand for locally produced hops increases, it is important that regional farmers are empowered with the tools to help them succeed. For example, currently there are no plant nutrient fertilizer recommendations for Virginia hops. Establishing fertilizer recommendations is important for the economic production of hops. Fertilizer application affects essential oil product in hops and is a component of overall hop quality (Keller, 1954). These essential oils vary from cultivar to cultivar and give hops their characteristic taste (Table 1.1).

Plant Nutrients

Essential nutrients are inorganic chemicals that plants require for adequate growth. For a nutrient to be considered essential, it must meet the following requirements:

1. The plant cannot complete its life cycle if the element is deficient.
2. The deficiency is specific to that element and cannot be replaced by another.
3. The element is directly involved in the plants growth as a component, an essential metabolite, or for the action of an enzyme system. (Arnon and Stout, 1939)

Plant nutrients are divided into macronutrients and micronutrients. Macronutrients are required in large quantities (e.g., N dry tissue concentration 1500 mg/kg), while micronutrients are required in far smaller amounts (e.g., Fe dry tissue concentration 100 mg/kg). Sixteen elements are considered essential for the growth of plants. The macronutrients are: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulphur (S), and magnesium (Mg) (Mengel and Kirkby, 2001). The micronutrients are: iron (Fe), chlorine (Cl), manganese (Mn), boron (B), zinc (Zn), copper (Cu), and molybdenum (Mo). Plants obtain oxygen, hydrogen, and carbon from the atmosphere. New research has shown that other elements such as silicon (Si), nickel (Ni), aluminum (Al), cobalt (Co), vanadium (V), selenium (Se), and platinum (Pt) may also be essential to plants. In legumes, for example, cobalt is used during the process of fixing nitrogen, while nickel is essential to urease production (Resh, 2016). In some plants such as cucumbers, silicone helps

with cell rigidity and plant defense against fungal infections. It is likely that some of these micronutrients may also be essential to hops.

Nutrient availability is highly influenced by pH. Optimum nutrient availability occurs around a neutral pH of 7.0. In acidic environments N, P, and K have limited availability (Mcfarland et al., 2015). In addition, extremely acidic soils can release metals such as Al, which can be toxic to plants.

Fertilizer Application and Nutrient Deficiencies

Understanding plant nutrient requirements is essential for correct fertilizer application. Problems such as verticillium wilt disease, sclerotinia wilt, powdery mildew, and spider mite infestations can be increased by over application of N (Neve, 2012; Gent et al., 2015; Takle and Cochrane, 2017). Excessive nutrient fertilization can not only cause disease problems but can lead to pollution and runoff. Best management practices (BMPs) are practices that have been shown to optimize production potential, input efficiency, while protecting the environment (Griffith and Murphy, 1991). Roberts (2007) summarizes the principles of BMP as right product, right rate, right time, and right place (Table 1.2). Eighty nine percent of total N inputs into the Mississippi River come from agriculture. Nutrient runoff contributes to dead zones in our rivers and bays which damages fishing industries and our local economies (Good, 2011). As a result, many countries have created regulation to control fertilizer runoff. Denmark, for example, have begun restricting nitrogen application rates in the 1980s and have now reduced usage by 52% (Good, 2011) They have done so without reducing yields.

Nutrient deficiency studies help growers determine when fertilizers are under- or over-applied. Under application of fertilizer can reduce plant vigor and production. It can also cause plants to prematurely mature and produce low quality products (Jones, 1998). Excessive fertilizer application can also increase crop production costs for farmers and quickly reduce their profits. In a hop yard, fertilizer can account for $618/hectare per year. In the first year this represents around 8% of costs (Sirrine et al., 2014). Nutrient restrictions can lead to low production and low- quality crops.

Fertilizer Recommendations

Current recommendations for fertilizer application for hop production are based on those from the Pacific Northwest. Only 84 $kg \cdot ha^{-1}$ of N is recommended for the first year plantings (Gingrich et al., 2000). Whole plant yields of 1121 $kg \cdot ha^{-1}$ can remove between 89.7 and 101 $kg \cdot ha^{-1}$ of N (Darby, 2011). This can reach as high as 168–191 $kg \cdot ha^{-1}$. Phosphorus recommendations range between 22.4–12 $kg \cdot ha^{-1}$ based on soil concentration. Potassium recommendations range between 44.8–168 $kg \cdot ha^{-1}$. Leaf tissue standards are poorly defined, and it is suggested that growers compare nutrient concentrations to previous years. Well defined ranges for tissue nutrient contents combined with soil tests can help growers avoid over fertilization of their crops. These amounts are significantly lower than those used for hemp, an annual crop in the same family as hops (Aubin et al., 2015). Fertilizer placement is also important when considering plant nutrient needs. Broadcasting fertilizer makes the nutrients more available for weeds and can encourage their growth and competition with crops (Kirkland and Beckie 1998). Fertilizer nutrient availability is affected by soil temperature, moisture, compaction, root diseases, and positional availability (Mahler and Mcdole, 2001).

Visual Deficiency Symptoms

Plant visual deficiency symptoms, such as leaf deficiency symptoms and plant structure can be helpful tools for field diagnosis of plant nutrient deficiencies. There are, however, issues with using visual symptoms alone. Some nutrient deficiencies may look similar, while others can mask the presence of additional deficiencies (McCauley et al., 2011). This can be especially problematic when several deficiencies are present. Nutrients may also interact in ways that cause multiple symptoms to appear simultaneously. For example, N is necessary for the production of chlorophyll (Table 1.3). This means that a reduction in N might lead to poor photosynthesis and thereby reduce the uptake of other nutrients. Pseudodeficiencies can also appear on plants. These false symptoms can be the result of herbicide or chemical damage, insect damage, and insects. Hidden hunger is when plants are nutrient deficient yet are asymptomatic. This can make it hard to diagnose nutrient deficiencies as they will not be immediately apparent (Tisdale et al., 1993).

Leaf Tissue Sampling and Analysis

Leaf tissue sampling is used to access plant nutritional status when deficiency symptoms appear (Table 1.4). The nutrient content of leaves can be a good indicator of overall plant nutritional status. The leaves taken should be recently matured leaves which have fully expanded (Barnes, 2012) since older leaves may have a decreased nutrient concentration, while younger leaves can have an increased nutrient concentration (Seefeldt, 2016; Bryson, 2014). Leaves should also be taken from good and poor areas (Self, 2014). This may help with deficiency diagnosis. Harvested leaves are washed with deionized water, followed by a 0.5 M HCl solution, before again being rinsed with DI water to remove external contaminants and pesticides. Rinsing samples with HCl may be unnecessary if leaves are clean and free of residue. Pitchay (2003), for example, rinsed leaves for 30 seconds with 0.2 M HCl before again rinsing with DI water. Excessive washing, however, may leach certain nutrients, such as B, from the leaf tissue. Diluted acids may increase the problem, while still failing to remove surface contaminants like Fe, Zn, and Cu (Marschner, 2012). Petiole testing is desirable for some crops but for crops such as grapes, leaves are the more desirable choice for testing for nutrient deficiency (Benito, 2013). This is especially true with respect to N, P, K that tend to show more variation in petiole testing.

Quantifying Nutrient Deficiencies

Plants are known to uptake elements that may not be essential for growth. As such, some plants can function as bio-accumulators of elements, such as lead. Tissue samples taken from plants grown in soil, therefore, can contain elements that are not essential for growth. One method for determining whether an element is essential for plant growth is to eliminate one-at-a-time. Hydroponic-based experiments allow scientists to carry out this process. Hoagland's solution is a liquid nutrient solution designed used in hydroponic systems to replace soil for plant growth (Hoagland and Arnon, 1950). It is considered one of the most balanced general-purpose solutions and has been modified and used extensively by growers and scientists. Hoagland used the term "nutriculture" to describe what we now

call hydroponics. The system Hoagland proposed called for plants to be suspended by their stems using corks into an aerated solution. It was noted that the acidity of solutions could increase and salts in the solution were taken up by the plants. Because of this, solutions must be changed regularly. Additional solution can be added as the nutrient solution is used but must be changed to prevent the buildup of certain salts to the point of toxicity. Ideal pH ranges for this type of system are between 5.0–6.5 and are adjusted using Sulfuric acid. Phosphoric acid is more commonly used in modern systems.

In experiments conducted by Pitchay (2003), all nitrate Hoagland's solutions were used for growing several crops including vinca (*Vinca rosea* L.) and marigolds (*Tagetes patula* L.) . Pitchay's adaptation changes the molybdenum source from molybdic acid to sodium molybdate and the Fe source from iron tartrate to FeDTPA. While plants were being established during the first week, solutions were topped off with deionized water as the solution evaporated. After the first week, plants were topped off using solution. Similar experiments have been conducted for olives (Fernández-Escobar et al., 2016), sorghum (*Sorghum bicolor* L.) (Zhao et al., 2005), and mealy-cup sage (*Salvia farinacea* L.) (Barnes, 2012). Additionally, nutrient solution-based experiments have been used for researching root:shoot ratios under reduced P conditions (Kim and Li, 2016).

Hydroponics Background

Hydroponic systems are those in which plants are grown without soil or traditional potting media. These systems can contain other forms of inert media such as gravel, sands, expanded clay, coco coir, and vermiculite. These are typically used to anchor plant roots and do not provide nutrients to the plant. Hydroponic systems have some benefits when compared to traditional farming. They can require less space. This can be important in greenhouses, when covered square footage is costly. Hydroponic systems can also be more water efficient when operated properly. Competition from weeds is eliminated in a hydroponic system. Growing plants in greenhouse hydroponic systems can allow for multiple crop cycles and extended growth times. (Resh, 2016)

For research purposes, hydroponic systems are desirable as they allow control over nutrient availability. They also reduce the influence of outside variables due to runoff, nearby crops which may harbor pests, and chemical applications to nearby crops. In a field setting, additional nutrients can leach in from outside areas and the surrounding soil. Additionally, nutrients are supplied directly to the rootzone at the exact quantities needed, at an ideal pH. Some hydroponic production of hops is being conducted in Armenia (Tadevosyan et al. 2008). Their research, which is focused on finding ideal cultivars for hydroponic production in the region, has shown that hydroponic production is viable for hops. Their research has also found that hop plants grow similarly in hydroponic systems compared to field production. The experiments relied on Davtyan solution (Davtyan, 1980), which is less ideal than Hoagland solution for deficiency research, due to the use of non-chelated iron and the restriction of micronutrients until bloom time (Tadevosyan, 2008). Additionally, Davtyan solution has a significantly higher nutrient concentration than Hoagland's solution.

Powdery Mildew Assay

Introduction

Podosphaera macularis (powdery mildew) is an important pest of hops both in the Pacific Northwest and in the eastern US. Infestations can lead to lowered alpha acid production and unusable cones. Alpha acids are important to brewers as they provide bitterness, which helps to balance the sweeter malt flavors in beer. More hop cones are required for producing beer when alpha acids are reduced. In one experiment, for every 1% increase in powdery mildew (PM) infested cones, alpha acids were reduced by 0.33% (Gent et al., 2014).

Importance

In the eastern United States, there are two mating types of PM, while in the PNW, there exists only one mating type. Because of this, there is a quarantine in effect in the PNW to prevent the

introduction of hop plant materials from outside of Idaho, Washington State, and Oregon. The only exception to this rule is kiln dried hops (Gent, 2015).

Powdery mildew requires two mating types to produce cleistothecia, a type of overwintering structure. Due to the presence of both in the eastern United States, PM can persist more easily in the environment, leading to earlier, more aggressive infestations. Additionally, due to both mating types existing in the east, PM may rapidly adapt resistance to fungicides and overcome plant defenses more quickly. Resistant strains of PM have been found in the PNW. These strains have begun to overcome the defenses of the popular cultivar, Cascade, and this is becoming a concern due to the popularity of this cultivar (Wolfenbarger et al., 2016).

Methods

Detached leaf assays are ideal for testing cultivar disease resistance in a controlled environment. They allow for the exclusion of other pests. In addition to field observations, this type of assay can help provide a better understanding of cultivar resistance. This method allows for rapid testing of cultivars.

Detached leaf assays are conducted by placing inoculated plant leaves inside of petri dishes and allowing the pathogen to grow. There are several ways inoculation can be accomplished, such as by placing spores on the leaves using a needle, spraying the leaves with a suspension of the pathogen, using a settling tower to coat the leaves, or by placing infested agar on the leaf (Reifschneider and Boiteux, 1988). Inoculation with a disease pathogen can also be carried using a settling tower, which allows for fast, even distribution of inoculum. Additionally, settling towers mimic the natural spread of disease by air. To extend the life of the plant tissue, the petri dishes can be filled with agar. In some cases, the petri dishes are filled with water or stacked so that the lower petri dish functions as a water reservoir (Quinn and Powell, 1982). Stacking petri dishes in this way not only extends the life of the leaf and allows for a longer disease progression time but can also be used as a way to control humidity to encourage powdery mildew growth. Some questions have been raised about the effectiveness of this type of assay. Ethylene

may build up in petri dishes causing plant diseases to develop differently than they would in the wild (Townley et al., 2001). However, some researchers who have raised questions over the effectiveness of this method still contend that it is a useful and quick method for assessing disease resistance (Warkentin et al., 1995). Mature leaves must be used for this type of assay as young tissue may be more susceptible, making plants appear more susceptible than they actually are. Ideally, plant resistance is tested rapidly using detached leaf assays. On the other hand, attached leaf assays may give a better picture of resistance, but they are more costly and time consuming (Twizeyimana et al., 2007).

Literature Cited

Arnon, D. I., and P. R. Stout. 1939. The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant physiology. 14:2:371.

Aubin, M. P., P. Seguin, A. Vanasse, G.F. Tremblay, A.F. Mustafa, and J.B. Charron. 2015. Industrial hemp response to nitrogen, phosphorus, and potassium fertilization. Crop, Forage & Turfgrass Management, 1:1.

Barnes, J., B. Whipker, I. McCall, and J. Frantz. 2012. Nutrient disorders of 'Evolution' mealy-cup sage. HortTechnology 22:4:502-508.

Barth, H.J., C. Klinke, and C. Schmidt. 1994. The hop atlas: The history and geography of the cultivated plant. Joh. Barth and Sohn, Nuremburg, DE.

Benito, A., I. Romero, N. Domínguez, E. García-Escudero, and I. Martín. 2013. Leaf blade and petiole analysis for nutrient diagnosis in Vitis vinifera L. cv. Garnacha tinta. Australian journal of grape and wine research. 19:2:285-298.

Bryson, G. M., H.A. Mills, D.N. Sasseville, J.B. Jones Jr., and A.V. Barker. 2014. Plant analysis handbook III: A guide to sampling, preparation, analysis, and interpretation and use of agronomic and horticultural crop plant tissue. Micro–macro Publishing Inc., Athens, GA.

Darby, H., L. Calderwood. 2016. Crowning and training: early season hop yard maintenance in the northeast. University of Vermont Extension.

Darby, H. 2011. Fertility guidelines for hops in the northeast. University of Vermont Extension Program.

Darby, H., J. Post, L. Calderwood, J. Cubins, E. Cummings, A. Gupta, S. Lewins, L. Ruhl, and S. Ziegler. 2017. Organic hop variety trial final report. University of Vermont Extension Program

Davtyan, G.S. 1980. Hydroponics. p. 352-365. Reference book on chemicalisation of agriculture. Moscow.

Eyres, G. and J.P. Dufour. 2008. Hop essential oil: Analysis, chemical composition and odor characteristics. p. 239-254. Beer in health and disease prevention. Academic Press. Cambridge, MA.

Fernández-Escobar, R., M. Guerreiro, M. Benlloch, and M. Benlloch-González. 2016. Symptoms of nutrient deficiencies in young olive trees and leaf nutrient concentration at which such symptoms appear. Scientia Horticulturae. 209:279-285.

Gent, D.H., J.D. Barbour, A.J. Dreves, D.G. James, R. Parker, D.B. Walsh, and S. O'Neal. 2015. Field guide for integrated pest management in hops. A cooperative publication produced by Oregon State University, University of Idaho, USDA-ARS, and Washington State University.

Gent, D. H., G.G. Grove, M.E. Nelson, S.N Wolfenbarger, and J.L. Woods. 2014. Crop damage caused by powdery mildew on hop and its relationship to late season management. Plant pathology. 63:3:625-639.

Gent, D. 2015. Hop quarantine important for hop powdery mildew control, USDA-ARS, Corvallis, Oregon.

Gingrich, G., J. Hart,and N. Christensen. 2000. Hops: fertilizer guide. Oregon State University Extension & Station Communications. FG 79.

Good, A. G., and P.H. Beatty. 2011. Fertilizing nature: a tragedy of excess in the commons. PLoS biology, 9:8.

Griffith, W.K. and L.S. Murphy. 1991. The development of crop production systems using best management practices. Potash & Phosphate Institute. Norcross, GA.

Hoagland, D. R., and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Circular. California agricultural experiment station, 347(2nd edit).

Karabin M, T. Hudcova, L. Jelinek, and P. Dostalek. 2016. Biologically active compounds from hops and prospective for their use. Comprehensive reviews-in food science and food safety. 15:3:542-567.

Kostelecky, T. 2016. Barth-Haas hops companion: Third Edition. John I. Haas Inc. Yakima, WA.

Van Holle, A., A. Van Landschoot, I. Roldán-Ruiz, D. Naudts and D. De Keukeleire. 2017. The brewing value of Amarillo hops (Humulus lupulus L.) grown in northwestern USA: A preliminary study of terroir significance. Journal of the Institute of Brewing, 123:3:312-318.

Jones, Jr., J.B. 1998. Plant nutrition manual. p. 149. Boca Raton, Fla. CRC Press.

Karabín, M., Hudcová, T., Jelínek, L. and Dostálek, P., 2016. Biologically active compounds from hops and prospects for their use. Comprehensive Reviews in Food Science and Food Safety, 15:3:542-567.

Keller, K. R., and R.A. Magee. 1954. The effect of application rates of nitrogen, phosphorus, and potash on some chemical constituents in two varieties of hops. Agronomy Journal. 46:8:388-391.

Kim, H.J. and Li, X., 2016. Effects of phosphorus on shoot and root growth, partitioning, and phosphorus utilization efficiency in lantana. HortScience, 51:8:1001-1009.

Kirkland, K.J. and Beckie, H.J., 1998. Contribution of nitrogen fertilizer placement to weed management in spring wheat (Triticum aestivum). Weed Technology. 12:3:507-514.

Kostelecky, T. 2016. Barth-Haas hops companion: Third Edition. John I. Haas Inc. Yakima, WA.

Mahler, R.L. and McDole, R.E., 2001. Fertilizer placement. University of Idaho, Cooperative Extension Service, Agricultural Experiment Station, College of Agriculture.

Marschner, H. 2012. Marschner's mineral nutrition of higher plants. Academic press. Cambridge, MA.

McCauley, A., Jones, C. and Jacobsen, J., 2011. Plant nutrient functions and deficiency and toxicity symptoms. Nutrient management module. 9:1-16.

McFarland, C.R., Huggins, D.R. and Koenig, R.T., 2015. Soil pH and implications for management: an introduction. Washington State University Extension.

Mengel, K., and E.A. Kirkby. 2001. Principles of plant nutrition. Kluwer Academic Publishers. Dordrecht, NL

Neve, R. A. 2012. Hops. Springer Science & Business Media. Berlin, DE.

Pitchay, D.S. 2003. Impact of 11 elemental nutrient deficiencies on shoot and root growth, and foliar analysis standards of 13 ornamental taxa with emphasis on Ca and B control of root apical meristem development.

Quinn, J.A., and C. Powell. 1982. Effects of temperature, light, and relative humidity on powdery mildew of begonia. Phytopathology. 72:5:480-484.

Reifschneider, F.J., and L.S. Boiteux. 1988. A vacuum-operated settling tower for inoculation of powdery mildew fungi. Phytopathology. 78:11:1463-1465.

Resh, H.M. 2016. Hydroponic food production: a definitive guidebook for the advanced home gardener and the commercial hydroponic grower. CRC Press. Boca Raton, FL.

Roberts, T.L., 2007. Right product, right rate, right time and right place… the foundation of best management practices for fertilizer. Fertilizer best management practices. 29. International Fertilizer Industry Association. Paris, FR.

Seefeldt. S, J. Walworth. University of Alaska Fairbanks. 2016. Plant tissue testing. Crop production and soil management. Cooperative Extension Service. 27 July 2018. <http://cespubs.uaf.edu/index.php/download_file/1025/>.

Self, J.R. 2014. Plant analysis. Colorado State University Extension. Fact Sheet No. 0.116

Siegle, L., and H. Scoggins. 2017. 2016 Virginia hop grower survey: results (United States, Virginia Cooperative Extension, Virginia State University).

Sirrine, R., E. Lizzote, D. Brown, T. O'Brien, A. Leach. 2014. Michigan State University. Estimated cost of producing hops in Michigan. Extension bulletin E3236.

Sirrine, R. 2014. Growing hops. Michigan State University. Extension bulletin E3210.

Tadevosyan, A.H., S.K. Mairapetyan, J.S. Alexanyan, H.M. Galstyan, R.J. Buniatyan, and B.T. Stepanyan. 2008. Introduction, hydroponic growing possibility and productivity of some hop varieties in the Ararat valley. II International Humulus Symposium. 848:141-148.

Takle, B. and D. Cochran. 2017. Evaluating the effects of nitrogen fertilization on hop yield. Farm Progress Reports: Vol. 2016. 1:28. 27 Oct. 2017 https://lib.dr.iastate.edu/farmprogressreports/vol2016/iss1/28

Tisdale, S.L., W.L. Nelson, J.D. Beaton and J.L. Havlin. 1993. Soil Fertility and Fertilizers (5th edn.). p. 408-409. Macmillan Publishing Company. New York, NY

Townley, A., J. Foundling, M. Corsten, and N.A. Pain. 2001. Mycosphaerella fijiensis disease development in leaves on whole plants and in a detached leaf assay. In Caribbean Division Meeting of the American Phytopathological Society. Publication no. P-2002-0119-CRA.

Twizeyimana, M., P.S. Ojiambo, A. Tenkouano, T. Ikotun, and R. Bandyopadhyay. 2007. Rapid screening of Musa species for resistance to black leaf streak using in vitro plantlets in tubes and detached leaves. Plant Dis. 91:308-314.

USDA – United States Department of Agriculture, 2017. Record high hop acreage in northwest region up 6 percent from 2017. 4 November 2017. <https://www.nass.usda.gov/Statistics_by_State/Regional_Office/Northwest/includes/Publications/Hops/HOP06_1_2017.pdf>.

USDA – United States Department of Agriculture, 2016. National hop report - 2016 hop production up 11 percent from last year. 20 June 2017 <http://usda.mannlib.cornell.edu/usda/current/hops/hops-12-16-2016.pdf>.

Virginia ABC. 2017. Annual Report. 20 June 2017. <https://rga.lis.virginia.gov/Published/2017/RD587/PDF>.

Warkentin, T.D., K.Y. Rashid, and R.C. Zimmer. 1995. Effectiveness of a detached leaf assay for determination of the reaction of pea plants to powdery mildew. Canadian journal of plant pathology, 17:1:87-89.

Wolfenbarger, S. N., L.M. Quesada-Ocampo, and D.H. Gent. 2016. Powdery mildew caused by Podosphaera macularis on hop (Humulus lupulus) in North Carolina. Plant Disease, 100:6:1245.

Zhao, D., K.R. Reddy, V.G. Kakani, and V.R. Reddy. 2005. Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. European Journal of Agronomy, 22:4:391-403.

Table 1.1 Cultivars of hops with associated storability rating and standard acid content range.

Cultivar	**Alpha Acid%**	**Beta Acid%**	**Storability**
Alpharoma	5.8-11	2.6-4.8	Very Good
Cascade	4.5-7.0	4.8-7.0	Poor
Centennial	9.5-11.5	3.4-4.5	Fair
Chinook	12.0-14.0	3.0-4.0	Good
Comet	9.4-12.4	3.0-6.1	-
Crystal	4.0-6.0	5.0-6.7	Very Poor
Glacier	3.3-9.7	5.4-9.5	Very Good
Mount Hood	4.0-7.0	5.0-8.0	Poor
Mount Rainier	8.0-10.8	7.6-9.3	Very Good
Nugget	11.5-14.0	3.0-5.0	Excellent
Sorachi Ace	12.0-16.0	6.0-7.0	-
Southern Brewer	8.0-12	2.5-5.0	-
Southern Cross	11.0-14.0	5.0-6.0	Good
Spalt Select	3.0-6.5	2.5-5.0	Good
Tahoma	7.2-8.2	8.5-9.5	Very Good
Ultra	2.0-3.5	3.0-4.5	Very Good
Yakima Gold	8.8-10.5	4.3-5.0	Excellent

(Kostelecky, 2016)

Table 1.2. BMP Principles and definitions.

BMP Guiding Principles	
Right Product	Fertilizer source needs to be matched to soil properties and crop needs. These needs can change seasonally. Nutrient interactions should be taken into account and balanced according to soil analysis and crop needs. Balanced fertilization is of key importance for increasing nutrient use and efficiency.
Right Rate	The amount of fertilizer applied should be matched to the crop needs and growth stage. Excess fertilizer leads to leaching and environmental losses. Under-fertilization results in low yields and poor crop quality. Crop nutrient budgets, realistic yield goals, tissue tests, and plant analysis are valuable planning tools for applying at the right rate. Omission plots, applicator calibration, crop scouting and record keeping are additional BMPs that will help to determine the correct fertilizer rates for application.
Right Time	Nutrients should be made available when needed by the plant and not during dormancy. Nutrients usage is most efficient, when availability is concurrent with crop demand. BMPs important for controlling the timing of nutrient availability are application timing (pre-plant or split applications), controlled release technologies (such as polymer coated fertilizers), stabilizers, and inhibitors.
Right Place	Nutrients should be applied and kept within the area of plant use (root zone). Application method is critical for efficient fertilizer use. Soil properties, specific crop, and cropping system are all important considerations when determining the correct method of fertilizer application. Fertilizer incorporation is often the best option for increasing fertilizer efficiency and keeping it in place. incorporation is usually the best option to keep nutrients in place and increase their efficiency. BMPS such as conservation tillage, buffer strips, cover crops, and irrigation management will keep nutrients in place and accessible for crop uptake. nitrate

(Roberts, 2007 and Griffith and Murphy, 1991)

Table 1.3 Plant nutrients and their functions.

Nutrient	Function	Structure/Location
Nitrogen	Ammonium depresses uptake of K, Ca, and Mg. Nitrate reduces uptake of P and S. Chlorine competes with nitrate reducing its uptake.	Component of multiple necessary organic compounds such as amino acids, proteins, coenzymes, nucleic acids, and chlorophyll
Phosphorus	N:P ratio of 10:1 considered optimum for most crops. Increased Ca increases P uptake. Iron may interfere with absorption due to formation of iron phosphates. High levels induce Zn deficiency symptoms.	Component of multiple important organic compounds such as sugar phosphates, Adenosine Tri-Phosphate, nucleic acids, phospholipids, and some coenzymes
Potassium	Na may replace K in instances where potassium levels are low. In situations with high K levels, plants may consume 2-4 times what they need.	Functions as a coenzyme or activator for many enzymes. High levels required for protein synthesis. Doesn't form stable structural parts of any molecules inside of plant cells.
Sulfur	Sulfur application reduces boron and molybdenum uptake. Additions of S will increase N content of plants.	Part of several organic compounds including amino acids and proteins. The vitamins thiamine and biotin contain sulfur. Coenzyme A also contains Sulfur and is important for the synthesis and oxidation of fatty acids.
Magnesium	K:Mg ratios of 8:1 are optimum for plant growth. High rates of K and Ca can cause plant to become Mg deficient. Mg depresses Mn uptake.	Part of the chlorophyll molecule and is required for activation of some enzymes. It is essential for enzymes involved in ATP bond breaking. Mg is essential for maintaining ribosome structure.
Calcium	Leaf tissue Ca:Mg ratios of 2:1 and K:Ca of 4:1 are optimum for plant growth. Calcium availability is affected by P and Cu. If Cu is deficient, adding P can lead to reduced Ca uptake.	Can precipitate as calcium oxalate crystals which are stored in vacuoles. An important structural component of cell walls in the form of calcium pectate, which holds together primary walls of neighboring cells. Maintains membrane integrity. Can interferes with magnesium's ability to activate enzymes.
Iron	High pH (>7.0) causes poor iron availability. Nitrate applications increase pH leading to poor availability. High P decreases Fe uptake. K increases mobility of Fe and may increase uptake.	Necessary component of chlorophyll synthesis and is an essential building block of the cytochromes. Cytochromes are electron carriers in photosynthesis and respiration. Iron activates some specific enzymes.
Chlorine	High Cl levels inhibit nitrate uptake and vice versa. Increased nitrate fertilization may reduce Cl toxicity.	Required for photosynthesis. Functions as an enzyme activator when producing oxygen from water.

Manganese	Uptake can be increased by nitrate anions. High Calcium leads to manganese deficiency, likely due to associated pH increase. Iron and manganese compete for absorbtion.	Activates enzymes in fatty acid synthesis which are responsible for DNA and RNA creation and the enzyme isocitrate dehydrogenase in the Krebs cycle. Participates in the photosynthetic production of O^2 from H_2O. May be involved in chlorophyll formation.
Boron	Uptake highly affected by pH. High pH reduces availability while low pH enhances it. High soil levels of Ca can reduce B uptake. Application of N while B is low, leads to B deficiency.	Role in plant nutrition is not well understood. May be involved in phloem carbohydrate transport.
Zinc	Ammonium applications tend to increase Zinc content of plants. High P levels reduce zinc absorption. Excessive levels of Zinc inhibit Fe uptake.	Required for the formation of indoleacetic acid. Needed to activate some enzymes such as alcohol dehydrogenase, lactic acid dehydrogenase, glutamic acid dehydrogenase, and carboxypeptidase.
Copper	Excess Cu induces iron deficiency and stunts root growth. Cu deficiency can be induced by addition of N, causing dilution effect. May also be caused by P additions.	Acts as an electron carrier and a component of some enzymes. A component of plastocyanin, which is involved in photosynthesis, polyphenol oxidase, and possibly nitrate reductase. Possibly involved in dinitrogen fixation.
Molybdenum	Strongly adsorbed by Fe and Al oxides. Soils high in these elements may lead to Mo deficiency.	Functions as an electron carrier in the conversion of nitrate to ammonium. Is essential for N^2 fixation.

Table 1.4. Nutrient mobility, sufficiency range, and deficiency symptoms.

Nutrient	Mobility	Sufficiency	Symptoms
Nitrogen	Mobile	1-6%	Poor growth, plants are spindly and undersized, poor root branching, leaves may be undersized and fall off early
Phosphorus	Mobile	0.2-0.5%	Small and stunted plants with rigid upright appearance, leaves may be brown and senesce early, roots may be long and slender, root versus shoot growth is favored (Kim and Lee, 2016)
Potassium	Mobile	1.5-4%	Chlorosis and necrosis in leaf margins and tips, under water stress plants may become flaccid, weak cuticles and poor lignification lead to weak plant stems
Sulphur	Immobile	0.15-0.5%	Growth rate reduced, shoots more affected, plants brittle with thin stems
Magnesium	Mobile	10-50ppm	Interveinal yellowing and chlorosis, in extreme cases intercostal regions become necrotic
Calcium	Immobile	0.5-1.5%	Growing shoot tips become deformed and chlorotic, affected tissues become soft due to dissolution of cells, leaf tissue becomes chlorotic at advanced stages
Iron	Immobile	50-75ppm	Interveinal chlorosis with reticulate pattern, dark green veins contrast against light yellow leaves, leaf growth is reduced as well as root growth
Chlorine	Mobile	50-200ppm	Wilted leaves that are chlorotic with necrosis, leaves turn bronze, roots become stunted and thickened near tip
Manganese	Immobile	0.15%-0.40%	Interveinal chlorosis with small yellow spots on leaves, tissues have small cell volume with thick cell walls, turgor of plants is reduced
Boron	Immobile	1-6ppm (monocots) 20-70ppm (dicots) 80-100ppm (dicots with latex system)	Abnormal or retarded growth in apical shoots, young leaves are wrinkled, thick, and dark green, leaves and stems become brittle, terminal shoot eventually dies
Zinc	Mobile	15-50ppm	Interveinal chlorosis, which may be pale green, yellow, or white. Internodal distance is shortened, shoots may die prematurely, and leaves fall off
Copper	Immobile	3-7ppm	Shortened internodes, leaf tips may be white and twisted
Molybdenum	Mobile	0.15-0.30ppm	Symptoms differ greatly between different species, interveinal mottling and marginal chlorosis, and leaf margins that curl upwards

(Bryson, 2014) (Resh 2012)

Chapter 2: Growth, Yield, and Quality Differ Among Hop Cultivars Grown in Virginia

Abstract

The number of craft breweries in the United State has increased substantially in the past decade, and the mid-Atlantic region is no exception, with an over 400% percent increase in number of breweries in Virginia between 2011 and 2016. As local breweries increase in number, so has the demand for local ingredients. Although the Atlantic Seaboard was historically a hop producing region, currently the vast majority of all hops produced in the U.S. are grown in the Pacific Northwest. To determine how hop cultivars would respond to growing conditions in the mid-Atlantic, thirteen cultivars were evaluated for growth and yield over the 2016 and 2017 growing seasons. Significant differences were observed for plant heights, side arm lengths, number of bines per plant, plant weights, total cone weight, cone weights per bine, and estimated yield per acre among cultivars. Total plant weights ranged from 0.30 kg for Southern Cross to 3.01 kg for Cascade, and 0.91 kg for Centennial to 2.72 kg for Cascade, in 2016 and 2017, respectively. The estimated yield per acre based on 1,000 plants at 8% dry matter ranged from 6.61 lbs. for Mt. Hood to 552.74 lbs. for Crystal in 2016, and 54.00 lbs. for Mt. Hood to 496.22 lbs. for Cascade in 2017. The most vigorous cultivars evaluated still had yields of only 25–30% of those reported from traditional hop producing regions. However, both growth and cone yield overall were significantly higher in the second season (2017), indicating as the plants mature, production may continue to be increasing. Disease, specifically downy mildew, was the greatest factor affecting many of the cultivars evaluated, and despite regular fungicide applications, production was likely impacted. In addition to downy mildew, several pests, and abiotic factors were identified as potentially impacting plant growth and production during this study,

supporting the need for future research into optimizing cultivation practices for hop production in the mid-Atlantic region.

Introduction

Between 2011 and 2016, the number of breweries in Virginia increased from 40 to 164 (Brewers Association, 2016). With the growing number of local craft breweries, interest in providing locally grown ingredients (hops and malted barley) has increased as well. Hops have a farm-gate value of $618 million (USDA, 2017), with the overwhelming majority of production occurring on large farms in the Pacific Northwest (PNW). These large farms benefit from a suitable climate, long summer day length, generations of production knowledge, and economies of scale in production and processing. Once harvested, hops grown in the PNW are dried, pelletized, and are shipped to breweries across the U.S. and the world.

Hops are dioecious plants that produce female inflorescences commonly called cones, which are the primary flavoring and aroma ingredient in beer, imparting the characteristic bitterness and floral, grassy, and earthy aromas, as well as the stability to beer (Keukeleire, 2000; Schonberger and Kostelecky, 2011). Within the cones are lupulin glands, which produce the three main flavoring components: alpha acids, beta acids, and essential oils. Heat during the brewing process results in the isomerization of alpha acids, forming iso-alpha acids, which provides the majority of the bittering flavor to beer. Beta acids on the other hand, provide only minor bittering to the beer, but do serve a role in beer stability (Karabin et al. 2016). Essential oils are responsible for beer aroma. They are highly volatile and only remain in beer from hops added near the end of the boil in the brewing process (Schonberger and Kostelecky, 2011). Due to the importance of these compounds to beer flavoring, selecting the appropriate cultivar, and

maintaining the highest quality through the production and postharvest process are critical for brewers to achieve the desired outcome.

Although the mid-Atlantic was historically a hop production region, currently little information exists on production protocols or variety performance in the Mid-Atlantic, which is a very different and challenging environment compared to traditional growing regions. To evaluate the suitability of hop cultivars for commercial production in the mid-Atlantic region, a research hop yard was constructed in 2015 in Blacksburg, VA, where 13 hop cultivars were evaluated for growth characteristics, yield, and quality during the 2016 and 2017 growing season.

Materials and Methods

Hop Yard Layout

A 0.70-acre tall trellis (5.4m) hop yard was constructed in Aug. – Oct. 2015 at the Urban Horticulture Center, managed by the Virginia Tech, School of Plant and Environmental Sciences (USDA Hardiness Zone 6b, 2100’ elevation, lat. 37°22’N, long. -80°46’W). The soil type at the site is Duffield, which is a well-drained, fine loamy mixture. Propagules were purchased from a commercial nursery as well rooted “field plants” (21 cell tray), and planted on 1.1 m spacing between plants, 3.0 m spacing between cultivars, and 3.6 m spacing between rows. Weed suppression fabric was installed bordering both sides of each row to retain soil moisture and limit weed growth. Within the planted strip, straw mulch was utilized for weed suppression. Two lines of drip irrigation were installed with emitters every 0.6 m, which were offset to deliver both water and liquid fertilizer to the plants every 0.3 m along the row.

Cultivars Evaluated

Thirteen publicly-available hop cultivars were planted in November 2015, including; Alpharoma, Cashmere, Cascade, Centennial, Comet, Crystal, Mt. Hood, Mt. Rainier, Nugget, Sorachi Ace, Southern Cross, Tahoma, and Ultra.

Fertility

The plants received two applications of ammonium sulfate ($(NH_4)_2SO_4$) granular fertilizer, as well as supplemental water-soluble fertilizer (15N-5P-15K Ca +Mg) through the irrigation system each season. Previous reports suggest hops required 100-150 lbs. per acre nitrogen (N) each season (Gingrich et al. 2000). Therefore, 100 lbs. per acre N was applied split evenly between granular applications, with an additional 5–10 lbs. per acre N applied through fertigation each season.

Pest and Disease Control

Pest and disease were controlled following recommendations in the Virginia Cooperative Extension, Pest Management Guide: Horticultural and Forest Crops (Day and Hong, 2018). Insecticides were applied when populations were observed that had the potential to cause economically significant damage. However, due to the potential yield impact from downy mildew and powdery mildew, preventative fungicide was applied on a regular 10 to 14-day schedule, or more often when conditions were favorable for infection.

Weed Control and Vegetative Burn- Down

In addition to the straw mulching within the rows, weeds were controlled using spot treatments of Roundup (Monsanto Company, Saint Louis, MO) during winter dormancy, and

were hand-pulled during the growing season. Once hop bines reached 2-3 m in height, Scythe (Gowan Company, Yuma, AZ) herbicide was applied at labeled rates to control basal shoot growth and weeds.

Training and Harvest

Due to the lack of growth data on hop for this region, all cultivars were trained at the same time, by wrapping two to four bines from each crown clockwise around a single coir sting which was secured to the top wire of the trellis. Previous work by Murphey and Probasco (1996) has shown that alpha acid content in hops peaks when cones reach between 22-24% dry matter. Therefore, when evaluating among cultivars in this study, hops were considered mature and harvest was targeted for cone dry matter ranging between 22-25% as determined by dehydration using a microwave oven following Madden and Darby (2012). At maturity, bines were severed at both the top wire and 1 meter from ground level. Cones were harvested by hand and separated into usable cones, which were characterized by cones with ≤30% damage from insect or disease and that measured ≥2 cm.

Data Collection

At harvest, plant height, mean side arm length, total plant weight, and cone weight was determined.

Quality Analysis

At harvest, 50 g subsample of cones from each cultivar was submitted to the Virginia Tech, Enology Analytical Service Laboratory where it was analyzed for percent moisture and

acid profile (Cohumulone, Humulone, Colupulone, and Lupulone) following ASBC method Hops-4B and Hops-14, respectively.

Experimental Design and Statistical Analysis

Trials were arranged in a randomized complete block design with subsampling. The field is divided into three blocks, with five plants (subsamples) of each cultivar per block. Cultivars are randomized within each block. Significant differences between traits among cultivars within each season were determined by ANOVA (Table 2.1), and mean separations among cultivars were determined by Tukey's HSD when probability was less than or equal to 0.05. All statistical analyses were performed using JMP® version 13.0 (SAS Institute Inc. Cary, NC)

Results

Significant differences were observed among hop cultivars for each of the traits examined during the 2016 and 2017 growing seasons; including plant height, side arm length, number of bines per string, plant weight at harvest, cone weight, cone weight per bine, and the calculated total yield per acre (Tables 2.2–2.4). Although no statistical differences were seen between plants heights between seasons, there were significant interactions between plants heights among cultivars and the growing season (Table 2.1). Mean side arm lengths differed significantly by year, block, cultivar, and all interaction among those factors. Number of bines per plant varied by year, cultivar, and the interaction between cultivars and seasons. Plant weight also varied significantly by year, cultivar, year and cultivar, as well as by the interaction of block and year, and block and cultivar.

During the two growing seasons, mean plant heights ranged from 3.01 m for Sorachi Ace, and 5.30 m for Southern Cross in 2016, and 2.93 for Southern Brewer and 5.17 m for Comet in 2017 (Table 2.2). In general, the mean plant height decreased from 2016 to 2017, with the exception of a few cultivars for which the opposite was true. Side arm lengths ranged from 6.53 cm for Tahoma to 28.7 cm for Crystal, and 12.4 cm for Sorachi Ace to 54.8 cm for Nugget, during the 2016 and 2017 seasons, respectively. Mean number of bines per plant also increased from 2016 to 2017, ranging from 1.40 for Centennial to 2.80 for both Cascade and Southern Cross, and from 1.80 for both Comet and Tahoma to 3.07 for Cascade, in 2016 and 2017, respectively. Overall plant weights ranged from 0.30 kg for Tahoma to 3.01 kg for Cascade in 2016, and 0.91 for Centennial and 2.72 kg for Cascade in 2017.

Mean cone weight per plant varied significantly between seasons, cultivars, and cultivars across seasons (Table 2.3). Cone weight per bine also varied by year, cultivar, and cultivar between years, as well as the interactions of cultivars across blocks within the hop yard. The mean yield calculated on a per acre basis showed similar response, with significant differences between year, cultivar, cultivar across seasons, and cultivars across blocks within the yard. Mean cone fresh weight per plant ranged from 12.00 g for Mt. Hood to 1002.87 g for Crystal, to 97.98 g for Mt. Hood to 900.33 g for Cascade, in 2016 and 2017, respectively (Table 2.4). Although several cultivars did show a decrease in the mean fresh weight of cones produced, the mean weight of cones per plant over all cultivars increased from 2016 to 2017 (298.44 g to 373.07 g, respectively). Cone weights per bine ranged from a mean of 6.77 g for Mt. Hood to 5.26.58 g for Crystal in 2016, and 73.65 g for Mt. Hood to 384.03 g for Comet in 2017. Similarly, the mean cone yield per acre adjusted to 8% moisture and based on a 1000 plants per

acre, ranged from 6.61 lbs. for Mt. Hood to 552.74 lbs for Crystal, and 54.00 lbs. for Mt. Hood and 496.22 lbs. for Cascade, in 2016 and 2017, respectively.

In 2016, only Alpharoma, Cascade, Mt. Rainier, and Southern Cross had alpha acid contents that fell within the published expected ranges for those cultivars, while all other cultivars had lower than expected alpha acid contents (Table 2.5). In 2017, again four cultivars fell within the expected range (Alpharoma, Centennial, Mt. Rainier, and Nugget), while three cultivars (Cascade, Crystal, and Ultra) had higher alpha acid contents than published data, and Comet and Sorachi Ace had alpha acid contents just below the lower published thresholds. Similarly, four cultivars (Alpha aroma, Cascade, Crystal, and Mt. Rainier) had beta acid content within the published ranges in 2016. While in 2017, the beta acid content of six cultivars (Alpharoma, Cascade, Mt. Hood, Mt. Rainier, Nugget, and Southern Brewer) was within the published ranges, and the beta acid content of Centennial and Comet was near the lower threshold of the published ranges.

Discussion

Many of the cultivars had mean plant heights over 4 m in the first year, and there was no statistical difference in plant heights between years. However, for all other traits analyzed (side arm length, number of bines, plant weights, cone weight, cones weight per bine, and overall yield) the second season (2017) had significantly higher values. This may indicate that as the plants matured and the crowns become more established, lateral growth and production were increasing.

A significant effect was observed for side arm length between blocks, years, cultivars, and all interactions among these factors. Upon inspection of the data, some cultivars had

significantly lower side arm lengths in block one in 2016. The hop yard was constructed on a slope (3-5%) with block one being the furthest down slope. Several of the plants in the lower portion of the hop yard did experience erosion following several heavy rain events during the study period, potentially impacting the growth of some of the plants in the lower end of the yard. However, no other growth or production traits measured followed this same pattern.

Additionally, we observed a significant cultivar and block interaction for mean plant weight, cone weight per bine, and overall yield per acre. However, there was no consistent pattern between the location of the plants and the differences between the growth characteristics observed, with the blocks in the most favorable for production varying among cultivar.

Over the two growing seasons, Cascade was the highest yielding cultivar overall at 945 g per plant fresh weight (average over the two seasons), which represents an estimated average production of 520 lbs. per acre, based on 1,000 plants per acre, and cones dried to 8% moisture. In contrast, Mt. Hood only averaged 55 g per plant, or 30.3 lbs. per acre at 8% moisture, in 2017. Although little published literature exists on hop performance in the Eastern US, these results are similar to those from the University of Vermont, which had an average yield for Cascade of 468 lbs. per acre over a 6 year period (Darby et al. 2017). Commercial producers in the Pacific Northwest; however, commonly report yields for Cascade between 1,600-2,000 lbs. per acre (USAHOPS, 2018), or 3-4 times higher than those seen here. However, Virginia has a very different climate and growing conditions compared to the PNW, and lacks the generational knowledge of production practices common in traditional hop producing regions.

In 2016, sixty-nine percent of samples tested from alpha and beta acid content were below expected values based on industry standards developed in the PNW, which indicates the hops had lower bittering potential and therefore of lower quality (Table 2.5). In the second year

(2017), the proportion of samples testing lower than reported ranges decreased to fifty-three percent, with several more samples just outside the lower threshold of reported values. This could indicate that as the plants mature, the quality of the hops (alpha and beta acids) may increase. Additionally, many of the cultivars with the lowest relative acid contents compared to their expected values, were also of the cultivars with the weakest growth, and most severely impacted by disease (Mt. Hood, Sorachi Ace, Southern Brewer, etc.).

Based on our observations and results, as well as comparing our yields to those common in the industry, it's clear that the growth of some of the cultivars were severely limited. There are many abiotic or cultural factors that may have contributed to this reduced plant growth and cone yield, including; inadequate or poorly timed irrigation or fertilizer applications, and/ or late training resulting in incomplete vegetative growth before reproductive growth was initiation, just to name a few. However, the most consistent factors to be observed affecting production were disease and pests. Despite regular fungicide applications, downy mildew was ubiquitous in the hop yard and had a severe impact on the growth of some cultivars. Through careful monitoring and control the severity of downy mildew impacts was constrained, but several of the cultivars never fully recovered from infection, and production was likely impacted. Several of the crowns were also impacted by Fusarium canker late in the 2017 season, killing fully mature bines which already contained cones. The European corn borer was an issue during the 2016 season, and resulted in the death of several mature bines late in the season. Additionally, Japanese beetles were the most detrimental pest in 2017. Although damage was highly localized within the hop yard, substantial injury was seen on individual plants, and was highly cultivar specific, with Sorachi Ace and Alphaaroma being the preferred targets. Therefore, developing a comprehensive IPM strategy before planting is essential for everyone interested in hop production, especially in

the mid-Atlantic region, which typically has high humidity and heavy rain events throughout the summer growing season.

Several hop cultivars have fared well, demonstrating at least moderate disease resistance and yield potential. These indicate promise for commercial production. Other cultivars evaluated have revealed poor disease tolerance, as well as weak growth and yield, and therefore would probably not be well suited for commercial application in Virginia. Of the cultivars evaluated, Cascade, Nugget, Crystal, and Comet have shown the most potential for this region. The yields to date have been relatively modest when compared to established yards in the PNW; however, they are consistent with other reports from the Eastern U.S. As these plants continue to mature and production practices begin to be optimized for the specific growing conditions of the mid-Atlantic region, it is hopeful that yields and quality will continue to increase. However, even after only two growing seasons, it's clear that some cultivars that are more susceptible to disease (specifically downy mildew), are likely not well suited for production in this region, due to the high disease pressure experienced as a result of the climate. Centennial, Mt. Hood, Southern Brewer, and Ultra, had high disease incidence and low yields, and would probably not be recommended for production.

Literature Cited

Brewers Association. 2016. Virginia craft beer sales statistics -2016. 26 September 2017. <https://www.brewersassociation.org/statistics/by-state/?state=VA>.

Darby H, J. Post, L. Calderwood, J. Cubins, E Cummings, A. Gupta, S. Lewins, L. Ruhl, and S. Ziegler. 2017. Organic hop variety trial final report. University of vermont extension. 25 September 2017. <http://www.uvm.edu/extension/cropsoil/wp-content/uploads/2016-Hop-Variety-Trial.pdf .>

Day, E.R. and C. Hong. 2018. Pest management guide: horticultural and forest crops. Virginia Polytechnic Institute. Blacksburg, Virginia. 2018.

Gingrich C, J. Hart, and N. Christensen. 2000. Hops. FG 79, Fertilizerguide. 5 October 2017. <http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/20648/fg79-e.pdf>.

Karabin M, T. Hudcova, L. Jelinek, and P. Dostalek. 2016. Biologically active compounds from hops and prospective for their use. Comprehensive reviews-in food science and food safety. 15:3:542-567.

Keukeleire D. 2000. Fundamentals of beer and hop chemistry. Quimica Nova. 23:1:108-112.

Madden R, and H. Darby. 2012. Hops harvest moisture determination. University of Vermont Extension. 10 October 2017. <http://www.uvm.edu/extension/cropsoil/wp-content/uploads/Hop_harvest_fact_sheet.pdf>.

Murphy JM, and G. Probasco G. 1996. The development of brewing quality characteristics in hops during maturation. Master Brewers Association of America, Technical Quarterly. 33:3:149-158.

Schonberger C, and T. Kostelecky. 2011. 125th anniversary review: the role of hops in brewing. Journal of the Institute of Brewing. 117:3:1-9.

USAHOPS. 2018. Varieties snapshot. 30 August 2018. <https://www.usahops.org/cabinet/data/USAHops_VarietyManual_2018_Web.pdf>.

USDA - United States Department of Agriculture. 2016. National hop report. 10 October 2017. <https://www.usahops.org/img/blog_pdf/75.pdf>.

Table 2.1 Mean squares and their significance for physiological traits for hop (*Humulus lupulus*)

Source	df	Plant Height (m)[z]	Side Arm Length (cm)[z]	# of Bines	Plant Wt. (kg)[z]
Year (Y)	1	0.70	4125.0**	5.11**	3.54**
Block (B)	1	0.99	628.0**	0.72	0.04
Y x B	1	0.13	425.0**	0.13	1.37*
Cultivar (C)	12	10.70**	1038.0**	3.99**	99.5**
C x Y	12	3.47**	422.8**	1.24*	21.42**
C x B	12	0.83	119.7**	0.76	8.17*
C x Y x B	12	1.18	101.5*	0.63	3.58
Error	321	0.77	56.7	0.56	11.47

*,**Indicate significance at 5% and 1%, respectively.

[a]Analyses was conducted on Box –Cox transformed data due to non-normal distribution as determined by data outside a skewness of -0.5 to 0.5, or kurtosis of -3.0 to 3.0.

[b]Means for each parameter with different letters with a single year indicate significant difference ($P \leq 0.05$) based on Tukey's HSD test.

Table 2.2. Mean values for physiological traits for hop (*Humulus lupulus*) cultivars grown at the Virginia Tech Urban Horticulture Center in Blacksburg, VA in 2016 and 2017.

	Plant Height (m)[z]		Side Arm Length (cm)[z]		# of Bines		Plant Wt. (kg)[z]	
Source	2016	2017	2016	2017	2016	2017	2016	2017
Alpharoma	4.25 abcd	4.73 ab	13.2 d	24.3 cd	1.80 bc	2.07 b	1.39 bc	1.33 bcde
Cascade	5.00 a	4.93 a	23.6 abc	31.5 bc	2.80 a	3.07 ab	3.01 a	2.72 a
Centennial	4.35 abcd	4.62 abc	9.8 d	15.1 de	1.40 c	2.40 ab	0.60 de	0.91 de
Comet	4.51 abc	5.17 a	14.2 bcd	20.2 cde	1.60 c	1.80 b	0.92 cd	1.72 abcd
Crystal	4.48 abc	3.87 bcd	28.7 a	26.8 cd	2.00 abc	2.33 ab	2.99 a	2.02 abc
Mt. Hood	3.21 de	3.59 d	7.7 d	22.0 cde	2.07 abc	1.80 b	0.74 cde	0.92 de
Mt. Rainier	3.51 cde	3.64 cd	15.4 abcd	16.2 de	1.60 c	1.93 b	1.07 bcd	0.92 e
Nugget	4.72 ab	4.82 ab	13.9 bcd	54.8 a	1.73 bc	2.07 b	2.08 ab	2.26 ab
Sorachi Ace	3.01 e	3.33 d	16.9 bcd	12.4 e	2.53 ab	2.13 ab	1.26 cd	0.95 e
Southern Brewer	3.71 bcde	2.93 bcd	11.8 bcd	12.7 de	1.64 bc	2.40 ab	0.34 de	1.29 cde
Southern Cross	5.3 a	3.85 bcd	24.6 ab	44.3 ab	2.80 a	2.71 ab	1.30 bc	1.10 cde
Tahoma	4.66 de	4.63 abc	6.5 d	17.6 de	2.07 abc	1.80 b	0.30 e	1.50 bcde
Ultra	3.57 cde	3.68 cd	12.2 cd	15.9 de	1.60 c	2.33 ab	0.64 cde	0.96 de
Means	4.34	4.14	15.4	24.6	1.90	2.18	1.20	1.35

[a]Analyses were conducted on Box –Cox transformed data due to non-normal distribution as determined by data outside a skewness of -0.5 to 0.5, or kurtosis of -3.0 to 3.0.

[b]Means for each parameter with different letters with a single year indicate significant difference ($P \leq 0.05$) based on Tukey's HSD test.

Table 2.3. Mean values of production traits for hop (*Humulus lupulus*) cultivars grown at the Virginia Tech Urban Horticulture Center in Blacksburg, VA in 2016 and 2017.

	Cone Wt. (g)[a,b]		Cone Wt. (g)[a] · bine^{-1}		Yield (lbs. · acre^{-1})[c]	
Source	2016	2017	2016	2017	2016	2017
Alpharoma	168.27 cd[d]	265.17 bcde	93.4 de	142.17 cde	92.74 bc	146.15 de
Cascade	989.67 a	900.33 a	381.28 ab	297.85 abc	545.46 a	496.22 a
Centennial	60.47 cde	177.63 e	47.37 de	83.39 de	33.33 c	97.90 e
Comet	190.07 bcd	566.01 abc	125.30 cde	384.03 a	104.76 bc	311.96 abcd
Crystal	1002.87 a	621.05 abc	526.58 a	255.77 abcd	552.74 a	342.29 abc
Mt. Hood	12.00 e	97.98 e	6.77 e	73.65 de	6.61 c	54.00 e
Mt. Rainier	235.11 bc	253.20 de	154.73 cde	146.70 cde	129.58 bc	139.55 de
Nugget	471.07 b	725.63 ab	285.23 bc	369.92 ab	259.63 b	399.94 ab
Sorachi Ace	456.35 bcd	311.39 cde	182.45 cde	170.05 cde	251.52 b	171.62 cde
Southern Brewer	39.13 cde	312.04 bcde	190.6 de9	113.77 bcde	21.57 c	171.98 bcde
Southern Cross	471.79 ab	237.15 e	184.29 cd	93.41 de	260.03 b	130.71 de
Tahoma	40.13 de	560.64 abcd	19.37 de	322.42 abc	22.12 c	309.00 abcd
Ultra	41.19 cde	156.70 e	32.93 de	65.79 e	22.70 c	86.37 e

[a]Weights of cones are reported on fresh weight basis.

[b]Analyses were conducted on Box –Cox transformed data due to non-normal distribution as determined by data outside a skewness of -0.5 to 0.5, or kurtosis of -3.0 to 3.0.

[c]Yield is calculated by converting the cone fresh weight (g) to 8% moisture based on an estimated 75% moisture at harvest, and 1,000 plants per acre.

[d]Means for each parameter with different letters within a single year indicate significant difference ($P \leq 0.05$) based on Tukey's HSD test.

Table 2.4. Mean squares and their significance for physiological traits for hop (*Humulus lupulus*)

Source	df	Cone Wt. (g)[a]	Cone Wt. (g)[a]bine^{-1}	Yield (lbs. · acre^{-1})[b]
Year (Y)	1	632101**	111139*	2811634**
Block (B)	1	40677	4382	650385
Y x B	1	1021	25212	71623
Cultivar (C)	12	505026**	393235**	10540342**
C x Y	12	127083**	156156**	2196211**
C x B	12	28920	48045*	1046566**
C x Y x B	12	15918	12812	496622
Error	321	18458	21456	375989

*,**Indicate significance at 5% and 1%, respectively.

[a]Weights of cones are reported on fresh weight basis.

[b]Analyses were conducted on Box –Cox transformed data due to non-normal distribution as determined by data outside a skewness of -0.5 to 0.5, or kurtosis of -3.0 to 3.0.

[c]Yield is calculated by converting the cone fresh weight (g) to 8% moisture based on an estimated 75% moisture at harvest, and 1,000 plants per acre.

[d]Means for each parameter with different letters within a single year indicate significant difference ($P \leq 0.05$) based on Tukey's HSD test.

Table 2.5. Comparison of alpha and beta acid contents among cultivars over the two-year study period to standard ranges published by the hops industry.

	Alpha Acids (%, dry weight)			Beta Acids (%, dry weight)		
Cultivar	**2016**	**2017**	**Expected Range[a]**	**2016**	**2017**	**Expected Range[a]**
Alpharoma	8.18[b]	9.83	5.8-10.9	2.73	3.61	2.4-4.8
Cascade	5.77	7.70	4.5-7.0	5.37	5.75	4.8-7.0
Centennial	8.58	10.78	9.5-11	2.90	3.43	3.5-4.5
Comet	8.90	10.21	11.3	2.91	3.67	4.6
Crystal	1.92	4.67	3.5-4.5	5.01	3.56	4.5-6.5
Mt. Hood	2.62	3.44	4.0-7.0	4.34	5.41	5.0-8.0
Mt. Rainier	4.47	6.23	3.0-8.0	6.38	6.87	5.0-8.0
Nugget	9.07	11.61	11.5-14.0	3.66	4.65	4.2-5.8
Sorachi Ace	8.25	12.87	13.0-16.0	3.03	6.56	8.8-9.9
Southern Brewer	7.42	5.04	9.0-10.5	2.3	2.78	2.8-5.0
Southern Cross	11.34	9.77	11.0-14.0	4.16	3.59	6.0-7.0
Tahoma	3.40	4.44	7.2-8.2	4.34	5.13	8.5-9.5
Ultra	1.50	4.87	2.0-3.5	0.85	2.00	3.0-4.5

[a]Expected ranges for alpha and beta acid content are based on industry reports (USAHOPS, 2018).

[b]Hop alpha and beta acid content were determined based on a composite sample collected at harvest and are reported on dry weight basis.

Chapter 3: Characterization of Macronutrient Deficiencies in Hops (*Humulus lupulus* L.)

Abstract

Hops (*Humulus lupulus* L.) are a dioecious rhizomatous perennial plant grown for their female flowers. The flowers are used mainly for brewing beer but also have herbal and medicinal uses. Existing descriptions and photographs of deficiency symptomology for macronutrients in hops are outdated, and deficiencies are poorly understood. For economical production of hops, growers must be able to recognize and define nutrient deficiencies. The goal of this experiment was to induce, characterize, and photograph nutrient deficiencies in hops. 'Cascade' hop plants were grown suspended in Hoagland solutions with either nitrogen (N), phosphorous (P), or potassium (K) excluded. The control treatment was a complete modified Hoagland's all-nitrate solution. Two experiments were conducted in greenhouses with the first occurring in fall, and the second occurring in the spring of the following year. Plant tissue analysis was conducted when deficiency symptoms were first observed (incipient deficiency) and once plant growth had halted. In experiment one, deficiency symptoms were observed at the following mean nutrient levels: N (2.88%), P (0.254%), and K (1.22%). In experiment two, deficiency symptoms were observed at the following mean levels: N (3.48%), P (0.360%), and K (1.20%). Nitrogen-deficient plants exhibited chlorosis, red petioles, and low plant mass. Nitrogen deficiency in hop plants was associated with lowered uptake of K, calcium (Ca), magnesium (Mg), and sulfur (S), but with increased molybdenum (Mo). Phosphorus-deficient plants exhibited necrotic spots, undersized leaves, and cupping. In both experiments, plants low in P experienced an increased root:shoot ratio during the experiment. This was likely due to nutrient seeking by the plant and resulted in prolific root growth when compared to the other treatments. Potassium deficient plants exhibited blue veins, marginal scorch, and rounded leaf tips. Potassium deficiency was linked to an uptake in Mg and P in both experiments. The information gathered in this study will reinforce current nutritional knowledge and will help hop growers understand how some nutrients may limit the uptake of others.

Currently, most hop production is restricted to the Pacific Northwest (PNW) due to long day length and regional Mediterranean or arid conditions that occur in the Willamette Valley or eastern Washington, respectively, resulting in low disease pressure. In total, PNW hop production covers 21,562 hectares with an annual value of production reaching $618 million (USDA, 2017). Hops are quickly becoming a crop of interest outside the PNW, driven by the increasing popularity of local craft beer. There are now 226 breweries in Virginia, many of which are interested in using locally produced hops (Virginia ABC, 2017). In 2017, there were 12 hectares of hops under production in the state of Virginia (Siegle and Scoggins, 2017).

Cones are the economically important female flowers of the dioecious perennial crop *Humulus lupulus* L. (Sirrine, 2014); produced on side-arms that extend from bines or twining stems (Darby and Calderwood, 2016). Cones are used predominantly for brewing beer. In addition to beer production, hops are also used as decoration and in herbal remedies and medicines. Hop cones produce what are known as lupulin glands, which contain essential oils, as well as alpha and beta acids and used as a flavoring and stabilizing ingredient in beer. The proportions of these components are what give each cultivar their characteristic smell, taste, and preservative effects (Eyres and Dufour, 2008).

Adequate fertilizer application is important for plant vigor, yield, and resistance to pests and pathogens. Excessive fertilization can lead to environmentally damaging runoff (Good and Beatty, 2011). Fertilization Best Management Practices (BMPs) involve using the proper rates, timing, source, and placement to reduce and minimize environmental impact while maximizing agricultural production (Roberts, 2007). Fertilizer costs for hops are estimated to be approximately $618 per hectare, which represents about 8% of the input costs in the first year (Sirrine et al., 2014).

Lack of availability or under-application of mineral nutrients, can cause decreased cone production with lower essential oils content (Keller and Magee, 1954) that decreases value and requires greater quantities for brewing. Fertilization rates should be based on soil and leaf tissue analysis. Soil pH

and immobile mineral nutrients (e.g. phosphorus) should be properly adjusted pre-plant based on regionally appropriate soil analysis; while mobile elements such as nitrogen should be applied during season, based on plant response at the correct growth stage. The addition of partially mobile mineral nutrients (e.g. potassium) should be applied at the farmers discretion taking into account soil type. Soil mobile or partially mobile mineral nutrients should also be efficiently placed so that it is readily available for the plant. Conventional broadcast fertilizer application is inherently less efficient than applying at the root zone (Mahler and McDole, 2001); therefore, producers should side-dress or fertigate nitrogen or potassium; whereas, phosphorus may need to be a knifed-in (i.e. incorporated) band if becoming deficient over the perennial crops life.

Little specific information exists as to nutrient requirements for hops with producers using dated, non-evidence based recommendations repeatedly disseminated by word of mouth and publications. Gingrich et al. (2000)., recommended 112 to 168 $kg \cdot ha^{-1}$ of N annually depending on age and cultivar of hop plant. Whole plant yields of 1121 $kg \cdot ha^{-1}$ can remove between 89.7 and 101 $kg \cdot ha^{-1}$ of N (Darby, 2011). Recently, Takle and Cochrane (2017) reported yield increases occur when N exceeds 168 $kg \cdot ha^{-1}$ N, with diminishing return observed at N rates greater than 224 $kg \cdot ha^{-1}$. However, a 252% increase in hop cone yield was observed when 336 $kg \cdot ha^{-1}$of N was applied versus no supplemental N regardless of N sources that included granular urea, granular calcium nitrate, and liquid urea ammonium nitrate (Takle and Cochrane, 2017). Nutrient fertilizers were applied as split applications with the first application at bine training and the second when bines had reached 2.74 m. Hop plants require significantly less additional phosphorus (P) at only 67.3-112 $kg \cdot ha^{-1}$ when soil concentrations are 0-30 $mg \cdot kg^{-1}$, 0-67.3 $kg \cdot ha^{-1}$ at 31-60 $mg \cdot kg^{-1}$ and no required additions when soil concentrations are over 60 $mg \cdot kg^{-1}$ (Gingrich et al., 2000). For comparison, hemp (*Cannabis sativa*), another member of the Cannabaceae, has been shown to remove similar amounts of nutrients. It can remove up to 81 $kg \cdot ha^{-1}$ of N, 19 $kg \cdot ha^{-1}$ of P, and 85 $kg \cdot ha^{-1}$ K. Recent research suggests that hemp, like hops, may be able to take up significantly more N

when available (Aubin et al., 2015). In soils with adequate P levels, neither hemp nor hop production is increased by P additions.

Excessive application of N can worsen some pest and disease problems. Hop aphid is a pest of both leaf and cone tissues that can be especially damaging late in the season. Late season aphid infestations can be problematic to treat due to pre-harvest intervals that prevent the application of certain pesticides. Over-application of N can cause plants to produce succulent growth, resulting in an aphid population explosion (Gent et al., 2015). The incidence of diseases such as verticillium wilt can be worsened with the over-application of N (Neve, 2012). Additionally, sclerotinia wilt, powdery mildew, and spider mite problems can also be linked to the over-application of N (Gent et al., 2015). Certain diseases can also be linked to nitrogen form, with fusarium canker favoring ammonium-based fertilizers and verticillium wilt favoring nitrate-based fertilizers.

Nutrient deficiencies can express similar visual symptomology or overlap chronologically (Self, 2014). Foliar analysis provides valuable information for troubleshooting mineral nutrition problems, particularly when combined with visual symptoms and soil testing, that provide needed insights to respond before symptoms appear (Bryson et al., 2014). Furthermore, routine foliar analysis can be advantageous in a perennial crop such as hops in which secondary metabolites are of economic importance, hop quality, and subsequent resulting beer flavor profile. Petiole mineral nutrient analysis may be an appropriate, preferred method for highly water-soluble nutrients (ie. N and P); however, it has been reported the use of petioles can produce variable, inconsistent results that is not best for assessing overall plant nutrition (Seefeldt and Walworth, 2016; Benito et al., 2013).

Complete Hoagland's solution provides all essential macro- and micro-nutrients needed for plant growth (Hoagland and Arnon, 1950) and is routinely used in plant nutrient deficiency studies across crop types by modifying the solutions salts by removal of a single element. Several approaches are used to bathe crop roots in Hoagland solution. Recently, Olive cuttings grown in sand and perlite have been drip-

irrigated with deionized water and only periodically fed with a nutrient solutions (Fernández-Escobar et al., 2016); mealy-cup sage rooted in sand and drip irrigated periodically with nutrient solutions (Barnes et al., 2012); sand anchored sorghum was continually irrigated with nutrient solutions (Zhao et al., 2005). Pitchay (2003) compared substrates with and without sand when he conducted all nitrate, modified Hoagland-based deficiency experiments. Sand substrates offered the benefit of anchoring plants, maintenance of soil-like moisture, and a reduction in the amount of solution required; however, sand drawbacks included increased system complexity, need for timed irrigation, and the potential for mineral nutrient contamination.

The objective of this research was to document visual symptoms with associated leaf tissue analysis of N, P, and K at or before first occurrence and when severe to aid producers in cultural management decisions of macro-nutrients in hops. The leaf tissue nutrient concentration means at which these visual symptoms appeared are also important as they will help to complement current sufficiency range information.

Materials and Methods

Humulus lupulus 'Cascade' plugs were grown in independent hydroponic units within climate controlled glass glazed greenhouse at Virginia Tech, Blacksburg, VA (N 37° 13' 12" W 80° 25' 12") to document mineral nutrient deficiencies. The cultivar 'Cascade' was chosen due to widespread commercial use and resultant economic importance. Rooting media was removed via washing with water from rooted cuttings from 105 cell trays (2.5 cm x 2.5 cm x 4 cm; Sandy Ridge farms, Zeeland, MI) that were supplied with 18N-2P-18K continuous feed fertilizer (Jack's Professional Boosted Base FeED, J.R. Peters, Inc., Allentown, PA) Cuttings were selected for uniformity and then pruned back to two nodes two weeks after being placed in the hydroponic system.

The hydroponic system consisted of an air pump attached to a Polyvinyl Chloride (PVC) pipe header to supply air via tubing to air stones placed in the bottom of 3.79 L buckets with a 16.4 cm bottom

outer diameter, 19.3 cm top outer diameter, and 19.1 cm height. (Leitica, Smart Seal, Rochester, MI) . The air pump (RBAT-1/4, JEHM Co., Inc., Lambertville, NJ) connected to two headers made of PVC, one for each bench. The headers were constructed of nominal pipe size NPS 1 ½ inch PVC. These had 0.64 cm holes drilled into them, which were tapped using a 1/16 inch 27 NPT tap. Valves were threaded into these holes. Air tubing was connected to these valves and run to individual buckets (Fig. 3.1). Inside the buckets, tubes were connected to air stones (Penn-Plax, AS6, 1 cm diameter, Hauppauge, NY). Each experimental unit consisted of a gray one-gallon bucket. This color was selected for its light blocking properties and to discourage the growth of algae. The lids of the buckets had one small hole drilled into the center so that air tubing could be passed through, with six larger holes drilled around the outer edge (Fig. 3.2). These large holes were used for suspending the plants in solution. Flexible polyurethane foam cut into 3.8 cm x 10.2 cm x 0.6 cm strips was used to suspend plants in solution. Plant stems were wrapped in foam and secured into the individual holes. Four plants were suspended in each bucket. Once plants had reached a height of approximately 8 cm, they were trained on strings that were suspended above the grow tables (Fig. 3.3).

All liners were established in a 6N-4P-17K complete hydroponic fertilizer (Jack's Oasis, J.R. Peters, Inc., Allentown, PA) in their individual hydroponic system. Hydroponic solution was transitioned from electrical conductivity (EC) of 1.18 to 1.30 $dS \cdot m^{-1} \pm 0.05$, while a pH of 6.0 ±0.2 was maintained. At 36 days, buckets were scrubbed and sanitized with Zerotol (BioSafe Systems, East Hartford CT) at a rate of 1:300 before being triple rinsed with deionized water and filled with complete and incomplete Hoagland nutrient solutions (Tables 2.1) using 18 megohm deionized water (US Water Systems, Indianapolis IN) indicating experiment initiation. For example, the -N solution had no N source available but was otherwise complete. Incomplete nutrient solutions were mixed and stored in 38 L containers. The complete, control solution was mixed and stored in a 114 L container. Experimental units were checked daily and brought to volume as needed. The entire solution was replaced weekly. The pH

of each hydroponic unit was adjusted as needed using 1M sodium hydroxide (NaOH) and 0.2M sulfuric acid (H_2SO_4).

The first (1) experiment was initiated on 7 July 2017 and completed 25 Sep. 2017 for a total of 76 days. The plants were pruned back to two nodes on 24th July 2017. The temperatures in the greenhouse reached an average high of 27.3°C and a low of 19.4°C. The second (2) experiment was initiated on 29 Mar. 2018 and severe deficiency harvest was completed 69 days later on 6 June 2018. Plants were pruned back to two nodes on 11 Apr. 2018. Four 400-watt high-pressure sodium lights were used during establishment due to low/short daily light integral. Lights were turned off on 1 May 2018 to avoid burning plants that had grown closer to the light. During the course of this experiment, temperatures in the greenhouse reached an average high of 28.2°C and a low of 18.3°C.

Both experiments were set up as a completely randomized design with a total of 30 individual aerated hydroponic units (buckets) utilized in each experiment. Treatments consisted of 12 control buckets and six buckets for each deficiency treatments (Fig. 3.4). Plants were destructively harvested at two points in time. The first harvest was randomly selected by researcher, in part based on the appearance of physical deficiency symptoms, but also timely enough to capture foliar mineral nutrient concentrations prior to nutrient deficiency resulted in altering plant physiology. Herein this will be referred to as 'incipient' stress. The second harvest took place when severe deficiency was apparent; confirmed by measuring plant height repeatedly to determine that vertical growth had stopped. Each harvest consisted of three randomly selected units from the treatment total of six. For each treatment harvest, two control units were also harvested. Each unit was then photographed, measured for height, and separated into root and shoot sections for drying. Newly opened, fully expanded leaves were then selected for tissue analysis. Roots and shoots were dried at 65°C and then weighed.

Plant weights and tissue analysis data were analyzed using REML and Tukey's HSD tests at $P \leq 0.05$ (JMP PRO 14, SAS Institute Inc.) with the experiment year treated as a random variable. Data

that were not normally distributed were transformed using a Box-Cox transformation. For nutrient concentrations this included nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn). Root weight, shoot weight, and ratio were transformed in the same manner. Root:shoot, plant height, and mass were evaluated in the same manner.

Results and Discussion

Nitrogen Deficiency

Physical Symptoms. Nitrogen deficient plants exhibited chlorotic leaves, reduced plant size, small leaves, and red petioles (Fig. 3.5-3.7). At the incipient stage, nitrogen deficient plant mass was 27% less than that of control plants. At the severe stage, it was 65% less. Similar symptoms were observed in previous studies (Wallace, 1962), which are consistent with nitrogen deficiency symptoms for many crops. McCauley et al. (2011) noted that nitrogen deficiencies result in general chlorosis, stunted growth, and necrosis of old leaves. This leads to crops that mature prematurely and have low yield (Jones, 1998). Nitrogen deficiencies result in reduced photosynthetic activity due to the loss of proteins and chlorophyll (Bryson et al., 2014). This affects the overall plant growth but also the assimilation of other nutrients.

Tissue concentration. Incipient deficiency symptoms were first observed at a mean of 3.18% dry weight in leaf tissue samples. This is comparable to the lower end of currently known sufficiency ranges, where nutrients are deficient. At incipient harvest, N deficient plant foliar K, Ca, Mg, and S concentrations were lower when compared to the control (Table 2.2) and micronutrients including B, Mn, Mo, and Zn were greater in the presence of N. At the second harvest when severe deficiency occurred, macronutrients K, Ca, Mg and S concentrations were again lower when roots were bathed in an incomplete Hoagland solution (-N) versus complete solution (+N); whereas only Mn and Mo concentration were greater in the -N treatment than in control (+N). These trends may be partially explained by the effect of pH on nutrient solubility. Potassium, Ca, Mg, and S are less soluble in a lower pH environment; although, B, Mn, and Zn are readily available at a low pH (McFarland et al., 2015 and

Truog, 1947). The pH of the N deficient solution had a tendency to become more acidic than in the other treatments that may have impacted mineral nutrient uptake. This acidic solution could have altered the availability of nutrients like K. Molybdenum was the only nutrient whose increase or decrease could not be explained by a low pH. There is evidence to suggest that Mo uptake under acidic conditions is enhanced by the increase of available P. While P concentrations were not significantly higher in this treatment than the control, they were higher at both the incipient and severe stage. This may indicate that more P was available to the plants and, therefore, more Mo was also available.

Root:Shoot. At incipient deficiency, root:shoot was 0.38 for hops receiving no N which indicated that shoot growth had begun to slow before leaves exhibited obvious visual symptoms (Table 2.3). Root weight of hop plants grown in absence of N remained comparable to the control. Root:shoot in –N Hoagland solution remained 0.37 which was only observed to be lower in the -P treatment. Plant height and overall growth was limited on N deficient plants indicating that plant growth can be significantly limited before N deficiency symptoms are apparent on individual leaves as suspected due to its ability to be remobilized within the plant.

Phosphorus Deficiency

Physical Symptoms. Phosphorus deficient plants exhibited chlorosis, undersized leaves, necrotic spots, and cupping in upper leaves (Fig. 3.5-3.7). Out of the three deficiency treatments, this one differed symptomatically from previous reports of P deficiency. Wallace (1962) noted small, pale green leaves, with red tinted petioles on hop leaves. Our experiment did not produce red petioles, and the experiments conducted by Wallace (1962) did not produce plants with necrotic spots. Leaf cupping can be the result of reduced leaf expansion and is a symptom often associated with P deficiency (McCauley et al., 2011).

Tissue concentration. Foliar P concentration of 0.307% dry weight was observed in -P treatment at the first, incipient harvest. (Table 2.2). This is comparable to the lower end of currently known leaf

tissue sufficiency ranges. Phosphorus deficiency did not have any significant association with increased or decreased concentrations of nutrients other than P in leaf tissue samples.

Root:Shoot. At incipiency, root:shoot was comparable to the control at 0.254 (Table 2.3). This changed once symptoms were severe, with root:shoot increasing beyond all other treatments to 0.462. Shoot weight was significantly less than that of the control while root mass was similar. This resulted in a high root to shoot ratio. High root to shoot ratios have been experienced with other crops such as lantana (Kim and Li, 2016). Root:shoot ratios in lantana were found to increase logarithmically when P levels were reduced. This was due to reduced shoot growth, while root growth was less inhibited. At adequate P concentration, lantana plants were found to have a ratio of 0.28 while lantana plants with a P solution concentration of 1mg•L had a ratio of 0.35.

Potassium Deficiency

Physical Symptoms. Potassium deficient plants exhibited yellowing towards leaf tips with bluish green veins, leaf cupping, marginal scorch, and leaf tips that were rounded (Figs. 3.5-3.7). Symptoms except for leaf cupping were consistent with those found by Wallace (1962). Potassium deficiencies are often not immediately apparent and may only become noticeable long after plant growth has slowed (McCauley et al., 2011).

Tissue concentration. Incipient deficiency symptoms were observed at a mean of 1.21 % dry weight in leaf tissue samples (Table 2.2). This is above the lower end of currently known sufficiency ranges recorded by Bryson (et al., 2014) from a research plot but below the range for samples taken from a production field. Physical symptoms on plant leaves were not apparent when the plant first began to suffer from deficiencies. This is referred to as “hidden hunger”, and occurs when plants are nutrient deficient but do not show obvious physical symptoms (Tisdale et al., 1993). Potassium deficiencies were associated with a significant increase in Mg and a decrease in Fe at incipient deficiency and severe deficiency. Calcium concentration was increased at incipient and severe deficiency but was only

significant at the severe deficiency level. There are antagonistic effects between K, Ca, and Mg, which affects the concentration of each of these cations with K being the most active. As a result, Ca and Mg levels are rarely high when K is at normal levels. The ratio of K to Ca+Mg tends to be consistent in plant tissue unless affected by a deficiency (Bryson et al., 2014). Variations may also occur at different growth stages, after the addition of lime, and by the source of N. In control plants this ratio was approximately 50%K:40%Ca:10%Mg while in the incipient or severely K deficient plants it was 18%K:62%Ca:20%Mg or 9%K:67%Ca:24%Mg, respectively. This clearly shows that when K is reduced, Ca and Mg concentrations increase.

Root:Shoot. Ratio was comparable to the control at both incipient and severe symptom levels (Table 2.3). Additionally, height and root weight were comparable to the control. Shoot weight was lower when compared to the control.

Summary and Recommendation

Proper diagnosis of nutrient deficiencies is important for the efficient production of crops. This experiment provides important data that can help to diagnose deficiencies in hop. We were able to produce symptom descriptions and images that are valuable tools for hop growers. These images provide trends in coloration, structure of plants, and leaf size that will allow growers to detect nutrient deficiencies and know when to conduct tissue tests. Our tissue analysis results can be used in conjunction with current sufficiency standards to interpret tissue analysis results. This information can be used to better understand hop nutrient uptake and the complexities of nutrient deficiencies. To further this information, additional deficiency experiments should be run for more macro and micronutrients. Boron deficiencies are known to be problematic with hop production and should be considered a priority. Growing hops in a larger hydroponic system for multiple years would also be beneficial for studying the change in long term plant health due to minor deficiencies, as well as its impact on production and quality.

Literature Cited

Aubin, M. P., P. Seguin, A. Vanasse, G.F. Tremblay, A.F. Mustafa, and J.B. Charron. 2015. Industrial hemp response to nitrogen, phosphorus, and potassium fertilization. Crop, Forage & Turfgrass Management, 1:1.

Barnes, J., B. Whipker, I. McCall, and J. Frantz. 2012. Nutrient disorders of 'Evolution' mealy-cup sage. HortTechnology, 22:4, 502-508.

Benito, A., I. Romero, N. Domínguez, E. García-Escudero, and I. Martín. 2013. Leaf blade and petiole analysis for nutrient diagnosis in Vitis vinifera L. cv. Garnacha tinta. Australian journal of grape and wine research, 19:2, 285-298.

Bryson, G. M., H.A. Mills, D.N. Sasseville, J.B. Jones Jr., and A.V. Barker. 2014. Plant analysis handbook III: A guide to sampling, preparation, analysis, and interpretation and use of agronomic and horticultural crop plant tissue. Micro–macro Publishing Inc., Athens, GA.

Darby, H., L. Calderwood. 2016. Crowning and training: early season hop yard maintenance in the northeast. University of Vermont Extension.

Eyres, G. and J.P. Dufour. 2008. Hop essential oil: Analysis, chemical composition and odor characteristics. In Beer in health and disease prevention (pp. 239-254).

Fernández-Escobar, R., M. Guerreiro, M. Benlloch, and M. Benlloch-González. 2016. Symptoms of nutrient deficiencies in young olive trees and leaf nutrient concentration at which such symptoms appear. Scientia Horticulturae, 209:279-285.

Gent, D.H., J.D. Barbour, A.J. Dreves, D.G. James, R. Parker, D.B. Walsh, and S. O'Neal. 2015. Field guide for integrated pest management in hops. A cooperative publication produced by Oregon State University, University of Idaho, USDA-ARS, and Washington State University.

Gingrich, G., J. Hart, and N. Christensen. 2000. Hops: fertilizer guide. Oregon State University Extension & Station Communications. FG 79.

Good, A. G., and P.H. Beatty. 2011. Fertilizing nature: a tragedy of excess in the commons. PLoS biology, 9:8.

Hoagland, D. R., and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Expt. Sta. Res. Bul. 347(2nd edit).

Jones, Jr., J.B. 1998. Plant nutrition manual. p. 149 CRC Press. Boca Raton, Fl.149 p.

Keller, K. R., and R.A. Magee. 1954. The effect of application rates of nitrogen, phosphorus, and potash on some chemical constituents in two varieties of hops 1. Agronomy Journal, 46:8:388-391.

Kim, H.J. and X. Li. 2016. Effects of phosphorus on shoot and root growth, partitioning, and phosphorus utilization efficiency in lantana. HortScience, 51:8:1001-1009.

Mahler, R.L. and R.E. McDole. 2001. Fertilizer placement. University of Idaho, Cooperative Extension Service, Agricultural Experiment Station, College of Agriculture.

McCauley, A., C. Jones and J. Jacobsen. 2011. Plant nutrient functions and deficiency and toxicity symptoms. Nutrient management module. 9:1-16.

McFarland, C.R., D.R. Huggins and R.T. Koenig. 2015. Soil pH and implications for management: an introduction.

Neve, R. A. 2012. Hops. Springer Science & Business Media. Berlin, DE.

Pitchay, D.S. 2003. Impact of 11 elemental nutrient deficiencies on shoot and root growth, and foliar analysis standards of 13 ornamental taxa with emphasis on Ca and B control of root apical meristem development.

Roberts, T.L., 2007. Right product, right rate, right time and right place… the foundation of best management practices for fertilizer. p. 29 Fertilizer best management practices.

Seefeldt. S, J. Walworth. University of Alaska Fairbanks. 2016. Plant tissue testing. Crop production and soil management. Cooperative Extension Service. 27 July 2018. < http://cespubs.uaf.edu/index.php/download_file/1025/>.

Self, J.R. 2014. Plant Analysis. Colorado State University Extension. Fact Sheet No. 0.116

Siegle, L., and H. Scoggins. 2017. 2016 Virginia hop grower survey: results (United States, Virginia Cooperative Extension, Virginia State University).

Sirrine, R., E. Lizzote, D. Brown, T. O'Brien, A. Leach. 2014. Michigan State University. Estimated cost of producing hops in michigan. Extension bulletin E3236.

Sirrine, R. 2014. Growing Hops. Michigan State University. Extension bulletin E3210.

Takle, B. and D. Cochran. 2017. Evaluating the effects of nitrogen fertilization on hop yield. Farm Progress Reports: Vol. 2016 : Iss. 1 , Article 28. 10 July 2018. <https://lib.dr.iastate.edu/farmprogressreports/vol2016/iss1/28>

Tisdale, S.L., W.L. Nelson, J.D. Beaton and J.L. Havlin. 1993. Soil Fertility and Fertilizers (5^{th} edn.). p. 408-409. Macmillan Publishing Company. New York, NY

Truog, E. 1947. Soil reaction influence on availability of plant nutrients. Soil Sci. Soc. Am. J. 11:C: 305–308.

USDA – United States Department of Agriculture, 2017. Record high hop acreage in northwest region up 6 percent from 2017. 4 November 2017 <https://www.nass.usda.gov/Statistics_by_State/Regional_Office/Northwest/includes/Publications/Hops/HOP06_1_2017.pdf>

Virginia ABC. 2017. Annual report. 20 June 2017<https://rga.lis.virginia.gov/Published/2017/RD587/PDF>

Wallace, T., 1962. The diagnosis of mineral deficiencies in plants by visual symptoms. Soil Science, 94:5:350.

Zhao, D., K.R. Reddy, V.G. Kakani, and V.R. Reddy. 2005. Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. European Journal of Agronomy, 22:4:391-403.

Tables and Figures

Table 3.1 Treatment nutrient solutions for inducing nutrient deficiencies. Adapted all nitrate Hoagland solutions (Pitchay, 2003)

Salt	**Molarity**	**Control**	**N**	**P**	**K**
		ml per 30 L final solution			
KNO_3	1M	150	0	150	0
$Ca(NO_3)_2.H_2O$	1M	150	0	150	150
KH_2PO_4	1M	30	30	0	0
$MgSO_4 \cdot 7H_2O$	1M	60	60	60	60
KCl	1M	0	150	30	0
$CaCl_2$	1M	150	150	0	0
$NaNO_3$	1M	0	0	0	150
NaH_2PO_4	1M	0	0	0	60
FeDTPA		30	30	30	30
$MnCl_2 \cdot 4H2O$	10mM	27	27	27	27
$ZnCl_2 \cdot 7H2O$	10mM	4.5	4.5	4.5	4.5
$CuCl \cdot 5H2O$	10mM	4.5	4.5	4.5	4.5
H_3BO_3	100mM	13.5	13.5	13.5	13.5
$Na_2 \cdot 2MoO_4 \cdot 2H_2O$	1mM	3	3	3	3

Table 3.2 Mean tissue nutrient concentration of macronutrients in percentage. Mean tissue nutrient concentration of micronutrients in ppm. Treatments represent control (C, N=6) and deficiency treatments lacking nitrogen (-N, N=3), phosphorus (-P, N=3), and potassium (-K, N=3). Incipient represents plants that were destructively harvested at incipient deficiency, while severe represents plants that were destructively harvested at severe deficiency (plant vertical growth halted). Tukey's HSD used for mean comparison within column. Significance levels are based on comparison with associated control. Example: -N (incipient) with Control. Connecting letters compare between all treatments and controls within the column at incipient or severe levels.

Macronutrient mean tissue nutrient concentration (% dry weight)							**Micronutrient mg•kg^{-1} dry wt**					
Treatment	**N**	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Mo**	**Zn**
Incipient												
C	6.51a	0.892b	4.75a	3.80ab	0.857b	0.379a	42.5c	7.28ab^{NS}	167ab	168b^{NS}	13.3b	42.6b
-N	3.18c*	1.01ab^{NS}	3.62b*	2.14d*	0.636d*	0.301b*	73.0a*	7.17ab^{NS}	254a^{NS}	248a*	32.7a*	58.8a*
-P	6.4a^{NS}	0.307d*	4.51a^{NS}	3.02c*	0.787bc^{NS}	0.35ab^{NS}	45.1bc^{NS}	7.98a^{NS}	110bc^{NS}	140bc^{NS}	12.4bc^{NS}	42.5b^{NS}
-K	6.70a^{NS}	1.05ab^{NS}	1.21d*	4.20a^{NS}	1.37a*	0.345ab^{NS}	53.8abc^{NS}	7.85a^{NS}	87.6cd*	169ab^{NS}	16.6b^{NS}	40.7b^{NS}
Severe												
C	5.43b	0.592c	4.53a	3.20c	0.753bc	0.353ab	61ab	5.7bc^{NS}	109c^{NS}	70.27d^{NS}	8.31c	27.9cd
-N	2.10c*	0.691c^{NS}	2.65c*	1.56d*	0.442e*	0.179c*	60.1ab^{NS}	4.08c^{NS}	118bc^{NS}	141bc*	20.5ab*	35.2bc^{NS}
-P	5.73b^{NS}	0.122e*	4.34a^{NS}	3.13bc^{NS}	0.696cd^{NS}	0.29b^{NS}	84.3a^{NS}	5.88abc^{NS}	113c^{NS}	79.2d^{NS}	5.42d*	25.2d^{NS}
-K	5.75b^{NS}	1.19a*	0.548d*	4.04a*	1.46a*	0.323ab^{NS}	72.5a^{NS}	5.62bc^{NS}	60.8d*	101cd^{NS}	7.85cd^{NS}	29.4cd^{NS}
Sufficiency	3.2-5.6	0.27-0.54	1.6-3.4	1.03-2.5	0.29-0.67	0.2-034	17.6-63.2	8-29	44.3-97.9	45-125	0.5-3	23.2-108

NS,*Nonsignificant or significantly different from the control at P≤0.05

Table 3.3 Treatment mean height, weights, and root:shoot compared. Treatments represent control (C, N=6) and deficiency treatments lacking nitrogen (-N, N=3), phosphorus (-P, N=3), and potassium (-K, N=3). Incipient represents plants that were destructively harvested at incipient deficiency, while severe represents plants that were destructively harvested at severe deficiency (plant vertical growth halted). Tukey's HSD used for mean comparison within column. Significance levels are based on comparison with associated control. Example: -N (incipient) with Control (incipient). Connecting letters compare between all treatments and controls.

Treatment	Height(cm)	Root Weight (g)	Shoot Weight (g)	Root:Shoot
Incipient Symptoms				
Control	80.7b	8.57d	37.2c	0.226b
-N	43.9c*	9.05d^{NS}	24.5d*	0.384a*
-P	71.7b^{NS}	7.71d^{NS}	32.3cd^{NS}	0.254b^{NS}
-K	82.1b^{NS}	8.54cd^{NS}	35.7c^{NS}	0.241b^{NS}
Severe Symptoms				
Control	123.4a	28.35a	114.7a	0.235b
-N	75.8b*	13.4bc*	37.1c*	0.368a*
-P	111.8a^{NS}	34.5a^{NS}	70.6b*	0.462a*
-K	118.7a^{NS}	21.6ab^{NS}	77.8b*	0.264b^{NS}

NS,*, Nonsignificant or significantly different from the control at $P \leq 0.05$

Figure 3.1. Air-stone and bucket connected to header in hydroponic system

Figure 3.2. Buckets connected to header with lids. Prior to plant installation.

Figure 3.3. Plants growing in system after being secured in foam (at four weeks). Plants have been trained on string trellising system.

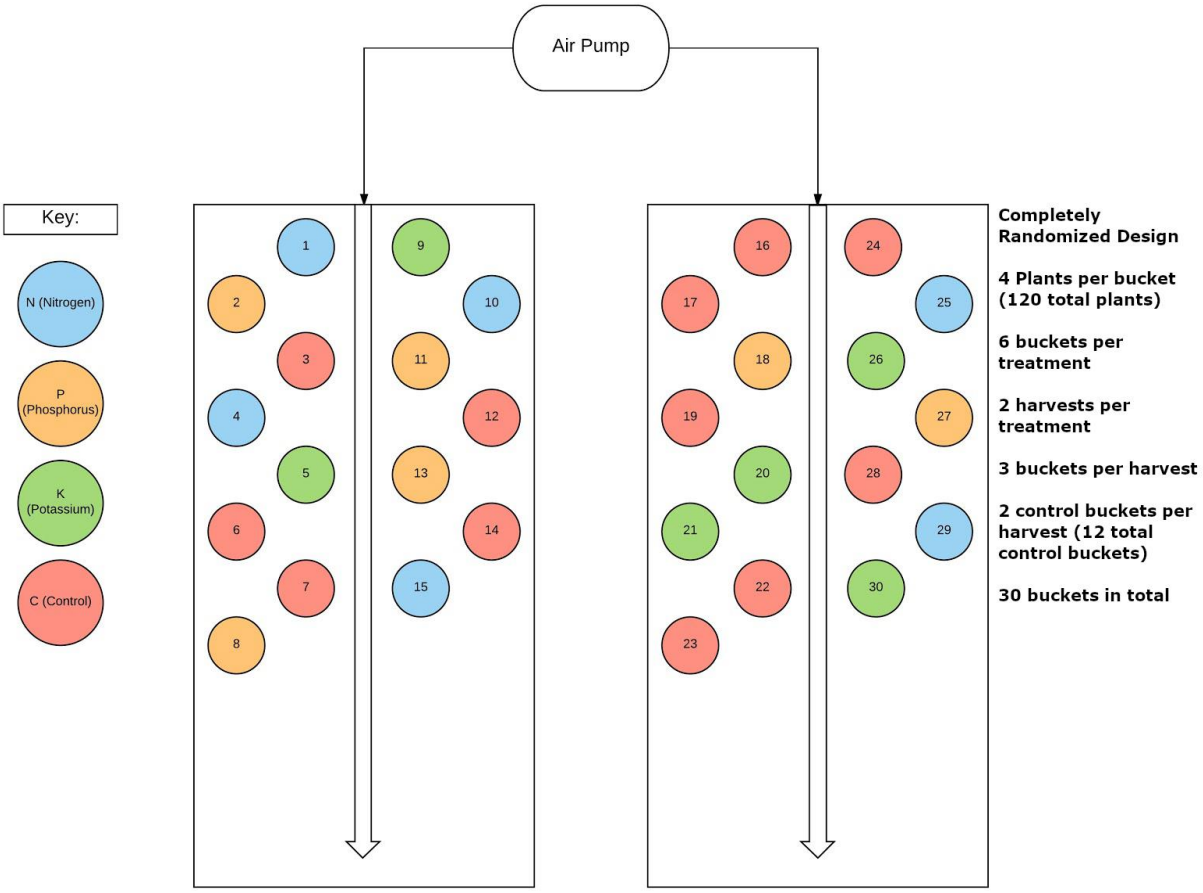

Figure 3.4. Example layout for experiment. Treatments were randomized for each run of experiment.

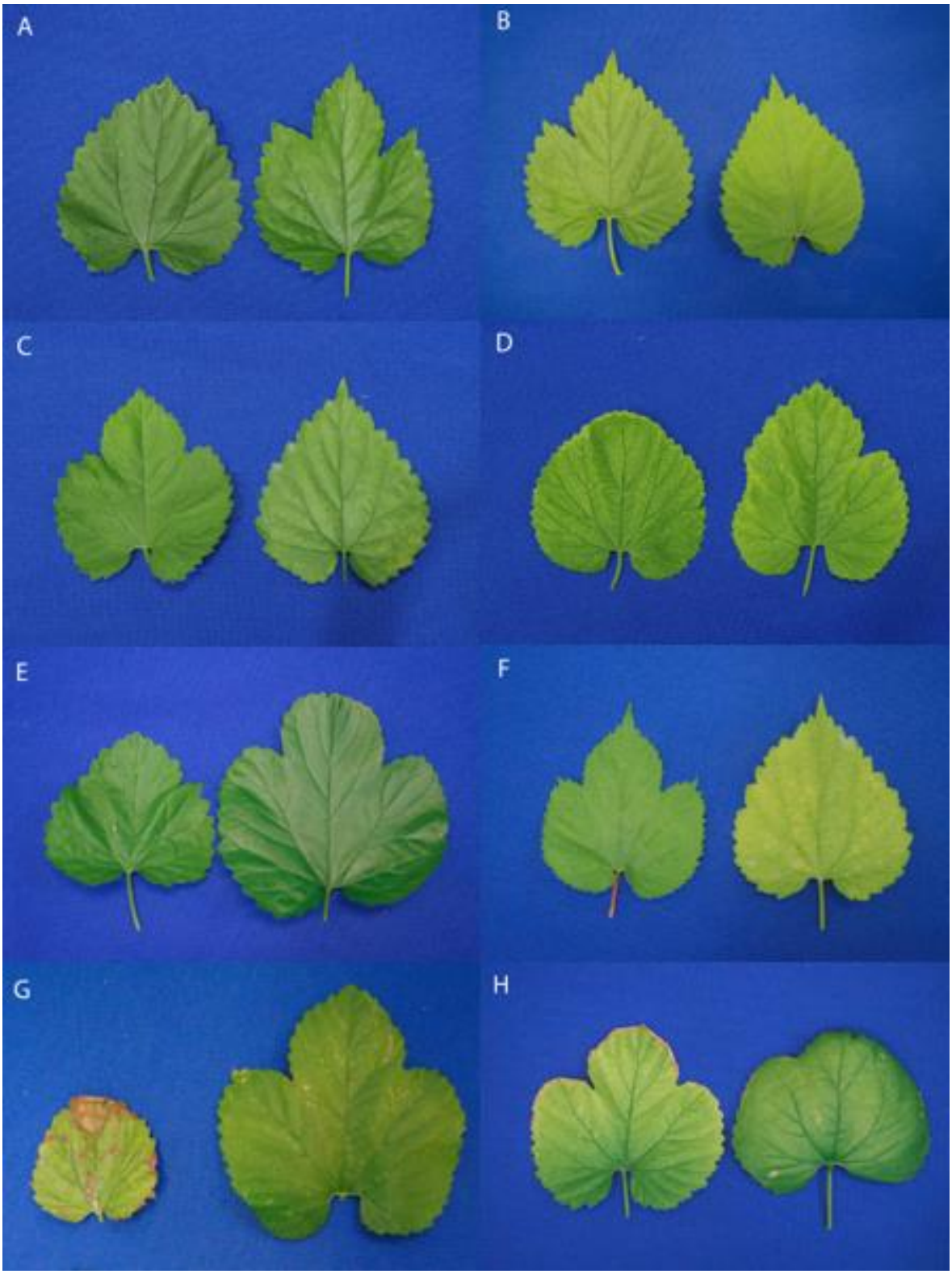

Figure 3.5. Representative leaves from each treatment, showing symptoms. Incipient deficiency: Control (A), Nitrogen (B), Phosphorus (C), Potassium (D). Severe deficiency: Control (E), Nitrogen (F), Phosphorus (G), Nitrogen (H).

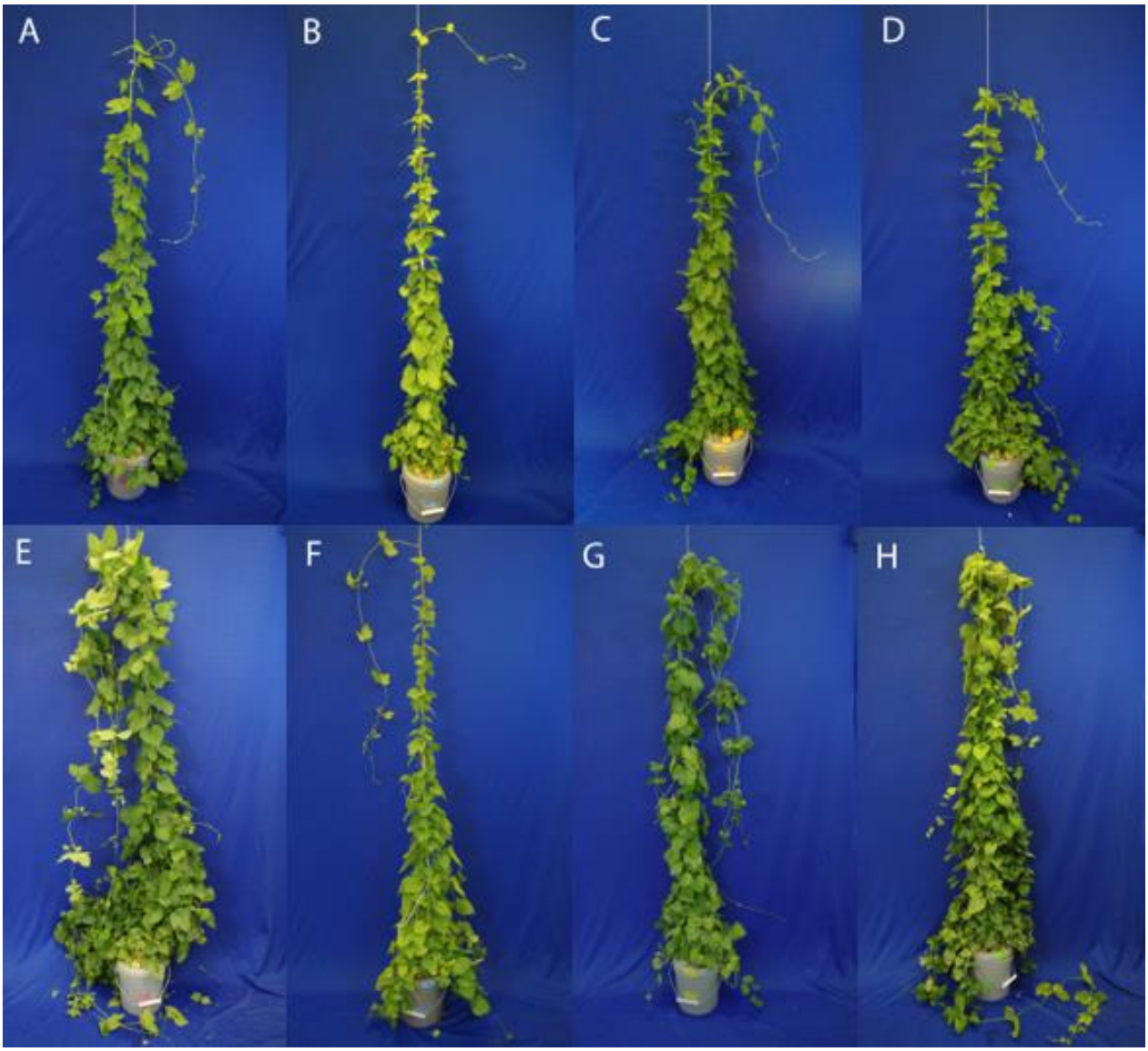

Figure 3.6. Representative plants from each treatment, showing symptoms. Incipient deficiency: Control (A), Nitrogen (B), Phosphorus (C), Potassium (D). Severe deficiency: Control (E), Nitrogen (F), Phosphorus (G), Nitrogen (H).

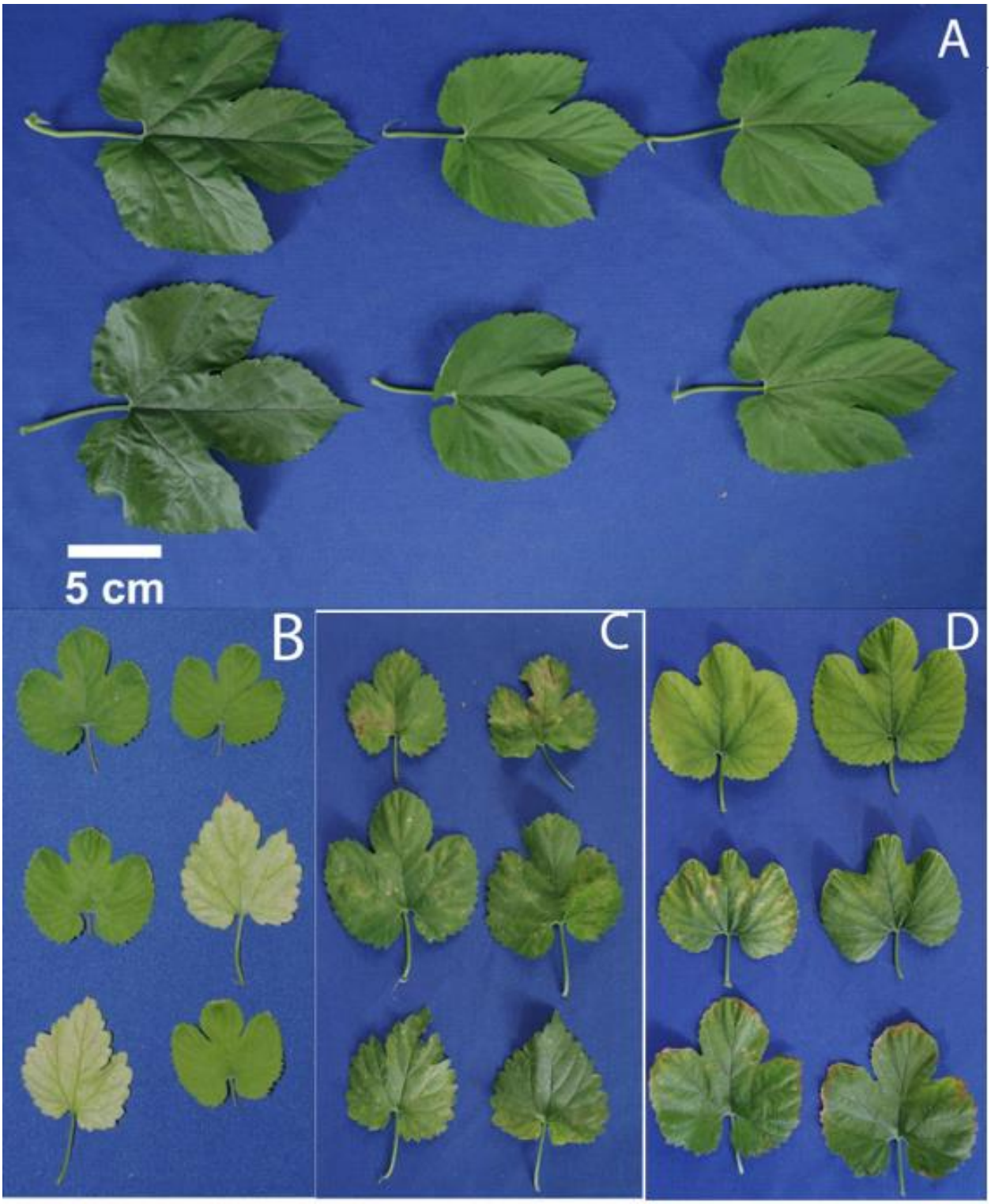

Figure 3.7. Comparison of representative leaves from each treatment at severe harvest. Control (A), nitrogen deficient (B), phosphorus deficient (C), potassium deficient (D). Images adjusted to the same scale for size comparison.

Chapter 4: A Method for Rapid Low Cost Detached Whole-Leaf Powdery Mildew Assay on Hops

Abstract

Powdery Mildew (*Podosphaera macularis*) is an important disease that affects the productivity of hops (*Humulus lupulus* L.). Infection from PM can cause severe damage and even crop failure as a result of reduced plant output and direct damage to cones. The disease typically appears as a white powdery coating on leaves, shoots, buds, and cones (Gent et al., 2015). Cone damage can result in undersized or distorted cones. Extensive damage results in necrotic brown spots on affected plant parts. Hop resistance to PM is an important factor in its control. Detached leaf assays allow for rapid testing of plant resistance to PM. The settling tower, flatbed scanner, and petri dishes are relatively low-cost to build and the software is freely available. Using this method, we were able to find a significant difference in PM leaf coverage between Comet, a hop cultivar known to be resistant to PM, and Tahoma. Tahoma had significantly more PM growth during the course of the experiment.

Introduction

The objective of this experiment is to compare powdery mildew resistance (*Podosphaera macularis*) among five publically-available hop cultivars: Alpharoma, Cascade, Comet, Sorachi Ace, and Tahoma. Comet and Cascade have shown field resistance to powdery mildew and were chosen to represent a known baseline of resistance for comparison between lab and field observations (Cerenak et al., 2009). This information can be important for growers when selecting resistant cultivars, leading to the reduction of fungicide inputs and possible development of resistance to certain chemistries. Plant resistance has been tied specific genes (Wolfenbarger et al., 2014). In many cases, this resistance is monogenic. This makes it easier for PM to overcome plant resistance and has led to PM strains which are adapted to specific resistance genes. The majority of hop production in the United States is currently restricted to the Pacific Northwest (PNW). As such, the majority of cultivar susceptibility data is based on

field observations in that region. Additionally, there are two mating-types of powdery mildew in the Eastern United States, while there is only one type in the PNW (Wolfenbarger et al., 2015). Overwintering structures can be formed in the Eastern United States as well as the possibility for more rapid adaptation to plant defenses and severe outbreaks (Gent et al., 2015).

Materials and Methods

Newly matured leaves were selected from plants of five cultivars of hops, Alpharoma, Cascade, Comet, Sorachi Ace, and Tahoma, at the Virginia Tech hop yard. Leaves were selected based on size and maturity. The selected leaves were excised from plants, placed without folding or bending inside the 100 mm petri dish, and brought back to the lab. Custom petri dish design was based on previous research by Quinn and Powell (1982). The custom petri disheswere constructed by stacking two 100 mm petri dishes, slightly offset, and gluing them together. A soldering iron was used to melt a hole between the two petri dishes. This allowed for the petiole of the leaves to pass through into the lower petri dish which was used as a water reservoir (Fig. 4.1). The reservoir increased humidity and allowed for water uptake through the leaf petiole, maximizing post-harvest life. This petri dish design has been used previously for begonias (Quinn and Powell, 1982), hops (Wolfenbarger et al., 2015), and grapes (Pearson and Gadoury, 1987). A settling tower was used for the inoculation of the leaves (Reifschneider and Boiteux, 1988). This tower (50cm x 50cm x 115cm) was constructed of plywood with a hinged bottom, allowing for the placement of petri dishes in the base (Fig. 4.2). A portable vacuum cleaner was connected to the tower by rubber tubing. For use, the vacuum cleaner is turned on creating suction inside the sealed tower. The top of the tower contains a platform on which the source inoculum is placed (Fig 4.3). Once the inoculum is placed on this platform, the vacuum is turned on, and the plug above the platform is quickly removed. The incoming air rushes past the plant inoculum, distributing the spores onto the plant tissue below. Source inoculum (*Podosphaera macularis*), which contained spores, was obtained from the cultivar Sorachi Ace. These samples were selected from the Virginia Tech hop yard on 17th Aug. 2017. Infested shoots and leaves with 100% visible coverage were chosen.

Inoculation

For each run, samples were randomly placed in a 4x4 grid inside the settling tower (Fig. 4.4). This allowed for 16 samples per run, 3 samples each Cascade, Alpharoma, Tahoma, and Sorachi Ace and 4 samples of Comet. Inoculum was applied to all samples. The process was replicated 6 times, with the petri dishes randomized each time. Inoculated samples were placed inside of a growth chamber (Environmental Growth Chambers, Q12194e, Chagrin Falls Ohio) for two weeks at 23 °C (Fig. 4.5). Lights in the growth chamber were set to a 16-hour light, 8 dark cycle. (4100k temp bulbs, 35 watts, 1650 lumens). The humidity was set to 70% and lower petri dish reservoirs were filled as needed. Plants were kept in chamber for two weeks to allow for PM growth.

Disease Analysis

After two weeks, leaves were removed from the growth chamber and analyzed for percentage of colony coverage. Images of the infested leaves were taken using a camera and a flatbed scanner on a blue background (Fig. 4.6). This allowed for quantification of coverage. Images were analyzed using Fiji, a distribution of Imagej that contains additional plugins (Schindelin et al., 2012). The trainable Weka Segmentation Plugin was used to identify and quantify areas with powdery mildew. This tool is typically used to measure areas of microscopy images that are a specific color (Arganda-Carreras et al., 2017). Coverage was determined by segmenting the image into three colors representing disease, leaf, and background (Fig. 4.7) using the Hoeffding Tree classifier. This classifier is “updateable” meaning that multiple images can be used to train the Weka Segmentation Plugin. A representative image was selected from each cultivar for training. This was done due to leaf coloration differences between cultivars. Each image was then processed using the Weka Plugin and the trained profile. This resulted in a segmented version of each image. Segmented areas were then selected using the versatile wand tool. Wand tool was set to select noncontiguous areas, and value tolerance was set to 40 as it most accurately differentiated among the three regions. The measure tool was used to determine the number of pixels that were

classified as disease and leaf. From this the percent coverage was calculated by adding together the pixel count for both the leaf area and disease area to get the number of pixels for the whole leaf. The disease pixel count was then divided by this area and multiplied by 100 for percentage. An ANOVA for percent mean coverage was done using JMP PRO 14 (SAS Institute Inc.). Treatment means were compared using Tukeys HSD at $p<0.05$.

Results and Discussion

Tahoma was found to be highly susceptible to PM when compared to the other cultivars in this experiment. Out of the cultivars tested, Tahoma had more leaves exhibit symptoms than any other. Additionally, PM colonies covered a mean of 19.5% leaf surface area (Table. 4.1). All other cultivars in this trial had a mean coverage of less than 1%. Comet and Cascade both showed minimal colony spread with PM failing to establish on many of the leaves. There is evidence to suggest that Comet possess R3 and R6 resistance genes (Wolfenbarger et al., 2014). Cascade also shows resistance to PM, although the exact method of its resistance is unknown (Gent et al., 2017). It is possible that the other cultivars may have some level of genetic resistance to PM. It is also possible that the specific PM strain in the Virginia Tech hop yard is adapted to the Tahoma cultivar. The number of colonies and inoculum concentration were not considered in this experiment. It is possible to count the number of individual colonies using other ImageJ plugins and may be of interest in future studies. These cultivars need additional trialing with known virulent strains of *P. macularis*. This is because cultivars may exhibit resistance to one PM strain while failing to resist another. Some virulent strains of PM are able to overcome the resistance of cultivars like Cascade. As PM continues to adapt to resistant cultivars, it becomes even more important to rapidly test cultivars for resistance. This method could be considered a first step in understanding cultivar resistance. It is not only rapid but relatively inexpensive and allows for the testing of multiple strains simultaneously.

Literature Cited

Arganda-Carreras, I., V. Kaynig, C. Rueden, K.W. Eliceiri, J, Schindelin, A, Cardona. and H. Sebastian Seung. 2017. Trainable Weka Segmentation: a machine learning tool for microscopy pixel classification. Bioinformatics. 33:15:2424-2426.

Cerenak, A., D. Kralj and B. Javornik. 2009. Compounds of essential oils as markers of hop resistance (Humulus lupulus) to powdery mildew (Podosphaera macularis). Acta agriculturae Slovenica. 93:3:267.

Gent, D.H., S.T. Massie, M.C. Twomey and S.N. Wolfenbarger. 2017. Adaptation to partial resistance to powdery mildew in the hop cultivar cascade by podosphaera macularis. Plant disease, 101:6:874-881.

Gent, D.H., J.D. Barbour, A.J. Dreves, D.G. James, R. Parker, D.B. Walsh and S. O'Neal. 2015. Field guide for integrated pest management in hops. A cooperative publication produced by Oregon State University, University of Idaho, USDA-ARS, and Washington State University.

Pearson R.C. and D.M. Gadoury. 1987. Cleistothecia, the source of primary inoculum for grape powdery mildew in New York. Phytopathology. 77:11:1509-1514.

Quinn, J. A. and C.C. Powell Jr. 1982. Effects of temperature, light, and relative humidity on powdery mildew of begonia. Phytopathology. 72:5:480-484.

Reifschneider, F. J. and L.S. Boiteux. 1988. A vacuum-operated settling tower for inoculation of powdery mildew fungi. Phytopathology. 78:11:1463-1465.

Schindelin, J., I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid and J.Y. Tinevez. 2012. Fiji: an open-source platform for biological-image analysis. Nature methods. 9:7:676.

Wolfenbarger, S. N., M.C. Twomey, D.M. Gadoury,B.J. Knaus, N.J. Grünwald, and D.H. Gent. 2015. Identification and distribution of mating-type idiomorphs in populations of Podosphaera macularis and development of chasmothecia of the fungus. Plant Pathology. 64:5:1094-1102

Wolfenbarger, S.N., Eck, E.B. and Gent, D.H., 2014. Characterization of resistance to powdery mildew in the hop cultivars Newport and Comet. Plant Health Progress. 15:2:55.

Table 4.1 Mean % powdery mildew coverage on leaves after two weeks in growth chamber.

Cultivar	Mean Coverage %[Z]	# Symptomatic Leaves (n)	Number of Samples
Alpha	0.130 b	3	18
Cascade	0.659 b	6	18
Comet	0.330 b	7	24
Sorachi	0.151 b	3	18
Tahoma	19.500 a	18	18

[Z] Significant at P≤0.05

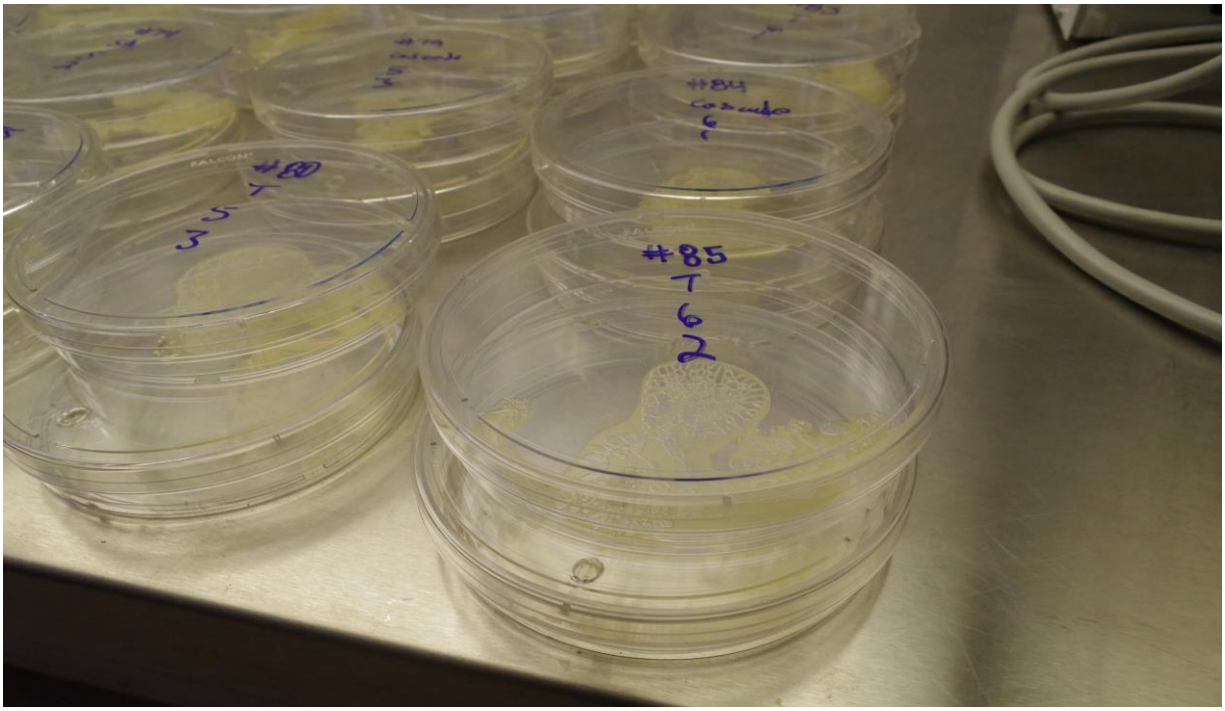

Figure 4.1. Stacked 100 mm petri dishes glued together. Samples are assigned a number and a code for cultivar and run.

Figure 4.2. Settling tower constructed from plywood with removable top and bottom. Vacuum cleaner attached to provide suction.

Figure 4.3. Inoculum platform in top of settling tower. This platform holds the petri dish with inoculum source. This hole is plugged until vacuum cleaner has created suction inside of box. Plug is removed causing air to rush across the inoculum, spreading spores.

Figure 4.4. Petri dishes filled with leaf samples awaiting inoculation. These dishes are placed in the base of the settling tower in a 4x4 grid. Lids are replaced after inoculation.

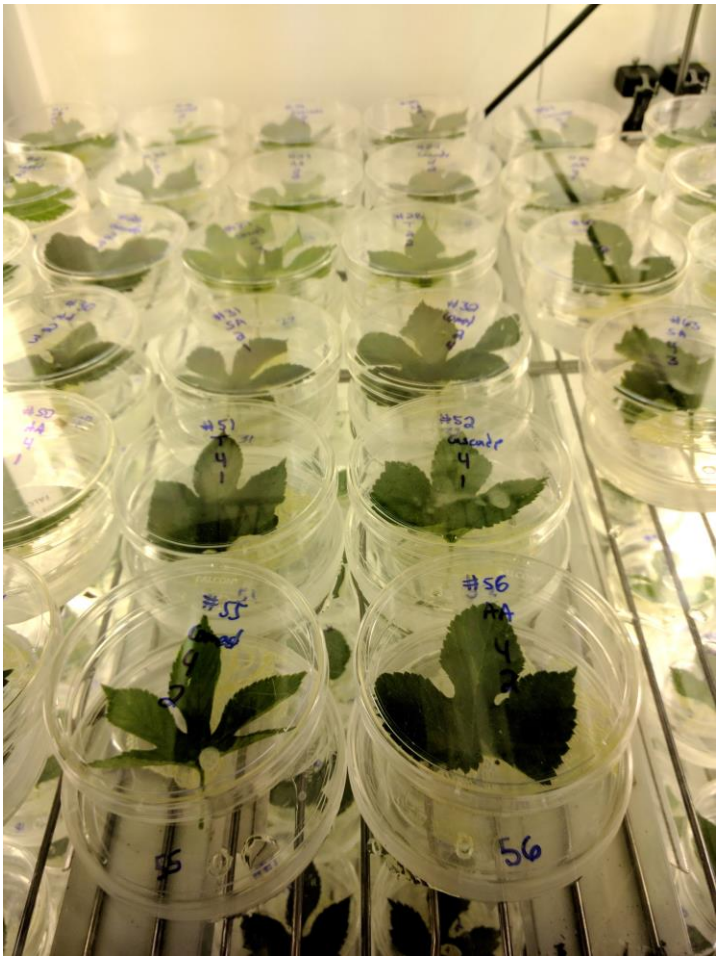

Figure 4.5. Inoculated samples placed in growth chamber for two weeks.

Figure 4.6. Image of leaf on flatbed scanner with blue background. This example is a Tahoma leaf, white areas are PM.

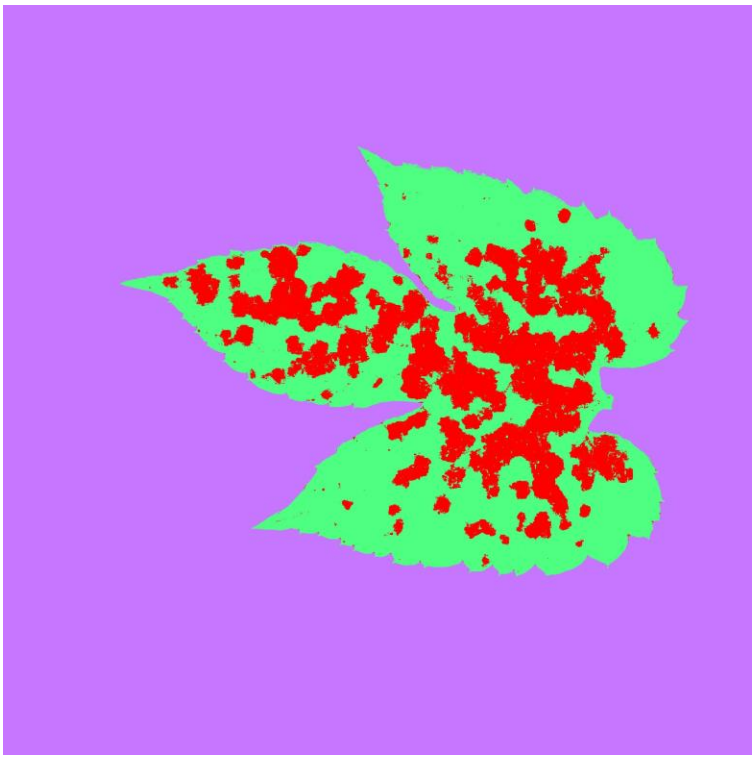

Figure 4.7. Same Tahoma leaf shown previously, now displayed as a segmented image. Background represented in purple, healthy leaf tissue in green, and diseased leaf tissue in red.

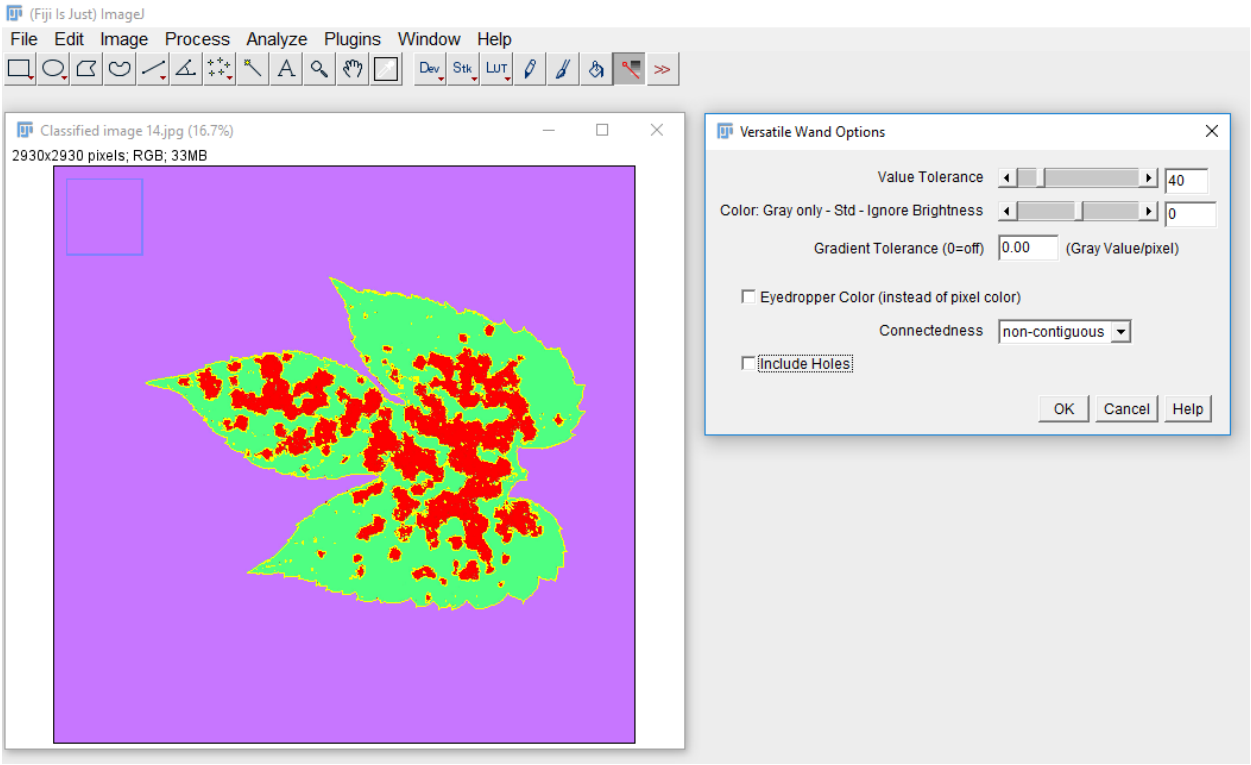

Figure 4.8. Versatile wand plugin used to select segmented areas and measure the number of pixels for each color.