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Investigating Hops Production in Arkansas to Support Specialty Crop Growth

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Investigating Hops Production in Arkansas to Support Specialty Crop Growth

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

by

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University of the Ozarks
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This thesis is approved for recommendation to the Graduate Council.

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OVERALL ABSTRACT

The hop plant (*Humulus lupulus* L.) is a perennial, climbing species within the *Cannabaceae* family that produces cones used for brewing. Hops are grown worldwide. In the United States most hops production occurs in the Pacific Northwest, but growth in the craft beer industry is driving efforts for hops production in other U.S. regions. Recommendations on hops cultivar suitability, fertility, and management are needed for the U.S. mid-south region. Objectives of this research on Arkansas-grown hops were to 1) assess the impact of cultivar and fertility rate on plant and cone attributes of six cultivars of Arkansas-grown hops and 2) determine impact of pruning timing on plant and cone attributes of Arkansas-grown 'Cascade'. For the first objective, six hop cultivars (Cascade, Cashmere, Centennial, Crystal, Nugget, and Zeus) were planted at the University of Arkansas System Division of Agriculture Fruit Research Station (UA System FRS) in Clarksville, AR in 2018. The six cultivars with three fertility rates (low, standard, and high) were grown in a completely randomized block design consisting of three replicates of three plant plots for each cultivar and fertility treatment combination in 2020 and 2021. Fertility rates consisted of low (97 kg/ha), standard (145 kg/ha), and high (193 kg/ha) rates of Triple 13 (13-13-13) applied in four evenly spilt applications in biweekly intervals on May 15, June 1, June 15, and June 30 of 2020 and 2021. Hops cones were harvested at 70-80% moisture content from August-September 2020 and 2021, dried, packaged, and frozen (-10 °C). Plant, cone, and sensory attributes were evaluated at harvest and compositional attributes of the cones were assessed postharvest. The cultivar x fertility interaction was not significant for any of the plant attributes at harvest while only the immature cone percentage was impacted in 2021. No differences were seen in acid content of the dried hops cones between the fertility rates in 2020, but all acid attributes were impacted in 2021. Cultivar impacted all harvest attributes except number of bines/plant (2.71) and number of nodes/plant (62.70) in both years. The total cone yield for all plants was 26.91% greater in 2020 (31 kg) for 48 plants compared to 2021 (24 kg) which had 45 hop plants that yielded cones. ‘Crystal’ (755.80 g), ‘Cascade’ (983.63 g) ‘Zeus’ (797.58 g), had the highest cone yield/plant while ‘Centennial’ (67.45 g) had the lowest. ‘Crystal’ and ‘Cascade’ had alpha acid levels within standard commercial ranges, while all ‘Zeus’ and ‘Cashmere’ were lower than typical levels. Regardless of year, ‘Cascade’ (5.50%), ‘Cashmere’ (5.41%), and ‘Zeus (4.75%) had the highest total alpha acids while ‘Crystal’ (7.74%), ‘Cascade’ (5.80%), and ‘Cashmere’ (4.91%) had the greatest total beta acids. A trained descriptive sensory panel (n=5-7) evaluated the aroma of dried, ground hops cones and found that aroma of the cones varied by year, and cones harvested in 2021 had a general increase in aromatic intensity, overall impact, and were more distinctive in defined attributes. For objective 2 in 2020 and 2021, ‘Cascade’ hops plants at the UA System FRS were pruned April 15 (Early), April 30 (Mid), or May 15 (Late) by removing all new plant growth from each crown at soil level. There were three replications per pruning timing. The impact of pruning timing and year on plant and cone attributes were evaluated. Pruning timing did not impact any of the plant attributes and most of the cone attributes except the percent of damaged cones/plant with the Mid pruning having the highest damage. Year impacted the number of laterals/plant, total cone yield/plant, and estimated dry cone yield/plant. Total cone yield/plant had a 60.1% reduction in 2021 (421.51 g) compared to 2020 (701.33 g). The ‘Cascade’ hops had total alpha acids (6.28-7.66%) and total beta acids (6.45-9.32%) that were slightly higher than commercially-grown hops. In 2020, the Early pruning had the highest level of alpha and beta acids, while in 2021, the Mid pruning had the highest level. This research indicated that ‘Crystal’, ‘Cascade’, and ‘Zeus’ cultivars have potential for commercial hops production in Arkansas, fertility rate had little to no impact on the measured plant and cone attributes, further pruning timing evaluations are needed

to determine best management practices, and cultivar had the most significant impact on plant and cone characteristics.

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DEDICATION

I would like to dedicate this thesis to my grandfather, Thomas Oliver Montgomery, who taught me to endeavor to persevere in all aspects of life.

This thesis work is also dedicated to my parents, J.E. and Leah McClellan, who have been a constant source of support and encouragement during the challenges of graduate school, work, and life. You two have always loved me unconditionally and your good examples have taught me to work hard for the things that I aspire to achieve. I would also like to dedicate this work to the memory of my grandmother, Harriett Walker Montgomery. Although she will be unable to see my graduation and completed thesis, she continues to be my inspiration to seek out knowledge, happiness, and fulfillment. To my friends, family, and loved ones from Pine Bluff and Clarksville, and to the acquaintances I have made during my graduate career, your constant support has helped me through this endeavor.

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OVERALL INTRODUCTION

The common hop plant (*Humulus lupulus*) is classified within the order *Rosales* and phylogenetically related to the *Cannabacea* family of perennial, short-day flowering plants (Melymuka and Bradtke, 2013). The species produces inflorescences that mature to form hop cones that are primarily used in the brewing industry to enhance the flavor and aroma profile of beer due to the phytochemical composition of the lupulin glands found within the cones.

While hops are grown commercially throughout the world, *H. lupulus* primarily grows between the 35th and 55th latitudes in the northern and southern hemispheres (Briggs et al., 2004; Turner et al., 2011). The International Hop Growers' Convention (IHGC) Economic Commission estimated the global area on which hops are harvested at approximately 62,000 ha with a global harvest around 123 million kg in 2019 (Hop Growers of America, 2019; IHGC, 2019). The primary hop production region in the United States derives almost entirely from three states, including Washington, Oregon, and Idaho which produced 71%, 17%, and 12% of the total 2020 U.S. hop crop, respectively, and was valued around $619 million (USDA, NASS 2020).

While hops acreage has increased over the last few decades, the United States has seen a fluctuation in total cone yield. Recent reductions in harvests have been caused by several biotic and abiotic factors, including environmental damage to hop yards, such as high winds in Washington State, pressure from pests and diseases, wildfires in and around the northwest, and a change in the production of cultivars based on brewers' preferences (Steiner, 2021). This has led to major price volatility and a turbulent supply chain for commercial and small-scale microbrewing operations around the country such that brewers' order whole or pelletized cones at premium prices months in advance. With increased demand for hop cones amid shortages in recent years, many locations around the United States have conducted hop studies to determine the plant's production potential and best management strategies for development outside of typical growing regions.

Arkansas' craft brewing industry has more than quintupled since 2011 with more breweries projected to open in the coming years (Magsam, 2018). Arkansas has seen growth from six breweries to over 50 in the past 10 years. However, brewers around the state have had to implement higher prices for products to mitigate the increased costs of inputs. Since hops cones are used as a primary ingredient, the limited supply, high demand, and restrained geography of production has forced local craft brewers to import hops from out of state, despite evidence that suggests hops can be a viable specialty crop in the southeastern and mid-southern United States.

The production potential, aromatic qualities, and plant characteristics of hops grown in Arkansas has garnered intrigue from local established farmers, craft brewers, and small-scale home growers. However, the best management practices for hops production, such as pruning timing, fertility recommendations, and other plant establishment methodologies have had little to no research verification for the state and surrounding regions. While northcentral Arkansas lies just within the cited latitude for optimal hop plant growth (around 35.3158°N), the state's higher humidity, warmer temperatures, and shorter daylight hours are quite dissimilar compared to typical growing regions. Therefore, providing accurate recommendations for crop management, cultivar performance and selection, fertility regimen, plant and cone composition, and other growing factors are difficult. Examining unique cultivar attributes, impacts of fertility practices, pruning timing, and other plant and cone characteristics for hops grown in northcentral Arkansas will help determine specialty crop production potential in the region, the unique aroma profile of selected cultivars, and several management strategies to increase productivity. Based on a review of literature regarding *H. lupulus* production in U.S. states along comparable latitudes with similar geography and climate, it was hypothesized that the first objective would indicate that increased fertility applications of 13N-13P-13K fertilizer would yield plants with greater vigor, total cone yield, and higher quality cones with more alpha and beta acids with only minor differences between the selected cultivars. The hypothesized outcome for the second objective was that an earlier shoot pruning date would result in a significant positive impact on the measured plant, cone, and quality attributes based on a higher number of growing degree days and increased maturity for the earliest pruning date rather than standard and late pruning times.

OBJECTIVES

1) Evaluate the Impact of Cultivar and Fertility Rate on Plant and Cone Attributes of Arkansas-grown Hops
2) Determine the Impact of Pruning Timing on Plant and Cone Attributes of Arkansas-grown ‘Cascade’ Hops

LITERATURE REVIEW

Evaluation of Production and Sensory Attributes of Arkansas-grown Hops

History of hops cultivation

The origin and utilization of *Humulus lupulus*, commonly referred to as the common hop, can be traced back thousands of years and is considered both historically and agriculturally significant because of worldwide cultivation. Although hops are currently grown for brewing, the first description of the plant was described as an appetizer and salad ingredient in a book from the 1st century called *Natural History* by Gaius Plinius Secundus, known as Pliny the Elder (Edwardson, 1952). Historians note that brewing dates back more than 5,500 years to Ancient Mesopotamia, but this ancient beverage was different than modern beer since it contained herbs and spices for bitter flavor instead of hops (Edwardson, 1952). Prior to the use of hops in brewing throughout Europe during the Middle Ages, *H. lupulus* was used as an ornamental plant and for medicinal and textile purposes (Briggs et al. 2004; Karabin et al., 2016). Individuals believed that the plant could cure illnesses, such as tumors and skin diseases, and fibers of the plant were used to manufacture twine and a fabric resembling cloth (Turner et al., 2011). The origin of hops cultivation in the United States occurred with the arrival of the first colonists from England during the mid-1600's in Virginia, and cultivation spread from the northeast to the Washington Territory and eventually to California by the late 1800's (Edwardson, 1952).

Hops Classification

The hop plant is in the order *Rosales* and family *Cannabacea.* Hops are phylogenetically related to hemp and other perennial, flowering plants within the *Cannabacea* family, including *C. sativa* (marijuana or hashish), that have been used as a source of textiles and as medicinals (Karabin et al., 2016; Pollio, 2016). There are three species within the *Humulus* genus, including

Humulus yunnanensis, *Humulus lupulus*, and *Humulus japonicas* (Briggs et al. 2004; Turner et al., 2011). The genus *Humulus* contains short-day plants indigenous to northern temperate climates (Mahafee and Pethybridge, 2009).

While all three species of *Humulus* contain similar phytochemical compounds and resemble the common hop, only *H. lupulus* has traits used for brewing. While there are numerous varieties within the *lupulus* species, like *H. lupulus* var. cordifolius, *H. lupulus* var. neomexicanus, and *H. lupulus* var. pubescens, the *H. lupulus* var. lupulus is the only taxonomic species responsible for imparting characteristic flavors and aromas favorable for brewing (Briggs et al., 2004; Turner et al., 2011). There are also wild hop and dwarf varieties native to North America, including *H. lupulus* var. lupuloides, and, although these varieties are unfavorable for brewing due to their chemical characteristics, the germplasm sources from these plants have notable benefits for breeding hops (Hampton et al., 2001).

Hops primary growing regions

Hops are grown commercially throughout the world. *H. lupulus* primarily grows between the 35th and 55th latitudes in the northern and southern hemispheres (Briggs et al., 2004; Turner et al., 2011). The plant can be grown in many climates, including semiarid, maritime, humid continental, and sub-tropical regions, with distinct cultivars better suited for specific climates (Turner et al., 2011).

The International Hop Growers' Convention (IHGC) Economic Commission estimated the global area on which hops are harvested at approximately 62,000 ha with a global harvest around 123 million kg in 2019 (Hop Growers of America, 2019; IHGC, 2019). The United States, Germany, and China were the three highest hop-producing countries in 2019 with a combined harvest of 101 million kg (222 million lbs) (IHGC, 2019). In addition to these countries, the Czech Republic, Slovenia, United Kingdom, Australia, Poland, New Zealand, and Spain are the top ten hop-producing countries (Hop Growers of America, 2019).

The primary hop production region in the United States derives from three states in the Pacific Northwest, including Washington, Oregon, and Idaho which produced 71%, 17%, and 12% of the total 2020 U.S. hop crop, respectively (USDA, NASS 2020). The remaining states had a commercial hop production estimated at one million pounds in 2019 (Hop Growers of America, 2019). According to the 2020 National Hop Report, hop production in the United States was valued around $619 million while the total production decreased 7% from 2019. The reduction in yield was caused by several factors, including high winds in Washington State, fires in Oregon that reduced sunlight, and a change in the production of cultivars based on brewers' preferences (Steiner, 2021). Aside from the three main hop-growing states which produce approximately 98% of the total hop crop, many locations around the United States are growing hops to meet the rising demand for cones. Other states that produce notable acreage of *H. lupulus* include Wisconsin (2.2%), Michigan (1.2%), New York (0.7%), Colorado (0.2%), California (0.2%), Minnesota (0.2%), and Ohio (0.1%) (Hop Growers of America, 2019).

Hops cultivars

For brewing purposes, hop cultivars are classified into three categories – bittering, aromatics, and dual purpose. Bittering hops are commonly higher in alpha-acid content and lower in essential oils (Giovanisci, 2019). The most popular hop cultivars by acreage in the United States include 'Citra®', 'CTZ' (also known as 'Columbus', 'Tomahawk', and 'Zeus'), 'Cascade', 'Simcoe', and 'Mosaic' with 75.4% of the cultivars grown in 2019 used for aroma (Hop Growers of America, 2019). While used in the United States, European-grown bittering cultivars often contain higher alpha-acids than the North American hops and are used more frequently in brewing (Giovanisci, 2019). Aromatic hops are typically higher in essential oil content than the bittering cultivars. These cultivars add notes of grass, fruit, honey, earth, flowers, and other spices, and include cultivars like ‘Amarillo’, ‘Brewers Gold’, ‘Cascade’, ‘Citra’, among many others. In general, the hops grown in North America tend to be more aromatic than European aromatic hops based on style preferences and breeding (Giovanisci, 2019; Briggs et al., 2004).

As the name implies, dual-purpose hops are considered balanced since these cultivars are used to add bitterness during the boiling process or incorporated after a beer is chilled for added flavor and aromas; common dual-purpose hop cultivars grown in the United States include ‘Cascade’, ‘Centennial’, ‘Chinook’, ‘Northern Brewer’, and ‘Willamette’ (Giovanisci, 2019). While classified as aromatic hops, there is an additional, smaller category of heirloom hop cultivars known as noble hops that refers to four of the oldest, most traditional hop cultivars, including ‘Czech Saaz’, ‘Tettnanger’, ‘Spalt’, and ‘Hallertau Mittelfruh’, which tend to have more essential oils (particularly humulene) than other aromatic cultivars (Giovanisci, 2019). Based on the timing of adding hops to a beer, the sheer number of hop cultivars, the differences in alpha- and beta-acid content, and various aroma and flavor qualities, a brewer can craft a wide array of different styles beers (Briggs et al, 2004).

Physiological development of hops

Hops plants are propagated by leaf or root cuttings and cultivated from rhizomes. The rapid, aboveground physiological development and structure of *H. lupulus*, especially the development of strobili (seed cones), is also a characteristic of the plant’s tenacious growth cycle. Unlike grapes, muscadines, and other vine-producing plants which use tendrils to facilitate growth and attachment to a trellis, hops produce large, twining bines that wrap around mesh, twine, or jute cord in a clockwise direction with the aid of small, hooked hairs on the hop bines called trichomes (Mahaffee and Pethybridge, 2009). Newly emerged shoots are initially trained onto suspended twine from large poles (6 m or 20 ft tall in typical commercial hop yards) that are designed to withstand over 9 kg (20 lb) of weight (Pyle, 1995). Two to five of the healthiest bines that remain after pruning the earliest shoots to the ground are trained as the primary bines for development, and these are important for optimal bine growth throughout the summer months.

The trained bines continue to grow rapidly with increasing daylight hours, and after the summer solstice, the plants react to the shortening day length by initiating the flowering process on the plant's lateral branches during which the inflorescence (cones) are formed and ultimately harvested while the discarded bines and leaves can be returned to the hop yard and composted to supplement future fertilizer input (Turner et al., 2011). Observational data indicated that hop bines are incapable of flowering unless 12–25 nodes were visible because hops require a cultivar-specific size effect (distance) between the roots and shoot apex to make the juvenile to adult transformation (Bauerle, 2019). The period following the summer solstice initiates the reproductive phase of phenological development for hops while the time period from shoot emergence to the solstice is referred to as the vegetative phenological stage.

Hops production costs

Initiating a hop yard requires a considerable investment in production costs based on equipment needed, labor, food safety requirements, and inflation in the cost of production inputs (Hop Growers of America, 2019). Previously established posts used for fencing or trellising other climbing plants (i.e., grapes, muscadines) are often used as a hops trellis by small-scale growers (Ha et al., 2017). The posts in a hopyard should support the heavy load of mature hops plants year after year. Trellis posts are 3.6-5.5 m (12-18 ft) tall and spaced 3.6 m (12 ft) apart, and a mesh netting or chord must be attached to the posts to facilitate the attachment and vertical development of *H. lupulus* (Ha et al., 2017; Teghtmeyer, S., 2018). Plants need to be spaced 0.6 m (2 ft) apart. While hop rhizomes can be used, transplants can be purchased ($3-$5 per unit) to ensure higher survival rates and minimize the likelihood of planting infected hops (Ha et al., 2017).

Integrated pest management protocols (IPM) (i.e., weeds, insects, and diseases) also need to be factored into total production costs based on the potential losses in yield and quality that pests inflict on hops production sites (Hop Growers of America, 2019; Turner et al., 2011). A 2015 Washington State University Pacific Northwest Hop Cost of Production Study estimated the annual cost of producing hops using a standard trellis with drip irrigation at $24,212/ha ($9,806 /acre) with an estimated net income of $1,896/ha ($768/acre) (Hop Growers of America, 2019). However, the cost of inputs and returns will vary depending on the production system used and the overall yield and quality of hops cones each season.

Hops production methods

Fertilizers in the form of granular nitrogen, phosphorus, and potassium (NPK) are also required for optimal *H. lupulus* growth, and a drip irrigation system is recommended for plant development. To determine nutrient needs, a grower will implement soil tests each season to estimate the amount of NPK required for a hop yard (Ha et al., 2017). A multi-year study conducted by Iskra et al. (2019) evaluated the influence of nitrogen fertilization rates and timing on cone quality and nitrate accumulation in hops cones grown in Oregon and Washington. Cone nitrogen content, alpha and beta acids, oil content, color at harvest, yield, and aroma were year dependent while increases in nitrogen addition rate linearly decreased hop acids and total oil volume and linearly increased cone color and nitrogen content (Iskra et al., 2019). The total yield per plant was unaffected by increasing nitrogen rates, hops acids decreased as nitrogen levels increased when the fertilizer was applied after bloom, and the timing of fertilization affected the aromatic qualities of the cones (Iskra et al., 2019). This analysis indicates that applying a minimal nitrogen fertilization regimen (160 kg mineral N/ha) during vegetation (before bloom) in typical growing regions is ideal for quality cone characteristics (Bavec et al., 2004; Iskra et al., 2019).

An additional factor of hops production that affects cone characteristics, such as the secondary metabolites and hop color, includes the timing of pruning the first shoots that emerge in a hop yard in early spring. A study conducted by Matsui et al. (2016) varied the pruning and harvest date for the ‘Saaz’ hop in four locations throughout the Czech Republic to determine if pruning timing affected overall yield, blooming time, hop secondary metabolites (acids and essential oils), and cone color. Fifteen combinations of pruning and harvest timing (three pruning conditions and five harvest times) were evaluated at each location over three years, and researchers recorded bloom, cone formation, yield, chemical analyses, sensory evaluations, and hop color to determine ideal pruning and harvesting methods (Matsui et al., 2016). Results indicated that there was no correlation between date of pruning and date of cone formation, and date of shoot pruning had no significant effects on hop essential oils, sensory score, and yield, yet the level of alpha-acids tended to be higher in the cones from the plants that were pruned later (mid to late April – Czech Republic) (Matsui et al., 2016). This effect may show that the length of the vegetative period (from pruning to blooming) does affect hop secondary metabolism, yet further research is needed for other cultivars over several years in different growing locations to better estimate the ideal timing of shoot pruning for optimal *H. lupulus* growth and quality.

Pest and disease pressure/management of hops

Depending on growing location and climatic conditions during a season, hop yards are susceptible to numerous pests, diseases, and invasive grasses that can affect cone quality. IPM strategies can be used to mitigate the risks of pests. Japanese beetles (*Popillia japonica*) and arthropods, like the two-spotted spider mite (*Tetranychus urticae*) and the damson-hop aphid (*Phorodon humuli*), are the most common pests for hops, particularly in the hop-growing regions of the northern hemisphere (Grasswitz and James, 2008; Turner et al., 2011). These insects thrive under hot, dry, and humid conditions and can devastate hop plants because the pests feed on bracts and bracteoles of the hops cones, which reduce cone quality, increase presence of mold, and transmit hop viruses (Pyle, 1995; Turner et al., 2011). To lessen the risks pests pose to hops, growers implement strategies to lessen an arthropod population, including introducing predatory insects (lady beetles, predatory mites, parasitic wasps, etc.), cover cropping, and the use of miticides and pesticides (Briggs et al., 2004; Lilley et al., 2010; Mahaffee et al., 2009; Pyle, 1995; Turner et al., 2011).

Several fungal diseases, such as downy mildew, powdery mildew, and verticillium wilt, along with viroid-caused diseases, like the hop latent virus, halo blight, and hop mosaic virus, also represent major threats to crop yield and quality. Downy mildew, caused by *Pseudoperonospora humuli*, and powdery mildew, produced by *Podosphaera macularis*, can have significant impacts on hop yield and quality (Pyle, 1995). Mildews are particularly prevalent in humid regions where diseases and viruses can flourish on plants. Halo blight (caused by *Diaporthe humulicola*), a disease that can be transmitted by insects in a hopyard, has been reported in recent years in Michigan and Connecticut; the virus can lead to brown leaf lesions, severe browning of cones, and a shatter of hop cones which can detrimentally impact total yield (Sirrine et al., 2022). Disease and viral pressures can be eased, though, with several cultural practices and timely fungicide applications. The removal of infected material, sanitation of pruning equipment, timely pruning and removal of basal shoots, along with water and fertility management are primary methods of reducing spread of fungal and viral diseases in plants (Gent et al., 2015; Sirrine et al., 2022).

Due to the harsh effects on bine development and cone quality, breeding specific cultivars that are impervious to diseases and viruses are a primary goal for growers, breeders, and agricultural researchers. In recent years, breeding resistant genes into *H. lupulus* plants with wild hop varieties have introduced cultivars that are resistant to diseases (Seigner et al., 2009). Future virus and disease prevention can rely on cultural practices and breeding efforts focused on producing disease-resistant cultivars to mitigate consequences that have affected growers and brewers worldwide.

Hop cone structure

The hop plant produces hop cones that are used for beer production. Hops cones are the inflorescences or strobili (seed cones) from the perennial female hop plant that are an essential component of industrial and craft brewing production (Briggs et al., 2004). A hop is composed of four primary structures including the strig, bract, bracteole, and inner lupulin glands – the component of the cones that growers and brewers value for brewing since these contain the complex phytochemical compounds (alpha acids, beta acids, and essential oils) that are imbued into beer for aroma and flavor (Hrnčič et al., 2019; Killeen et al., 2016; Patrick, 2013).

The blossoms of the hop plant initially appear as large sand burrs, but the inflorescences slowly take on a characteristic cone shape as they grow. The final size and shape of the cones depends on the cultivar and varies in size from 2.5 to 5 cm long by 1.3 to 2.5 cm in diameter (1 to 2 in. long by 0.5 to 1 in. in diameter) (Pyle, 1995).

Harvesting hop cones

The growth, development, and handling of the strobili produced by *H. lupulus* are crucial for growers since these affect hop cone qualities. Hops harvest in the United States usually occurs between mid-August through late September, and the final yield is dependent on many factors, including cultivar, age of the plant, soil characteristics, pest and disease pressure, growing location, and weather conditions throughout the growing season (Briggs et al., 2004; Gingrich et al., 1994; Lilley et al., 2010; Morcol et al., 2020; Rodolfi et al., 2019; Santagostini et al., 2019; Sharp et al., 2014).

While it can be difficult to determine when to harvest the cones from *H. lupulus*, there are several characteristics used to decide the ideal timing and method of collection. Growers typically assess the tactile and aromatic qualities of cones while still attached to lateral branches to aid in determining ripeness. Immature cones have a damp, soft feel when squeezed while mature hops have a distinguishing paper-like, light texture, and hops spring back when compressed (Pyle, 1995). Another method for determining cone maturity entails picking a hops sample and cutting it lengthwise down the center with a knife. When fully mature, the internal resin (lupulin sacs containing the essential oils and bitter compounds) will appear golden yellow and emit a pungent aroma reminiscent of a “hoppy” beer (Pyle, 1995).

Determining when to harvest cones is important for quality purposes since overly-ripe cones tend to brown and oxidize if left on bines too long while immature cones contain a smaller quantity of lupulin. In the northern hemisphere, the first traces of lupulin resin can be detected in early August (beta acids develop several days prior to alpha acids), and resin synthesis is nearly complete by the end of the month (Biggs et al., 2014). Once cones are mature/ripe, hop bines are cut down from the trellis and are transported for cone harvest by machine or hand depending on the size of the hop yard.

Commercial hop producers with large acreage often use these machines to facilitate and hasten harvesting. Growers place bines within a trackway and, depending on the design, mature plants enter the machine either horizontally or vertically. The hops and leaves are stripped from the bine by numerous moving wire hooks and then passed over screens to separate hop cones from debris (Briggs et al., 2004). Debris can be composted and returned to the hop yard as a supplement for mulch or fertilizer (Briggs et al., 2004; Turner et al., 2011)

Hops cones drying and storage

To maintain the valuable components of hop cones, proper drying and storage methods after harvest must be implemented to ensure optimal hop quality for brewing and to allow for long-term use. Freshly harvested hops are approximately 80% water, and if left untreated will spoil rapidly. To prevent rapid oxidation and spoilage, most of the water is removed from the hops cones by drying the cones for several hours using kilns, oasts, or drying rooms until a final moisture content of 7-10% is achieved (Briggs et al., 2004; Pyle, 1995). The air speed, temperature, and humidity must be controlled and monitored throughout drying as alpha acids are destroyed with high air temperature (above 60°C or 140°F) (Briggs et al., 2004). Once the hops are dried, the cones must be stored at low temperatures in an oxygen-depleted receptacle such as a jute or polypropylene sack. Growers often place harvested cones in commercial freezers (0.2 °C or 33 °F) once the dried product is vacuum sealed or canned to prolong freshness. Cold storage can prevent rapid deterioration of the secondary metabolites, but loss of both alpha acids and beta acids can be expected after several months of storage depending on hop cultivar (Briggs et al., 2004).

Chemical constituents of hops cones

The commercial and brewing value of hops cones lies in phytochemically complex substances within the bracts, particularly within the lupulin glands. The cones from the female plants contain various secondary metabolites, including alpha acids, like humulone and cohumulone, monoterpenes, sesquiterpenes, beta bitter acids, essential oils, and polyphenols. These compounds are collectively known as phloroglucinol-derived bitter acids, which impart characteristic flavors and floral scents to beer once thermal isomerization has occurred – a process completed during the brewing process (Killeen et al., 2016). Hops have an exceptionally high content of alpha acids, up to 25% or more, of the dry weight of the cones (De Keukeleire, 2000).

Hop alpha (*α)* acids and beta (*β*) acids

The most substantial component of dried hops are the alpha acids, and these compounds are structurally complex enolic acids that contain a six-carbon ring with several substituent groups. While there are more than seven prominent alpha acids within the lupulin glands of the hops, humulone, cohumulone, and adhumulone constitute 98-99% of the alpha-acids (Killeen et al., 2016; Mahaffee and Pethybridge, 2009). Humulone is the primary alpha acid found in many hop cultivars and is known to impart a "soft" bitter flavor during brewing; this chemical, also known for its anti-bacterial, anti-cancer, and antioxidant properties, imparts most of the bitter flavor that is characteristic of a beer's taste (Karabin et al., 2016; Patrick, 2013). Like humulone, cohumulone is another alpha-acid that imparts flavors into beer during isomerization, but this compound is often described by brewers as being much harsher in bitter flavor comparatively (Briggs et al., 2004; Patrick, 2013). The remaining alpha acids – adhumulone, posthumulone, and prehumulone – also add to the overall flavor profile of beers, yet additional research is still needed to ascertain the specific effects these acids have on a person's taste perception of a beer (Morcol et al., 2020; Patrick, 2013).

The beta acids found within the hops cone are only a minor contribution to a beer's flavor, but they are a crucial component in the brewing process, especially for preservation. The beta acids are another secondary metabolite that are characteristic of hop cultivars, and quantities vary with cultivar and cone maturity (Mahaffee and Pethybridge, 2009). While the number of analogues is the same in alpha acids, beta acids are chemically disparate from alpha acids due to the isopentenyl side chain in place of the second hydroxyl group at ring position 6. Previous studies regarding these compounds have noted that the ratio of alpha to beta acids varies depending on the stage of development, growing location (terroir), and cultivar, but it often ranges from 1:1 to 4:1 (Forteschi et al., 2019; Mahaffee and Pethybridge, 2009; Rodolfi et al., 2019; Santagostini et al., 2019).

Essential oils in hops cones

The essential oil group of hop constituents are generally thought to be the source of the characteristic "hoppy" aroma found in beer. The oil is a complex mixture of compounds that contains over 300 different chemical entities composed of hydrocarbons, oxygenated and sulfur-containing compounds which can be aliphatics, monoterpenes, and sesquiterpenes (Mahaffee and Pethybridge, 2009). Typical yield of oil relative to dried hop cones is approximately 0.3% for most cultivars (Malizia et al., 2011). The ratios of specific volatile oils (alpha humulene, myrcene, and beta caryophyllene) as well as the various floral and fruit aromas imbued into beer by the volatile oils usually define brewing quality, and while these proportions vary among cultivar, the level of oils increases logarithmically with cone ripening (Briggs et al., 2004; Danenhower et al., 2008; Killeen et al., 2016; Steenackers et al., 2015). A decrease in these essential oils is particularly concerning for brewers, and this can occur during the storage process through oxidation, polymerization, or resinification of components, as well as machine picking, drying, and inadequate baling and pelleting techniques (Mahaffee and Pethybridge, 2009).

Polyphenolic compounds found in hops cones

Unlike alpha and beta acids, which are often considered the most important resins to brewers due to their flavor and anti-microbial properties, polyphenols are also vital for many aspects of a beer's quality. Phenolic substances found in the lupulin glands act as anti- or pro-oxidants, flavor precursors, and react with other phytochemicals that influence several quality characteristics of beer, such as flavor, color, colloidal, and flavor stability (Wannenmacher et al., 2018). While phenolic acids are unlikely to influence beer flavor, they act as flavor precursors in beer, and recent studies purport that polyphenol extracts affect mouthfeel, bitterness, and astringency (Goiris et al., 2014). Like other iso-alpha acids, phenolic compounds undergo structural changes as they are extracted or enzymatically released throughout the brewing process. As phenolic compounds precipitate with proteins and adsorb to hot and cold trub (sediment), yeast cells and stabilization agents decrease in concentration during brewing (Briggs et al., 2004; Wannenmacher et al., 2018). While the exact influence that hop polyphenols have on quality, flavor, and aroma of beer are still debated and researched, their antioxidant nature and influence on stability of beer will likely ensure ongoing research into the role that phenolic constituents have in brewing (Briggs et al., 2004; Wannenmacher et al., 2018).

How hops are used in brewing

The art and science of brewing, while simplistic in theory since most brews consist of only four ingredients (water, malt, yeast, and hops), is considered by brewers to be one of the most complex fermented beverages in the world since the flavor, color, mouthfeel, and alcohol content can vary in more ways than any other beverage (Trosset, 2020). The brewing process often begins with milling (crushing) malt, which creates a larger surface area on the grain's endosperm and generates various enzymes that convert starches into sugars (maltose) and dextrins (Trosset, 2020). While barley is generally the most commonly used whole grain in craft brewing, wheat and rye are also used frequently (Thesseling et al., 2019; Trosset, 2020). After milling, a brewer will start the mashing phase which entails adding hot water (62-70°C or 144-159°F) to the malted grain for 30-120 minutes in which time the starches become converted to sugars – known as the body of the beer (Trosset, 2020). When finished, the sugary liquid (called wort) is separated and transferred to a large kettle. The size of the boiling kettle varies depending on the amount of beer being made.

The wort is then brought to a boil for 60-90 minutes depending on the brewer's preference for bitter flavor; this process is responsible for two main objectives: pasteurizing the wort (sterilization to kill bacteria and extend shelf life) and adding flavoring ingredients (i.e., hops, ginger, molasses, etc.) (Trosset, 2020). As hops are boiled in wort, the alpha acids (humulone, cohumulone, and adhumulone) are isomerized into bitter iso-alpha-acids while the beta acids (lupulone, colupulone, and adlupulone) are oxidized and contribute less to the overall flavor profile of the beer (Danenhower et al. 2008). The altered alpha compounds not only impact the bitter taste in beer, but they strongly contribute to the foam stability and inhibit growth of gram-positive bacteria, in particular *Lactobacillus*, in beer (Steenackers et al., 2015). After the wort is boiled, a brewer will transfer the liquid through a heat exchanger into a fermenter to cool the liquid to 16-21°C (60-70°F), which facilitates use of brewer's yeast. The cooled wort and yeast remain in the kettle for 4-6 days in which time the yeast will convert the sugars into alcohol and carbon dioxide. When the brewer determines that fermentation is complete, the liquid (now referred to as beer) is cooled to -1°C (30°F) for conditioning. This step supports yeast flocculation (known as settling), which not only aids in the clarification of beer, but it allows the brewer to collect yeast for re-use (Trosset, 2020). Once conditioned, the beer is typically filtered into a "bright tank," a pressure-rated temperature-controlled vessel used to hold a finished beer prior to packaging, where the beer is carbonated and kept for kegging, canning, bottling, or barrel aging depending on the type of beer created (Trosset, 2020).

Different styles of beer, craft brewing vs. micro-brewing

To create different styles of beer, a brewer can modify two of the main ingredients – hops and yeast – to manipulate the aroma and flavor of the beverage. The timing, quantity, and cultivar of hops and yeast added to wort during the brewing process contribute to the sensorial perceptions of a specific brew (Briggs et al., 2004; Trosset, 2020). The United States produces more styles and brands than any other market in the world – over 100 types – and this is accomplished by the type of yeast used during fermentation (Froehlich, 2013). Based on the yeast used, a beer can be categorized into two main types: ales and lagers. To produce an ale, a brewer uses specific yeast that can settle and ferment at the top of the kettle (applicably known as "top fermented") that can withstand higher temperatures (Froehlich, 2013; Salanță et al. 2020; Vaidyanathan, 2019). Ales typically ferment within a shorter period of time and have a rich, complex, more yeast-derived flavor than lagers. The most commonly consumed ales include pale ales, India pale ales (IPA's), brown ales, stouts, hefeweizen (wheat-based beer), porters, among many other varieties that are characterized by their golden or copper colors and intense, hoppy aromas and flavors (Froehlich, 2013).

Conversely, lagers are made by using yeast that ferments at the bottom of a fermentation vessel. The yeast converts sugar to alcohol at a colder temperature which produces fewer esters (flavoring compounds) to create a mild-tasting beverage; typical lagers include amber or red lager, pilsner, bock, doppelbock, Oktoberfest, and many other beers that are darker in color, richer in flavor, and have less of a hop flavor and aroma compared to ales (Froehlich, 2013). Additionally, a brewer can alter when hops are added to wort to create a more complex flavor profile of the beer being made.

When dried hops are added to cooled wort near the end of the fermentation process, brewers refer to this method as dry hopping. Adding undried hops to cooled wort requires the addition of as much as six times the typical dried quantity, and this process also changes the flavor perceptions of a brew (Briggs et al., 2004; Pyle, 1995). While the ingredients used to make ales and lagers are nearly identical, the implementation of different types of yeast and hops used during brewing create beers with vastly differing aromas and flavors.

While the terms microbrewery, domestic brewery, and craft brewery are often used interchangeably, the subtle differences in the scale of beer production require further explanation. “Domestic” beer generally refers to American lagers produced from major brewing companies while craft brewing entails a production volume of 6 million barrels or less a year (Froehlich, 2013). The latter is generally thought to produce beer made from higher quality ingredients with more distinctive flavor and aroma qualities. The accepted definition of microbrewing, recently re-defined by the Brewers Association, is a market segment of the craft beer industry that produces less than 15,000 barrels of beer per year (75% or more of the product sold off-site) (Froelich, 2013). Homebrewing is aptly named since this type of brewing is completed by individuals at small breweries or at residential sites for personal consumption. Regardless of the quantity of beer produced by a company, craft brewery, or at home, quality, ingredients, and preference for specific beers varies among beer aficionados.

Bitterness in beer

Depending on the desired flavor and aroma qualities desired in a beer, a brewer can add specific quantities of hops based on the cultivar used and the alpha and beta acid content within the lupulin glands since these ultimately influence the taste perception of the brew. A beer's bitterness is often expressed using the International Bitterness Units scale (abbreviated IBU) that measures the parts per million of isohumulone found in a beer (Dykstra, 2015). Although this scale is often used as a general guideline for flavor, with lower IBU's corresponding to less bitterness and vice versa, the implementation of other ingredients (i.e., malt, spices, etc.) can alter the bitterness of a beer. The scale is often regarded as unreliable to some brewers since the style and ingredients used in the beverages can alter the perceived bitterness (Dykstra, 2015). With approximately 150 *H. lupulus* L. cultivars grown around the world today with more developed every year through breeding programs, a brewer can select one or more hop cultivars to use in a brew to add desirable flavor and aroma notes based on the chemical characteristics of a given variety (Giovanisci, 2019).

Prices for hops cones

According to the National Agricultural Statistics Service (NASS) which publishes annual national hop reports regarding U.S. hops acreage, yield, price, and total estimated value, the country's hops market can fluctuate significantly from year to year based on many factors, including the size of the yearly harvest, environmental impacts (i.e., drought, wildfire, smoke, disease, and pest pressure), and consumer demand which can affect the average price of cones (USDA, NASS 2020). For example, the 2020 hop report noted that the Pacific Northwest produced approximately 47 million kg (104 million lbs - dry) of hops cones which was 7% below the previous year's total harvest (USDA, NASS 2020). This drop in total yield occurred simultaneously with the record for greatest total harvested acreage for the United States with nearly 25,000 ha (60,000 ac) of farmland used for hops production.

The U.S. hop yield averaged 1.983 kg/ha (1,770 lbs/acre) of hops cones in 2020, and this production was down nearly 12%, a 235 kg/ha (211 lbs/acre) loss nationally (USDA, NASS 2020). While the number of plants per hectare varies widely depending on growing location, conventional tall-trellis hop yards typically average 2,099 plants/ha (850 plants/acre), and typical mature plant yields (4-5 years old) vary between 1,343 kg/ha to 1,904/ha (1,200 lbs/acre to 1,700 lbs/acre) or 0.34-0.45 kg (0.76- 1.0 lb) per plant (dried cones) depending on the previously mentioned factors (Ha et al., 2017). Cultivar yield differences also affect the final price of hops cones. Previous data indicates that varieties such as 'Cascade', 'Chinook', and 'Citra' (high alpha-acid cultivars) can yield as much as 771 kg to 862 kg (1,700 lbs to 1,900 lbs) per acre while 'U.S. Northern Brewer' (bittering variety) and 'Willamette' (a triploid aroma-type hop) yield between 1,343 kg/ha to 1,679 kg/ha (1,200 lbs/acre to 1,550 lbs/acre) (Brewers Association, 2021).

This fluctuation in harvested cones per year can consequently affect the price of hops cones, and specific cost by weight of cones varies depending on the amount purchased, location of purchased hops, form of cones (pelletized or whole cone), and the cultivar selected for use in brewing. According to a market research study for commercial hop production in New England, some brewers prefer to purchase locally sourced hops and will pay a higher price (as much as

$33/kg or $15/lb) if the alpha and beta acid profiles of the cones are within a desirable range, and the average price for bittering hops were significantly less than aroma-hops ($2.2-6.6/kg or $1-$3/lbs of bittering hops compared to $55/kg $25+ per pound of aroma dried cones) (Wilson, 2010). During one instance of adverse environmental factors, the price of whole hops cones increased from approximately $4.4/kg ($2/lbs) to nearly $66/kg ($30/lbs) in one year (Wilson, 2010). The form of hops cones used in brewing – whole cone or pelletized – is also a factor that brewers consider when making a beer. Pelletized hops cones are made by milling, crushing, and compacting whole hops cones through an extruder which makes the surface area of the pellet smaller than a whole hop cone, yet the ratio of whole cones to pellets used for brewing is generally a 5:1 ratio where 142 g (5 oz) of whole cones is roughly equivalent to 28 g (1 oz) of pelletized cones (Carr, 2014). The pelletized form of hops cones is also significantly more expensive than unaltered cones. One cost production study estimated that pelletized hops average 30%-40% higher cost than whole cones (Sirrine et al., 2014). However, both forms of hops cones are used frequently by commercial and craft brewers, and both offer advantages and disadvantages according to preference, beer style, equipment used, and cone availability.

Chemical analysis of hops cones

While many hops growers determine cone maturity by assessing lupulin gland color, smell, moisture content, and strobili size, there are several analytical methods that can be used to more accurately determine the proportions of secondary metabolites – alpha and beta acids – that ultimately help determine the quality and quantity of hops used in brewing (Danenhower et al., 2008; Killeen et al, 2016). The role that alpha and beta acids play during the brewing process is crucial for brewers since the ratio of these acids are responsible for the bitterness of a brew (alpha acids) as well as the antiseptic qualities (beta acids). The Association of Brewing

Chemists (ASBC) has many standard procedures for hops analysis including spectrophotometry (SPEC) and high-performance liquid chromatography (HPLC) for alpha and beta acid analysis (Lilla, 2018). The spectrophotometric and HPLC methods involve pH-regulated ultraviolet light absorption through the different hop resins, and the maximum absorption values for alpha and beta acids have been defined at 325 and 355 nm respectively, while the minimum (background) absorption is 275 nm (Killeen et al., 2016; Mahaffee and Pethybridge, 2009). These tests analyze the three acid compounds in alpha acids (cohumulone, humulone, and adhumulone), and the three acid compounds in beta acids (colupulone, lupulone, and adlupulone).

The use of an HPLC has become the preferred method for analytical testing due to the ability to quantify individual alpha and beta acid content. The device injects a portion of a sample into a liquid stream (known as the mobile phase) which is pumped through a steel column or tube filled with sand-like particles (Lilla, 2018). Under pressure, the interaction of the sample with these particles separates the alpha and beta acids while a spectrophotometer within the machine detects the specific compounds.

Regardless of the method used to quantify the alpha and beta acid content within a sample of hops cones, substantiating these compounds is essential for brewers since the levels of these metabolites not only determine the quality and value of cones but also the aroma and flavor characteristics they impart in a beer. The ASBC has other methods established for measuring the moisture content, phenolics, essential oils, and sensory of hops. The moisture content (or dry matter) of the hops cones need to be calculated for analysis.

Sensory techniques used to assess hops or beer

Another technique used by brewers, food scientists, and extension departments to assess the quality and potential likeability of hops and the impact hops has on beer involves sensory analysis, which entails using consumer volunteers or trained panelists to rate the sensory perceptions (Missbach et al., 2017). Descriptive sensory analysis involves a trained panel that uses a lexicon (terms to describe the product) and references to evaluate products on a line scale. In consumer sensory studies, a large number of consumers (over 75 panelists) are needed to ensure a representative population, and the consumer panels assess the acceptability of a sample usually in terms likeability or preference. For hops, sensory panelists note the specific olfactory characteristics of whole or ground hop cones or beer and are asked to describe and rate the perceived qualities on a scale. The samples presented to the panelists are typically “blind coded” (marked with three-digit codes to prevent biases). Specific flavor profiles can also be reported by sensory panelists when given different beer samples, and a general aroma or flavor lexicon can be made to give specific attributes, such as “fruity,” “herbal,” “floral,” and “citrus” that aid in the identification of distinct profiles imparted from the hop cones into a beer during brewing (Missbach et al., 2017).

There are several consumer sensory methods that a brewer or sensory scientist can implement to obtain a real-time flavor profile of a specific product, and these include time intensity (TI), temporal dominance of sensations (TDS), and drinking profile (DP), and each provides information regarding a panelist’s impression of specific flavor attributes (Vázquez-Araújo et al., 2013). TI entails recording the evolution over time of the intensity of a single sensory attribute, the DP methodology investigates how consumers drink or eat a product, recording the intensity of several attributes during multiple samplings, and TDS is a descriptive multi-attribute methodology that deals with the interaction among attributes (Vázquez-Araújo et al., 2013). To determine which descriptive sensory analysis method is optimal for the complex flavor profiles found in beer, one study conducted by Vázquez-Araújo et al. (2013) presented twelve panelists with three commercial beer samples with different flavor profiles (a traditional English ale, a North American-style lager, and a European-style lager), and trained volunteers were asked to assess many of the notable attributes in each sample, including flavors of alcohol, hops, grass, malt, floral, estery, fruity, bitter, and other common sensory qualities associated with beer. After testing and data analysis occurred, the researchers noted that the TI results showed significant differences ($P \leq 0.05$) in the duration of the flavors of the three studied beers, with estery and fruity notes fading first, and bitterness lasting longest than all other attributes (Vázquez-Araújo et al., 2013). The DP evaluations showed the main gustatory differences among beers, but the evolution of the samples was not revealed after five sips, and the TDS gave useful information about the order of appearance of dominant attributes and took far less time to implement compared to TI which needed at least eight minutes per sample. While a beer's flavor profile can be challenging to assess given the variability of complex flavor attributes, this study showed that each methodology could provide useful information to brewers and can be used to assess the likelihood that the general public will purchase a beer.

Expansion of brewing industry in the United States

According to the Brewers Association, many parts of the country have experienced a dramatic rise in the consumption and production of craft beer within the last decade (Brewers Association, 2019). In 2017, while total beer sales were down, reports indicate that craft beer increased 5% by volume and represented 12.7% of all beer production (8% growth from the previous year) and had a total economic value of $26 billion generated from the 6,266 craft breweries in operation that year (Magsam, 2018).

While the upward trend is prevalent across the country, Arkansas in particular set notable production records within the last few years; "Arkansas breweries produced more than 4.5

million liters (1.2 million gallons) of beer in 2017, a better than 35% increase over 2016, according to data from the state's Alcoholic Beverage Control Division" (Magsam, 2018). Within the same year, the state ranked 38th in the number of craft breweries per capita and had a total of 31 active breweries and 16 microbreweries that collectively produced 4,598,438 L (1,214,779 gal) of beer, or 39,186 barrels, which equated to an economic value of over $406 million for the state (Magsam, 2018).

Due to the often-fluctuating hop production market, a typical brewery contracts hop orders many months or even years ahead of the harvest season to secure hops for brewing. This high demand can cause a dramatic increase in the price of hops cones. Therefore, the increased demand for high quality hops by Arkansas brewers alongside a limited global supply poses the question of whether hops are feasible to grow in the southeastern and mid-southern regions of the United States and if the quality is acceptable. If feasible, the production of hops in Arkansas could potentially ameliorate the cost brewers pay for hops cones, increase regional diversification of specialty crop production, create marketing opportunities for local hop producers, and bolster Arkansas' brewing industry.

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Chapter I

Impact of Cultivar and Fertility Rate on Plant and Cone Attributes of Arkansas-grown Hops

Abstract

The hop plant (*Humulus lupulus*) is a perennial, climbing species within the *Cannabaceae* family that produces cones that contribute to the quality and flavor of beer. Most hops production occurs in the Pacific Northwest of the United States, but growth in the craft brewing industry is driving efforts for hops production in other regions. Recommendations for hops cultivar suitability and fertility management are needed for the U.S. mid-south region. Six hop cultivars (Cascade, Cashmere, Centennial, Crystal, Nugget, and Zeus) were planted at the University of Arkansas System Division of Agriculture Fruit Research Station in Clarksville, AR in 2018. The six cultivars with three fertility rates (low, standard, and high) were grown in a completely randomized block design consisting of three replicates of three plant plots for each cultivar and fertility treatment combination in 2020 and 2021. Fertility rates consisted of low (97 kg/ha), standard (145 kg/ha), and high (193 kg/ha) rates of Triple 13 (13-13-13) applied in four evenly spilt applications on May 15, June 1, June 15, and June 30 of 2020 and 2021. Hops cones were harvested at 70-80% moisture content from August-September 2020 and 2021, dried, packaged, and frozen (-10 °C). The plant and cone attributes evaluated at harvest included number of bines/plant, number of nodes/plant, number of laterals/plant, bine length (m), cone yield/plant (g), and individual cone weight (g). The quality attributes of the dried hops cones assessed post-harvest included cohumulone (%), n+-adhumulone (%), total alpha acids (%), colupulone (%), n+-adlupulone (%), and total beta acids. The cultivar x fertility interaction was not significant for any of the plant attributes at harvest while only the immature cone percentage

was impacted in 2021. No differences were seen in acid content of the hops between the fertility rates in 2020, but all acids attributes were impacted in 2021. Cultivar impacted all harvest attributes except number of bines/plant (2.71) and number of nodes/plant (62.70) in both years. While the number of laterals increased in the third harvest year in general, ‘Cascade’ (105.78), ‘Crystal’ (102.39), and ‘Nugget’ (86.31) produced more laterals/plant than ‘Centennial’ (54.03). ‘Crystal’ had longer bines (12.26 m) than ‘Zeus’ and ‘Centennial’ with the shortest bines at 9.87 m and 8.59 m, respectively. The total cone yield for all plants was 26.91% greater in 2020 (31 kg) for 48 plants compared to 2021 (24 kg) which had 45 hop plants that yielded cones. ‘Crystal’ (755.80 g), ‘Cascade’ (983.63 g) ‘Zeus’ (797.58 g), had the highest cone yield/plant while ‘Centennial’ (67.45 g) had the lowest. ‘Cashmere’ (0.36 g) had a lower individual cone weight at harvest than the other cultivars. ‘Crystal’ and ‘Cascade’ had alpha acid levels within standard commercial ranges, while all ‘Zeus’ and ‘Cashmere’ were lower than typical levels. Regardless of year, ‘Cascade’ (5.50%), ‘Cashmere’ (5.41%), and ‘Zeus (4.75%) had the greatest total alpha acid levels while ‘Crystal’ (7.74%), ‘Cascade’ (5.80%), and ‘Cashmere’ (4.91%) had the highest total beta acids. The descriptive sensory panel (n=5-7) evaluated the aroma of dried, ground hops cones and found that aroma of the cones varied by year, and cones harvested in 2021 had a general increase in aromatic intensity, overall impact, and were more distinctive in defined attributes. This research indicated that ‘Crystal’, ‘Cascade’, and ‘Zeus’ cultivars have potential for commercial hops production in Arkansas, and that fertility rate has little to no impact on the measured plant and cone quality attributes.

Introduction

The hop plant (*Humulus lupulus* L.) is a perennial, climbing plant that produces hops cones used for beer production. Hops plants are grown commercially throughout the world, primarily between the 35th and 55th latitudes in the northern and southern hemispheres. The International Hop Growers' Convention Economic Commission estimated there were 55,000 ha of hops acreage with a global harvest weight of 800,000 kg in 2019 (Hop Growers of America, 2019). The United States, Germany, and China were the three highest hop-producing countries in 2019 with a combined harvest of 106 million kg (Hop Growers of America, 2019).

The primary hop production region in the United States is in the Pacific Northwest, including Washington, Oregon, and Idaho which produced 71%, 17%, and 12% of the total 2020 U.S. hop crop, respectively (USDA, NASS 2020). The remaining states had a commercial hop production estimated at 454,000 kg in 2019 (Hop Growers of America, 2019). While the three main hop-growing states produce 98% of the total U.S. hop crop, many locations around the states are growing hops to meet the rising local demand for cones. Other locations that produce notable acreage of hops include Wisconsin (2.2%), Michigan (1.2%), New York (0.7%), Colorado (0.2%), California (0.2%), Minnesota (0.2%), and Ohio (0.1%) (Hop Growers of America, 2019).

The hop plant is dioecious which means that there are separate male and female plants with female plants cultivated for hop cone production and male plants used for breeding. The shoots are known as bines and can grow supported by a trellis system from 4.6-6.1 m in height. The bines produce lateral shoots and use trichomes (hair-like structures) that attach to twine or rope that run vertically up the trellis to support the plant structure.

Hop bines are incapable of flowering unless 12–25 nodes are visible because hops require a cultivar-specific size effect (distance) between the roots and shoot apex to make the juvenile-adult transformation (Bauerle, 2019). Hops plants mature 3-5 years after establishment depending on location, climactic conditions, cultivar, and cultural management (Chechourka, 2018). Ha et al. (2017) found that hops plants grown in Indiana take 4-5 years to reach full production with yields in the first, second, third, and fourth years estimated at 20%, 40%, 60%, and 80% of mature yields, respectively. Similarly, Sirrine et al. (2015) noted that the yield estimate for the first year is negligible in Michigan-grown hops, but 50%, 75%, and 100% of production are expected in the second, third, and fourth year, respectively.

Hops cones are the inflorescences or strobili (seed cones) from the perennial female hop plant that are an essential component of industrial and craft brewing production. A hop is composed of four primary structures, including the strig, bract, bracteole, and inner lupulin glands. The lupulin glands contain the complex phytochemical compounds valued for aroma and flavor of beer (Hrnčič et al., 2019; Killeen et al., 2016; Patrick, 2013). The cones from the female plants contain various secondary metabolites, hard and soft resins, including alpha acids and beta acids, monoterpenes, sesquiterpenes, essential oils, and polyphenols. There are over 300 compounds found in the inner lupulin glands that contribute to the flavor and aroma profiles of hops cones (Farber and Barth, 2019). The most substantial component of dried hops are the alpha acids, and these compounds are structurally complex enolic acids that contain a six-carbon ring with several substituent groups. While there are more than seven prominent alpha acids within the lupulin glands of the hops, humulone, cohumulone, and adhumulone constitute 98-99% of the alpha acids (Killeen et al., 2016; Mahaffee et al., 2009). The beta acids found within the hop cones are only a minor contribution to a beer's flavor, but they are a crucial component in the brewing process, especially for preservation.

To maintain the valuable components of hop cones, proper drying and storage methods after harvest must be implemented to ensure optimal hop quality for brewing and to allow for long-term use and storage. Freshly harvested hops are approximately 80% water and, if left untreated, will spoil rapidly. Hops are dried after harvest to 8-12% moisture for use in brewing. Dried hops are typically placed in food-grade bags, vacuum sealed to maintain quality, and then frozen (-2 °C) or refrigerated (4 °C).

For brewing purposes, hop cultivars are classified into three categories – bittering, aromatics, and dual purpose. The most popular hop cultivars by acreage in the United States include 'Citra®', 'CTZ' (also known as 'Columbus', 'Tomahawk', and 'Zeus'), 'Cascade', 'Simcoe', and 'Mosaic' with 75.4% of the cultivars grown in 2019 used for aroma (Hop Growers of America, 2019). Aromatic hops are typically higher in essential oil content than the bittering cultivars. These cultivars add notes of grass, fruit, honey, earth, flowers, and other spices, and include cultivars like 'Amarillo,' 'Brewers Gold,' 'Cascade,' 'Citra,' among many others. 'Cascade', 'Chinook', and 'Citra' (high alpha acid cultivars) can yield as much as 771-862 kg/ha while 'U.S. Northern Brewer' (bittering cultivar) and 'Willamette' (a triploid aroma-type hop) yield between 1,343-1,679 kg/ha (Brewers Association, 2019; 2021).

Commercially grown U.S. hop cultivars typically originate from European cultivars adapted to the U.S. terroir (i.e., climactic growing conditions). While over 80 unique hops plants exist today, there has been an increase in the U.S. breeding programs for hops cultivars suitable for the U.S. climate. The tests, procedures, and development required to release a new hop cultivar takes 9-10 years before it can be cultivated industrially (Kerckhoven et al., 2020).

One of the most prominent and widely grown U.S. hop cultivars is ‘Cascade’, the most popular hop by acreage since 2013 (International Hop Growers Commission, 2019). This cultivar was the first hop released from the United States Department of Agriculture (USDA) in 1972. ‘Cascade’ was created from ‘English Fuggle’ and ‘Russian Serebrianker’ through wind pollination by an unknown male variety (BarthHaas, 2021). This was primarily due to the plant’s unique citrus aroma profile, brewing qualities, and adaptation to U.S. hop yards (Kerckhoven et al., 2020). ‘Cascade’ is considered medium in maturity (early September) but retains brewing qualities and a bright appearance for about three weeks after maturity, and the cultivar is resistant to downy mildew while susceptible to *Verticillium* wilt and to Prunus Necrotic Ringspot Virus (Carter et al., 1990). The lateral side branches that emerge from the plant’s nodes are 60.9-76.2 cm, and the cones are compact, medium sized, and easily harvested (Brooks et al., 1972). Commercial producers in the Pacific Northwest reported that ‘Cascade’ can yield 1,792-2,240 kg/ha with an alpha and beta acid levels 4.0-7.0% and 4.8-7.0%, respectively (Idahohops, 2011; Judd, 2018).

Like ‘Cascade’, other high acreage U.S. hop cultivars tend to have greater aromatic qualities due to their distinct aroma and flavor attributes and are used extensively in the craft beer sector. Two aroma hops cultivars that are considered high alpha acid hops are ‘Centennial’ and ‘Zeus’, and these have accounted for a cultivation area of approximately 2,132 ha and 1,976 ha, respectively (Kerckhoven et al., 2020). Characterized by floral, citrus, and medium intensity aromas, ‘Centennial’ was released by Washington State University in 1990 and was derived from a three-quarters cross between ‘Brewer’s Gold’ with contributions from ‘Fuggle’, ‘East Kent’, and ‘Golding’ with 9.5-11.5% alpha acids and 3.5-4.5% beta acids (Judd, 2018). ‘Zeus’, referred to as a super high alpha cultivar, has reported alpha acids between 14.5-16.5% and beta acids near 4-5% with aroma qualities comparable to citrus fruits and plants, such as nettle, aniseed, and fennel (USAHOPS, 2018). The commercial yield potential for ‘Centennial’ and ‘Zeus’ are 1,700-2,000 kg/ha and 2,800-3,249 kg/ha, respectively.

‘Nugget’, another successful high aroma cultivar released by the USDA was commercialized in 1983 and considered a dual-purpose hop with alpha acid and essential oil profiles that impart bitter flavors and a mild herbal aroma in craft beers (Dodds, 2017; Idahohops, 2011). The cultivar’s low proportion of cohumulone (unwanted by brewers for harsh bitterness), good storage stability, and high yield (2,016-2,464 kg/ha) supported the high acreage cultivation of ‘Nugget’ in Washington and Oregon (Idahohops, 2011). ‘Cashmere’, another dual-purpose hop that was released by Washington State University in 2013, has been described as unique, pleasant, complex, and powerful with sweet and citrus fruit aromas like coconut, melon, pineapple, lemon, and lime peel (Healey, 2021; Idahohops, 2011; USAHOPS, 2018). The cultivar has ‘Cascade’ and ‘Northern Brewer’ genetics with alpha acids near 7.7-9.1%, beta acids between 6.4-7.1%, and quality analyses have shown that it contains twice as much humulene as ‘Cascade’ (USA HOPS, 2018).

Hop breeding programs have focused on the development of genetic crosses to increase plant and quality characteristics that are better suited for regional diversification, which can be seen in ‘Crystal’ and ‘Cashmere’. ‘Crystal’ is a triploid cultivar that was bred by the USDA from a cross between the noble hop ‘Hallertau Mittelfrüh’, ‘Cascade’, ‘Northern Brewer’, and ‘Early Green’ and regarded as the most pungent of the triploid ‘Hallertau’ family of hops with alpha acids between 3.5-5.5% (USA Hops, 2018). Studies indicate that triploid hop plants have a higher yield potential, increased alpha acid content, and absence of seeds (Trojak-Goluch and Skomra, 2018).

Irrigation management practices during hops vegetative growth, especially during mid to late season, are crucial for optimal plant vigor, cone, and quality development. While hops plants are somewhat drought tolerant and produce quality cones with standard yields under limited irrigation, research conducted at the Washington State University noted that commercial hops require approximately 610-715 mm (24 to 28 in) of water per year (75-80% of the total annual water use occurs after mid-June) (Evans, 2003). However, water administered at a constant rate could reduce cone production and quality, promote root rot and fungal diseases, and nutrient leaching into local groundwater systems (Jackson et al., 2019).

The physical and chemical properties of the soil are also considerable for a crop's irrigation requirement since both influence nutrient and water-holding capacity (Jackson et al., 2019). The soil quality in Yakima Valley, the primary hops production region in the Pacific Northwest, has notably less clay content than other production regions, and was deemed better suited growing hops (Miller and Highsmith, 1950). Well-drained, deep sandy loam or alluvial soils are recommended due to hops plants significant root system which can develop taproots around 2.5 m (8 ft.) and cover large areas (Adams, 2018; Evans, 2003). Although plants extract 50-60% of water from the top 0.6 m of soil, hops grown in the Pacific Northwest are located on sites with deep soils that hold more water to compensate for years with lower-than-average precipitation (Evans, 2003). Hops perform optimally in soils relatively neutral to slightly acidic in pH (5.6-7.5), and significant variations outside this range could result in nutrient deficiencies and toxicity symptoms (Jackson et al., 2019; Owen and Whipker, 2020; Sirrine, 2010). To produce a substantial hop cone crop, growers must consider the growing location, expected yield, likelihood of drought, water availability, irrigation system, and soil quality to ensure that plants are not water or nutrient deprived.

Climate, seasonal weather conditions, and other environmental factors can significantly influence the quality and production potential of hops plants. Hops are especially sensitive to sun, wind, rain, heat, insects, and diseases (Miller and Highsmith, 1950). Hops are considered short-day, photosensitive plants that thrive in yards with direct sunlight and in locations with a long day length (15 hours or more) as studies have shown a shortage of daylight can diminish hop yield (Viljem et al., 2010). Additionally, evidence has suggested that optimal plant growth has a regional limit based on latitudes between 35 and 55 degrees north that receive approximately 15-16 hrs of daylight prior to the summer solstice, at which time the shorter days initiate flowering (Agehara, 2020; Bauerle, 2019; Krebs, 2019; Sirrine, 2010). Hops have shown optimal growth when specific climactic variables are met, including chill hours below 4.4°C (40°F) for 1□2 months, a vegetative growth season of at least 120 days, and 1700°C (3,092°F) (total daily accumulation of temperatures above a threshold) in a season, otherwise known as growing degree days (GDD) above 10°C (50°F) (American Public Gardens Association, 2019; Sirrine, 2010; Viljem et al., 2010). These metrics are uncommon for locations below 35 degrees latitude north. The primary hops growing region in Washington, located at latitude and longitude 46.602070°N – 120.505898°E, respectively, reported day length at approximately 15 hours and 50 minutes at the solstice, 1,799°C (3,238°F) cumulative GDD from April 1 – October 31, 2021, and an average precipitation of 70 mm (2.8 in) between January 1 – October 31, 2021 (Timeanddate, 2022; Washington State University, 2021). Aside from a fluctuation in rainfall year to year and risks from biotic and abiotic variables (e.g., wildfires, pests, and diseases), the terroir of this region has geographical characteristics that are ideal for optimal hop plant development.

There are many cultural management practices that can impact plant and cone quality in a hop yard including the implementation, timing, and quantity of fertilizer during hop plant growth and development. To accurately determine hop nutrient needs, annual soil tests prior to a plant's uptake of nutrients during late spring and early summer are recommended as nutrient needs vary according to plant age, soil quality and organic matter content, cultivar, biomass accumulation, yield potential, and growing region (Darby, 2011; Dodds, 2017; Judd, 2018; Sirrine et al., 2010). Evaluation of petiole and leaf samples can also identify macronutrient deficiencies throughout critical growth stages of hops. Leaves and petioles are taken when the plants are halfway to the top of the trellis from 1.5-1.8 m above the ground, and results are often compared to previous years' testing to note nutrient variation (Darby, 2011). Owen and Whipker (2020) and Mahler (2001) found that substrate fluctuations, soil temperature, moisture, compaction, and root diseases can influence leaf nutrient availability. Specifically, when the substrate pH is above a species-specific pH, nutrients such as phosphorous (P), iron (Fe), manganese (Mn), boron (B), zinc (Zn), and copper (Cu) are less available for plant uptake that can result in leaf deficiencies. However, a soil pH below the optimal threshold can cause calcium (Ca) and magnesium (Mg) to become less available that could lead to other deficiency symptoms, while Fe, Mn, B, Zn, and Cu are more available for plant uptake that can lead to toxicity symptoms (Owen and Whipker, 2020). To prevent nutrient deficiencies, a grower must implement a fertility and nutrient management regimen specific to cultivar and growing region and these can be employed with natural or synthetic nutrients.

The macronutrients needed for hops production include nitrogen (N), phosphorous (P), and potassium (K), while other minerals such as B, Fe, and Mn are needed in smaller amounts. Previous literature regarding fertilizer recommendations in the Pacific Northwest indicate that soil N is one of the most vital macronutrients for bine development and cone production, and first year plants need approximately 84 kg/ha (75 lb/acre) of N while roughly 112-168 kg/ha (100-150 lbs/ acre) are added in subsequent years as plants mature (Dodds, 2017; Gingrich et al., 2000; Sirrine, 2010). It was found that N fertility may influence yield, arthropod pests, disease, cone aroma and quality, cone chemistry, cone color, and nitrate accumulation in the cones (Iskra et al., 2019). Research has shown that N deficiency can lead to a yellowing effect on hop leaves, cones, and other plant organs, while a plant with optimal N levels will exhibit a bright green hue (Havlin et al., 2016). The increase in plant biomass and N accumulation in hop plants occur at similar rates and are rapid between mid-June until the latter part of July in commercial hopyards with maximum biomass accumulation by the end of July (Sullivan et al., 1999). The N application rates are suggested during the rapid uptake period and adjusted accordingly for soils with lower or higher organic matter levels, with as high as 230 kg/ha on nutrient deficient soils that contain 6 parts per million (ppm or mg/kg) or less of N (Dodds, 2017; Grant, 2021). Previous assessments from N level testing from petioles from the Pacific Northwest reported that a nitrate level range between 6,000-10,000 mg/kg (0.6-1%) is normal, a result of 0-6,000 mg/kg (0-0.6%) is considered deficient, and a value above 10,000 mg/kg (1% or higher) is excessive (Darby, 2011). While this macronutrient addition will vary based many biotic and climactic factors and plant phenological stage at the time of analysis, a grower should ensure that N requirements are met annually to ensure the development of plant matter and cones during production.

Another major macronutrient required for optimal bine growth, leaf development, plant-water balance, and cone production is K. Reports have indicated that hops grown in the Northwest utilize nearly as much K as N at 45-168 kg/ha depending on soil quality, plant age, and growing location with an optimum soil content around 200 mg/L or above (Gingrich et al., 2000; Judd, 2018). According to Gingrich et al. (2000), soil quality in the Pacific Northwest has been cited as high in K, so applications of this macronutrient are often lower compared to N. One notable sign of K deficiency in hops can be visualized by marginal leaf scorch and poor growth (Dodds, 2017). Hops plants at all growth stages have a low P requirement for growth. According to growers, one can apply as much as 22 kg/ha if the soil has an optimal P concentration of 16 mg/L or higher, while deficient soils require 67-112 kg/ha (Darby, 2011). While considered less important than macronutrients, trace minerals such as B, Fe, Mg, Zn, S, and Mn along with specific soil pH (potential of hydrogen) levels are needed for hop plant growth, and these micronutrients have considerable effects on plant and cone development especially when deficient. Inadequate levels of these micronutrients can cause several visible defects in plant and cone properties during hop plant growth. Although these micronutrients are needed to a lesser degree than the macronutrients N, P, and K, they are essential for plant development and can be mixed with other fertilizers in granular form or dissolved in a solution to apply through irrigation lines.

While there are many attributes that impact hops plant and cone quality, the sensory profiles of beer can be “fruity,” “herbal,” “floral,” and “citrus” and result from the distinct profiles imparted from the hop cones into a beer during brewing (Missbach et al., 2017). Other research has been done to determine which descriptive sensory analysis method is optimal for the complex flavor profiles found in beer (Vázquez□Araújo et al., 2013). Although beer flavor profiles can be challenging to assess due to the variability of complex flavor attributes, sensory analysis can provide useful information to brewers that concern the cultivars and quantities of cones that are used in beer. This two-year project by the UA System is important to determine the potential for hops production in Arkansas. Thus, the objectives of this research are to determine the impact of cultivar and fertility rates on plant and cone attributes of Arkansas-grown hops.

Materials and Methods

Hops study

Hops production studies were established at the University of Arkansas System (UA System) Division of Agriculture Fruit Research Station, Clarksville, AR in September 2018 [West-Central Arkansas, lat. 35.3158°N and long. 93.2412°W; U.S. Dept of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]. The cultivar/fertility study was composed of nine 1.2 m wide x 7.3 m long plots divided into three blocks with three replications of six hop cultivars/block (Fig. 1). Equal plant spacing (76.2 cm) was maintained with a “low”, “standard”, or “high” fertility rate (97 kg, 145 kg, and 193 kg 13N-13P-13K granular fertilizer/ha, respectively) using a biweekly, hand broadcasting protocol. Daily maximum and minimum temperatures at the Fruit Research Station were recorded using a Nimbus Digital Thermometer (Sensor Instrument Co. Inc., Center Point, OR). Rainfall was measured using a rain gauge.

Hops trellis and plants

The hops were grown on a trellis that was 3.66 m high with equal spacing between plants. Each hop bine was trained using a landscape fabric staple that had three lines of bailing twine attached that were suspended to the top of the horizontal trellis wire. Prowl® pre-emergent herbicide (BASF Corporation, Durham, NC) was used in early September 2018 prior to planting, and on September 14, 2018, each hop plug plant was planted using a hand trowel and each plant was immediately watered. Plug plants for ‘Cascade’, ‘Cashmere’, and ‘Crystal’ were sourced from Agristarts (Apopka, FL), and ‘Centennial’, ‘Nugget’, and ‘Zeus’ were sourced from Great Lakes Hops (Zeeland, MI). Cultivar selection for this study was based on several factors that included public availability, success rates from local growers, and locations along the similar latitudes that had previously grown these cultivars. A shallow layer of mulch 10.2-15.2 cm deep was placed around each plant shortly after implanting to conserve soil moisture and reduce invasive grasses. One line of drip irrigation was installed with drip emitters (Rain Bird® PCEM20SPB 1.0 GPH) spaced every 76.2 cm to deliver water directly to each plant along the fertility trial row. The hops entered dormancy during the winter months (November through March), and all above ground growth died back to the ground. Bines from all cultivars that survived dormancy emerged from the perennial crowns around mid-March through early April.

Hop fertilizer application

Fertility treatments for the hops plants included three rates, low, standard, and high. The plants received four applications of 13N-13P-13K (Oakley Fertilizer, Inc., Beebe, AR) granular fertilizer that consisted of equal parts N, P, and K. The applications were applied by hand broadcast methods in biweekly intervals on May 15, June 1, June 15, and June 30 in 2020 and 2021 (applied a day early on May 14, 2020, due to rain conditions on the planned date). Previous reports suggest hops require approximately 168 kg/ha (150 lbs/acre) N each season (Gingrich et al., 2000). Therefore, 168 kg/ha was used as the standard rate, with low (112 kg/ha) and high rates (224 kg/ha) set at 66%, and 133% of the standard rate amount, respectively. After converting to the size of the trial (0.0001 ha, 0.0022 ac), each treatment plot received 192.06 g (low), 288.12 g (standard), or 383.19 g (high) per six plants in a treatment for each application in 2020 and 2021. Drip irrigation was used after each application to ensure fertilizer was dissolved into soil unless rain was expected within the subsequent 24-hour period.

Leaf and nutrient sampling for hops plants

Plant tissue nutrient samples were taken from the hopyard on July 6 and July 20 in 2020 and 2021, respectively, prior to hop cone harvest to measure the concentration of macronutrients and micronutrients in the hop leaf tissue. 30 mature leaves and 30 petioles were removed from each cultivar x fertility treatment (18 samples total). Samples were collected from the main bine stem 1.5-1.8 m from the soil level. Leaves and petioles were separated by cultivar and fertility treatment and placed into pre-labeled paper bags, then the bags were placed into coolers for transport. Samples were sent for tissue analysis at the UA System Agriculture Diagnostic Laboratory, Fayetteville. Dried leaves and petioles were ground until able to pass through a 1 mm screen, and analytical tests for nutrient levels involved high-temperature combustion with an Elementar Vario MAX Cube™ (Ronkonkoma, NY), and UV-Vis Spectrophotometry with a Spectro ARCOS ICP (Precision, Centennial, CO). The P, K, Ca, Mg, S, and Na were measured as percent (%) of dry matter while Fe, Mn, Cu, Zn, and B were measured in (mg/kg), and these nutrients were analyzed by nitric acid (HNO_3) digestion while total N carbon levels were obtained by combustion.

Irrigation during growing season

Irrigation management was implemented during the spring and throughout the vegetative growing season (April-August) to ensure plants were not deprived of water. Drip emitters were installed approximately 0.5 m (1.5 ft) above each plant and evenly spaced along a drip irrigation line that was equivalent to the plant spacing used during establishment. Emitters were rated for 1 mm^3 per hour (1 gph) and used anywhere from 6-8 hours 1-3 times per week during the peak summer months (June-August).

Weed control and invasive grass removal during growing season

In addition to the layer of mulch that was added around plant crowns during establishment, invasive weeds and grasses were controlled using spot treatments of Roundup weed and grass killer (Monsanto Company, Saint Louis, MO) during winter dormancy, and were removed by hand or hoe during the growing season. A biweekly weed control protocol was used during the vegetative and reproductive phenological stages of development.

Cultural management, scouting, and pest management methods

Hop plants underwent management practices to promote plant health and cone production. Once bines reached 1.8 m in height, the lowest leaves and lateral branches (below 15.2 cm to soil level) were removed by hand or with sheers to dissuade common pests and diseases and promote airflow throughout the hop yard. The lateral branches of the vigorously growing bines were untangled by hand or pruned to separate the plants from one another as needed throughout the primary vegetative growth stage (June-August). During a ten-week period from (June through early August) in 2020 and 2021, hops plants were assessed visually on several characteristics that included morphology (lateral length, internode spacing, foliage growth and development), signs of disease presence, nutrient deficiency symptoms, and inflorescence time. All visual attributes were reassessed and noted at harvest prior to bine removal from the hopyard.

Certain pests and diseases, such as the two-spotted spider mite (*Tetranychus urticae*), several Lepidoptera species (*Spodoptera frugiperda* and *Polygonia interrogationis*, or army worms and question mark caterpillars, respectively), damson hop aphid (*Phorodon humuli*), downy mildew (*Peronospora sparsa*), and powdery mildew (*Golovinomyces orontii*) can negatively impact the vigor and quality attributes of hops cones. To determine when these pests' affected hops and to what extent their presence had on hop plant health throughout cultivation, weekly scouting with the use of a handheld lens was performed during bine development until harvest (May through August). The scouting method entailed randomly selecting 2-3 plants per plot and examining approximately five leaves/plant (selected from different locations along the length of the bines in a figure V formation) using the magnified lens. When damage was spotted due to insect, disease, or nutritional deficiency, the extent was noted, and an integrated pest management strategy was followed. The use of several brands of insecticides, miticides, and fungicides were implemented in routine backpack spray applications to deter further damage from fungal diseases and common pests (weekly spray schedules were followed when insect populations were significant). Coragen (Dupont™, Wilmington, DE), Thuricide BT (Southern Agriculture Insecticides Inc., Palmetto, FL), and Delegate (Corteva Agriscience, Indianapolis, IN) chemicals were used to exterminate most insects known to affect hops. Acramite (Chemtura, Middlebury, CT) was sprayed when significant mite populations were found, and Forum (BASF Corporation, Durham, NC) and Ranman (ISK Biosciences Corporation, Concord, OH) were implemented as a fungicide to deter mildews. Label rates and safety protocols (the use of protective Tyvek® clothing during spray application) were followed closely to prevent injury or adverse health risks associated with chemical exposure.

Hop harvest

The moisture content and ripeness of the hop cones were assessed during late summer and early fall to determine the ideal time of harvest. Hops were harvested when the moisture content of the cones were 75-80%, the color and texture of the bracts were light and papery to the touch, and the internal lupulin glands were dark yellow and pungent. A sample of 30 cones per plant were picked one to two weeks prior to harvest, weighed, dried until devoid of moisture, and reweighed to determine the moisture content of the cones. All plants were harvested between mid-August through mid-September. At harvest, the hops plant was cut at the base of the plant at soil level and transported to a table located in a tented area to evaluate plant and cone attributes at harvest.

Plant attributes evaluated at harvest. Hops plants were assessed for plant attributes prior to cone harvest. The hops plants were evaluated for the number of bines/plant, number of nodes/plant, number of laterals/bine, and bine length (m).

Cones attributes evaluated at harvest. The hops cones were hand harvested from the bines at 70-80% moisture content and separated into mature, immature, or diseased/damaged. The cones were weighed on an electronic scale (ArlynGuard S, model MKE-5-IS, East Rockaway, NY). The total cone yield (g)/plant was calculated as the sum of weights of the mature + immature + diseased/damaged cones. The percent of mature cones was calculated as the weight of the mature cones/total weight. The percent of immature cones was calculated as the weight of the immature cones/total weight. The percent of diseased/damaged cones was calculated as the weight of the diseased/damaged cones/total weight. The individual cone weight (g) was calculated by dividing the total weight of the 30-cone moisture content sample by 30. The estimated dry cone yield/plant (g) was calculated as 10% of the total yield/plant.

Drying and storing hop cones

The mature and immature cones from each plant were combined and then placed into paper bags (17.8 cm wide x 11.4 cm long x 34.9 cm long) labeled with wet cone weight/bag. The cones in the bags were placed in a dehydrator custom built for this site (Herrera, et al.,2021). The temperature of the dehydrator was 43-49 °C, and a dehumidifier was used to remove moisture from the air. The hops were removed when the cones reached 8-10% moisture content. To ensure the cones were dried to these specifications, the individual bags were weighed every 2-4 hrs after

14-16 hrs elapsed until the intended moisture level was achieved. Additionally, other visual indicators were used to evaluate if hops were sufficiently dried. These included the presence of yellow powdery lupulin when handled and the texture of the bracts (springy, papery, and light in color).

After the cones were dried, the hops were packaged and vacuum sealed in food-grade plastic bags (UltraSource Vacuum Chamber Pouches, 4 mil, 20.3 x 30.4 cm). A Floor Model Chamber Vacuum Packaging Machine (VacPak-It VMC20FGF, Clark Associates, Lancaster, PA) was used to vacuum seal the bags with about 95% air removal from each package. This vacuum strength (removal of air from pouches) varied depending on the number of hops in the package. If the vacuum strength was too high, the cones were crushed, and the lupulin would fall from the cones and settle at the bottom of the plastic bag. The bags of hops were placed into a freezer at -2 °C for later analysis.

Dried hops analysis

Dried hops were taken to the UA System Food Science Department for analysis. For the analysis of the dried hop cones, hops bags were removed from the freezer, samples were removed, and the unused hops were resealed with the vacuum sealer and returned to the freezer. The whole-cone hops were ground for analysis using a Magic Bullet blender (MBR - 1101, Los Angeles, CA) with cross blades in a 473-mL container. Analysis of dried hops included moisture content and alpha and beta acids by High Pressure Liquid Chromatography (HPLC) using American Society of Brewing Chemists (ASBC) methods. The extractions of alpha and beta acids were done in analytical triplicate per sample. The moisture content of the hops was done in analytical duplicate per sample.

Moisture content analysis. The moisture content of the dried hops must be analyzed because the moisture content after drying can deviate from the optimal 8-10%. The hops were dried 100% to determine the moisture content for the hops cones to calculate alpha and beta acids levels using the ASBC method Hops-4C (Moisture by Routine Air Oven Method). Approximately 2.5 g of unground hops were placed in an aluminum dish. The dish was covered with aluminum foil, then the dishes with hops were weighed on a precision scale (0.001 g) and placed in a Fischer Scientific Isotemp Oven Model 655F (Houston, TX) at 103-104 °C. The dish covers were removed, the hops were dried for 1 hr, then the covers were replaced while the dish was in the oven. The dishes were transferred to a desiccator containing Drierite Absorbent (8 mesh DX2515-1, Millipore Corporation, Burlington, MA). The lid was placed on the desiccator and sealed with high vacuum silicone grease. The hops were cooled in the desiccator and reweighed. After weighing, the percent moisture of the hops was calculated using the formula:

$$\text{moisture in hops (\%)} = (\text{loss in weight}*100)/(\text{weight of undried sample})$$

Dry weight of the samples can also be calculated from the moisture content.

Alpha and beta acid analysis. Dried hops were analyzed by HPLC using the ASBC Hops-14 (alpha acids and beta acids in Hops and Hop Extracts by HPLC) procedure. This procedure was modified because of the limited amount of sample. A 2-g sample of dried hops were placed in 50-mL centrifuge tubes and weighed. Then, 4 mL of methanol and 20 mL of diethyl ether were added to each tube. The tube was capped and placed on a shaker for 30 min. After 30 min., flasks were opened and 8 mL of 0.1M hydrochloric acid was added. The original method for Hops-14 instructs to use 10 g of hops with 20 mL of methanol, 100 mL of diethyl ether and after shaking 40 mL of hydrochloric acid. So, for this project, the HPLC extraction was downscaled by a factor of five as compared to the original procedure. The flasks were capped and placed on the shaker for 10 min. After this, the flasks were kept in the dark for 10 min as the phases separated. After the phases separated, 1.0 mL of the supernatant phase was pipetted in a 10 mL volumetric flask and brought up to volume with methanol. The contents of the flask were sealed with parafilm and mixed. The solution was syringe filtered using a 25 mm 0.45 nylon membrane filters (VWR, Radnor, PA) before injection into the HPLC.

Samples (50 µL) were analyzed using a Waters HPLC system equipped with a model 600 pump, a model 717 Plus autosampler and a model 996 photodiode array detector. Separation was carried out using a Phenomenex (Torrance, CA) Nucleosil-5 C18 chromatographic column (250 × 4 mm, 5-µm ODS RP18). The mobile phase was a combination of methanol, water, and phosphoric acid in an 85:17:0.25 ratio (v/v) that was mixed and filtered through a 0.45-µm filter. To achieve adequate resolution, the column was conditioned with mobile phase for 1 hr prior to use. The flow rate was 0.8 mL/min, and the detection wavelength was 314 nm at an ambient temperature. Each sample was injected and analyzed in duplicate with a run time of 30 minutes. Samples were either run on the HPLC immediately or stored at 2 °C and protected from light for analysis within 24 hours. After analysis, the HPLC peak areas were converted to levels of the alpha and beta acids using the standard curves. The percentage of the fraction per gram of hops was calculated using the following formula:

% w/w= (HPLC conc (mg/ml) *methanol volume (mL)*(mL methanol+mL ether+mL hydrochloric acid))/(mL supernatant taken*1000*starting weight of sample (g)).

<u>Standards and Calibration.</u> The calibration curve was made using Standard hop extract ICE-4 (ASBC, Saint Paul, MN) for HPLC analysis. This is a hop extract containing a specified concentration of alpha and beta acids. ICE-4 contains cohumulone (10.98%), n+adhumulone

(31.60%), colupulone (13.02%), and n+adlupulone (13.52%) with total alpha acids levels of 42.58% and total beta acids levels of 26.54%.

Alpha acids can be subdivided in three main individual acids: cohumulone, n-humulone, and adhumulone. The procedure of ASBC Hops-14 that was used to separate cohumulone as an individual fraction and n-humulone and ad-humulone together as a fraction. This gives two fractions: "cohomulone" and "n+-adhumulone". The same applies to the beta acids. Colupulone was separated from the other beta acids, n-lupulone and adlupulone.

From the ICE-4 standard, 1.500 ± 0.001 g was weighed and diluted in 25 mL of toluene in a 25-mL volumetric flask. The standard was first diluted (dissolved) with toluene. The toluene dilution was then diluted by a factor of 10 volumetrically with methanol (standard A) followed by subsequent dilutions. The calibration curve of each of the standards was achieved by plotting the levels of cohumulone, n+adhumulone, colupulone, and n+adlupulone in the standard against the acquired area.

Sensory Analysis

Descriptive sensory analysis was performed at the Sensory and Consumer Research Center at the UA System, Fayetteville, AR in 2020 and 2021. The descriptive sensory panelists (n=5-7) evaluated the aroma of dried, ground hops for each cultivar in triplicate. Only four cultivars (Cascade, Cashmere, Crystal, and Zeus) were evaluated due to limited availability of 'Centennial' and 'Nugget'. The samples for sensory analysis for each cultivar were from the standard fertility rate and field replications were combined for sensory analysis, but panelists evaluated the hops in triplicate. The hops were ground and served to the panelists one at a time at room temperature (25°C) in Snap-Seal™ translucent polypropylene containers (45 mL) labeled with three-digit codes. Serving order was randomized across each replication to prevent presentation order bias. Panelists were trained to use the Sensory Spectrum method, an objective method for describing the intensity of attributes in products using references for the attributes. Intensities of the aroma were based on the Universal Scale, where a saltine cracker was equal to 2.0, applesauce was equal to 5.0, orange juice was equal to 7.5, grape juice was equal to 10.0, and Big Red Gum® (Mars, Inc., MeLean, VA) was equal to 15.0. The panelists developed a lexicon of descriptive sensory terms through consensus during orientation and practice sessions for the aroma attributes of dried, ground hops (Table 1). The descriptive panel evaluated the hops for 23 aroma attributes using a 15-point scale, where 0 = less of an attribute and 15 = more of an attribute.

Design and statistical analysis

This study analyzed as a full factorial with cultivar (Cascade, Cashmere, Crystal, Centennial, Nugget, and Zeus) and fertility rate (low, standard, and high) as the main effects and cultivar x fertility rate as the interaction by year (2020 and 2021). The fertility rate treatments were in triplicate each year. The alpha and beta acid attributes were evaluated in analytical triplicate while the moisture content analysis was assessed in analytical duplicate. Pest data was presented as observations. Leaf and petiole nutrient levels of the cultivars and fertility rates were presented as means and standard deviations by year. Statistical analyses were conducted using JMP® (version 16.0.0; SAS Institute, Cary, NC). To determine if there was a significant difference among the fertility rates, a univariate analysis of variance (ANOVA) was used to analyze the levels of variance. Tukey's honest significant difference (HSD) test was used to detect significant differences ($p < 0.05$) among means and verify interactions at 95% significance level. For descriptive sensory evaluation, four cultivars from the standard fertility rate were evaluated in triplicate using a univariate ANOVA to detect the significance of the cultivar main effect for each attribute. The panelist main effect and genotype x panelist interaction were included in the model to account for the error explained by between-panelist and within-panelist variation.

Results and Discussion

Average monthly temperature and rainfall at the Fruit Research Station in Clarksville, AR were tracked, recorded, and reported from January to September, the end of hops harvest (Fig. 2.) through reports generated by the Southern Regional Climate Center (Texas A&M University, 2022) and with a Nimbus Digital Thermometer (Sensor Instrument Co. Inc., Center Point, OR). The 2020 hops season in Clarksville, AR was relatively mild in terms of temperature and rainfall. The 2021 season had notable weather events in February and April. There were record cold temperatures (-5 °C) with 178 mm of snow in February of 2021 at the Fruit Research Station followed by a freeze in late April (-1 °C overnight). Shoots of the hops plants emerged in the spring in mid-March and early April both years. The average high temperature was 22 °C and low temperature was 12 °C in 2020 and 2021. Average (January-September) rainfall in 2021 (103 mm) was less than rainfall in 2020 (139 mm). The total precipitation from January to September was 1,247 mm and 929 mm in 2020 and 2021, respectively. During July to September in 2021, there was less rainfall each month with 239 mm less during these months compared to 2020 (445 mm). Maximum day length for both years occurred June 20 with 14 hours and 36 minutes of daylight (1 hour and 18 minutes less than commercial regions in the Pacific Northwest). The average day length was 12 hours and 48 minutes during the measured time interval. Growing degree day was calculated by totaling the number of hours above the minimum degree threshold for hops (10 °C). Between May 1 – September 1, there were 4,718 GDD in 2020 and 4,910 GDD in 2021.

Leaf and petiole tissue nutrient analysis

According to Sallato (2020), leaf and petiole tissue nutrient data is a valuable and standardized tool to diagnose nutrient levels and monitor the nutritional status of plants. Results from these analyses determines whether a plant has adequate, deficient, or excessive amounts of each element and what nutrients were taken up after fertility applications. Data was compared to optimal ranges of nutrients for hop petioles and leaves reported in research from hops grown in similar growing conditions as Arkansas and sampled a similar phenological stage (Table 2).

The results from plant foliage and petiole sampling (Table 3) indicated that foliage and petiole macronutrients and trace elements varied between cultivars and fertility with some levels within and below comparable nutrients from hops grown in North Carolina (Table 2). Davis et al. (2012) noted that hop leaves sampled at four sites on July 15 during the reproductive phenological stage and full bloom had between 2.64-3.22% N in leaves while Sirrine (2019) stated that typical petiole N varied between 2.13-3.93% in commercial yards. The leaf and petiole nutrient ranges for N for nearly all cultivars and fertility rates were within optimal levels in both years except for 'Crystal' in 2020 which had slightly lower petiole N at 1.62%. Except for 'Centennial' (4.07%), both 'Crystal' (3.30%) and 'Cascade' (4.42%) had lower leaf N levels in 2020 compared to the other cultivars, but both cultivars were further along in their phenological development at the July 6 sampling date as it was observed that they emerged from the soil and produced flowers one to two weeks prior to the other four cultivars. Leaf sample results from both years indicated that increased fertility had higher levels of leaf N as the low rate had the lowest N levels while the standard and high rates had higher N accumulation. While within a comparable range, the leaf N results from 2021 were lower on average when compared to the previous year, but the sampling date of July 20 in 2021 compared to July 6 in 2020 likely attributes to the lower leaf N levels. Rodriguez (2018a) noted that excess N can promote excess foliage growth, but other plant characteristics suffer consequently. Specifically, the energy for flower growth can be redirected to foliage proliferation which could cause plants to fail to produce the necessary reproductive organs during the growing season. High-nitrogen fertilizer rates have also been reported to increase the mineral salts (Na) content in soil which can subtract water from the plant while leaving the salts behind that can cause severe leaf deterioration (burned appearance), chlorosis (yellowing), browning, and wilting (Rodriguez, 2018b). Results for P levels in both years in the Arkansas study indicated that all plants were within the standard range when compared to the North Carolina study. Davis et al. (2012) showed that typical P levels the leaves of hops plants at sites in North Carolina had between 0.21-0.33% P, and all plants sampled in northcentral Arkansas in July 2020 and 2021 had between 0.21-0.37% P. The values for K of leaves for all cultivars and fertility rates were generally within optimal leaf nutrient ranges (1.10-1.84%) in both years while several cultivars in 2020 and 2021 (‘Cashmere’, ‘Centennial’, ‘Nugget’, and ‘Zeus’) had higher K nutrient accumulation in leaves compared to the cultivars grown in North Carolina. In general, it was seen that leaf K levels were lower in 2021 and increasing fertility did not have a correlation to increased K nutrients in the sampled leaves.

All plants in the fertility trial had lower levels of several micronutrients when compared to the North Carolina research conducted by Davis et al. (2012), including Ca, Mg, Zn, and B, while other micronutrients (S and Fe) were within a sufficient range. The Ca levels of the leaves of plants grown in North Carolina fluctuated between 3.57-5.11% while the northcentral Arkansas-grown hops varied between 1.12-4.07% in both years. Similarly, Mg levels, which were between 0.85-1.29% in the leaves sampled by Davis et al. (2012) were found to be lower in the Arkansas hops leaves that had between 0.21-0.38% Mg in 2020 and 2021. B nutrient ranges were 79.20-126.20 mg/kg in the Davis et al. (2012) study while the B levels in the Arkansas-grown hops were substantially lower (8.37-23.71 mg/kg). Zn levels between 24.43-41.63 mg/kg were seen in leaves sampled in North Carolina during the reproductive stage while the Arkansas fertility study leaves had 16.38-23.33 mg/kg leaf Zn. Darby (2020) noted that leaf nitrate levels in hops grown in Vermont decreased over the course of the sampling period (early to late July) when hops transition from the stage of bine and lateral development to the production of inflorescences and cones, so it can be presumed that the N concentration in the leaves and petioles from Arkansas-grown hops would have decreased when sampled later in July or early August. The S and Fe levels between all cultivars and fertility applications were within the comparable nutrient ranges seen in the North Carolina.

Morphological, plant vigor, pest, and flowering assessments

Multiple plants, regardless of fertility rate and cultivar, had leaf chlorosis (yellowing), burnt and scorched leaf coloration, foliage distortions (cupping, non-uniform shapes), and several cultivars (Centennial, and Nugget) had plants that either failed to reach the top of the trellis or produced an inadequate number of nodes for inflorescence to occur. These visual signs are characteristic of nutrient deficiency, particularly Zn and B (Judd, 2018; Mahler, 2001; Owen and Whipker, 2020). Judd (2018) noted that Zn deficiency symptoms include interveinal chlorosis (pale green, yellow, or white color) with shortened internodal distance, and premature leaf and shoot deterioration while B deficiency symptoms entail abnormal or delayed apical shoot development, terminal shoot death, and deformed, brittle leaves. Halo blight, a bacterial disease caused by *Pseudomonas syringae*, was identified in hops leaf samples collected in 2020. According to Sirrine et al. (2022), the disease can lead to brown leaf lesions with a halo of chlorotic tissue, brown bracts, fragile cones that shatter when handled, and substantial yield loss. Added pest pressure from Japanese beetles (*Popillia japonica*), arthropods, like the two-spotted spider mite (*Tetranychus urticae*) and the damson-hop aphid (*Phorodon humuli*), army worms (*Spodoptera frugiperda*), and question mark caterpillars (*Polygonia interrogationis*) were prevalent during the two-year trial, but the most notable insect outbreaks in the northcentral Arkansas hop yard occurred in July and August in 2020. Both nutrient deficiency, insect pressure, drier weather conditions in 2021, and halo blight were likely two factors that contributed to harvest losses and cone deterioration regardless of cultivar in both years.

Flower emergence occurred within a 3-week time frame in 2020 and 2021, and the date was cultivar-specific. 'Cascade' and 'Crystal' showed signs of inflorescence that occurred during the first two weeks of June in 2020 and 2021, followed by 'Nugget' and 'Zeus' (late June through early July), 'Cashmere' (mid-June through mid-July), while 'Centennial' exhibited the most variable flowering dates (mid-June through early August) in both years.

Plant attributes at harvest

Hops plants were assessed for plant attributes at harvest, including number of bines/plant, number of nodes/plant, number of laterals/plant, and bine length (Tables 5 and 6). There was not a significant cultivar x fertility rate interaction and fertility rate did not affect any of the cone attributes in both years. Cultivar did not impact the number of bines/plant or the number of nodes/plant in 2020 or 2021, yet the number of laterals/plant and bine length was significantly impacted in both years. Regardless of year, the number of bines/plant ranged from 2.4-3.0, the number of nodes/plant varied from 46-74, number of laterals/plant ranged from 41.2-129.7, and the bine length fluctuated between 8.4-14.2 m.

2020. In 2020, the number of bines/plant was 2.63, and the number of nodes/plant was 56.66. 'Cascade' (81.89), and 'Cashmere' (84.67) had significantly more laterals than 'Centennial' (41.22). 'Cashmere' (12.16 m) had the longest bine length, while 'Nugget' (8.4) had the shortest total bine length.

2021. In 2021, the number of bines/plant was 2.80, and the number of nodes/plant was 68.73. 'Cascade' (129.67), 'Crystal' (126.89), and 'Nugget' (126.67) produced significantly more laterals than 'Centennial' (66.83). 'Centennial' (8.63) had the shortest bine length along with 'Zeus' (9.70 m) and both were significantly shorter than 'Crystal' (14.22 m) which had the longest bine length.

While many factors could be attributed to lateral number and bine development, cultivar nutrient uptake, growth after establishment, and plant establishment date are likely attributable to the differences in lateral development and plant growth variation seen among cultivars. A standard training protocol was used in which three bines per plant were trained after the initial shoot pruning date (April 30), but mechanical pruning/weeding, wind damage, or weak bine development that caused tissue injury led to the variation in bine number of some of the plants. Plug plants for 'Cascade', 'Cashmere', and 'Crystal' were sourced from a different location than 'Centennial', 'Nugget', and 'Zeus'. 'Centennial' and 'Nugget' struggled after establishment (September 14, 2018). Two of the nine 'Centennial' plants and five of the nine 'Nugget' plants did not survive winter dormancy or spring pruning prior to harvest in 2020 and were replanted later in the spring. Throughout the vegetative growth stage in both years, 'Centennial', 'Nugget', and 'Zeus' were among the shortest bines at harvest in 2020 and 2021. Previous literature regarding planting date indicates that commercially-grown hops are typically planted in early spring (late April to early May) (Sirrine, 2010). A lower soil temperature at the time of planting and other weather-related conditions during the hop yard establishment in the Fall of 2018 may have affected survival rates and development.

A study conducted by Davis et al. (2014) assessed hop vigor, overall visual health, and plant height throughout the growing season in North Carolina and compared the morphological observations of 10 cultivars (Sterling, Northern Brewer, Centennial, Willamette, Mt. Hood, Newport, Cascade, Chinook, Nugget, and Zeus) for three years. The site (Mills River, NC) was in a mountainous region that had a primarily sandy loam soil, and the cultivars were planted on a high-trellis system (6 m tall). Davis et al. (2014) noted that the plant development varied widely among cultivars. In general, 'Zeus', 'Nugget', 'Chinook', and 'Cascade' were the top performers in terms of length, vigor, and overall visual health, 'Centennial' had a moderate bine length yet weak bine growth, 'Sterling' and 'Northern Brewer' emerged late, remained poor performers, and suffered considerable pest damage from Japanese beetles, and the high-alpha acid cultivars performed the best regarding vigor.

The results from the North Carolina study differ in comparison to the results found in the Arkansas-grown hops fertility trial with some exceptions. In terms of bine length and vigor, the cited highest alpha acid cultivars (Zeus, Centennial, and Nugget) had shorter bine lengths at harvest ranging from 10.7 m for 'Nugget', 9.9 m for 'Zeus', and 8.6 m for 'Centennial' between years compared to 'Cascade', 'Crystal', and 'Cashmere' which had 12.7 m, 12.3 m, and 11.8 m, respectively. In the North Carolina study, 'Zeus' had a bine length around 18.1 m while 'Cascade', 'Nugget', and 'Centennial' had lengths at 14.2 m, 13.9 m, and 13.0 m, respectively. Although trellis length (61% taller) can account for some variation between cultivar bine lengths between both trials, the Mills River, NC trial location (lat. 35.3884) compared to Clarksville, AR site (lat. 35.3158N) indicated that day length was not attributable to the variability. Afonso et al.

(2020) examined hop vigor and yield in northeast Portugal, and an analysis of soil properties was determined and related to the plant nutritional status and dry matter yield of different parts of the plant (hops, leaves, stems). Results suggested that crop yield was reduced mostly due to poor soil aeration and excessive soil and tissue Mn and Fe levels. While lower Fe levels were seen in hop leaves collected in mid to late July in 2020 and 2021, the plots that were rated with weak vigor were likely affected by other biotic and abiotic variables during the vegetative and reproductive growth stages.

Studies regarding fertilizer recommendations in the Pacific Northwest indicated that N is one of the most vital macronutrients for bine development and cone production, and first year plants need approximately 84 kg per hectare of N while roughly 112-168 kg per hectare are added in subsequent years as plants mature (Dodds, 2017; Gingrich et al., 2000; Sirrine, 2010). The application rates in 2020 were equivalent to the fertility rates applied in 2021, and were comparable to the rates used for hops plants grown in the Pacific Northwest (Dodds, 2017; Gingrich et al., 2000; Sirrine, 2010). Gent et al. (2015) noted that excessive nitrogen fertilization can increase incidence of several diseases and arthropod pests, including powdery mildew, Verticillium wilt, spider mites, and hop aphids.

Darby (2011) noted that hops plants grown in Vermont receive N applications around 30 to 45 days after shoot emergence or mid-May to mid-June for the northeastern U.S climate. While the primary N uptake period for hops occurs during the vegetative stage (May through early to mid-July), reports have indicated that a grower should refrain from adding fertility treatments after inflorescence as this can lead to unwanted vegetative growth (Darby, 2011). Other studies have also recommended earlier N fertilizer applications to avoid times in the season when pests are highly evident since large doses of N applied later in the summer (e.g., late June to early July) may induce spider mite outbreaks (Gent et al. 2015). Ford et al. (2021) indicated that fertility applications after burr detection have the potential to increase the risk of pests and diseases while potentially reducing cone quality and hop yield.

Cone attributes at harvest

The cone attributes evaluated at harvest included total cone yield/plant, percent of mature, immature, or damaged/diseased cones per plant, cone moisture content (%), individual cone weight, and estimated dry cone yield/plant (Table 6 and Table 7). Cones were harvested at a moisture content of 67%-84% for both years (data not shown). There was not a significant cultivar x fertility interaction for any of the cone attributes at harvest in 2020, while a significant cultivar x fertility rate interaction was seen for the immature cones/plant in 2021. Fertility rate effects did not impact any of the cone attributes except mature cones/plant and damaged cones/plant in 2020. Cultivar had a significant impact on all cone attributes in 2020 and 2021. The results from Tables 7 and 8 indicated that cultivar had more of a significant impact than the fertility rate on the measured plant and cone attributes at harvest. The total cone yield for all plants was 26.91% greater in 2020 (30.90 kg) for 48 plants compared to 2021 (24.35 kg) which had 45 hop plants that yielded cones. Regardless of year, total cone yield/plant ranged from 52.6-1,175.3 g, mature cone percent fluctuated between 54.3-93.4%, immature cone percent varied from 1.7-39.5%, damaged cone percentage differed from 0.2-52.5%, and individual cone weight ranged from 0.3-0.7 g. Estimated dry cone weight was 10% of the value of total cone yield/plant. The disparity in rainfall from July through September in 2021 (206 mm) compared to 2020 (445 mm) was considerable and likely resulted in lower cone production and quality attributes measured in 2021.

2020. In 2020, ‘Cascade’, ‘Crystal’, and ‘Zeus’ had the highest total cone yield/plant (1,175.28 g, 656.42 g, and 1,072.09 g, respectively). ‘Cashmere’, ‘Nugget’, and ‘Centennial’ had the lowest cone yield/plant in 2020 (442.86 g, 187.42 g, and 78.28 g, respectively). ‘Cascade’ and ‘Zeus’ had significantly more total cone yield/plant compared to ‘Cashmere’, ‘Nugget’, and ‘Centennial’. ‘Zeus’ (92.47%) had the highest mature cone percentage among all cultivars and significantly more than ‘Cascade’ (57.15%), ‘Centennial’ (44.85%), and ‘Nugget’ (41.69%). ‘Nugget’ (39.45%) and ‘Cascade’ (36.45%) had the greatest percentage of immature cones/plant and significantly more than ‘Centennial’ (20.61%), ‘Crystal’ (10.77%), ‘Cashmere’ (8.46%), and ‘Zeus’ (5.77%). ‘Centennial’ (34.54%) had a significantly higher percentage of damaged cones/plant compared to ‘Cashmere’ (15.26%), ‘Crystal’ (12.85%), ‘Cascade’ (6.40%), and ‘Zeus’ (1.76%). ‘Cashmere’ (0.39 g) had lower individual cone weight than ‘Nugget’, ‘Crystal’, ‘Zeus’, and ‘Cascade’ (0.64 g, 0.63 g, 0.62 g, and 0.58 g, respectively). The standard fertility rate had a significantly greater percentage of mature cones (74.98%) compared to the low rate (55.17%). The standard (5.58%) and high (14.32%) fertility rates had significantly fewer damaged cones/plant than the low fertility rate (24.99%). ‘Zeus’ (84.48%) had the highest moisture content at harvest followed by ‘Crystal’ (78.49%), ‘Nugget’ (76.55%), ‘Cascade’ (74.36%), ‘Centennial’ (73.67%), and ‘Cashmere’ (73.53%) (data not shown).

2021. In 2021, the cultivar x fertility rate interaction was significant only for immature cones/plant (Figure 3). ‘Cascade’, ‘Crystal’, and ‘Zeus’ has the highest total cone yield/plant in in (791.972.0 g, 855.17 g, and 523.06 g, respectively). ‘Cashmere’, ‘Nugget’, and ‘Centennial’ had the lowest cone yield/plant (360.73 g, 385.03 g, and 52.61 g, respectively). ‘Crystal’ had significantly higher total cone yield/plant compared to ‘Nugget’, ‘Cashmere’, and ‘Centennial’. ‘Zeus’ (93.36%), ‘Crystal’ (88.54%), and ‘Nugget’ (79.92%) had the greatest mature cone percentage per plant which were significantly greater than ‘Cascade’ (54.26%) and ‘Centennial’ (41.01%). Significant differences in cone immaturity percentage were evident from the cultivar x fertility interactions seen in Fig. 3. In general, all ‘Cascade’ cultivar x fertility rate combinations had higher percentages of immature cones compared to the other cultivars with 43.49% of the total cones considered immature. Alternatively, ‘Cashmere’, while not significantly different between fertility rates, had the lowest percentage of immature cones postharvest (1.71%). ‘Zeus’ cones were not impacted by fertility rate for cone immaturity and were seen to have one of the lowest amounts of immature cones (5.56%). ‘Nugget’ standard fertility rate (37.18%) had significantly more immature cones at harvest compared to the cones grown using the high fertility rate (8.40%). While not significant, ‘Crystal’ high fertility rate (16.80%) had a greater total immature cone quantity compared to the low (4.20%) and standard (8.60%) fertility rates. The ‘Centennial’ plants grown using the standard rate of fertilizer did not produce immature cones. ‘Centennial’ (52.53%) had a significantly greater damaged cones/plant percentage compared to all other cultivars. Of the three ‘Nugget’ plants grown using the standard fertility rate, one plant survived to harvest and produced no damaged cones while the two of the high rate ‘Nugget’ plants had no damaged or low (0.04%) damaged cones. ‘Cashmere’ (0.33 g) had significantly smaller and lighter cones compared to each of the other cultivars that fluctuated between 0.56 g (‘Cascade’) and 0.72 g (‘Nugget’) per cone. ‘Zeus’ (79.4%) had the highest moisture content at harvest followed by ‘Centennial’ (74.2%), ‘Crystal’ (73.72%), ‘Nugget’ (71.93%), ‘Cashmere’ (70%), and ‘Cascade’ (67.06%) (data not shown).

Iskra et al. (2019) conducted a multi-year field study in Oregon and Washington to evaluate the influence of N fertilization rate and timing on cone quality and nitrate accumulation in cones. Results showed that the impact of N rate on cone yield, levels of hop acids, total oil content, color, and nitrate level were year dependent. However, when data were aggregated over years and analyzed using a mixed effect model, yield was not improved with the highest N rate. Additionally, a year-by-year analysis indicated that N rate application timing had no significant effect on yield. The application of equal part 13N-13P-13K in the northcentral Arkansas hop fertility trial using varied rates were similar to the findings of Iskra et al. (2019) that reported no significant increase in yield when a higher nitrogen rate was used. While the high fertility rate had the greatest total cone yield/plant (646.21 g) in 2020, it was not significantly different than the other fertility rate effects on yield. In 2021, the high fertility rate had the lowest total cone yield/plant (474.72 g) compared to the standard (511.48 g) and low (498.08 g) rates, yet these were not significantly different in terms of yield.

High-yielding plants such as hops require adequate nutrition to grow optimally and produce higher yields with standard quality cones. Many of the nutrients required by hops may be deficient or excessive compared to the crop’s needs. It can be difficult to ascertain the specific cause of lower yields and abnormal plant symptoms, especially if multiple production factors can lead to the same symptom. Gent et al. (2015) reported that hop plants are sensitive to Zn deficiency. Plants deficient in Zn have weak growth, short lateral branches, and poor cone production. B deficiency can result in delayed emergence of shoots, stunting, distortion, and crinkling of young leaves and it was found to be most common in acidic and/or sandy textured soils. Gent et al. (2015) noted that P deficiency can cause thin and weak bines, and brown discoloration on hop cones. Lower Zn, B, Ca, and Mg leaf nutrient levels compared to hops grown in North Carolina and sampled during a similar time frame were noted in samples in both 2020 and 2021, and several signs of nutrient deficits were seen in several cultivars during production. ‘Centennial’ and ‘Nugget’ struggled to grow and produce after establishment. Regardless of cultivar, fertility rate, and year, many plants throughout the fertility study demonstrated characteristics of micronutrient deficiencies, particularly Zn and B, water and heat stress, and cone browning due to weather conditions and halo blight.

A cultivar trial conducted by Davis et al. (2012) assessed fresh hop cone yield for 10 cultivars (Sterling, Northern Brewer, Centennial, Willamette, Mt. Hood, Newport, Cascade, Chinook, Nugget, and Zeus) at 2 farm locations (Raleigh, NC and Mills River, NC) that were referred to as the Piedmont and Mountain sites, respectively, and the climates differed by temperature, elevation, annual precipitation, and soil texture. Researchers noted that the Piedmont study hop yard had a sandy clay loam soil, hot and humid summers, and higher than average rainfall. The mountain study locale had a sandy clay loam soil texture with slightly cooler summer weather and less precipitation comparatively. The 10 cultivars were planted in 0.1 ha hop yards in both trials except for ‘Zeus’ and ‘Centennial’ at the piedmont site which had 30 and 25 plants, respectively. The mountain study was planted in 2011 on a high-trellis system (6 m tall) while the Piedmont study was planted in 2010 on a short-trellis system (3.7 m tall) that was almost identical in height to the northcentral Arkansas fertility trial (3.6 m tall). Hop cones were hand-harvested on four dates from mid-July through early September for the second year of production at the piedmont hop yard while the mountain study was hand-harvested on eight dates from late July through early September for the first year of production. Results showed that 85% of the total yield was harvested from just two cultivars (Zeus and Cascade) at the Piedmont site that had a cone yield/plant of 147 g and 145 g, respectively, followed by ‘Nugget’ (11 g) and

‘Centennial’ (6 g). Depending on cultivar, the yields at the Mountain site were 3-30 times greater than in the Piedmont site. ‘Zeus’ (499.0 g) and ‘Cascade’ (464.9 g) had the highest yield per plant while ‘Nugget’ and ‘Centennial’ yielded 340.2 g and 59.5 g, respectively. The yields from these cultivars at the Mountain site were more comparable to the third-year yields from the northcentral Arkansas fertility study (Table 7), and it indicated that total cone yield was highly variable and dependent on cultivar which suggests that certain cultivars are better suited and more adaptable for specific regions.

Judd (2018) evaluated 13 publicly-available hop cultivars (Alpharoma, Cashmere, Cascade, Centennial, Comet, Crystal, Mt. Hood, Mt. Rainier, Nugget, Sorachi Ace, Southern Cross, Tahoma, and Ultra) in Virginia for two years. Plants were trellised and trained using a 5.4 m-trellis in a 0.7-acre hop yard with Duffield soil – a well-drained, fine loamy soil mixture (USDA Hardiness Zone 6b, 2100’ elevation, lat. 37°22’N, long. -80°46’W) (Judd, 2018). Judd (2018) used two applications of ammonium sulfate ((NH4)2SO4) granular fertilizer using Pacific Northwest recommended levels on a per ha basis along with 5.6-11.2 kg/ha of water-soluble fertilizer (15N-5P-15K Ca +Mg) through the irrigation system each season. The results from the Arkansas-grown fertility trial were comparable to the results reported by Judd (2018), including significant differences among hop cultivars for plant height, cone weight, and other traits examined during growing seasons. Although several cultivars showed a decrease in the mean weight of cones produced, the mean weight of cones/plant over all cultivars increased from 2016 to 2017 (298.44 g to 373.07 g, respectively). Among the cultivars assessed at both trials, Judd et al. (2018) noted that ‘Cascade’ (945 g) and ‘Crystal’ (812 g) produced the greatest cone yield/plant followed by ‘Nugget’ (598 g) and ‘Centennial’ (119 g). Although the hops grown in Virginia were trained on a taller trellis, the Arkansas cultivar and fertility trial had either greater or similar total cone yields for ‘Cascade’ (983.63 g) and ‘Crystal’ (755.8 g) while ‘Nugget’ (286.23 g) and ‘Centennial’ (67.45 g) produced 48% and 57% less yield, respectively.

Hop cone yields from fully mature plants grown in the Pacific Northwest, where the latitude, soil texture and quality, growing degree days, and other climatic factors are optimal, have shown that yields for the studied cultivars are around ten times greater compared to the highest yielding cultivars in the northcentral Arkansas fertility study that were harvested in their second and third year of production in 2020 and 2021, respectively. Based on reported observations from hop yards in the Pacific Northwest, the 6 cultivars assessed in Arkansas produced considerably less total cone yield/plant. When adjusted for total cone yield/ha to per/plant, previous literature from typical commercial hop yards indicated that ‘Zeus’ (12.3 kg/plant), ‘Nugget’ (9.0 kg/plant), and ‘Cashmere’ (8.6 kg/plant) had the greatest total potential cone yield/plant between the studied cultivars followed by ‘Centennial’ (8.4 kg/plant), ‘Crystal’ (8.2 kg/plant), and ‘Cascade’ (8.2 kg/plant) which equates to 9.1 kg/plant (Idahohops, 2011; USAHOPS, 2018). The average of the total cone yield for all cultivars in the Arkansas fertility trial (wet weight) was 548.42 g/plant (0.55 kg/plant) which is approximately 6.03% of the expected yield from the cultivars had they been grown in typical commercial hop yards. ‘Cascade’, the highest producing cultivar in the study, yielded 983.63 g/plant for both years, which is 88% less than commercial ‘Cascade’ standards. ‘Zeus’ (797.6 g/plant) and ‘Crystal’ (755.8 g/plant) were the second and third highest yielding cultivars in the Arkansas fertility trial which produced 6.48% and 9.22% of expected commercial yields, respectively. ‘Cashmere’ (401.8 g/plant), ‘Nugget’ (286.2 g/plant), and ‘Centennial’ (65.5 g/plant) produced 4.67%, 3.18%, and 0.78% of typical yields, respectively. While these higher yields can be attributed to several factors, such as climate, trellising methods, soil quality and texture, production experience, and plant age, the cone yield disparity indicates that hops grown outside of typical regions in the Pacific Northwest, especially in hot and humid environments like the mid-south U.S. and northcentral Arkansas, will have significantly fewer cones per plant regardless of fertility application rates. The Arkansas fertility trial's total cone yields were more comparable to the Virginia and North Carolina research studies that were located at sites within 1-2 degrees N latitudinally which indicates the daylight dependence of hops plants for optimal yield. However, while yield potential is likely to be substantially lower, the alpha and beta acid levels and overall quality of the cones produced in northcentral Arkansas is another primary factor in assessing the potential of hops production in the region.

Alpha and beta acid attributes of dried hop cones

The quality attributes of dried hops cones evaluated postharvest included individual and total alpha acids (combined fraction of cohumulone and n+-adhumulone) as well as the individual and total beta acids (combined fraction of colupulone and n+-adlupulone). The quality of the cones grown in northcentral Arkansas were evaluated and compared to commercial standard alpha and beta acid levels from cultivars grown in the Pacific Northwest (Table 8). Due to a lack of cones, 'Centennial' and 'Nugget' were excluded from quality analysis in 2020 and 2021 (Tables 9 and 10). The cultivar x fertility rate interaction did not significantly impact any of the measured attributes for cone quality in 2020 but impacted all the attributes in 2021. In 2020 and 2021, alpha acids varied by cultivar including total alpha acids (3.49-6.31%), cohumulone (1.36-3.47%), and n+-adhumulone (1.39-2.96%), in addition beta acids also varied including total beta acids (3.72-8.31%), colupulone (2.10-3.37%), and n+-adlupulone (1.35-5.74%). Regardless of year, 'Cascade' (5.50%), 'Cashmere' (5.41%), and 'Zeus (4.75%) had the greatest total alpha acid levels while ‘Crystal’ (7.74%), ‘Cascade’ (5.80%), and ‘Cashmere’ (4.91%) had the highest total beta acids.

2020. In 2020, cultivar impacted all the acid attributes while fertility only impacted total beta acids (Table 9). Although not significant, the low fertility rate had the highest values for the alpha and beta acids. For total beta acids, hops grown using the low fertility rate (5.95%) were greater than the standard fertility rate (5.02%). ‘Zeus’ (3.47%) and ‘Cascade’ (3.37%) had a higher cohumulone than ‘Cashmere’ (2.38%) and ‘Crystal’ (1.48%). ‘Cascade’ (2.95%) and ‘Cashmere’ (2.67%) had higher n+-adhumulone than ‘Crystal’ (2.18%) and ‘Zeus’ (1.89%). ‘Cascade’ (6.31%) had higher total alpha acids than ‘Cashmere’ (5.06%) and ‘Crystal (3.67%). ‘Cascade’ (3.37%) had greater levels of colupulone than ‘Zeus’ (2.73), ‘Crystal’ (2.64%), and ‘Cashmere’ (2.10%). N+-adlupulone levels were more variable (1.61-5.72%) than colupulone (2.1-3.37%). ‘Crystal’ (5.72%) had greater n+-adlupulone compared to ‘Cascade’ (3.98%), ‘Cashmere’ (3.12%), and ‘Zeus’ (1.61%). ‘Crystal’ (7.17%) and ‘Cascade’ (6.45%) had higher total beta acid levels than ‘Cashmere’ (4.43%) and ‘Zeus’ (3.83%).

2021. Figures 4 and 5 show the significant cultivar x fertility interactions for the alpha and beta acids. the low and standard ‘Cashmere’ fertility rates had the highest total alpha acids and n+-adhumulone levels. For total alpha acids, ‘Cascade’ low (5.58%) had significantly greater quantities than the standard (4.06%) and high (4.38%) rates. ‘Zeus’ high (3.17%) had significantly lower total alpha acids compared to the low (4.59%) and high (4.63%) fertility rates. In general, ‘Cashmere’ had the highest total alpha acid in the standard (6.12%) and low (5.78%) rates, and all ‘Cashmere’ cultivar x fertility rate combinations were higher in total alpha acid compared to all ‘Crystal’ fertility rate combinations.

For all alpha acids, ‘Cascade’ low fertility rate had significantly higher levels than the standard and high rates while ‘Zeus’ low fertility rate had lower total alpha acids and cohumulone than the low or standard rates. ‘Zeus’ cones grown with the high fertility rate had one of the lowest values for n+ adhumulone. The cones from each ‘Crystal’ fertility combinations (1.36%) did not differ significantly between rates, and all were significantly lower in cohumulone levels than all other cultivar x fertility rate interactions.

The individual and total beta acid levels were also variable between cultivars and fertility rates. Fertility rate did not impact any of the individual or total beta acids except ‘Zeus’ standard rate (2.64%) that had significantly greater levels of colupulone compared to the high rate (2.00%). All ‘Crystal’ cultivar x fertility rate combinations had significantly more total beta acids and n+ adlupulone compared to all other cultivar fertility interactions. In general, ‘Zeus’ had lower total beta acids as compared to the other fertility rates but ‘Zeus’ high (3.18%) had the lowest total beta acids among all cultivar x fertility interactions. All ‘Zeus’ fertility rates had the lowest levels of n+-adlupulone.

The dried hops cones from the cultivars assessed in the fertility study had a broad range of alpha and beta acid levels depending on cultivar, and results indicated that several cultivars had acids that were within or above commercial standards (Table 8). While beta acids generally vary less between the selected cultivars (3-7%), the alpha acids range between 3.5-16.5% depending on cultivar. Commercial acid values for each cultivar can fluctuate between relatively high alpha acid cultivars, such as ‘Zeus’ (14.5-16.5%), ‘Nugget’ (11.5-14%), ‘Centennial’ (9.5-11.5%), and ‘Cashmere’ (6.9-10.1%) to low alpha acid cultivars, such as ‘Cascade’ (4.5-7%) and ‘Crystal’ (3.5-5.5%) (BarthHaas, 2021; Brooks et al., 1972; Judd, 2018, Idahohops, 2018). Beta acid levels for each cultivar were equal to or lower than alpha acids and less varied. The total beta acid levels tend to be higher in several of the cultivars when grown in commercial yards, such as ‘Cascade’ (4.8-7.0%), ‘Cashmere’ (3.5-7.0%), and ‘Crystal’ (4.5-6.5%), while ‘Nugget’ (3.0-5.8%), ‘Zeus’ (4.0-5.0%), and ‘Centennial’ (3.5-5.5%) have relatively low levels of the bitter acid. For Arkansas-grown hops, the highest total alpha acid cultivars were ‘Cascade’ (5.5%) and ‘Cashmere’ (5.41%) followed by ‘Zeus’ (4.75%) and ‘Crystal’ (3.58%), while the greatest total beta acids were found in ‘Crystal’ (7.74%) and ‘Cascade’ (5.8%) followed by ‘Cashmere’ (4.91%) and ‘Zeus’ (3.78%). Reported quality levels of alpha and beta acids from the same cultivars grown in the Pacific Northwest indicated that ‘Cascade’ was the only cultivar to have alpha and beta acid levels within optimal commercial standards with 5.5% and 5.8% alpha and beta acids, respectively. ‘Cashmere’ (5.41% alpha acid and 4.91% beta acid) had alpha acid values that were around 1.5% below commercial standards and beta acids that were within the standard ranges. Alternatively, ‘Crystal’ (3.58% alpha acid and 7.74% beta acid) had alpha acid levels within standard commercial ranges and approximately 1.3% greater total beta acids compared to the same cultivar grown in the Pacific Northwest. ‘Zeus’ had beta acids that were close to commercial standard (0.2% less), yet the total alpha acids were much lower in the Arkansas-grown ‘Zeus’ plants (4.75%) compared to the commercial standard alpha acids (14.5-16.5%) that are touted for their high alpha acid levels.

A multi-year study in Oregon and Washington showed that the impact of N rate on cone yield, levels of hop acids, total oil content, color, and nitrate level were year dependent (Iskra et al., 2019). Alpha acids, beta acids, and total oil volume decreased linearly with increasing N. Since the alpha and beta acids decreased, and nitrate concentration increased when N was applied after bloom, the researchers recommended the use of the lowest feasible N rate and to cease nitrogen applications before or at bloom to optimize certain cone quality factors while minimizing nitrate accumulation (Iskra et al., 2019). Another study conducted by Likens and Nickerson (1967) found that excessive commercial N application reduced alpha acids and total oil but did not influence oil composition. These results were similar to the results for Arkansas-grown hops. In 2020 and 2021, the dried cone analysis of individual and total acids indicated that some cultivars had lower quality attributes when grown using the high fertility compared to the plants grown using the low fertility rate. ‘Zeus’ high fertility rate had significantly less colupulone, total alpha acids, and cohumulone than the standard fertility rate while ‘Cascade’ low had higher levels of total alpha acids and cohumulone compared to the other rates. While significant in these cases, fertility rate did not seem to have as significant an influence on the quality attributes of the cones compared to the quality differences between cultivars.

For hops grown in Virginia, Judd (2018) found that regardless of ammonium sulfate additions, the alpha and beta acid levels changed by year and were lower than commercial cultivars. Judd (2018) noted that nearly 70% of the samples tested for alpha and beta acid content were below Pacific Northwest industry standards which indicates that the Virginia-grown and Arkansas-grown hops had a lower bittering potential and lower quality in terms of acid development. While the Virginia research trial showed quality improvements in the second year of growth, 53% of samples had lower than average acid values yet some cultivars had levels similar to commercial cultivars which occurred in several samples in the Arkansas fertility study. While Judd (2018) noted slight increases in alpha and beta acid levels for some cultivars, the cultivars grown in northcentral Arkansas generally decreased in acids from 2020 to 2021 which could be attributed to poor bine development from nutrient deficiency and lower rainfall during the vegetative growing season in June-August 2021.

Sensory attributes of ground, dried hop cones

For descriptive sensory analysis, the trained panel evaluated 22 dried, ground hops aroma attributes on a 15-point scale (0 is less of the attribute; 15 is more of the attribute) in triplicate in 2020 and 2021 (Tables 11 and 12). The ages of the descriptive panelists varied with four females and one male on the panel in 2020 and five females and two males on the panel in 2021. In general, panelists ascribed higher aroma attributes to the cones grown in 2021 regardless of cultivar compared to the cones harvested in 2020. There was a higher variation between cultivars in terms of aroma attributes from cones grown in 2020 (14 significant attributes) compared to the same cultivars that were tested in 2021 (7 significant attributes). However, the overall impact rating of the cones grown in 2021 were lower than the cones assessed in 2020. While the overall impact scaling of all cultivars grown in 2020 were higher by 1 rating point, nearly all the other attributes from 2021 were scaled higher particularly dill, floral, mint, citrus, and herb. The only exceptions of aroma attributes that declined between years was the garlic aroma attribute in ‘Cashmere’ and ‘Crystal’, overall citrus for ‘Cascade’, and terpene aromas in ‘Cashmere’.

2020. The descriptive sensory panel (n=5) evaluated 22 hops aroma attributes in 2020. Crystal (6.7) and Cashmere (6.6) had a higher overall aroma impact than Cascade (6.1) and Zeus (5.9). The hops cultivars differed in overall green herb complex (2.5-3.2), overall citrus complex (2.0-3.4), and overall pepper complex (1.6-2.8). Cascade had the highest overall citrus complex, and Crystal had the highest overall green herb and pepper complexes. Cultivars also differed for fruity, terpenes, aged cheese, umami, lemon, lemongrass, other citrus, floral, mint, and garlic, but these levels were less than 2.7. The panelists could not differentiate grass, foliage, sage, thyme, green herb, other, dill, white pepper or black pepper attributes for these cultivars. The panelists were asked to use one word to define the aroma for each cultivar, and Cascade was “citrusy”, Cashmere was “terpene”, Crystal was “savory”, and Zeus was “grass/foliage”.

2021. The descriptive sensory panel (n=7) evaluated 22 hops aroma attributes in 2021. The overall aroma impact (5.1-5.7) did not differ between cultivars, however the hops cultivars differed in overall green herb complex (3.3-4.7), overall pepper complex (2.8-3.4), overall citrus complex (2.5-3.2), and thyme (2.5-3.8). ‘Cascade’ had the greatest overall pepper complex, and ‘Cashmere’ had the lowest overall green herb and citrus complexes. Cultivars also differed for aged cheese, lemon, and black pepper, but these levels were less than 3.0. The panelists could not differentiate grass, foliage, fruity, terpenes, umami, lemongrass, citrus other, sage, green herb other, floral, mint, garlic, dill, or white pepper for these cultivars. The panelists were asked to use one word to define the aroma for each cultivar, and ‘Cascade’ was “herbal”, ‘Cashmere’ was “foliage”, ‘Crystal’ was “herbal/citrus”, and Zeus was “herbal”.

Previous literature concerning the sensory and quality of hop cones and beer-derived products described that hop quality varies between seasons and cultivars due to the climate, cultivation method, soil conditions, cone maturity at harvest, root condition, and other abiotic factors (Lafontaine, et al., 2019; Matsui et al., 2013). The higher aroma intensity ratings given to the cones in 2021, regardless of cultivar, along with less variation in intensity between cultivars indicated that the aroma profiles increased in impact and differed in defining attributes between years. Data for GDD, precipitation, and significant weather occurrences showed that the 2021 season had 4,910 degree days compared to 4,718 in 2020 in addition to less precipitation, and a period of sub-freezing temperatures in February which may have had some influence on the quality attributes and sensory characteristics.

Matsui et al. (2013) conducted a study to elucidate the influence of hop plant age on beer quality and aroma by examining differences in hop aroma for sensory evaluation and chemical analysis in the Czech Republic. In addition, to assess how the vegetative and reproductive stages of growth were dependent on hop plant age, the length of the vine and leaf size, stem diameter and flowering were monitored throughout the growing season. The hop samples used were selected from eight hopyards in the Saaz region during the 2010 and 2011 growing seasons. A comparison of vegetative growth, leaf size, and stem diameter showed that hop plants that were younger were larger and flowered later. Results indicated that the alpha acid content was higher in the younger hops, and significant differences in hop aroma characteristics were observed in beer made from the different hop samples based on plant age. It was concluded that the more vigorous vegetative growth and late flowering associated with the younger roots changed secondary metabolism which also affected the generation of terpenes and hop aroma quality in the beer produced. Similar results were found in the Arkansas hops fertility trial in that second-year hop plants (2020) generally had greater vigor during bine assessment and higher alpha acids except for ‘Cashmere’ while flowering timing was slightly earlier in third year plants. Typical aroma profiles for ‘Cascade’ and ‘Crystal’ have been regarded as savory, herbal, floral, and mild in citrus while the higher alpha acid cultivars (Zeus and Cashmere) have been characterized as having fruity and spicy characteristics with more herbal aromas (Idahohops, 2011; USAHOPS, 2018). While similar aromas were attributed to the lower alpha acid cultivars grown in Arkansas, both ‘Zeus’ and ‘Cashmere’ had lower alpha acids compared to commercial standards which may have attributed to the less distinctive aromas given to the two cultivars by the Arkansas sensory panel. A comparison between Matsui et al. (2013) indicated that the significant differences attributed to the aromas by the sensory panel for Arkansas-grown cones in 2020 and

2021 were likely due to multiple factors, such as plant age, cultivar, growing conditions, inflorescence timing, and secondary metabolism. This data suggests that the terroir effects and sensory attributes of Arkansas-grown hops are unique for this region, and the cones were similar to commercial standards for the medium intensity, less aromatic, and lower alpha acid cultivars (Crystal and Cascade). The cultivars that typically have higher alpha acids (‘Zeus’ and ‘Cashmere’) were considered less pungent, fruity, and aromatic in general compared to commercial cones.

Lafontaine et al. (2019) assessed the influence of cone ripening on the dry-hop aroma potential and acid development using ‘Cascade’ hops. The process of dry-hopping has been a popular practice by brewers to add more of a hop aroma to beer without imparting a bitter taste. Since the cones are not boiled when added at the end of the brewing process, the oils are not imbued into the beverage while the flavor and aroma increase perceptibility. In that study, 5–6 weekly hop samples were collected over three years, analyzed, and used to dry-hop an “unhopped” beer base for sensory panel analysis. While the results indicated that the development of humulones did not change because of harvest date, the essential oil content, perceived hop aroma intensity, and citrus quality increased as a function of harvest date. The results from Lafontaine et al. (2019) suggested that later-harvested hops (dry matter content >26%) might be better suited for dry-hopping and beer production because they attribute the most citrusy aroma to beer. The 2020 and 2021 harvest seasons at the northcentral Arkansas production trial occurred over a period of 2-3 weeks in 2020 and 2021 in mid-August through early September. While the cones gleaned during the 2020 season were generally given lower aromatic impact ratings, it can be assumed that a lengthened harvest period would heighten the aromatic sensory qualities of the harvested cones.

Lafontaine et al (2018a; 2018b) conducted two sensory studies to examine the impact of dry-hopping rate and mixed cultivar dry-hopping effects on the sensorial and analytical characteristics of beer. One study involved descriptive sensory testing five beers (including an 'unhopped' control) that were prepared by statically dry-hopping an 'unhopped' beer with ground, whole cone 'Cascade' hops from a single harvest with rates at 200, 386, 800, and 1,600 g/hL. The results indicated that the addition of more hops by static dry-hopping does not lead to increased aroma intensity. Dry-hopping rates >800 g/hL were described as more herbal/tea in quality rather than the typical citrus aroma ascribed to the cultivar. The study suggested that to maintain a more balanced hop aroma a static dry-hopping rate between 400 and 800 g/hL was preferred by panelists. The second sensory study (Lafontaine et al., 2018b) involved dry-hopping unhopped control beers with blends of 'Cascade', 'Chinook', and 'Centennial' hops cones to understand the contribution that each of these hops make to the brewed aroma. Trained panelists used descriptive analysis for each of the treatments, and the response variables used by the panel encompassed the sensory attributes that defined the unique aromatic features of the three hop cultivars, (i.e., citrus, tropical/fruity, tropical/catty, and herbal). Lafontaine et al. (2018b) suggested that it is possible to produce a beer that can exhibit similar aroma profiles when dry-hopped with varied blends of the three cultivars and some tested blends achieved aromas that were similar to a single cultivar. This data indicated that ideal aroma intensity and quality can be achieved in a dry-hopped product with multiple cultivars that can afford a brewer the ability to make substitutions when faced with hop cone shortages.

Conclusion

There has been minimal research on the best management practices for fertility rate application, timing, amount, and effects on plant and cone attributes in the mid-southern region of the United States. Specific fertilization recommendations vary widely in published literature, differing among production regions, cultivar, irrigation methods, soil types and quality, and production objectives. The six hop cultivars grown in northcentral Arkansas were not as productive in terms of plant and cone attributes compared to the same cultivars grown commercially in the Pacific Northwest and at several research trials with similar latitude. Disease and pest along with other biotic and abiotic factors during vegetative growth, trellis height, number of bines/plant, and age of the plants contributed to the diminished plant vigor, yields, cone quality, and uncharacteristic sensory attributes that were noted in 2020 and 2021. Although the Arkansas-grown hops plants were limited in terms of trellising height, shorter day length, unideal soil texture, insect pressure, halo blight, and other detrimental climactic factors, several cultivars (Cascade, Crystal, and Zeus) have potential for small-scale commercial production in northcentral Arkansas based on yield and quality assessments.

After two growing seasons, ‘Cascade’, ‘Zeus’, and ‘Crystal’ produced the most total cone yield/plant and were generally more vigorous compared to ‘Cashmere’, ‘Nugget’, and ‘Centennial’. While ‘Zeus’ and ‘Cascade’ generally had better acid qualities when grown with a lower fertility rate, the three fertilizer rates showed little to no impact on the measured plant and cone attributes at and post-harvest. ‘Cascade’, ‘Zeus’, and ‘Crystal’ had alpha and beta acid levels that were either within or near commercial ranges except for ‘Zeus’ that had considerably lower alpha acids compared to commercially-grown ‘Zeus’ plants. A lower than commercial standard fertility regimen and the cessation of fertility prior to bloom would be recommended for growers in the region based on results from 2020 and 2021 along with research from other cultivar fertility studies conducted in regions with comparable latitudes. While dependent on many biotic and abiotic factors, the cone, plant, and compositional attributes measured for the second- and third-year hops plants are likely to increase for the fourth- and fifth-year results in terms of sensory, yield, and other plant and cone characteristics. Further production trials for low alpha acid, triploid, and noble cultivars (e.g., Pacific Gem, Willamette, Hallertau Mittelfruh, Ultra, Saaz, Liberty, Tettnang, and Fruggle) should be assessed given the higher yield and quality of ‘Cascade’ and ‘Crystal’ that were genetically related and bred for lower alpha acid content, high essential oils, and distinct aromatic compounds. While not ideal for large-scale commercial production and sale, the Arkansas-grown fertility trial showed that several cultivars would be better suited for small-scale home and microbrewery production for the mid-south region that would offer unique aromas and flavors to beers. While some compositional attributes were lower than commercial standards, Arkansas-grown hops could offer characteristic terroir-specific flavors and aromas that would allow local brewers the opportunity to craft specialty beers made with local hops.

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Table 1: Descriptive sensory lexicon used to evaluate aroma attributes from dried, ground hop cones harvested from hop plants grown in Clarksville, AR (2020 and 2021)

Aroma attributes [z]	Aroma definition
Grass	Green, slightly sweet aroma associated with cut grass/dry grass/hay
Green plant (foliage)	Freshly cut leaves or weeds
Citrus complex	General impression of citrus fruits
Lemon	Lemon
Lemongrass	Lemongrass
Other	Citrus, other than lemon and lemongrass
Fruity	Mixture of nonspecific fruits: berries, apples/ pears, tropical, melons; usually not citrus fruits
Green herb complex	General impression of dried herbs
Sage	Sage
Thyme	Thyme
Other	Green herbs, other than sage and thyme
Pepper complex	General impression of pepper, peppercorns
White pepper	Freshly ground white pepper
Black pepper	Freshly ground black pepper
Terpenes/skunk/off-note	Hemp or Cannabis, also reminiscent of skunk like character
Aged cheese	Aged (ripened) cheese
Umami/savory	General impression of cooked meat
Garlic	Garlic
Dill	Dill seeds
Floral	Sweet, fragrant aroma associated with flowers
Mint	Mint family (sweet, green and menthol): peppermint, spearmint, wintergreen
Overall impact	Intensity associated with overall aroma of the sample
Defining attribute	Term that can be used to characterize the sample

[z] The Universal Aromatic Scale was used as the reference for the aroma attributes. The aroma attribute definition is aromas associated with the attribute listed.

Intensity 2.0 = Soda note of saltine cracker (Nabisco Premium Unsalted Tops Saltine Crackers, Nabisco, East Hanover, NJ)

Intensity 5.0 = Cooked apple note of applesauce (Dr. Pepper Snapple Group, Plano, TX)

Intensity 7.5 = Orange note of orange juice (Minute Maid Frozen Concentrate Orange Juice (Coca-Cola, Atlanta, GA), reconstituted with 36 oz of filtered water

Intensity 10.0 = Grape note of grape juice (Welch's, Concord, MA)

Intensity 15.0 = Big Red Gum® (Mars, Inc., MeLean, VA)

Table 2: Optimum nutrient levels[z] for hop petioles and leaves sampled at the reproductive stage and full bloom

Nutrients	Petioles (commercial)	Leaves (North Carolina)
Nitrogen (%)	2.13 – 3.93	2.64 – 3.22
Phosphorous (%)	0.18 – 0.43	0.21 – 0.33
Potassium (%)	0.97 – 2.55	1.10 – 1.84
Calcium (%)	3.09 – 6.05	3.57 – 5.11
Magnesium (%)	0.55 – 1.71	0.85 – 1.29
Sulfur sampled basis (%)	0.18 – 3.0	-
Sulfur dry matter basis (%)	0.18 – 0.3	0.21 – 0.27
Iron (mg/kg)	35.4 – 151	42.91 – 96.11
Manganese (mg/kg)	50 – 150	-
Zinc (mg/kg)	19.4 – 57.1	24.43 – 41.63
Copper (mg/kg)	5.7 – 16.6	-
Boron (mg/kg)	48 – 150	79.2 – 126.2
Molybdenum (mg/kg)	1 – 5	-
Nitrate (mg/kg)	-[y]	1,551 – 10,197

[z] Nutrient levels reported in other research; Davis et al. (2022): NC hops 2010-2012 research project observations. https://newcropsorganics.ces.ncsu.edu/specialty-crops/nc-hops/observations/#tissue. Sirrine (2019): Recommended nutrient ranges for hop petiole samples. USA Hops: Hop Growers of America. https://www.usahops.org/growers/recommended-nutrient-ranges-for-hop-peti/.

[y] Data not listed

Table 3: Petiole and leaf nutrient levels[z] for hops cultivars with different fertility[y] rates sampled in Clarksville, AR during reproductive phenological stage and full bloom (2020 and 2021)

	Petiole	**Leaf**											
Cultivar/ fertility[y]	**Nitrogen (%)**	**Nitrogen (%)**	**Phosphorus (%)**	**Potassium (%)**	**Calcium (%)**	**Magnesium (%)**	**Sulfur (%)**	**Sodium (mg/kg)**	**Iron (mg/kg)**	**Manganese (mg/kg)**	**Zinc (mg/kg)**	**Copper (mg/kg)**	**Boron (mg/kg)**
							2020						
Cultivar													
Cascade	2.17±0.23	4.42±0.50	0.26±0.01	1.77±0.06	2.53±0.33	0.30±0.06	0.19±0.02	6.98±0.37	65.90±5.79	164.45±40.35	17.32±0.74	6.28±2.43	19.89±3.95
Cashmere	2.68±0.66	4.48±0.75	0.35±0.11	2.13±0.16	1.87±0.36	0.23±0.02	0.18±0.02	1.94±1.12	73.18±23.28	274.20±90.38	18.87±2.98	3.92±0.48	23.71±6.77
Centennial	2.55±0.64	4.07±0.48	0.30±0.06	2.08±0.10	1.59±0.46	0.28±0	0.18±0.02	1.30±0	61.64±2.07	209.49±34.51	17.35±3.48	2.96±0.61	13.95±3.68
Crystal	1.62±0.13	3.30±0.31	0.24±0.03	1.95±0.20	2.02±0.32	0.24±0.04	0.16±0.01	1.30±0	55.35±5.95	154.56±8.08	15.60±1.96	4.16±0.26	20.37±1.49
Nugget	3.08±0.76	4.45±1.13	0.37±0.12	2.35±0.41	1.12±0.12	0.21±0.04	0.20±0.05	10.39±3.44	53.92±9.96	131.62±29.93	22.84±5.29	4.55±0.75	13.30±1.73
Zeus	2.89±0.24	5.16±0.21	0.33±0.03	2.29±0.14	1.12±0.08	0.24±0.02	0.23±0.02	1.3±0	97.31±7.02	142.14±47.71	21.42±1.26	6.76±0.67	8.37±1.17
Fertility													
Low	2.09±0.38	3.79±0.78	0.27±0.04	2.02±0.20	1.72±0.57	0.24±0.04	0.17±0.03	3.04±2.70	64.40±16.87	170.48±46.47	17.05±2.15	5.15±2.22	17.56±5.84
Standard	2.74±0.69	4.55±0.66	0.32±0.09	2.09±0.33	1.63±0.54	0.27±0.05	0.20±0.03	4.04±4.54	73.87±23.05	159.04±61.65	20.21±4.54	4.80±1.53	17.59±8.45
High	2.66±0.73	4.60±0.78	0.33±0.09	2.17±0.28	1.72±0.57	0.24±0.04	0.20±0.03	4.53±4.63	65.38±14.06	208.72±82.14	19.45±3.59	4.36±1.33	14.65±4.10
							2021						
Cultivar													
Cascade	-[x]	3.44±0.23	0.24±0.03	1.81±0.08	4.07±1.21	0.37±0.06	0.18±0.01	0.80±0	52.68±4.05	181.35±101.90	20.08±4.55	4.39±0.33	19.53±2.91
Cashmere	-	2.60±0.38	0.26±0.06	1.99±0.27	2.85±0.54	0.20±0.03	0.14±0.02	0.80±0	36.52±3.28	232.81±98.10	21.94±0.70	3.31±0.23	18.94±3.16
Centennial	-	2.87±0.50	0.21±0.02	1.74±0.04	3.01±0.71	0.38±0.07	0.16±0.02	3.06±2.80	47.39±5.60	239.21±56.10	16.38±1.09	3.54±0.61	15.25±2.97
Crystal	-	2.55±0.33	0.21±0.01	1.85±0.09	4.04±0.71	0.35±0.06	0.15±0.02	0.91±0.18	44.13±6.14	256.66±65.24	20.89±3.90	3.89±0.53	23.51±2.75
Nugget	-	2.84±0.04	0.26±0.01	2.03±0.30	2.64±0.43	0.25±0.05	0.16±0.01	4.57±2.24	57.00±11.00	211.67±55.64	23.33±5.69	4.13±0.51	17.67±2.52
Zeus	-	3.63±0.40	0.27±0.03	2.09±0.12	3.15±0.42	0.32±0.06	0.20±0.02	1.63±1.44	57.53±7.71	214.74±126.04	19.66±3.10	6.79±0.15	12.80±1.32
Fertility													
Low	-	2.70±0.50	0.24±0.03	1.84±0.15	2.81±0.35	0.28±0.05	0.15±0.02	2.42±2.14	47.41±9.87	169.47±74.89	19.61±3.25	4.05±1.32	20.06±4.97
Standard	-	3.00±0.44	0.23±0.03	1.99±0.25	3.19±0.59	0.32±0.10	0.17±0.02	2.12±2.45	52.71±12.84	207.84±60.13	19.74±4.57	4.47±1.35	17.45±4.03
High	-	3.28±0.49	0.26±0.05	1.94±0.20	3.87±1.11	0.34±0.09	0.18±0.03	1.35±1.35	47.50±5.05	290.91±74.89	21.81±3.68	4.50±1.22	16.34±2.92

[z] Hop leaves and petioles were analyzed with combustion and UV-Vis spectrophotometry

[y] For fertility, Triple 13 (13-13-13) was added at three rates (low=192 g; standard= 288 g; high= 383 g per 6 plants in a treatment) May 15, June 1, June 15, and June 30 with the rate split into four applications.

[x] Data not obtained

Table 4: Main effects and interaction on plant attributes of hop cultivars with different fertility rates grown in Clarksville, AR (2020)

Effects [z]	**Number bines/ plant**	**Number of nodes/plant**	**Number of laterals/ plant**	**Bine length (m)**
Cultivar				
Cascade	3.00 a [x]	60.89 a	81.89 a	13.14 a
Cashmere	2.72 a	63.28 a	84.67 a	12.16 a
Centennial	2.44 a	59.11 a	41.22 b	8.55 a
Crystal	2.56 a	58.44 a	77.89 ab	10.29 a
Nugget	2.39 a	52.22 a	45.94 ab	8.40 a
Zeus	2.67 a	46.00 a	62.56 ab	10.03 a
P-value	*0.4634*	*0.5094*	*0.0038*	*0.0394*
Fertility				
Low	2.67 a	55.78 a	66.72 a	10.46 a
Standard	2.44 a	59.72 a	59.08 a	9.98 a
High	2.72 a	54.47 a	71.28 a	10.84 a
P-value	*0.6099*	*0.7383*	*0.4106*	*0.7748*
Cultivar x Fertility				
(P-value)	*0.7490*	*0.2332*	*0.1009*	*0.2844*

[z] Means with different letters for each attribute are significantly different ($p<0.05$) according to Least Square Means Student's t-test

Table 5: Main effects and interaction on plant attributes of hop cultivars with different fertility rates grown in Clarksville, AR, AR (2021)

Effects [z]	**Number of bines/ plant**	**Number of nodes/plant**	**Number of laterals/ plant**	**Bine length (m)**
Cultivar				
Cascade	2.78 a	71.67 a	129.67 a	12.24 ab
Cashmere	2.83 a	68.78 a	108.72 ab	11.50 ab
Centennial	2.89 a	66.06 a	66.83 b	8.63 b
Crystal	3.00 a	74.00 a	126.89 a	14.22 a
Nugget	2.83 a	73.00 a	126.67 a	12.92 ab
Zeus	2.44 a	58.89 a	90.56 ab	9.70 b
P-value	*0.2092*	*0.2826*	*0.0014*	*0.0025*
Fertility [y]				
Low	2.94 a	69.64 a	114.97 a	12.58 a
Standard	2.72 a	70.39 a	113.17 a	11.36 a
High	2.72 a	66.17 a	96.53 a	10.67 a
P-value	*0.3573*	*0.6916*	*0.2155*	*0.1806*
Cultivar x Fertility				
(P-value)	*0.4622*	*0.0837*	*0.5173*	*0.2179*

[z] Means with different letters for each attribute are significantly different ($p<0.05$) according to Least Square Means Student's t-test

Table 6: Main effects and interactions of cultivar and fertility rate on hop cone (fresh, wet) attributes at harvest of hop cultivar plants with different fertility rates grown in Clarksville, AR (2020)

Effect	Total cone yield/plant[z] (g)	Mature Cones/ plant (%)	Immature Cones/plant (%)	Damaged Cones/plant (%)	Individual cone weight (g)	Estimated dry cone yield/plant (g)
Cultivar						
Cascade	1175.28 a	57.15 bc	36.45 a	6.40 bc	0.58 ab	117.53 a
Cashmere	442.86 b	76.28 ab	8.46 bc	15.26 bc	0.39 c	44.29 b
Centennial	78.28 b	44.85 c	20.61 b	34.54 a	0.42 bc	7.83 b
Crystal	656.42 ab	76.38 ab	10.77 bc	12.85 bc	0.63 a	65.64 ab
Nugget	187.42 b	41.59 c	39.45 a	18.96 ab	0.64 a	18.74 b
Zeus	1072.09 a	92.47 a	5.77 c	1.76 c	0.62 a	107.21 a
P-value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*0.0005*	*<0.0001*
Fertility[y]						
Low	592.38 a	55.17 b	19.84 a	24.99 a	0.54 a	59.24 a
Standard	567.57 a	74.98 a	19.45 a	5.58 b	0.55 a	56.76 a
High	646.21 a	64.21 ab	21.47 a	14.32 b	0.54 a	64.62 a
P-value	*0.8501*	*0.0014*	*0.8224*	*<0.0001*	*0.9564*	*0.8501*
Cultivar x Fertility (*P-value*)	*0.9604*	*0.1452*	*0.5981*	*0.0913*	*0.6003*	*0.9604*

[z] Total cones= mature + immature + damaged (diseased, sunburned, insect damage)

[y] Fertility rates consisted of low (112 kg/ha), standard (168 kg/ha), and high (224 kg/ha) rates of Triple 13 (13-13-13) applied in four evenly spilt applications on May 15, June 1, June 15, and June 30.

[x] Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test

Table 7: Main effects and interactions of cultivar and fertility rate on hop cone (fresh, wet) attributes at harvest of hop cultivar plants with different fertility rates grown in Clarksville, AR (2021)

Effect [y]	**Total cone yield/plant** [z] **(g)**	**Mature cones/plant (%)**	**Immature cones/plant (%)**	**Damaged cones/plant (%)**	**Individual cone weight (g)**	**Estimated dry cone yield/plant (g)**
Cultivar						
Cascade	791.97 ab	54.26 bc	43.49 a	2.25 b	0.56 a	79.20 ab
Cashmere	360.73 cd	88.64 a	1.71 c	9.65 b	0.33 b	36.07 cd
Centennial	52.61 d	41.01 c	6.46 c	52.53 a	0.61 a	5.26 d
Crystal	855.17 a	88.54 a	9.87 bc	1.59 b	0.58 a	85.52 a
Nugget	385.03 bcd	79.92 ab	19.93 b	0.15 b	0.72 a	38.50 bcd
Zeus	523.06 abc	93.36 a	5.56 c	1.08 b	0.68 a	52.31 abc
P-value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Fertility [x]						
Low	498.08 a	76.18 a	16.88 a	10.13 a	0.65 a	49.81 a
Standard	511.48 a	75.47 a	15.03 a	9.68 a	0.54 a	51.15 a
High	474.72 a	71.22 a	16.21 a	13.82 a	0.55 a	47.47 a
P-value	*0.9192*	*0.6840*	*0.8488*	*0.7293*	*0.0706*	*0.9192*
Cultivar x Fertility (*P-value*)	*0.4050*	*0.6904*	*0.0031*	*0.9451*	*0.0572*	*0.4050*

[z] Total cones = mature + immature + damaged (diseased, sunburned, insect damage)

[y] Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test

[x] Fertility rates consisted of low (112 kg/ha), standard (168 kg/ha), and high (224 kg/ha) rates of Triple 13 (13-13-13) applied in four evenly spilt applications on May 15, June 1, June 15, and June 30

Table 8: Range of alpha and beta acid levels[z] for hop cultivars grown commercially in the Pacific Northwest in the United States

Cultivars	Total alpha acid (%)	Total beta Acid (%)
Cascade	4.5 – 7.0	4.8 – 7.0
Cashmere	7.7 – 9.1	6.4 – 7.1
Centennial	9.5 – 11.5	3.5 – 4.5
Crystal	3.5 – 5.5	4.5 – 6.5
Nugget	11.5 – 14.0	4.2 – 5.8
Zeus	12.0 – 16.5	4.0 – 6.0

[z] Alpha and beta acid levels reported in cultivar guide; USAHOPS (2018): Varieties snapshot. 21 Feb 2022. https://www.usahops.org/cabinet/data/USAHops_VarietyManual_2018_Web.pdf.

Table 9: Main effects and interaction for individual and total alpha and beta acids levels[z] for cultivars of hop plants with different fertility rates grown in Clarksville, AR (2020)

Cultivar	Cohumulone (%)	n+-adhumulone[y] (%)	Total alpha-acids (%)	Colupulone (%)	n+-adlupulone (%)	Total beta-acids (%)
Cultivar						
Cascade	3.37 a[x]	2.95 a	6.31 a	3.37 a	3.98 b	6.45 a
Cashmere	2.38 b	2.67 a	5.06 b	2.10 c	3.12 b	4.43 b
Centennial	-[w]	-	-	-	-	-
Crystal	1.48 c	2.18 b	3.67 c	2.64 bc	5.72 a	7.17 a
Nugget	-	-	-	-	-	-
Zeus	3.47 a	1.89 b	5.36 ab	2.73 b	1.61 c	3.83 b
P-value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Fertility[v]						
Low	2.93 a	2.49 a	5.52 a	2.91 a	3.87 a	5.95 a
Standard	2.48 a	2.38 a	4.86 a	2.58 a	3.42 a	5.02 b
High	2.63 a	2.40 a	4.98 a	2.64 a	3.52 a	5.44 ab
P-value	*0.0868*	*0.7305*	*0.2376*	*0.2408*	*0.4170*	*0.0340*
Cultivar x Fertility (*P-value*)	*0.1328*	*0.5608*	*0.2491*	*0.1743*	*0.8013*	*0.5857*

[z] Hop cones were analyzed with high performance liquid chromatography analysis using American Society of Brewing Chemists (ASBC) methods

[y] n+adhumulone refers to the level of n-humulone and ad-humulone combined in one fraction, analogue for n+-adlupulone for n-lupulone and ad-lupulone combined

[x] Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test

[w] Data not available for Centennial and Nugget due to missing plants or low amount of cones for analysis.

[v] For fertility, Triple 13 (13-13-13) was added at three rates (low=192 g; standard= 288 g; high= 383 g per 6 plants in a treatment) May 15, June 1, June 15, and June 30 with the rate split into four applications.

Table 10: Main effects and interaction for individual and total alpha and beta acids levels[z] for cultivars of hop plants with different fertility rates grown in Clarksville, AR (2021)

Effect	Cohumulone (%)	n+-adhumulone[y] (%)	Total alpha acids (%)	Colupulone (%)	n+-adlupulone (%)	Total beta acids (%)
Cultivar						
Cascade	2.44 b[x]	2.24 b	4.68 b	2.42 ab	2.72 c	5.14 b
Cashmere	2.80 a	2.96 a	5.76 a	2.26 b	3.12 b	5.38 b
Centennial	-[w]	-	-	-	-	-
Crystal	1.36 c	2.13 b	3.49 d	2.58 a	5.74 a	8.31 a
Nugget	-	-	-	-	-	-
Zeus	2.74 a	1.39 c	4.13 c	2.37 b	1.35 d	3.72 c
P-value	*<0.0001*	*<0.0001*	*<0.0001*	*0.0196*	*<0.0001*	*<0.0001*
Fertility[v]						
Low	2.57 a	2.35 a	4.92 a	2.56 a	3.42 a	5.97 a
Standard	2.31 b	2.12 b	4.44 b	2.38 ab	3.13 a	5.51 ab
High	2.12 b	2.07 b	4.18 b	2.28 b	3.14 a	5.42 b
P-value	*<0.0001*	*0.0029*	*0.0002*	*0.0068*	*0.0388*	*0.0185*
Cultivar x Fertility (*P-value*)	*<0.0001*	*<0.0001*	*<0.0001*	*0.0007*	*0.0452*	*0.0116*

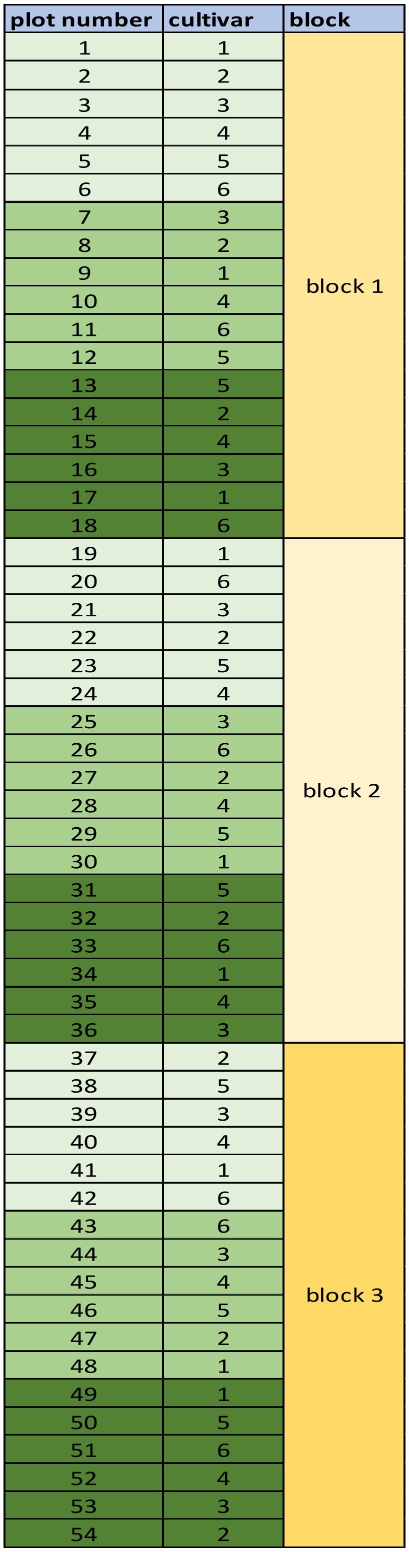
[z] Hop cones were analyzed with high performance liquid chromatography analysis using American Society of Brewing Chemists (ASBC) methods

[y] n+adhumulone refers to the level of n-humulone and ad-humulone combined in one fraction, analogue for n+-adlupulone for n-lupulone and ad-lupulone combined

[x] Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test

[w] Data not available for Centennial and Nugget due to missing plants or low amount of cones for analysis.

[v] For fertility, Triple 13 (13-13-13) was added at three rates (low=192 g; standard= 288 g; high= 383 g per 6 plants in a treatment) May 15, June 1, June 15, and June 30 with the rate split into four applications.



plot number	cultivar	block
1	1	block 1
2	2	
3	3	
4	4	
5	5	
6	6	
7	3	
8	2	
9	1	
10	4	
11	6	
12	5	
13	5	
14	2	
15	4	
16	3	
17	1	
18	6	
19	1	block 2
20	6	
21	3	
22	2	
23	5	
24	4	
25	3	
26	6	
27	2	
28	4	
29	5	
30	1	
31	5	
32	2	
33	6	
34	1	
35	4	
36	3	
37	2	block 3
38	5	
39	3	
40	4	
41	1	
42	6	
43	6	
44	3	
45	4	
46	5	
47	2	
48	1	
49	1	
50	5	
51	6	
52	4	
53	3	
54	2	

Cultivars

1=Cascade

2=Nugget

3=Zeus

4=Cashmere

5=Centennial

6=Crystal

Fig. 1. Plot map of hops grown in Clarksville, AR divided in three blocks with three replicates of each cultivar planted in a completely randomized block design (2020 and 2021)

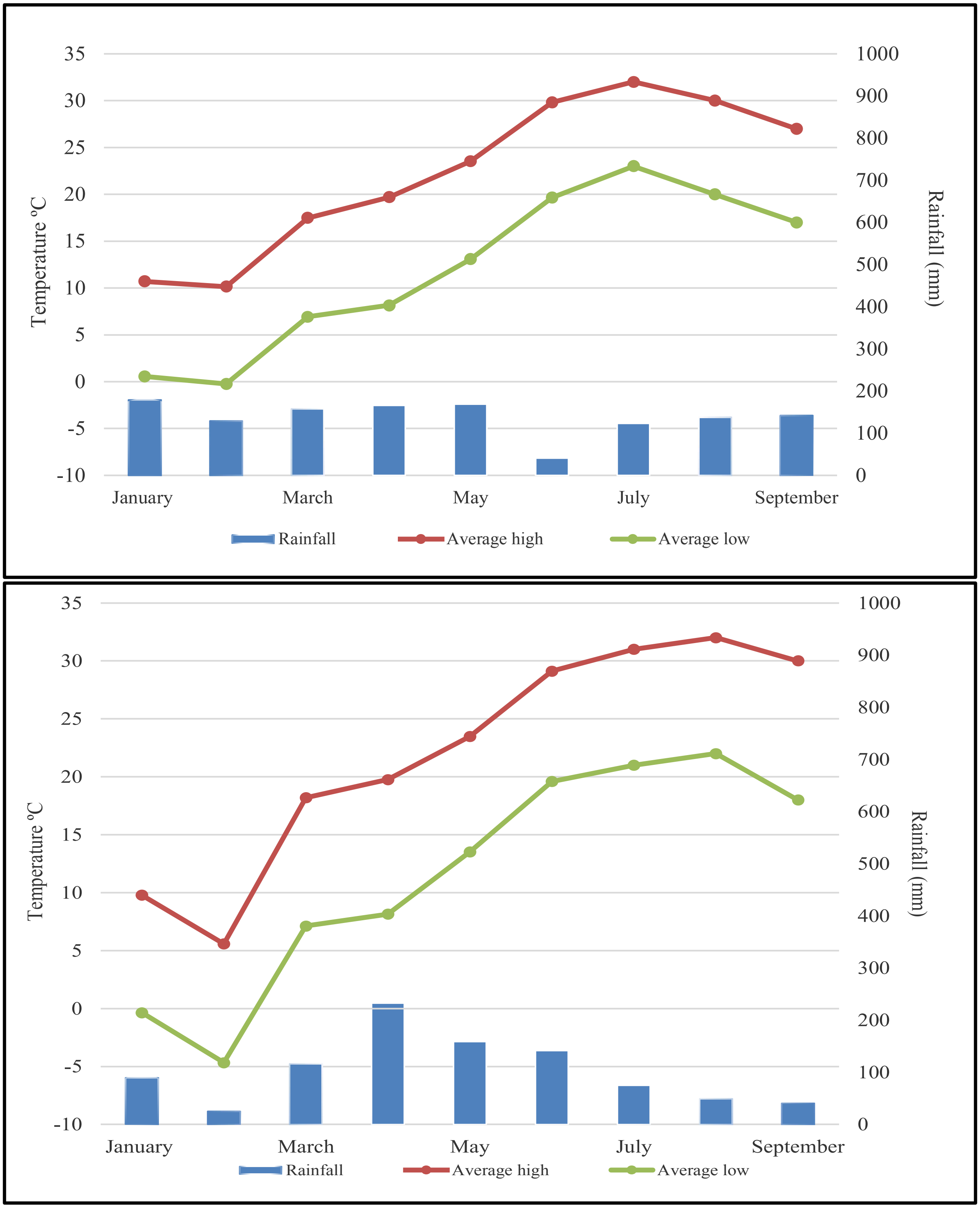


Fig. 2. Temperature and rain conditions at the University of Arkansas System Division of Agriculture Fruit Research Station, Clarksville, AR (2020 top and 2021 bottom)

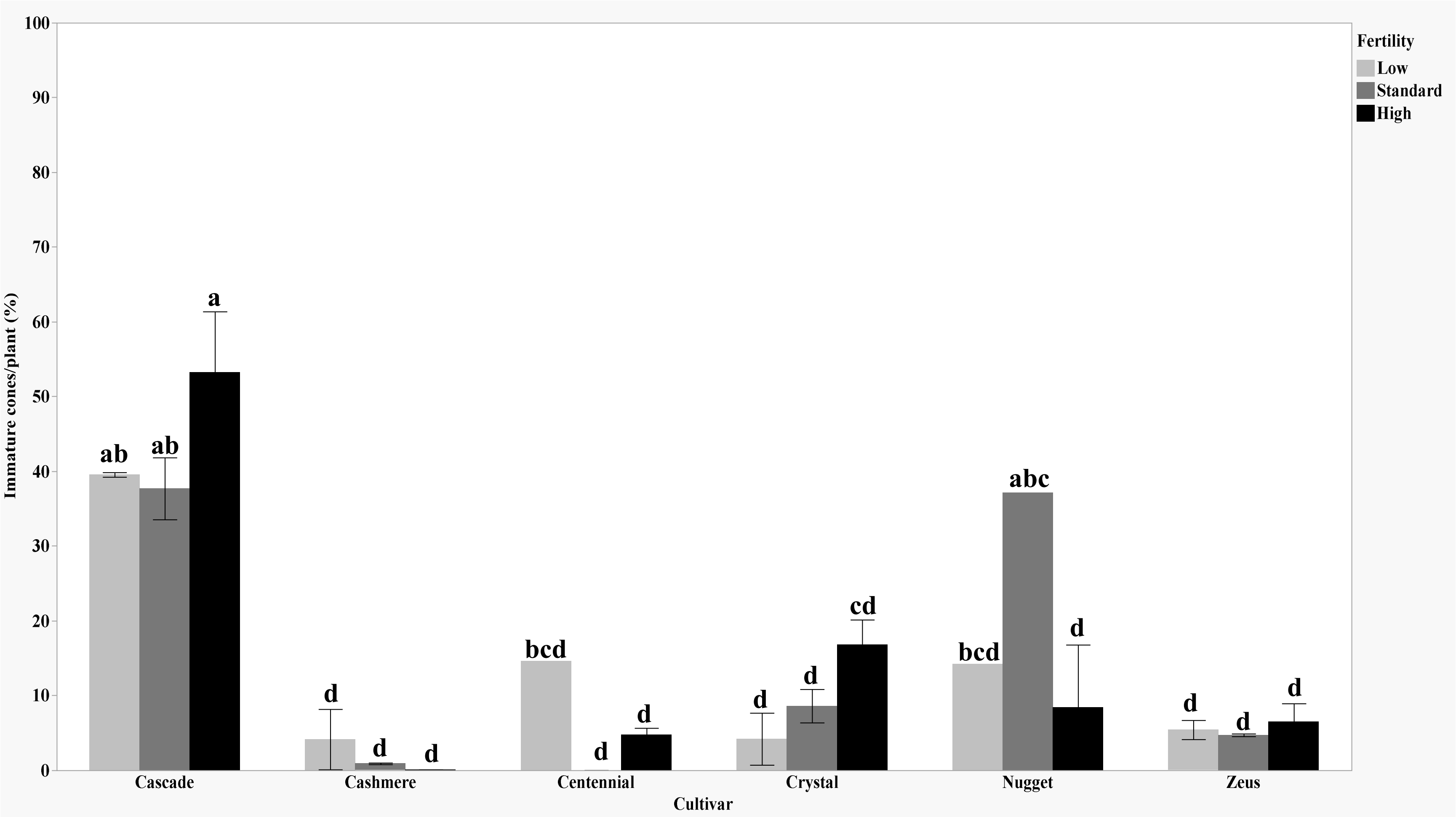


Fig. 3. Interactions of fertility rate (Low, Standard, and High) and cultivar on percentage of immature hops cones harvested from hops plants grown in Clarksville, AR (2021)

Means with different letters for each attribute are significantly different ($p<0.05$) according to Least Square Means Student's t-test

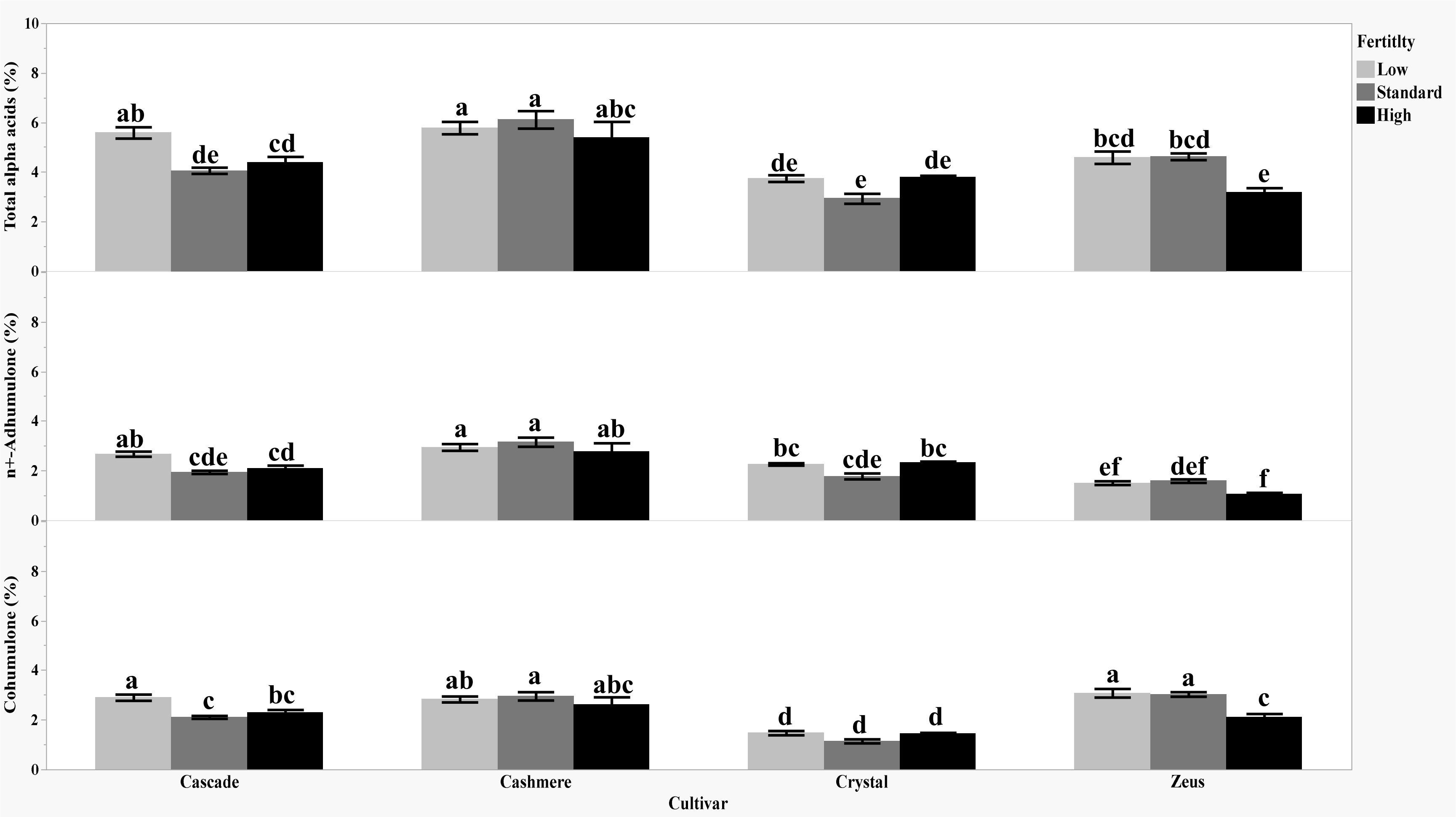


Fig. 4. Interactions of fertility rate (Low, Standard, and High) and cultivar on individual and total alpha acids of hops plants grown in Clarksville, AR (2021)

Hop cones analyzed with high performance liquid chromatography using American Society of Brewing Chemists (ASBC) Hop-14 method

Means with different letters for each attribute are significantly different ($p<0.05$) according to Least Square Means Student's t-test

n+-adhumulone refers to the level of n-humulone and adhumulone combined in one fraction

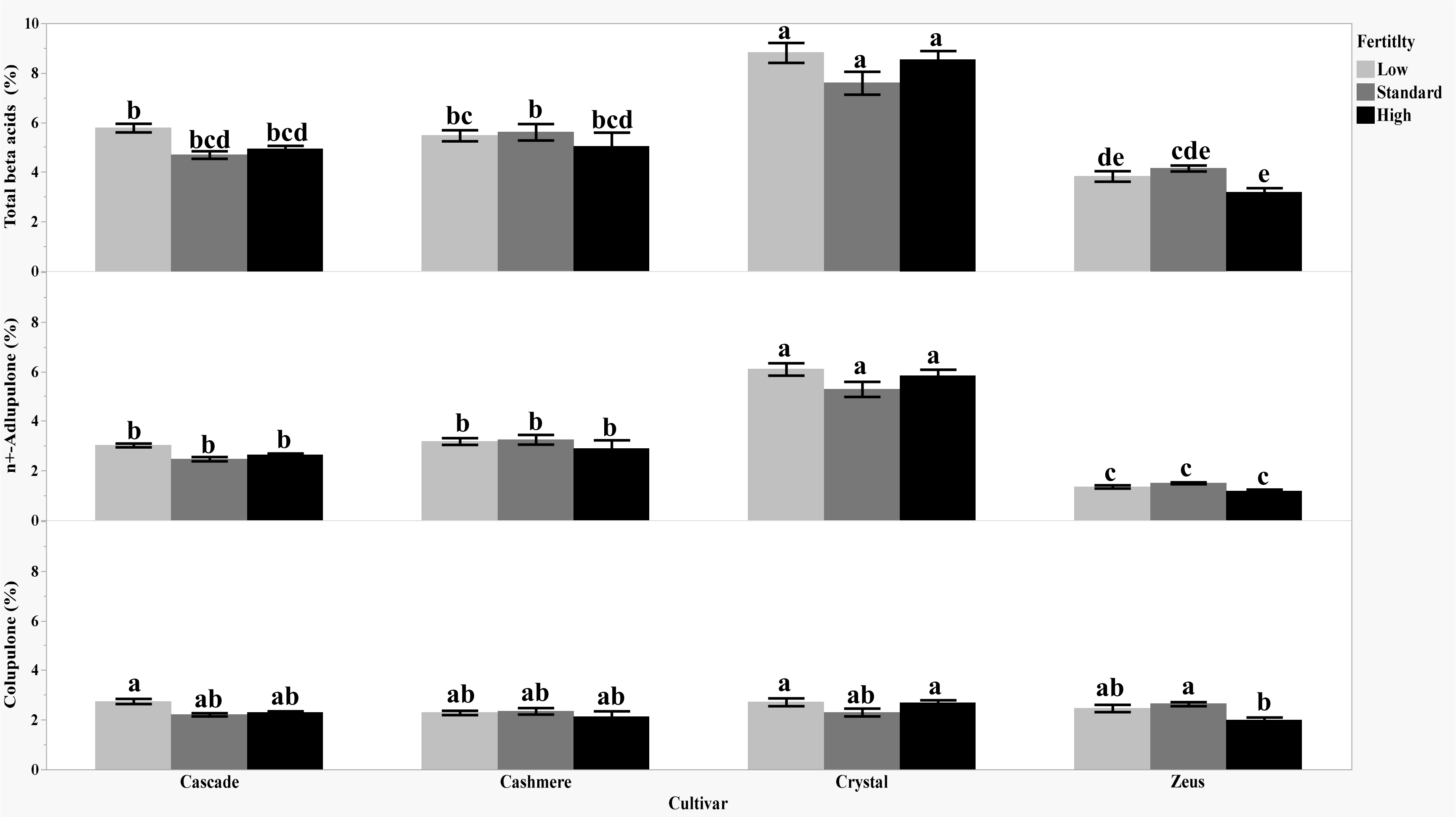


Fig. 5. Interactions of fertility rate (Low, Standard, and High) and cultivar on individual and total beta acids of hops plants grown in Clarksville, AR (2021)

Hop cones analyzed with high performance liquid chromatography using American Society of Brewing Chemists (ASBC) Hop-14 method

Means with different letters for each attribute are significantly different ($p<0.05$) according to Least Square Means Student's t-test

n+-adhumulone refers to the level of n-humulone and adhumulone combined in one fraction

Table 11. Descriptive sensory evaluation[z] of dried, ground hop cones from cultivars of hop plants grown in Clarksville, AR (2020), the second year of production

Attributes[z]	**Cascade**	**Cashmere**	**Crystal**	**Zeus**	*P-value*
Grass	2.0 a[y]	1.9 a	1.5 a	2.4 a	*0.145*
Foliage	1.9 a	1.2 a	1.4 a	1.9 a	*0.451*
Fruity*	**1.1 ab**	**0.1 c**	**0.3 bc**	**1.5 a**	***0.005***
Terpenes off note skunk*	**0.5 c**	**3.2 a**	**1.4 b**	**0.3 c**	***<0.0001***
Aged cheese*	**0.3 b**	**1.4 a**	**1.0 a**	**0.2 b**	***<0.0001***
Umami savory*	**0.6 bc**	**1.1 ab**	**1.3 a**	**0.3 c**	***0.001***
Overall citrus complex*	**3.4 a**	**2.0 b**	**2.7 ab**	**2.7 ab**	***0.013***
Lemon*	**1.8 a**	**1.0 b**	**1.3 ab**	**1.5 a**	***<0.0001***
Lemongrass*	**2.7 a**	**0.9 b**	**1.6 b**	**1.6 b**	***0.002***
Other*	**0.3 ab**	**0.0 b**	**0.0 b**	**0.8 a**	***0.016***
Overall green herb complex*	**2.7 b**	**2.6 b**	**3.2 a**	**2.5 b**	***0.034***
Sage	1.5 a	2.0 a	2.3 a	1.5 a	*0.076*
Thyme	1.6 a	1.1 a	1.0 a	0.8 a	*0.255*
Other	0.2 a	0.1 a	0.5 a	0.7 a	*0.053*
Floral*	**0.6 b**	**0.5 b**	**0.4 b**	**1.5 a**	***0.001***
Mint*	**0.4 a**	**0.1 bc**	**0.0 c**	**0.2 ab**	***0.005***
Garlic*	**0.3 c**	**1.9 a**	**1.2 b**	**0.3 c**	***<0.0001***
Dill	0.5 a	0.4 a	0.2 a	0.5 a	*0.466*
Overall pepper complex*	**2.2 b**	**2.3 ab**	**2.8 a**	**1.6 c**	***0.001***
White pepper	1.1 a	1.4 a	1.7 a	1.0 a	*0.096*
Black pepper	1.3 a	1.4 a	1.5 a	0.7 a	*0.105*
Overall impact*	**6.1 b**	**6.6 a**	**6.7 a**	**5.9 b**	***0.001***
Defining attribute[x]	**Citrusy**	**Terpenes/off-note**	**Savory**	**Grass/foliage**	

[z] The Universal Aromatic Scale (0 to 15 points) was used as the reference for the aroma attributes. The aroma attribute definition is aromas associated with the attribute listed as Intensity 2.0 = Soda note of saltine cracker; Intensity 5.0 = Cooked apple note of applesauce; Intensity 7.5 = Orange note of orange juice; Intensity 10.0 = Grape note of grape juice; Intensity 15.0 = Big Red Gum®

[y] Means with different letters for each attribute are significantly different ($p<0.05$) according to Tukey's Honest Significant Difference (HSD) test, highlighted row are significant attributes

[x] Defining attribute is the term used to characterize the sample

Table 12. Descriptive sensory evaluation[z] (n=7) of dried, ground hop cones from cultivars of hop plants grown in Clarksville, AR (2021), the third year of production

Attributes	**Cascade**	**Cashmere**	**Crystal**	**Zeus**	*P-value*
Grass	2.7 a	3.1 a	2.6 a	3.0 a	*0.179*
Foliage	3.7 a	4.1 a	3.4 a	3.5 a	*0.099*
Fruity	1.2 a	1.2 a	1.5 a	1.5 a	*0.47*
Terpenes off note skunk	2.5 a	2.1 a	2.4 a	2.6 a	*0.585*
Aged cheese*	**2.3 a**	**2.0 ab**	**2.4 a**	**1.4 b**	***0.03***
Umami savory	1.8 a	2.0 a	1.9 a	1.5 a	*0.111*
Overall citrus complex*	**3.2 a**	**2.5 b**	**3.2 a**	**3.2 a**	***0.006***
Lemon*	**2.7 a**	**1.9 b**	**2.8 a**	**2.8 a**	***0.026***
Lemongrass	2.0 a	1.5 a	1.8 a	1.9 a	*0.134*
Other	0.6 a	0.7 a	0.6 a	0.5 a	*0.734*
Overall green herb complex*	**4.7 a**	**3.3 b**	**4.1 a**	**4.5 a**	***0.001***
Sage	3.3 a	2.4 a	3.0 a	3.2 a	*0.062*
Thyme*	**3.8 a**	**2.5 b**	**3.5 a**	**3.7 a**	***0***
Other	1.3 a	1.4 a	1.7 a	1.5 a	*0.051*
Floral	1.9 a	1.6 a	2.2 a	2.1 a	*0.08*
Mint	1.9 a	1.5 a	1.9 a	2.0 a	*0.097*
Garlic	1.0 a	1.2 a	0.8 a	1.2 a	*0.391*
Dill	2.0 a	1.7 a	2.0 a	2.1 a	*0.204*
Overall pepper complex*	**3.4 a**	**2.8 b**	**2.9 b**	**2.8 b**	***0.019***
White Pepper	2.7 a	2.3 a	2.4 a	2.0 a	*0.067*
Black Pepper*	**2.9 a**	**2.2 b**	**2.5 ab**	**2.6 ab**	***0.042***
Overall Impact	5.7 a	5.2 a	5.3 a	5.1 a	*0.236*
Defining attribute	**Herbal**	**Foliage**	**Herbal/citrus**	**Herbal**	

[z] The Universal Aromatic Scale (0 to 15 points) was used as the reference for the aroma attributes. The aroma attribute definition is aromas associated with the attribute listed as Intensity 2.0 = Soda note of saltine cracker; Intensity 5.0 = Cooked apple note of applesauce; Intensity 7.5 = Orange note of orange juice; Intensity 10.0 = Grape note of grape juice; Intensity 15.0 = Big Red Gum®

[y] Means with different letters for each attribute are significantly different (p<0.05) according to Tukey's Honest Significant Difference (HSD) test, highlighted row are significant attributes

[x] Defining attribute is the term used to characterize the sample

Chapter II

Impact of Pruning Timing on Plant and Cone Attributes of Arkansas-grown ‘Cascade’ Hops

Abstract

The hop plant (*Humulus lupulus* L.) is a perennial, climbing species that produces cones used to contribute quality and flavor in beer production. In the United States, most hops are grown the Pacific Northwest, but growth in the craft brewing industry is driving efforts for hops production in other U.S. regions. Recommendations on cultural management practices are needed for the U.S. mid-south region as previous research indicated that spring pruning affects the hops plants and cones. Nine ‘Cascade’ hops plants were planted at the University of Arkansas System Division of Agriculture Fruit Research Station in Clarksville, AR in the fall of 2018 and grown on a 3.66 m high trellis with 76.2 cm spacing between plants. ‘Cascade’ hops plants were pruned April 15 (Early), April 30 (Mid), or May 15 (Late) in 2020 and 2021 (the second and third year after establishment) by removing all new plant growth around each crown at soil level. There were three plants (replications) per pruning timing. The impact of pruning timing and year on plant attributes, cone attributes, and cone quality of ‘Cascade’ hops plants were evaluated. Plants were evaluated for attributes at harvest, including number of bines/plant, number of nodes/plant, number of laterals/plant, and bine length. The cone attributes evaluated at harvest included total cone yield/plant, percent of mature, immature, or damaged/diseased cones/plant, cone moisture content, individual cone weight, and estimated dry cone yield/plant. The quality attributes of dried hops cones evaluated postharvest included individual and total alpha and beta acids. Pruning timing did not impact any of the plant attributes and most of the cone attributes except the percent of damaged cones/plant with the Mid pruning having the highest damage. The year impacted the number of laterals/plant, total cone yield/plant, and estimated dry cone yield/plant. The number of laterals/plant were lower in 2020 (82.22) compared to 2021 (117.56), a 69.9% increase by year. Total cone yield/plant had a 60.1% reduction in 2021 (421.51 g) compared to 2020 (701.33 g). The Arkansas-grown 'Cascade' yield/plant was 9-14% of the production potential in commercial hop producing regions. The pruning x year interaction was significant for the individual and total alpha and beta acids of the dried 'Cascade' hops grown in Arkansas. The 'Cascade' hops had total alpha acids (6.28-7.66%) and total beta acids (6.45-9.32%) that were slightly higher than commercially-available hops. In 2020, the Early pruning had the highest level of alpha and beta acids, while in 2021, the Mid pruning had the highest level. The fluctuation in plant attributes, cone attributes, and cone quality between pruning timing and year supports the need for future research for optimizing shoot pruning practices for hop production in the U.S. mid-southern region.

Introduction

The hop plant (*Humulus lupulus* L.) is a perennial, climbing bine species within the *Cannabaceae* family that produces hops cones used for beer production. The origin and utilization of hops can be traced back thousands of years and is considered both historically and agriculturally significant because of worldwide cultivation. Hops are herbaceous climbing plants that send shoots up in early spring that later die back to a cold-hardy rhizome in the fall. The plant is dioecious which means that there are separate male and female plants. Female plants are cultivated for hop cone production, while male plants are used for breeding. The shoots are known as bines and can grow supported by a trellis system from 4.6-6.1 m in height. The bines produce lateral shoots and use trichomes (hair-like structures) that attach to twine/rope that run vertically up the trellis to support the plant structure.

In comparison with small stature perennial species that require vernalization (seed cooling during germination to accelerate flowering when planted), hop bines have a protracted juvenile phase during which they are incapable of flowering unless 12–25 nodes are visible (Bauerle, 2019). This is due to hops requiring a variety-specific size effect (distance) between the roots and shoot apex to make the juvenile-adult transformation. Hops plants typically mature 3-5 years depending on location, climactic conditions, cultivar, and cultural management (Chechourka, 2018). Ha et al. (2017) also observed that hops plants grown in temperate climates take 4-5 years to reach full production in Indiana. Yields in the first, second, third, and fourth years are estimated at 20%, 40%, 60%, and 80% of mature yields, respectively (Ha et al., 2017). Similarly, Sirrine et al. (2015) noted that the conservative yield estimate for the first year is negligible in Michigan-grown hops, but 50%, 75%, and 100% of production are expected in the second, third, and fourth year, respectively.

Hops cones are the inflorescences or strobili (seed cones) from the perennial female hop plant that are an essential component of industrial and craft brewing production. A hop is composed of four primary structures, including the strig, bract, bracteole, and inner lupulin glands. The lupulin glands contain the complex phytochemical compounds valued for aroma and flavor of beer (Hrnčič et al., 2019; Killeen et al., 2016; Patrick, 2013). The cones from the female plants contain various secondary metabolites, hard and soft resins, including alpha acids and beta acids, monoterpenes, sesquiterpenes, essential oils, and polyphenols. There are over 300 compounds found in the inner lupulin glands that contribute to the flavor and aroma profiles of hops cones (Farber & Barth, 2019).

The most substantial component of dried hops are the alpha acids, and these compounds are structurally complex enolic acids that contain a six-carbon ring with several substituent groups. While there are more than seven prominent alpha acids within the lupulin glands of the hops, humulone, cohumulone, and adhumulone constitute 98-99% of the alpha acids (Killeen et al., 2016; Mahaffee et al., 2009). The beta acids found within the hop cones are only a minor contribution to a beer’s flavor, but they are a crucial component in the brewing process, especially for preservation. Previous studies regarding these compounds have noted that the ratio of alpha to beta acids varies depending on the stage of development, cultural management, growing location (terroir), and cultivar, but it often ranges from 1:1 to 4:1 (Forteschi et al., 2019; Mahaffee et al., 2009; Rodolfi et al., 2019; Santagostini et al., 2019).

To maintain the valuable components of hop cones, proper drying and storage methods after harvest must be implemented to ensure optimal hop quality for brewing and to allow for long-term use and storage. Freshly harvested hops are approximately 80% water and, if left untreated, will spoil rapidly. Hops are dried after harvest to 7-10% moisture for use in brewing. Dried hops are typically placed in food-grade bags, vacuum sealed to maintain quality, and then frozen (-2 °C) or refrigerated (4 °C).

Hops plants are grown commercially throughout the world, primarily between the 35th and 55th latitudes in the Northern and Southern hemispheres. The International Hop Growers’ Convention Economic Commission estimated there were 62,000 ha of hops acreage with a global harvest weight of 123 million kg in 2019 (Hop Growers of America, 2019). The United States, Germany, and China were the three highest hop-producing countries in 2019 with a combined harvest of 106 million kg (Hop Growers of America, 2019).

The primary hop production region in the United States is in the Pacific Northwest, including Washington, Oregon, and Idaho which produced 71%, 17%, and 12% of the total 2020 U.S. hop crop, respectively (USDA, NASS 2020). The remaining states had a commercial hop production estimated at 454,000 kg in 2019 (Hop Growers of America, 2019). While the three main hop-growing states produce 98% of the total U.S. hop crop, many locations around the states are growing hops to meet the rising local demand for cones. Other locations that produce notable acreage of hops include Wisconsin (2.2%), Michigan (1.2%), New York (0.7%), Colorado (0.2%), California (0.2%), Minnesota (0.2%), and Ohio (0.1%) (Hop Growers of America, 2019).

For brewing purposes, hop cultivars are classified into three categories – bittering, aromatics, and dual purpose. The most popular hop cultivars by acreage in the United States include 'Citra®', 'CTZ' (also known as 'Columbus', 'Tomahawk', and 'Zeus'), 'Cascade', 'Simcoe', and 'Mosaic' with 75.4% of the cultivars grown in 2019 used for aroma (Hop Growers of America, 2019). Aromatic hops are typically higher in essential oil content than the bittering cultivars. These cultivars add notes of grass, fruit, honey, earth, flowers, and other spices, and include cultivars like 'Amarillo,' 'Brewers Gold,' 'Cascade,' 'Citra,' among many others. 'Cascade', 'Chinook', and 'Citra' (high alpha acid cultivars) can yield as much as 771-862 kg/ha while 'U.S. Northern Brewer' (bittering cultivar) and 'Willamette' (a triploid aroma-type hop) yield between 1,343-1,679 kg/ha (Brewers Association, 2019; 2021).

Commercially grown U.S. hop cultivars typically originate from European cultivars adapted to the U.S. terroir (i.e., climactic growing conditions). There has been an increase in the U.S. breeding programs for hops cultivars suitable for the U.S. climate. One of the most prominent and widely used U.S. hop cultivars is 'Cascade'. This cultivar was the first hop released from the United States Department of Agriculture (USDA) in 1972. This was primarily due to the plant's unique aroma profile, brewing qualities and adaptation to U.S. hop yards. 'Cascade' is considered medium in maturity (early September) but retains brewing quality and bright appearance for about three weeks after maturity. The lateral side branches that emerge from the plant's nodes are 60.9-76.2 cm, and the cones are compact, medium sized, and easily harvested (Brooks et al., 1972). 'Cascade' was created from 'English Fuggle' and 'Russian Serebrianker' through wind pollination by an unknown male variety (BarthHaas, 2021).

'Cascade' hop plants can reach the top of a 3.7-4.6 m trellis within a brief vegetative growing season (May-July in the Southeastern United States) with fragrant cones that emerge and mature between August and September. 'Cascade' plants have shown a tolerance to drought and a resistance to insect and disease pressure, thus making the cultivar an ideal candidate for U.S. hop farms around the Pacific Northwest and Southeastern United States. 'Cascade' is considered an aromatic cultivar and characterized by dark green, elongated cones with a relatively low quantity of alpha acids. 'Cascade' has alpha acid profiles between 4.5-7.0% and beta acids around 4.8-7.0% (BarthHaas, 2021; Brooks et al., 1972, USAHOPS, 2018).

There are many cultural practices that can impact plant and cone quality including the timing of pruning hops plants. Pruning involves the removal of the first shoots on the plant that emerge in early Spring. Matsui et al. (2016) varied the pruning and harvest date for 'Saaz' hop in four locations throughout the Czech Republic with fifteen pruning conditions and five harvest times evaluated at each location over three years. Results indicated that there was no correlation between date of pruning and date of cone formation, and date of shoot pruning had no significant effects on hop essential oils, sensory scores, and yield, yet the level of alpha acids tended to be higher in the cones from plants pruned later (mid to late April in Czech Republic) (Matsui et al., 2016). While this research study indicated that the length of the vegetative period (from pruning to blooming) does not significantly affect hop secondary metabolism, a similar study conducted in the Czech Republic reported significant differences in cone yield and alpha acid content in hops depending on pruning timing and method (Křivánek et al. 2008).

Křivánek et al. (2008) compared two methods of pruning – shallow and deep cutting – and timing (late March, mid-April, and late-April) over three years for the hybrid cultivar ‘Agnus’ to determine if the timing and method of shoot pruning influenced yield and quality of cones. The deep pruning method entailed pruning the newly emerged spring shoots to just above the crown at the surface of the soil, and the shallow method involved leaving 5-8 cm of shoots above ground after pruning. Cone harvest for all ‘Agnus’ plants occurred during the final week of September. The results showed that the shallow pruning method increased cone yield, and the quality assessments indicated a higher alpha acid content (an increase of 11% on average over three years) in addition to higher quality shoots for training with a faster growth rate (Krivanek et al. 2008). The late term pruning interval had a greater alpha acid content and the highest yield compared to the early pruning dates.

Similarly, Lafontaine et al. (2018) conducted a three-year study that assessed cone quality and harvest timing for the aroma potential and chemical development of ‘Cascade’ hops throughout the vegetative growing period. While the concentrations of humulones did not change as a function of harvest date, the total hop essential oil content indicated a positive trend with increasing cone maturity (Lafontaine et al. 2018). According to Lafontaine et al. (2018), the aroma intensity and citrus quality of the ‘Cascade’ hops also increased as a function of harvest date. These results suggest that for brewers to utilize hops at their peak brewing potential, early harvested ‘Cascade’ cones might be ideal for bittering, while, later harvested ‘Cascade’ hops might be better for dry-hopping or aroma additions (Lafontaine et al. 2018). The disparity of the results between both trials could indicate that other factors, such as agrometeorological influences and location, aside from the timing and method of pruning, have a significant effect on cone yield and quality of harvested cones.

Cultural practices such as pruning can also impact hops diseases such as downy mildew (caused by *Pseudoperonospora humuli*), powdery mildew (caused by *Podosphaera macularis*), and halo blight (caused by *Diaporthe humulicola*) depending on timing and method (i.e., mechanical, chemical dessication, or combination). Mildews and bacterial viruses have the potential to devastate hop yards in the United States. Gent et al. (2012) assessed Spring pruning quality and timing with severity of downy mildew and powdery mildew through analysis of survey data collected from commercial hop yards in Oregon and Washington. Severity of powdery mildew and cone yield was similar between plots that received the delayed or standard pruning timing treatment (Gent et al., 2012; 2015). Probst et al. (2016) found that delayed pruning offered a low-cost means of reducing incidence of powdery mildew but did not impact the cone yield, levels of bittering-acids, and color of the hops. The halo blight viroid disease can cause brown leaf lesions, cone and bract browning, and cone shatter that can result in severe yield loss. Sirrine et al. (2022) suggested that mechanical control (scratching) and chemical options may help growers control halo blight. Regardless of pruning methodology, the date of pruning has been shown to be a critical cultural method since pruning date has an effect on training date and ultimately yield (Sirrine et al., 2022).

Since 'Cascade' is one of the most widely used hops cultivars in the United States and because there is limited information about growing 'Cascade' in Arkansas, the objectives of this research were to determine the impact of pruning timing on plant and cone attributes of Arkansas-grown 'Cascade' hops.

Materials and Methods

Hops study

Hops production studies were established at the University of Arkansas System (UA System) Division of Agriculture Fruit Research Station, Clarksville, AR in September 2018 [West-Central Arkansas, lat. 35°31'58"N and long. 93°24'12"W; U.S. Dept of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]. The study included a cultivar/fertility study and a pruning study. The cultivar/fertility study composed of nine 1.2 m wide x 7.3 m long plots divided into three blocks with three replications of six hop cultivars per block (plant numbers 1-54). For the pruning study, an additional 6.9 m plot of nine ‘Cascade’ hop plants were established at the end of the cultivar/fertility study row (plant numbers 55-63). Equal plant spacing was maintained and the “standard” fertility rate (48.02 g 13-13-13 granular fertilizer/plant) using the biweekly, hand broadcasting protocol was used.

Hops trellis and plants

The hops were grown on a trellis that was 3.66 m high with 76.2 cm spacing between plants. Each hop bine was trained using a landscape fabric staple that had three lines of bailing twine attached that were suspended to the top of the horizontal trellis wire. Prowl® pre-emergent herbicide (BASF Corporation, Durham, NC) was used in early September 2018 prior to planting, and on September 14, 2018, each hop plug plant was planted using a hand trowel and each plant was immediately watered. Plug plants for ‘Cascade’, ‘Cashmere’, and ‘Crystal’ were sourced from Agristarts (Apopka, FL), and ‘Centennial’, ‘Nugget’, and ‘Zeus’ were sourced from Great Lakes Hops (Zeeland, MI). A shallow layer of mulch 10.2-15.2 cm deep was placed around each plant shortly after implanting to conserve soil moisture and reduce invasive grasses. The hops entered dormancy during the winter months (November through March), and all above ground growth died back to the ground. Bines from all cultivars emerged from the perennial crown around mid-March through early April.

Hop pruning

'Cascade' hops (plants 55-63) in this study were pruned post dormancy (mid-March) at three dates in 2020 and 2021. The first date of shoot pruning was April 15 (Early) for plants 55-57, the second pruning occurred April 30 (Mid) for plants 58-60, and the final date was May 15 (Late) for plants 61-63. The method used for cutting the bines back consisted of the removal of all new plant growth around each perennial crown which entailed cutting all shoots to soil level with hand sheers.

Cultural management, scouting, and pest management methods

Hop plants underwent management practices to promote plant health and cone production. Once bines reached 1.8 m in height, the lowest leaves and lateral branches (below 15.2 cm to soil level) were removed by hand or with sheers to dissuade common pests and diseases and promote airflow throughout the hop yard. The lateral branches of the vigorously growing bines were untangled by hand or pruned to separate the plants from one another as needed throughout the primary vegetative growth stage (June-August).

Certain pests and diseases, such as the two-spotted spider mite (*Tetranychus urticae*), several Lepidoptera species (*Spodoptera frugiperda* and *Polygonia interrogationis*, or army worms and question mark caterpillars, respectively), damson hop aphid (*Phorodon humuli*), downy mildew (*Peronospora sparsa*), and powdery mildew (*Golovinomyces orontii*) can negatively impact the vigor and quality attributes of hops cones. To determine when these pests' affected hops and to what extent their presence had on hop plant health throughout cultivation, weekly scouting with the use of a handheld lens was performed during bine development until harvest (May through August). The scouting method entailed randomly selecting 2-3 plants and examining approximately five leaves per plant (selected from different locations along the length of the bines in a figure V formation) using the magnified lens. When damage was spotted due to insect, disease, or nutritional deficiency, the extent was noted, and an integrated pest management strategy was followed. The use of several brands of insecticides, miticides, and fungicides were implemented in routine backpack spray applications to deter further damage from fungal diseases and common pests (weekly spray schedules were followed when insect populations were significant). Coragen (Dupont™, Wilmington, DE), Thuricide BT (Southern Agriculture Insecticides Inc., Palmetto, FL), and Delegate (Corteva Agriscience, Indianapolis, IN) chemicals were used to exterminate most insects known to affect hops. Acramite (Chemtura, Middlebury, CT) was sprayed when significant mite populations were found, and Forum (BASF Corporation, Durham, NC) and Ranman (ISK Biosciences Corporation, Concord, OH) were implemented as a fungicide to deter mildews. Label rates and safety protocols (the use of protective Tyvek® clothing during spray application) were followed closely to prevent injury or adverse health risks associated with chemical exposure.

Hop harvest

The moisture content and ripeness of the hop cones were assessed during late summer and early fall to determine the ideal time of harvest. Hops were harvested when the moisture content of the cones were 75-80%, the color and texture of the bracts were light and papery to the touch, and the internal lupulin glands were dark yellow and pungent. 'Cascade' plants in this study were harvested on August 19, 2020 and August 16, 2021. At harvest, the hops plant was cut at the base of the plant at soil level and transported to a table located in a tented area to evaluate plant and cone attributes at harvest.

Plant attributes evaluated at harvest. Hops plants were assessed for plant attributes prior to cone harvest. The hops plant was evaluated for the number of bines/plant, number of nodes/plant, number of laterals/bine, and bine length (m).

Cones attributes evaluated at harvest. The hops cones were hand harvested from the bines and separated into mature, immature, or diseased/damaged. The cones were weighed on an electronic scale (ArlynGuard S, model MKE-5-IS, East Rockaway, NY). The total cone yield (g)/plant was calculated as the sum of weights of the mature + immature + diseased/damaged cones. The percent of mature cones was calculated as the weight of the mature cones/total weight. The percent of immature cones was calculated as the weight of the immature cones/total weight. The percent of diseased/damaged cones was calculated as the weight of the diseased/damaged cones/total weight. The cone moisture content (%) was determined from the weight of 30 cones collected at harvest. The individual cone weight (g) was calculated by dividing the total weight of the 30-cone sample by 30. The estimated dry cone yield/plant (g) was calculated as 10% of the total yield/plant.

Drying and storing hop cones

The cones from each plant were combined and then placed into paper bags (17.8 cm wide x 11.4 cm long x 34.9 cm long) labeled with wet cone weight/bag. The cones in the bags were placed in a dehydrator custom built for this site (Herrera et al., 2021). The temperature of the dehydrator was 43-49 °C, and a dehumidifier was used to remove moisture from the air. The hops were removed when the cones reached 8-10% moisture content. To ensure the cones were dried to these specifications, the individual bags were weighed every 2 hrs after 14-16 hrs elapsed until the intended moisture level was achieved. Additionally, other visual indicators were used to evaluate if hops were sufficiently dried. These included the presence of yellow powdery lupulin when handled and the texture of the bracts (springy, papery, and light in color).

After the cones were dried, the hops were packaged and vacuum sealed in food-grade plastic bags (UltraSource Vacuum Chamber Pouches, 4 mil, 20.3 x 30.4 cm). A Floor Model Chamber Vacuum Packaging Machine (VacPak-It VMC20FGF, Clark Associates, Lancaster, PA) was used to vacuum seal the bags with about 95% air removal from each package. This vacuum strength (removal of air from pouches) varied depending on the number of hops in the package. If the vacuum strength was too high, the cones were crushed, and the lupulin would fall from the cones and settle at the bottom of the plastic bag. The bags of hops were placed into a freezer at -2 °C for later analysis.

Dried hops analysis

Dried hops were taken to the UA System Food Science Department for analysis. For the analysis of the dried hop cones, hops were removed from the freezer, samples were removed, the unused hops were resealed with the vacuum sealer, and returned to the freezer. The whole-cone hops were ground for analysis using a Magic Bullet blender (MBR - 1101, Los Angeles, CA) with cross blades in a 473-mL container. Analysis of dried hops included moisture content and alpha and beta acids by High Pressure Liquid Chromatography (HPLC) using American Society of Brewing Chemists (ASBC) methods. The extractions of alpha and beta acids were done in analytical triplicate per sample. The moisture content of the hops was done in analytical duplicate per sample.

<u>Moisture content analysis.</u> The moisture content of the dried hops must be analyzed because the moisture content after drying can deviate from the optimal 8-10%. The hops were dried 100% to determine the moisture content for the hops cones to calculate alpha and beta acids levels using

the ASBC method Hops-4C (Moisture by Routine Air Oven Method). Approximately 2.5 g of unground hops were placed in an aluminum dish. The dish was covered with aluminum foil, then the dishes with hops were weighed on a precision scale (0.001 g) and placed in a Fischer Scientific Isotemp Oven Model 655F (Houston, TX) at 103-104 °C. The dish covers were removed, the hops were dried for 1 hr, then the covers were replaced while the dish was in the oven. The dishes were transferred to a desiccator containing Drierite Absorbent (8 mesh DX2515-1, Millipore Corporation, Burlington, MA). The lid was placed on the desiccator and sealed with high vacuum silicone grease. The hops were cooled in the desiccator and reweighed. After weighing, the percent moisture of the hops was calculated using the formula:

moisture in hops (%) = (loss in weight*100)/(weight of undried sample)

Dry weight of the samples can also be calculated from the moisture content.

<u>Alpha and beta acid analysis.</u> Dried hops were analyzed by HPLC using the ASBC Hops-14 (alpha acids and beta acids in Hops and Hop Extracts by HPLC) procedure. This procedure was modified because of the limited amount of sample. A 2-g sample of dried hops were placed in 50-mL centrifuge tubes and weighed. Then, 4 mL of methanol and 20 mL of diethyl ether were added to each tube. The tube was capped and placed on a shaker for 30 min. After 30 min., flasks were opened and 8 mL of 0.1M hydrochloric acid was added. The original method for Hops-14 instructs to use 10 g of hops with 20 mL of methanol, 100 mL of diethyl ether and after shaking 40 mL of hydrochloric acid. So, for this project, the HPLC extraction was downscaled by a factor of five as compared to the original procedure. The flasks were capped and placed on the shaker for 10 min. After this, the flasks were kept in the dark for 10 min as the phases separated. After the phases separated, 1.0 mL of the supernatant phase was pipetted in a 10 mL volumetric flask and brought up to volume with methanol. The contents of the flask were sealed with

parafilm and mixed. The solution was syringe filtered using a 25 mm 0.45 nylon membrane filters (VWR, Radnor, PA) before injection onto the HPLC.

Samples (50 µL) were analyzed using a Waters HPLC system equipped with a model 600 pump, a model 717 Plus autosampler and a model 996 photodiode array detector. Separation was carried out using a Phenomenex (Torrance, CA) Nucleosil-5 C18 chromatographic column (250 × 4 mm, 5-µm ODS RP18). The mobile phase was a combination of methanol, water, and phosphoric acid in an 85:17:0.25 ratio (v/v) that was mixed and filtered through a 0.45-µm filter. To achieve adequate resolution, the column was conditioned with mobile phase for 1 hr prior to use. The flow rate was 0.8 mL/min, and the detection wavelength was 314 nm at an ambient temperature. Each sample was injected and analyzed in duplicate with a run time of 30 minutes. Samples were either run on the HPLC immediately or stored at 2 °C and protected from light for analysis within 24 hours. After analysis, the HPLC peak areas were converted to levels of the alpha and beta acids using the standard curves. The percentage of the fraction per gram of hops was calculated using the following formula:

% w/w= (HPLC conc (mg/ml) *methanol volume (mL)*(mL methanol+mL ether+mL hydrochloric acid))/(mL supernatant taken*1000*starting weight of sample (g)).

Standards and Calibration. The calibration curve was made using Standard hop extract ICE-4 (ASBC, Saint Paul, MN) for HPLC analysis. This is a hop extract containing a specified concentration of alpha and beta acids. ICE-4 contains cohumulone (10.98%), n+adhumulone (31.60%), colupulone (13.02%), and n+adlupulone (13.52%) with total alpha acids levels of 42.58% and total beta acids levels of 26.54%.

Alpha acids can be subdivided in three main individual acids: cohumulone, n-humulone, and adhumulone. The procedure of ASBC Hops-14 that was used to separate cohumulone as an individual fraction and n-humulone and ad-humulone together as a fraction. This gives two fractions: “cohomulone” and “n+-adhumulone”. The same applies to the beta acids. Colupulone was separated from the other beta acids, n-lupulone and adlupulone.

From the ICE-4 standard, 1.500 ± 0.001 g was weighed and diluted in 25 mL of toluene in a 25-mL volumetric flask. The standard was first diluted (dissolved) with toluene. The toluene dilution was then diluted by a factor of 10 volumetrically with methanol (standard A) followed by subsequent dilutions. The calibration curve of each of the standards was achieved by plotting the levels of cohumulone, n+adhumulone, colupulone, and n+adlupulone in the standard against the acquired area.

Statistical design and analysis

This pruning study on ‘Cascade’ was analyzed as a full factorial with year (2020 and 2021) and pruning timing (Early, Mid, and Late) as the main effects and year x pruning timing as the interaction. The pruning treatments were in triplicate each year. The alpha and beta acid attributes were evaluated in analytical triplicate while the moisture content analysis was assessed in analytical duplicate. Statistical analyses were conducted using JMP® (version 16.0.0; SAS Institute, Cary, NC). To determine if there was a significant difference among the pruning dates, a univariate analysis of variance (ANOVA) was used to analyze the levels of variance. Least Square Means Student’s t-test was used to detect significant differences ($p < 0.05$) among means and verify interactions at 95% significance level.

Results and Discussion

Average monthly temperature and rainfall at the Fruit Research Station in Clarksville, AR were tracked, recorded, and reported from January to September, the end of hops harvest (Fig. 1.) The 2020 hops season in Clarksville, AR was relatively mild in terms of temperature and rainfall. The 2021 season had notable weather events in February and April. There were record cold temperatures (-5 °C) with 178 mm of snow in February of 2021 at the Fruit Research Station followed by a freeze in late April (-1 °C overnight). Shoots of the hops plants emerged in the spring in mid-March and early April both years. The average high temperature was 22 °C and low temperature was 12 °C in 2020 and 2021. Total rainfall in 2021 (103 mm) was less than rainfall in 2020 (139 mm). Maximum day length for both years occurred June 20 with 14 hours and 36 minutes of daylight (1 hour and 18 minutes less than commercial regions in the Pacific Northwest). The average day length was 12 hours and 48 minutes during the measured time interval.

Main effects and interaction of different pruning treatments and year were evaluated for plant and cone attributes of 'Cascade' hop plants grown in Clarksville, AR in 2020 and 2021, the second and third year after planting.

Plant attributes at harvest

Hops plants were assessed for plant attributes at harvest, including number of bines/plant, number of nodes/plant, number of laterals/plant, and bine length (Table 1). There was not a significant pruning x year interaction for any of the plant attributes at harvest. Pruning timing did not impact any of the plant attributes. Year only impacted the number of laterals/plant. The plants in 2021 (117.56) had more laterals/plant than the plants in 2020 (82.22), a 69.9% increase. All plants had three bines/plant with 63 nodes/plant with a bine length of 12 m.

Acosta-Rangel et al. (2021) evaluated hops in west-central Florida and noted that the average number of lateral shoots/plant varied considerably depending on cultivar and the hop plants country of origin. In the first of two experiments regarding hop growth and yield rates, seven cultivars were evaluated for bine height, bine number, lateral shoot number, and dry cone yield (10% moisture). The top three yielding cultivars, ‘Cascade’, ‘Zeus’, and ‘Nugget’, were U.S. cultivars, while two European landrace cultivars (Perle and Tettnanger) and the Japanese cultivar (Sorachi Ace) did not produce any cones. Yield had positive correlations with bine number and lateral shoot number, but it had no significant correlation with bine height (Acosta-Rangel et al., 2021). Correlation analysis revealed that bine number and lateral shoot number were important yield-determining traits, whereas bine height was not a good yield indicator. It was concluded that low genetic diversity of European cultivars, such as ‘Tettnanger’ and ‘Saaz’ (Murakami et al., 2006), may explain their poor performance and acclimation to subtropical climatic conditions in this study while ‘Cascade’ and ‘CTZ’ have shown success in their adaptation to different soil types and pH levels.

Judd (2018) analyzed plant height among other cone and physiological attributes at harvest for 13 publicly-available cultivars (Alpharoma, Cashmere, Cascade, Centennial, Comet, Crystal, Mt. Hood, Mt. Rainier, Nugget, Sorachi Ace, Southern Cross, Tahoma, and Ultra) for two years (2016 and 2017) in Blacksburg, Virginia. While pruning timing was disregarded in this study, results from the Virginia-grown hops trial showed no statistical difference in ‘Cascade’ plant heights between years (Judd, 2018). Although not significant, the total bine length in our pruning study decreased from 12.24 m in 2020 to 11.38 m in 2021 which was also seen in Judd (2018) (5.00 m to 4.93 m between 2016 and 2017, respectively).

Cone attributes at harvest

The cone attributes evaluated at harvest included total cone yield/plant, percent of mature, immature, or damaged/diseased cones per plant, cone moisture content, individual cone weight, and estimated dry cone yield/plant (Table 2). There was not a significant pruning x year interaction for any of the cone attributes at harvest. Pruning timing did not impact any of the cone attributes except for the percentage of damaged cones/plant. The Mid pruning date had a greater number of damaged cones (11.68%) compared to the Early and Late treatments (4.23% and 1.80%, respectively). Year only impacted total cone yield/plant and estimated dry cone yield/plant. The 'Cascade' plants had a total cone yield/plant of 701.33 g in 2020, while the 2021 plants had 421.51 g, a reduction of 39.90%. Consequently, the estimated dry cone yield/plant was significantly lower in 2021 (42.15 g) than in 2020 (70.13 g). 'Cascade' had an individual cone weight of 0.56 g at 75.16% moisture content at harvest. The harvested cones were 64.74% mature and 33.24% immature. Comparatively, commercial producers in the Pacific Northwest report yields for 'Cascade' between 1,792-2,240 kg/hectare (Judd, 2018; USAHOPS, 2018) or roughly 4.98 kg of dried cones per plant (estimated at 405 plants/ha). This commercial harvest contrast would equate to an significantly higher yield compared to the Arkansas-grown 'Cascade' hops which had an estimated production of 284.04 kg/ha (2020) and 170.71 kg/ha (2021), based on cones dried to 10% moisture.

This 'Cascade' pruning study had cone and plant attributes lower than ranges found in previous studies that assessed the effects of pruning timing for hops plants. Krivanek et al. (2008) reported that plants in Czech pruned later in the spring had a higher total cone yield compared to earlier pruning dates. The Arkansas-grown plants pruned at the later interval had a greater yield, but not significantly. The Czech study averaged the six cultivars grown in the study and concluded that early pruning (end of March) yielded 2,160 g of cones/plant, while the second and third pruning treatments (first and second half of April) produced 2,290 g and 2,650 g/plant, respectively. However, Křivánek et al. (2008) used 4-6 bines/plant compared to the three bines/plant used in the Arkansas-grown plants. The Czech plants were also fully mature (seven years old), so a higher yield was expected based on plant maturity and growing region. In the

Arkansas pruning study, there were some differences but not at a significant level where the Late treatment had the greatest total percentage of immature cones at 36.7%, though, followed by the Mid and Early pruning treatments which yielded 36.1% and 26.9% of immature cones, respectively. The shorter length of time between shoot pruning and harvest date for the later pruning treatments is the probable reason for the disparity in cone maturation. Matsui et al. (2016) showed that there was no correlation between pruning dates on cone formation, essential oils, and total yield. The staggered pruning treatments (10-day intervals throughout early to late April) used for the 'Saaz' hop by Matsui et al. (2016) showed that yield fluctuated by year (2010-2012) and location (four regions in the Czech Republic) with an average yield of 607 g/plant with slightly higher yields in the later pruning dates, but not at a significant level. Similar results regarding yield were shown for this Arkansas pruning study.

The Arkansas-grown 'Cascade' plants in the pruning study had a lower total cone yield/plant when compared to plants of the same cultivar that were grown in the Pacific Northwest (USDA NASS, 2020). After averaging the overall 'Cascade' yield/acre for each state (Idaho, Oregon, and Washington) and dividing by the number of plants/ha for most commercial hop yards, it was shown that each plant of the cultivar yielded approximately 816.5 g, which was 268.7 g or 49.1% more than the 'Cascade' yield/plant produced in Arkansas (547.8 g/plant). The report also indicated a 7% decrease in the total yield from those states in 2020. The reason for this yield disparity can be attributed to numerous biotic and abiotic factors. The northwestern hops plants were fully mature and grown on a taller trellis, thus adding to the overall yield of the cultivar. Although a nearly 20% decrease in cone yield/plant was found for the 'Cascade' pruning study plants grown in Arkansas, the shorter trellis, fewer number of daylight hours, and more immature plants (2-3 years in Arkansas compared to 5+ years in Pacific Northwest) in Arkansas suggest that the total cone yield/plant were not as disparate from typical yields.

Alpha and beta acid attributes of dried hop cones

The quality attributes of dried hops cones evaluated postharvest included individual and total alpha acids (combined fraction of cohumulone and n+-adhumulone) as well as the individual and total beta acids (combined fraction of colupulone and n+-adlupulone) (Table 3). Commercial, dried 'Cascade' hops cones typically contain 4.5-7.5% alpha acid and 4.8-7% beta acid (BarthHaas, 2021; Brooks et al., 1972; Judd, 2018, USAHOPS, 2018). There were significant pruning x year interactions for all individual and total alpha and beta acids (Fig. 2, Fig. 3, and Table 3). The total alpha acids ranged from 6.28-7.66%, cohumulone ranged from 3.26-4.12%, and n+-adhumulone varied from 2.86-3.63%. The total beta acids ranged from 6.45-9.32%, colupulone ranged from 3.14-4.32%, and n+-adlupulone ranged from 3.61-5.00%. In general, the Early pruning timing had the highest level of alpha and beta acids in 2020, while in 2021 the Mid pruning had the highest levels.

For total alpha acids, the 2021 Mid pruning (7.66%) had the highest percentage between pruning dates and years and was significantly higher than the 2021 Early (6.46%) and Late (6.28%) pruning and the 2020 Mid (6.46%) pruning time. For cohumulone, the 2020 Early pruning (4.12%) and the 2021 Mid pruning (4.03%) were higher than the 2021 Early and Late pruning (3.29% and 3.26%, respectively) which did not differ from each other. For the n+-adhumulone, the 2021 Mid pruning (3.63%) was higher than the 2021 Late pruning (3.02%) and the 2020 Mid pruning (2.86%).

The 2021 Mid pruning had the highest total beta acids, colupulone, and n+-adlupulone (9.32%, 4.32%, and 5.00%, respectfully) but was only significantly higher than the other pruning times and years for total beta acids and colupulone. These results contradict Matsui et al. (2016) which noted that in some locations within the Czech Republic, hops pruned later in the spring vegetative season had significantly higher alpha acids. Yet, the previously mentioned study was derived from only one year of observations, so a multi-year analysis would offer more conclusive results.

De Keukeleire et al. (2003) evaluated the accumulation of alpha acids and beta acids during stages of flowering from five hops cultivars (Wye Challenger, Wye Target, Golding, Admiral, and Whitbread Golding Variety). The researchers examined the inflorescences at three stages of flowering, including the first appearance of inflorescences (stage 1; harvest date in early August) to the formation of small flowers (stage 2; harvest date mid-August), and finally to mature hop cones (stage 3; harvest date early September). The results showed that all hop acids were present from the first stages of flowering with increasing concentrations during cone development (De Keukeleire et al., 2003). Beta acids (colupulone and n+-adlupulone) reached a maximum concentration at the second stage (small cones) for all hop cultivars except ‘Wye Challenger’. The beta acids were present during the first stage of flowering, and the ratios of both alpha to beta acids fluctuated during hop flowering. It was concluded that the production of co-compounds proceeded more efficiently during later stages of flowering since the concentrations of alpha acids and beta acids did not differ significantly when these medium-sized cones were compared to the final cones harvested two weeks later (De Keukeleire et al., 2003). It was also noted that beta acids decreased during the last 2 weeks of flowering, which concurred with the results found in the Arkansas pruning study in 2021 since total beta acids decreased significantly from 9.32% (Mid treatment) to 6.75% (Late pruning) in 2021. The total beta acid percentage also decreased from 6.81% (Mid pruning) to 6.45% (Late pruning) in 2020, but not significantly.

A study conducted by the University of Vermont collected ‘Cascade’ hop cones by hand on five dates in weekly intervals during harvest and were termed as Early (late August), Normal (early September), or Late (mid-September) (Darby, 2019). The Early sampling dates were harvested 1 and 2 weeks prior to the standard harvest time while the Late samples were taken 1 and 2 weeks after harvest. Researchers noted that the Normal and Late harvest dates (harvest dates 3-5) had the highest alpha and beta acids compared to the earlier harvest dates (Darby, 2019), and major increases in both alpha and beta acids occurred after Early harvest dates (harvest dates 1-2). The later-harvested cones had the highest values for brewing qualities with alpha acid ratios around 7.5% and beta acids at 8.2% compared to the earlier harvest dates (4.2% and 5.5% alpha and beta acids, respectively). These results indicated that Vermont-grown hops harvested too early disrupted the flavor and aroma constituents of hops since the oils and secondary metabolites had less time to develop while later-harvested cones reduced brewing quality and aroma through degradation and increased exposure to pests, diseases, and various weather conditions (Darby, 2019). Darby (2019) also noted that hops harvested later in the season were susceptible to accelerated oxidation in storage through the loss of volatile aroma compounds and usually suffer from shortened storability as do cones damaged by diseases and or pests. However, by reviewing the results from the Arkansas hops pruning study, the Mid pruning date (April 30) had higher individual and total alpha and beta acids in 2021, and higher total beta acids and individual acids in 2020. The greater level of acids in the Mid pruning treatment for the two years along with the fluctuations in quality indicated that shoot pruning timing greatly influenced the secondary metabolites in a mature hop plant. However, further quality and physiological analysis of fully mature plants (4-5 years after establishment) would need to be studied for more conclusive results.

Conclusion

There is limited information on the pruning timing of *H. lupulus* shoots during spring emergence for hops grown outside of the major hop-growing locations. Previous results have shown that the removal of shoots at the ground level prior to training influenced the hop plant and cone attributes, but with varying results. Pruning timing recommendations vary depending on growing regions, number of daylight hours, growing degree days, training dates, rainfall, and soil quality, along with other cultural management practices, during the vegetative growing season and can influence the cone, plant, and quality attributes of hops.

The impact of pruning timing (Early, Mid, and Late in the spring) and year (2020 and 2021) on the plant and cone attributes of ‘Cascade’ hops plants grown in Arkansas were evaluated. The pruning timing did not impact any of the plant attributes and most of the cone attributes except the percent of damaged cones/plant with the Mid pruning having the highest. Year impacted the number of laterals/plant (69.9% increase from 2020 to 2021), total cone yield/plant (60.1% reduction from 701.33 g in 2020 to 421.51 g in 2021) and estimated dry cone yield/plant.

The pruning timing x year interaction was significant for individual and total alpha and beta acids of the dried ‘Cascade’ hops grown in Arkansas. The ‘Cascade’ hops had total alpha acids (6.28-7.66%) and total beta acids (6.45-9.32%) that were slightly higher than commercially-available hops (4.5-7.5% alpha acids and 4.8-7.0% beta-acids). If hops cones require a certain number of daylight hours for optimal quality, pruning too late in the vegetative growing season could impact alpha and beta acid formation. However, pruning too early could lead to degraded metabolites and a greater risk of disease and insect pressure that would impact cone yield and quality.

Pruning timing in the southeast and, more specifically, northcentral Arkansas had some impacts on plant and cone attributes, particularly for the alpha and beta acid levels, and this will be crucial information for growers in similar regions who are currently growing hops or intend to cultivate hops. Further examination of pruning timing with more mature plants (4-5 years after establishment) and increased number of bines will be needed to adequately infer the impact of pruning on hop plant and cone attributes.

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Table 1: Main effects and interaction of pruning timing and year on plant attributes of 'Cascade' hop plants grown in Clarksville, AR

Effects [z]	Number of bines/plant	Number of nodes/plant	Number of laterals/plant	Bine length (m)
Pruning				
Early (April 15)	3.00 a	61.67 a	94.83 a	11.61 a
Mid (April 30)	3.00 a	65.67 a	102.17 a	12.69 a
Late (May 15)	3.00 a	62.83 a	102.67 a	11.13 a
P-value	*0.9999*	*0.6064*	*0.4782*	*0.3423*
Year				
2020	3.00 a	60.33 a	82.22 b	12.24 a
2021	3.00 a	66.44 a	117.56 a	11.38 a
P-value	0.9999	*0.0878*	*<.0001*	*0.3293*
Pruning x Year				
P-value	*0.9999*	*0.1614*	*0.2173*	*0.4002*

[z] Means with different letters for each attribute are significantly different ($p<0.05$) according to Least Square Means Student's t-test

Table 2: Main effects and interaction of pruning timing and year on cone attributes at harvest of 'Cascade' hop grown in Clarksville, AR

Effects [z]	**Total cone yield/plant** [y] **(g)**	**Mature cones/ plant (%)**	**Immature cones/plant (%)**	**Damaged cones/plant (%)**	**Cone moisture content (%)**	**Individual cone weight (g)**	**Estimated dry cone yield/plant (g)**
Pruning							
Early (April 15)	544.08 a	71.90 a	26.93 a	4.23 b	75.07 a	0.53 a	54.43 a
Mid (April 30)	552.08 a	60.53 a	36.12 a	11.68 a	77.55 a	0.53 a	55.21 a
Late (May 15)	587.90 a	61.77 a	36.68 a	1.80 b	72.87 a	0.60 a	58.79 a
P-value	*0.9481*	*0.5749*	*0.5834*	*0.0215*	*0.3512*	*0.4015*	*0.9481*
Year							
2020	701.33 a	61.12 a	34.83 a	4.05 a	76.64 a	0.59 a	70.13 a
2021	421.51 b	68.35 a	31.65 a	7.76 a	73.68 a	0.53 a	42.15 b
P-value	*0.0329*	*0.4591*	*0.7118*	*0.1733*	*0.2638*	*0.2366*	*0.0329*
Pruning x Year							
P-value	*0.8558*	*0.7812*	*0.6551*	*0.1790*	*0.2573*	*0.5982*	*0.8558*

[z] Means with different letters for each attribute are significantly different (p<0.05) according to Least Square Means Student's t-test

[y] Total cones= mature + immature + damaged (diseased, sunburned, insect damage)

Table 3: Main effects and interaction of pruning timing and year on individual and total alpha and beta acids^z of dried hops cones from 'Cascade' hop plants grown in Clarksville, AR

Effects ^{y}	**Cohumulone (%)**	**n+-adhumulone^x (%)**	**Total alpha acids (%)**	**Colupulone (%)**	**n+-adlupulone (%)**	**Total beta acids (%)**
Pruning						
Early (April 15)	3.70 a	3.17 a	6.87 a	3.65 a	4.55 a	7.77 a
Mid (April 30)	3.82 a	3.25 a	7.06 a	3.88 a	4.63 a	8.07 a
Late (May 15)	3.37 a	3.07 a	6.45 a	3.25 b	4.05 a	6.60 b
(*P-value*)	*0.1459*	*0.6249*	*0.2959*	*0.0024*	*0.0092*	*0.0019*
Year						
2020	3.74 a	3.05 a	6.78 a	3.54 a	4.52 a	7.01 b
2021	3.53 a	3.27 a	6.80 a	3.65 a	4.30 a	7.95 a
(*P-value*)	*0.2658*	*0.1298*	*0.9647*	*0.4358*	*0.1645*	*0.0073*
Pruning x Year						
(*P-value*)	*0.0285*	*0.0397*	*0.0383*	*0.0015*	*0.0003*	*0.0065*

[z] Hop cones were analyzed with High Performance Liquid Chromatography analysis using American Society of Brewing Chemists (ASBC) Hop-14 method

[y] Means with different letters for each attribute are significantly different ($p<0.05$) according to Least Square Means Student's t-test

[x] n+adhumulone refers to the level of n-humulone and ad-humulone combined in one fraction, analogue for n+-adlupulone for n-lupulone and ad-lupulone combined

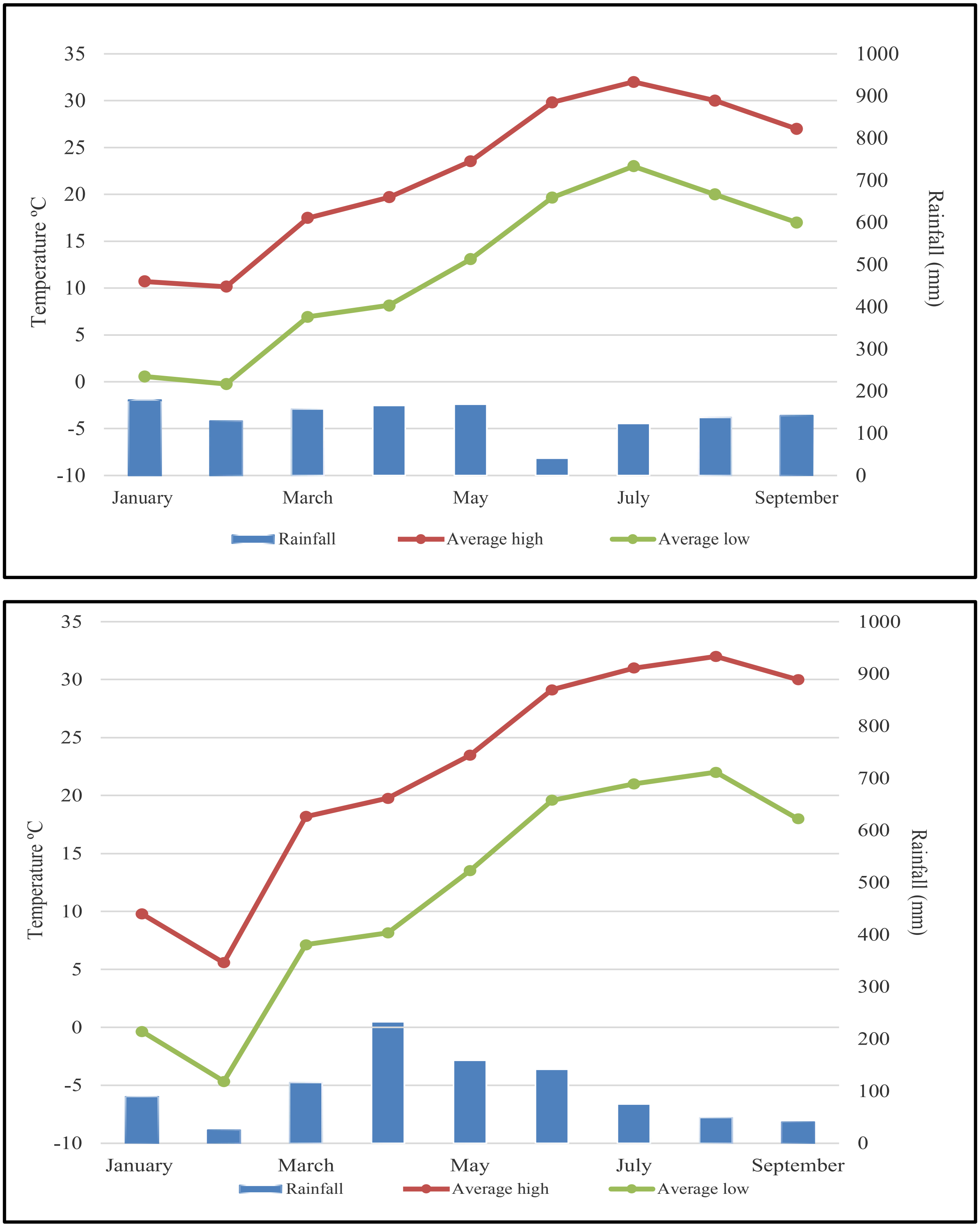

Fig. 1. Temperature and rain conditions at the University of Arkansas System Division of Agriculture Fruit Research Station, Clarksville, AR (2020 top and 2021 bottom)

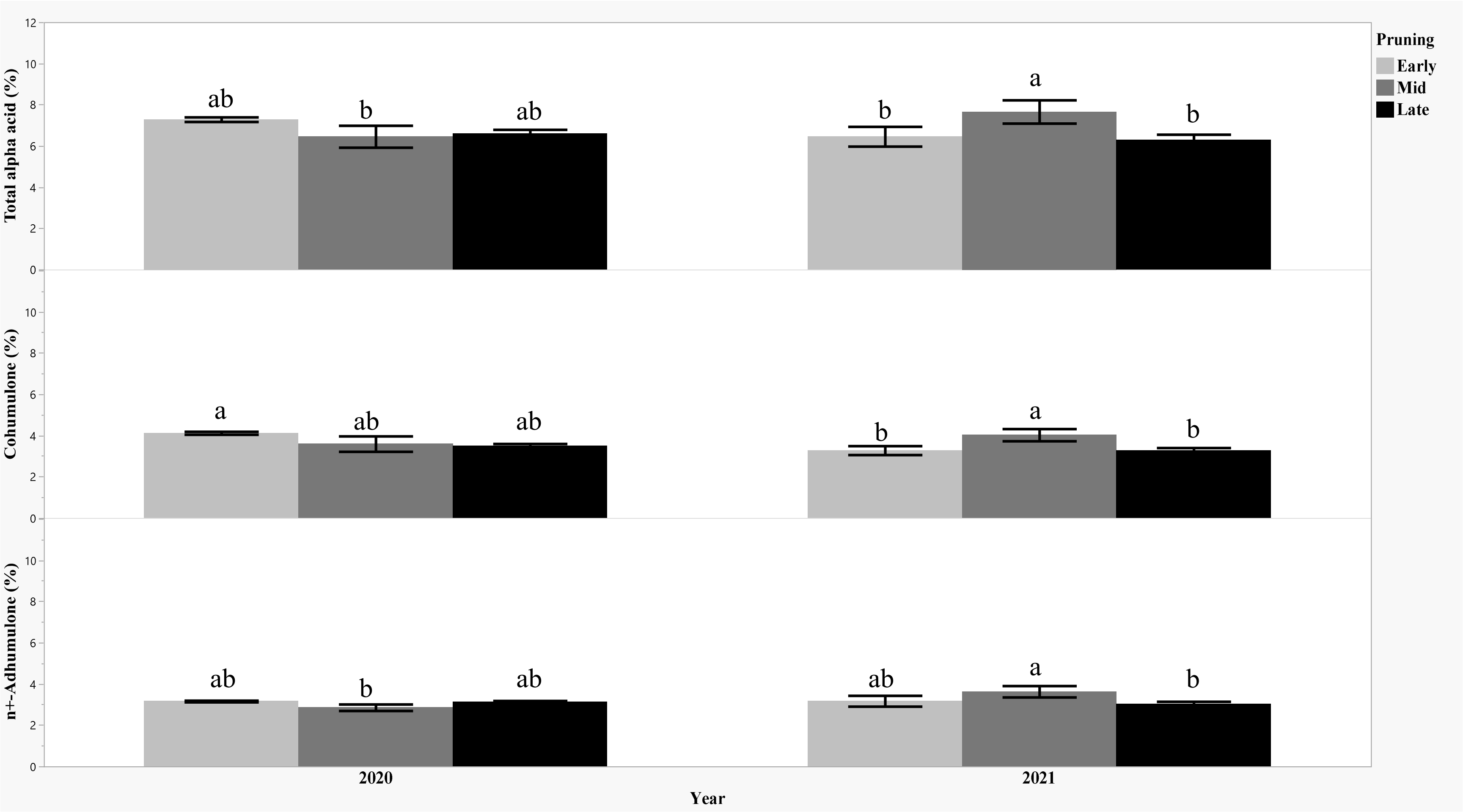

Fig. 2. Interactions of pruning timing (Early=April 15, Mid=April 30, and Late=May 15) and year (2020 and 2021) on individual and total alpha acids of 'Cascade' hop plants grown in Clarksville, AR

Hop cones analyzed with high performance liquid chromatography using American Society of Brewing Chemists (ASBC) Hop-14 method

Means with different letters for each attribute are significantly different ($p<0.05$) according to Least Square Means Student's t-test

n+-adhumulone refers to the level of n-humulone and adhumulone combined in one fraction

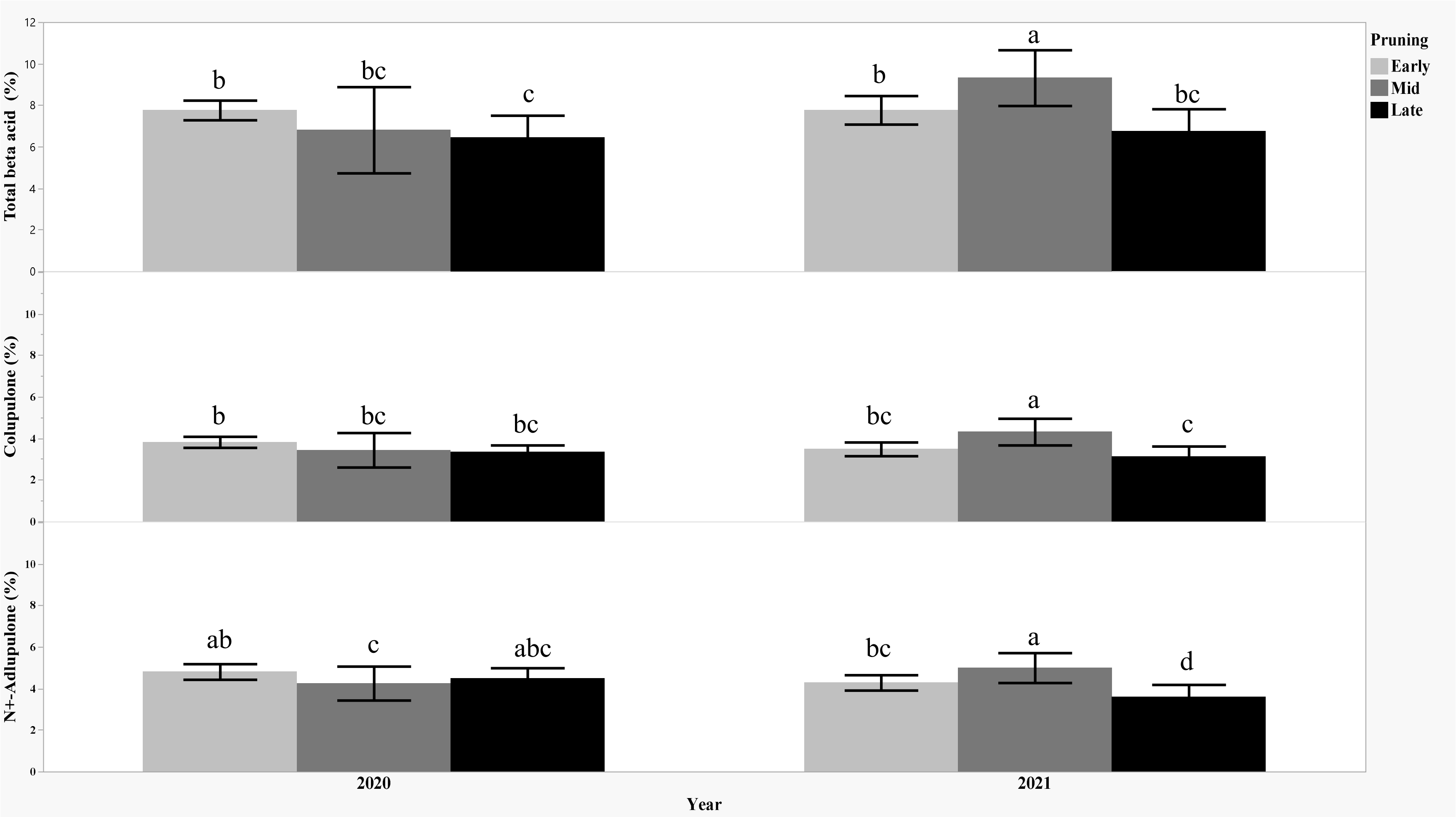

Fig. 3. Interactions of pruning timing (Early=April 15, Mid=April 30, and Late=May 15) and year (2020 and 2021) on individual and total beta acids of 'Cascade' hop plants grown in Clarksville, AR

Hop cones analyzed with high performance liquid chromatography using American Society of Brewing Chemists (ASBC) Hop-14 method

Means with different letters for each attribute are significantly different ($p<0.05$) according to Least Square Means Student's t-test

n+-adlupulone refers to the level of n-lupulone and adlupulone combined in one fraction

OVERALL CONCLUSIONS

The main objectives of this research on the viability of hops production in Arkansas were to 1) evaluate the impact of cultivar and fertility rate on plant and cone attributes of Arkansas-grown hops, and 2) determine the impact of pruning timing on plant and cone attributes of Arkansas-grown 'Cascade' hops. To accomplish the first objective, six hops cultivars (Cascade, Cashmere, Centennial, Crystal, Nugget, and Zeus) were harvested in 2020 and 2021 from the University of Arkansas System Division of Agriculture Fruit Research Station in Clarksville, AR to evaluate plant, cone, compositional, and sensory attributes of the tested cultivars grown with three fertility rates (low, standard, and high). For objective two in 2020 and 2021, pruning timing was assessed at three intervals for three three-plant plots of 'Cascade' hops to determine the best management practices for plant pruning and the effects that timing and year had on harvest attributes. 'Crystal', 'Cascade', and 'Zeus' cultivars have potential for specialty crop production in Arkansas, the fertility rates used had little to no impact on the measured plant and cone attributes, and pruning timing varied by year while the Early and Mid pruning had the highest alpha and beta acid levels in 2020 and 2021, respectively. This project determined that it is feasible to grow *H. lupulus* L. plants in the northcentral Arkansas and the mid-south region, the cone attributes showed distinct sensory and compositional attributes depending on cultivar, and the compositional and sensory attributes for several of the cultivars would make them ideal for local brewers to implement into their products. While the yield, sensory, and chemical attributes were generally lower and dissimilar to the same cultivars grown in typical commercial hop yards, the Arkansas-grown hops cones still have potential for small-scale specialty crop production. However, further trials are needed for other cultivars and regions in the Arkansas for cultivar selection and adaptation assessments, harvest attributes, and fertility and pruning effects to provide more definitive results regarding the unique sensory and compositional attributes for hops grown in Arkansas.

Appendix

To: From:

Date: Action: Action Date: Protocol #: Study Title:

Renee Terrell Threlfall FDSC B-3

Douglas James Adams, Chair IRB Committee

06/11/2019

Exemption Granted

06/11/2019 1905199069

Identifying Marketable Attributes of Hops Cultivars Grown Commercially in Arkansas and Other Regions

The above-referenced protocol has been determined to be exempt.

If you wish to make any modifications in the approved protocol that may affect the level of risk to your participants, you must seek approval prior to implementing those changes. All modifications must provide sufficient detail to assess the impact of the change.

If you have any questions or need any assistance from the IRB, please contact the IRB Coordinator at 109 MLKG Building, 5-2208, or irb@uark.edu.

cc: Amanda L. McWhirt, Key Personnel