APPLE ORCHARD MANAGEMENT FOR HARD CIDER PRODUCTION: INFLUENCE OF NITROGEN FERTILIZATION AND CARBOHYDRATE AVAILABILITY ON TANNIN SYNTHESIS, YEAST ASSIMILABLE NITROGEN, AND FERMENTATION KINETICS

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Abstract

Two areas of interest were identified to investigate how orchard management can improve cider apple orchard management: the influence of nitrogen fertilization on yeast assimilable nitrogen (YAN) concentrations, and when tannins are synthesized in apples and what factors influence their synthesis. Two experiments were carried out using soil and foliar applied nitrogen fertilizers to investigate how nitrogen influences the concentrations and composition of YAN; juice from these experiments were fermented and the production of hydrogen sulfide (H_2S) tracked. Different rates of foliar urea application beginning six weeks before harvest increased YAN by as much as 319% compared to the Control. A high rate of soil applied calcium nitrate fertilizer increased juice primary amino nitrogen (PAN) by 103% relative to the Control. In both fertilizer studies PAN constituted over 90% of YAN. Fertilization increased fermentation rate, but no consistent relationship was found with fertilization rate and H_2S synthesis. There was no influence of nitrogen fertilization on polyphenol concentrations. The increases in YAN demonstrate that nitrogen fertilization is an effective means of increasing juice YAN while not impacting important sensory attributes such as polyphenols. In order to investigate when polyphenols are produced in cider apples, and the influence of carbohydrate availability, light, temperature, and location within the tree canopy on fruit and juice polyphenol

concentrations, five separate experiments were conducted over three years. Analysis of polyphenol concentrations in cortex tissue in developing fruit showed that most polyphenol synthesis occurred in the first five weeks after full bloom (WAFB). Shading whole trees or individual branches in the first five WAFB reduced total polyphenol concentrations by as much 23%. Bagging fruit three WAFB had variable effects on polyphenol concentrations depending on cultivar. Shading branches from four WAFB through harvest resulted in a 16% reduction of polyphenols relative to the Control. Fruit from the tops and exposed lateral sides of tree canopies had lower total polyphenol concentrations in juice than the interior of the canopy. These results suggest that most polyphenols are synthesized early in fruit development and that carbohydrate supply during this period likely influences their development.

BIOGRAPHICAL SKETCH

Adam was born and raised in Lancaster, Pennsylvania. There he developed a passion for biology, the outdoors, and good food from his family and the patchwork of small farms and forests that encompass the landscape. He attended Bowdoin College, in Brunswick, Maine and focused his studies on ecology and population biology. Adam graduated in 2008, majoring in biology and environmental studies, with a minor in Spanish. After college, he worked for the Cornell Lab of Ornithology researching songbird reproductive strategies in South and Central America, and co-founded a language institute in Lima, Peru.

While living in Lima, Adam became interested in viticulture and enology through his introduction to pisco, the clear aromatic brandy and national beverage of Peru. He then returned to the United States to work in the vineyards and cellars of the Napa Valley for two years before starting his masters with Dr. Justine Vanden Heuvel at Cornell University. After completing his masters studying the impact of under-vine cover crops on grape vine physiology and soil quality, Adam received the Frederick Dreer Award to study management practices of dry-farmed vineyards in Spain. He then began his PhD at Cornell researching management of apple orchards for the production of hard cider with Dr. Gregory Peck in the spring semester of 2015. During his studies at Cornell, Adam met his wife, Juana, who was also a PhD student in plant science. They plan to move to Colombia, Juana's native country, after they graduate to study and work with sustainable coffee production.

To all of the teachers and mentors throughout my life who challenged me, taught me to think critically, and continue to provide me guidance.

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TABLE OF CONTENTS

CHAPTER ONE

Hard Cider in the United States: Opportunities and Challenges in Cider Apple Production, Tannin Synthesis in Apples, and the Influence of Yeast Assimilable Nitrogen in Alcoholic Fermentation and Cider Organoleptic Properties

Cider Production in the United States

The United States and Western Europe have a long history of fermented "hard" cider production. In colonial times through the mid-19th century, most farms contained apple orchards for cider production, and production and consumption was prolific in the United States (Watson, 2013). During the 1700's in Massachusetts, average per capita cider consumption was more than 200 liters a year, suggesting most adults drank several liters of cider per day (Watson, 2013). However, as industrialization in North America and Europe increased urban populations in the latter half of the 19th century, beer making became more practical, and more popular than cider making to meet demand. The transport and storage of barley for beer production was cheaper than that of apples for cider making; beer could also be made year-round from stored grain, while cider was only made in the fall and winter after harvest. The temperance movement of the late 19th and early 20th century and prohibition in the United States further drove cider production and consumption to a minor role in the alcoholic beverage industry (Merwin et al., 2008).

Hard cider is currently experiencing a resurgence in popularity. The industry has grown rapidly in the United States; annual production has increased from 23.1 million liters in 2007 to 173.4 million liters in 2017, making cider the fastest growing beverage sector in the country during this period (Alcohol and Tobacco Tax and Trade Bureau, 2008; Alcohol and Tobacco Tax and Trade Bureau, 2018). In 2015, approximately 18 million bushels, equivalent to 7% of the US

apple crop, would be necessary to produce the volume of cider produced in the country that year (Alcohol and Tobacco Tax and Trade Bureau, 2016; USDA National Agricultural Statistics Service, 2015). However, a considerable amount of cider is produced from imported juice concentrate. Much of this production with imported juice concentrate stems from a global oversupply of apples that have driven down the cost of concentrate from fresh eating and processing apples (USDA National Agricultural Statistics Service, 2015; US Apple Association, 2016). Limited domestic production of bitter cider varieties has also driven the importation of concentrate made from bitter cider apples from Europe.

The growth of the cider industry offers an opportunity for apple growers in the United States to expand and diversify production, and potentially increase their profitability with the cultivation of bittersweet and bittersharp cider cultivars. Many high tannin cultivars sought after by cider producers for the sensory attributes of their juice are not available in sufficient quantities to meet demand within the United States (Pashow, 2018). A 2012 survey of Virginia cider makers found that two thirds of cidermakers were willing to pay 50% or more for European cider apples than culinary varieties (Peck et al., 2013). An economic analysis of New York cider orchards also found that under a number of operational models and horticultural practices, growing cider-specific apple cultivars can be profitable (Peck and Knickerbocker, 2018)
Many of the bitter cider varieties most sought after by cidermakers for the sensory attributes of their juice have not been produced in substantial quantities in high-density commercial orchards within the United States. Furthermore, many of these varieties possess horticultural traits that pose challenges to their production in commercial orchards; many are susceptible to fireblight (*Erwinia amylovora*), apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*), are prone to pre-mature fruit drop, and are heavily biennially bearing (Merwin et

al., 2008). Planting a high-density orchard is an expensive endeavor costing between $37,000 and $62,000 per hectare (Farris et al., 2013). Growers considering planting orchards of cider apples must consider which varieties and planting systems will perform best for the 20-25-year anticipated lifespan of the orchard. Additionally, growers need information on which cider apple cultivars are suitable for different geographic regions, and how management impacts fruit and cider quality in order to be commercially successful. Beyond the rapid growth of the cider industry, part of the undersupply of high tannin apple cultivars stems from a reticence of growers to make large long-term investments in growing new cultivars that can only be used in a young cider industry, and cannot be diverted for fresh eating or other apple products (Becot et al., 2016). The aim of the research in this dissertation is to help understand how orchard management can impact the quality and profitability of apples for cider production. Of particular interest is understanding how nutritional and environmental factors impact the development of yeast assimilable nitrogen (YAN), tannins in bitter cider varieties, and alcoholic fermentation kinetics of juice from these apples.

Nitrogen in Apple Trees and Yeast Assimilable Nitrogen in Alcoholic Fermentation

The role of nitrogen in plant metabolisms and its management in fresh-market apple production is well studied (Wargo et al., 2003; Lea and Beech, 1978; Santos et al., 2016; Cheng and Fuchigami, 2002; Merwin and Stiles, 1994). In the spring, apple trees utilize stored nitrogen reserves to support vegetative growth, flowering, and fruitset (Cheng and Raba, 2009). Shortly after fruitset, apple trees utilize nitrogen acquired from the soil. Both ground and foliar applications of nitrogen fertilizers are common means of sustaining sufficient nitrogen for commercial apple orchards (Merwin and Stiles, 1994). A number of different nitrogen fertilizers

are used in apple orchards, including compost, and inorganic fertilizers such as calcium nitrate or urea that can be applied to the soil or via fertigation; foliar nitrogen applications in the fall are common to ensure sufficient reserves for the following spring (Agnello et al., 2019).

When nitrogen is limited, apple trees often have decreased fruit size, yield, and soluble solid concentrations (Xia et al., 2009). Sufficient nitrogen resources are also important to support vegetative growth in young apple trees (Cheng and Fuchigami, 2002). Conversely, excess application of nitrogen has been correlated with decreased red and increased green coloration in apples, lower flesh firmness, and reduced fruit quality after long-term storage (Wargo et al., 2004; Wargo et al., 2003; Raese et al., 2007; Fallahi, 1997). Excessive nitrogen concentrations in apple trees can also increase susceptibility to fireblight, and apple scab (*Venturia inaequalis*), and reduce bud cold hardiness (Fallahi and Mohan, 2000; Rühmann et al., 2002; Stiles and Reid, 1991). Greater susceptibility to apple scab in trees with higher nitrogen statuses has been associated with decreased phenolic content in vigorously growing vegetation (Leser and Treutter, 2005). As a diagnostic tool, orchard managers test mature leaves for total nitrogen concentration once terminal shoot growth has subsided and target total leaf nitrogen content between 2.2 and 2.4% for mature hard apples and processing cultivars (Stiles and Reid, 1991). Currently, there are no nitrogen fertilization guidelines specifically for cider apple production in the U.S.

While high nitrogen fertilizer rates are used, there may be a decrease in fruit quality for fresh market apples. However, increasing fruit nitrogen concentration in cider apples may be beneficial for cider fermentation. Yeast assimilable nitrogen is comprised of the nitrogen compounds that can be metabolized by *Saccharomyces cerevisiae* yeast for cell division and growth during alcoholic fermentation of fruit juice, and is typically the limiting factor in fermentation rate (Bell and Henschke, 2005). Yeast assimilable nitrogen is essential for yeast cell

metabolism; maintaining appropriate YAN concentrations is important for sustaining healthy yeast populations to complete alcoholic fermentation, and their concentration can influence the production of both positive and undesirable aromatic secondary metabolites (Boudreau et al., 2017a; Tahim and Mansfield, 2019; Waterhouse et al., 2016).

Yeast assimilable nitrogen is composed of primary amino nitrogen (PAN), ammonia ions, and some short oligopeptides (Bell and Henschke, 2005). Unlike grapes (*Vitis vinifera*) that contain a variable, but large proportion of YAN as ammonia, the vast majority of YAN in apple fruit is composed of PAN (Bell and Henschke, 2005; Ma et al., 2018). While composition of amino acids vary by apple cultivar, asparagine is typically the most abundant amino acid in the fruit (Ma et al., 2018; Zhang et al., 2010). However, ammonia is preferentially utilized and more rapidly metabolized by *S. cerevisiae* compared with organic nitrogen sources (Crépin et al., 2012). There are no studies that specify the target YAN concentration for the cider industry; however, recent studies have shown that apple juice is often deficient in YAN according to standards developed for the wine industry (Ma et al., 2018; Peck, et al., 2016; Tahim and Mansfield, 2019). Current winemaking practices often cite 140 mg/L YAN as the minimum concentration for the successful completion of most fermentations, and recommend a range between 200 and 350 mg/L YAN depending on initial sugar concentration, yeast strain, and wine style (Bell and Henschke, 2005; Ugliano et al., 2011; Torrea et al., 2011). Apples typically have YAN concentrations under 100 mg/L. For example, a survey of 12 cultivars grown in Virginia over two growing seasons found a mean YAN concentration of 59 mg/L (Boudreau et al., 2018).

Deficiencies in YAN result in slow or incomplete fermentations (Bell and Henschke, 2005; Vilanova et al., 2007). The composition of YAN can impact chemical and sensory attributes of finished ciders and wines, as well (Boudreau et al., 2017a; Herraiz and Ough, 1993).

In particular, low YAN is known to increase the production of hydrogen sulfide (H_2S), a reduced sulfur compound and common defect in ciders because it smells like cabbage or rotten eggs (Boudreau et al., 2017a; Jiranek et al., 1995). Hydrogen sulfide is an intermediary compound formed from the reduction of sulfate or sulfite in order to synthesize the sulfur containing amino acids cysteine and methionine (Ono et al., 1999). Hydrogen sulfide production during alcoholic fermentation is most commonly associated with insufficient YAN to provide amino acid precursors to sequester sulfide, resulting in excess H_2S permeating the yeast cell membrane (Ugliano et al., 2011;Jiranek et al., 1995). However, cases of greater YAN concentrations increasing H_2S production have been observed in other studies (Boudreau et al., 2017b; Ugliano et al., 2009). The activity of sulfite reductase in different yeast strains also has a strong impact on H_2S synthesis during alcoholic fermentation (Cordente et al., 2009). Other nutrients deficiencies, such as thiamin and pantothenic acid can also lead to an accumulation of H_2S (Wainwright, 1971; Wang et al., 2003).

While some volatile aromatics in cider are derived from compounds produced in the fruit, most are secondary metabolites produced by yeast during alcoholic fermentation in hard cider (Xu et al., 2007). In particular, fusel alcohols and acetate esters can be formed from the byproducts of amino acid metabolism (Sumby et al., 2010). Sufficient YAN is also critical for catalyzing the fatty acid metabolic pathways in yeast that produce ethyl esters as a byproduct (Saerens et al., 2010). Increasing YAN concentration in wine and cider fermentations has been found to increase both ethyl and acetate ester concentrations, which contribute a large proportion of the fruity aroma of finished wines and ciders (Santos et al., 2016; Garde-Cerdán and Ancín-Azpilicueta, 2008;Tahim and Mansfield, 2019). Supplementation with amino acids, versus ammonia, yields a different volatile aromatic profile in grape-based wines, but the causal

mechanisms have not been well described, and whether these changes are positive or negative in terms of sensory characteristics remains an area of current research (Torrea et al., 2011; Tahim and Mansfield, 2019).

Proprietary exogenous nitrogen supplements are commercially available and commonly added to fruit juice fermentations to address YAN deficiencies. Diammonium phosphate (DAP) is probably the most commonly used. However, many wine and cider producers prefer adding yeast nutrient supplements with greater PAN than ammonia concentrations in order to limit initial alcoholic fermentation rates, temperature increases from increased metabolic rates that can stress yeast cells, and changes to sensory character imparted by high ammonia additions (Charoenchai et al., 1998; Tahim and Mansfield, 2019). These high PAN supplements are commonly composed of inactivated dry yeast cells and contain minerals, sterols, and vitamins important for yeast metabolism (Ángeles et al., 2009).

Increased nitrogen fertilization of vineyards have successfully increased YAN concentration in grape wine must (Moss, 2016; Neilsen et al., 2010). Moss (2016) found a cumulative application of 30 kg/ha of nitrogen applied as urea to foliage was capable of more than doubling the YAN concentration in *V. vinifera* cv 'Sauvignon blanc' and 'Petit Manseng' must. These foliar applications were also more effective in increasing juice YAN than a 60 kg/ha ground application of calcium nitrate. Foliar urea applications may therefore also be an effective means of increasing apple fruit YAN for cider production. Additionally, if orchard nitrogen fertilization is capable of increasing apple YAN, it is more likely to be as PAN than ammonia, which would be preferred for subsequent fermentation by most cider makers. While the addition of urea is forbidden as a nitrogen supplement directly to alcoholic fermentations due to the formation of ethyl carbamate, urea is metabolized to ammonia and carbon dioxide by urease

enzymes in plant tissue, and is a common and safe fertilization practice (Butzke and Bisson, 1997; Witte, 2011).

Tannin Synthesis in Apples

Most artisanal style ciders are made from blends of several cultivars that collectively provide target levels of sugar, acidity, and tannins (Lea and Drilleau, 2003). Many commercially produced fresh eating and processing apples can be used in cider blends to provide appropriate sugar and acid levels, but do not contribute bitterness and astringency (Thompson-Witrick et al., 2014). Cider-specific bittersweet and bittersharp apple cultivars contain high levels of tannins that contribute bitterness and astringency to ciders (Barker, 1903). These varieties are not marketed to eat fresh due to their bitterness, but are essential for producing traditional and artisanal style ciders (Lea and Drilleau, 2003). Understanding how orchard design and management impacts the concentration of tannins in the juice from these cultivars offers an opportunity for growers to maximize this quality attribute for cider production.

Tannins are a diverse group of molecules defined as water-soluble phenolic compounds that have the ability to precipitate alkaloids, gelatins, and other proteins (Bate-Smith, 1962). They provide mouthfeel in beverages such as red wine and cider by binding with and precipitating proteins in saliva out of solution. This decreases the lubricating properties of saliva, giving the perception of "dryness" by increasing the coefficient of friction in the mouth (Prinz and Lucas, 2000). In apples, catechin and epicatechin (flavan-3-ol monomers) will polymerize with catechin initiators and epicatechin elongation units, to form procyanidins, also known as condensed tannins (Delage et al., 1991). As monomers and polymers shorter than four elongation units, epicatechins and catechins are bitter tasting. Longer procyanidin polymers do not have

taste or aroma but are more reactive with proteins and provide astringent organoleptic characteristics to ciders (Lea and Arnold, 1978).

Both the bitter and astringent characteristics of epicatechins, catechins, and their polymers are important and positive quality attributes in ciders. There are many other phenolic compounds in apples such as dihydrochalcones, hydroxycinnamic acids, and flavonols, but these compounds provide a smaller contribution to the organoleptic properties of ciders (Lea and Timberlake, 1974). All apples contain catechins, epicatechins, and their polymers in the peel and flesh, but they are typically in very low concentrations in commercial fresh eating and processing cultivars (Thompson-Witrick et al., 2014; Zhang et al., 2010). Flavan-3-ol concentrations are typically under 100 mg/L in apple juice from fresh eating and processing varieties (Kahle et al., 2005). Apples high in tannins concentrations have been selected specifically for the hard cider industry; they possess flavan-3-ol concentrations many fold greater than fresh eating apples, with concentrations in juice that frequently exceed 2 g/L (Guyot et al., 2003).

The phenolic composition of apples has been well described (Burda et al., 1990; Henry-Kirk et al., 2012; Lea and Timberlake, 1974; Renard et al., 2007; Zhang et al., 2010). However, the factors influencing tannin development and accumulation are not well understood. The vast majority of flavan-3-ol synthesis occurs during the cell division phase of fruit growth in apples, approximately in the first six weeks after bloom (Renard et al., 2007). Studies of other apple cultivars have also found that the majority of polyphenol synthesis in apple fruit occurs during the early stages of fruit development as well (Zhang et al., 2010; Ju et al., 1995; Henry-Kirk et al., 2012). While some polyphenols, such as anthocyanins and flavonols, are influenced by light exposure to fruit, flavan-3-ol synthesis does not seem to be as sensitive to light exposure as these other polyphenols (Chen et al., 2012; Takos et al., 2006; Ju et al., 1997; Awad et al., 2000).

Enzyme concentrations in the metabolic pathway for polyphenol synthesis are greatest in young fruit, including phenylalanine ammonia-lyase, chalcone-synthase, and dihydroflavonol reductase, which are all involved in the metabolic pathway for anthocyanidin and flavan-3-ol synthesis (Ju et al., 1997; Ju et al., 1995). While some of the enzymes involved in flavan-3-ol metabolic pathway are light sensitive, their concentrations have not been found to be rate limiting for the synthesis of flavonoids and anthocyanidins in the 60 days after full bloom, when most flavan-3-ol synthesis occurs (Ju et al., 1997; Ju et al., 1995; Lister et al., 1996).

There may be cultivar dependent variation in the influence of light exposure on fruit and the synthesis of flavan-3-ols in apple tissue. Chen et al. (2012) found fruit bagging to decrease concentrations in flavan-3-ol concentrations in 'Red Delicious' and 'Golden Delicious' fruit cortex tissue while not having any impact on flavan-3-ol synthesis in 'Royal Gala'. Additionally, concentrations of flavan-3-ols have not been found to be affected by light exposure in fruit peels of 'Elstar' and 'Jonagold' apples (Awad et al., 2000).

Some phenolic compounds such as anthocyanins and flavonols in the skin are heavily impacted and regulated by sunlight exposure (Chen et al., 2012; Feng et al., 2014; Treutter, 2000). However, both of these classes of compounds provide protection from UV/light damage (Reuber et al., 1996). Tannins do not provide photo-protective services to the fruit but are produced to deter herbivores. In plant tissue eaten by mammals, tannins reduce the digestibility of proteins by binding to them, decreasing the forage quality of the plant tissue (Robbins et al., 1987). They can also be toxic to insect herbivores with high pH guts by forming peroxides and quinone free radicals (Barbehenn and Constabel, 2011).

Resultantly, herbivory can stimulate tannin production in plant tissues. Mechanical simulated insect feeding on trembling aspen (*Populus tremuloides*) leaves resulted in condensed

tannin concentrations increasing by nearly a third in both wounded and unwounded leaves (Peters and Constabel, 2002). Insect feeding on leaf tissue has also been found to stimulate tannin production in other parts of the plant. Japanese beetle (*Popillia japonica*) feeding on the leaves of evening primrose (*Oenothera biennis*) stimulated a 37% increase in tannin production in reproductive tissues compared with control plants, and resulted in a 77% reduction in seed predation by three lepidopteran larvae species (McArt et al., 2013).

In fruit, several factors have been found to impact epicatechin, catechin, and procyanidin production. Lower accumulations of polyphenols in tissues of fertilized plants have been documented in strawberries (*Fragaria ananassa*) and trembling aspen as well (Anttonen et al., 2006; Bryant et al., 1987). Greater nitrogen concentrations in apples have been correlated with lower concentrations of catechins and tannins in skin and flesh (Awad and de Jager, 2002; Lea and Beech, 1978). Lea and Beech (1978), showed that juice from unfertilized Dabinett trees had 17% greater tannin concentrations than juice from unfertilized trees. However, unfertilized trees were also visibly nitrogen stressed and yielded 35% less than fertilized trees. Other field studies of cider apples have not found total polyphenol concentrations to be influenced by nitrogen fertilization (Valois, 2007).

Fruit position within the canopy has also been found to impact catechin, epicatechin, and procyanidin concentrations. Awad et al. (2000) found greater concentrations of catechins in the skins of ‘Elstar’ and ‘Jonagold’ apples located on one-year-old terminals than one-year-old laterals or spurs. Feng et al., (2014) found greater concentrations of catechins, epicatechins, and procyanidins B1 and B2 in both the skin and flesh of fruit in the exterior, versus the interior canopy of three dessert apple cultivars. However, other studies have found no impact of exposed versus shaded fruit positions on catechin concentrations (Awad et al., 2001; Jakopic et al., 2009).

Fruit from exposed parts of the canopy receive greater carbohydrate resources from more photosynthetically active leaves on proximate shoot tips and spur leaves than shaded fruit during the first several weeks of fruit development (Grappadelli et al., 1994); this disparity in resource availability may provide an explanation for variation of flavan-3-ol concentrations in fruit. Supporting this source/sink relationship, increasing CO_2 air concentrations to 680 ppm increased tannin leaf concentration by 25% from ambient conditions in young tomatoes (*Lycopersicon esculentum*), and lowering CO_2 concentrations to 170 ppm decreased tannin concentrations by 25% (Bialczyk et al., 1999). Crop thinning experiments have found fruit from trees with lower crop loads to have greater concentrations of catechins and epicatechins, lending support to this argument, as well (Stopar et al., 2002). However, adjusting crop load 50 days after full bloom has been found not to influence total polyphenol concentrations in apples (Peck et al., 2016). Additionally, varying the harvest date of apples by four weeks at the end of fruit development has not been found to influence polyphenol concentrations (Ewing et al., 2019).

In addition to variation in procyanidin concentrations in apple tissue impacting the astringency of apples, both protein and polysaccharide content can impact the extractability of phenolic compounds during juicing. Polyphenols are primarily stored in the vacuoles of apple cells; these compounds come into contact with other cell components when the cells are disrupted, such as during milling and juicing (Le Bourvellec et al., 2009). Procyanidins bind non-covalently with proteins and polysaccharides in cell walls via hydrogen bonds and hydrophobic interactions (McManus et al., 1985). Among the major groups of polysaccharide classes in cell walls, pectin is the most reactive with procyanidins, followed by xyloglucans, starch, and cellulose (Le Bourvellec et al., 2005). Binding is influenced by procyanidin concentration and molecular size. Increased procyanidin concentrations result in greater binding until cell wall

binding sites are saturated, and binding rates plateau (Renard et al., 2001). Procyanidins with greater degrees of polymerization are also more selectively bound to cell walls due to a greater number of potential binding sites. A study of French cider apples found that procyanidins with a mean polymerization length of 70 were bound to cell wall materials at a rate 3.5 greater than procyanidins with a mean polymerization length of three (Le Bourvellec et al., 2004). Epicatechin monomers do not bind readily with cell wall materials, so astringency, not bitterness is primarily affected by interactions of polyphenols and cell walls (Renard et al., 2001).

Cider and winemakers that wish to maximize the tannin concentrations in their products must therefore be aware of not only the concentration of procyanidins in fruit, but also limiting contact time with pomace after milling to reduce the potential of binding to cell wall constituents. A study of wines made from *Vitis vinifera* and *Vitis* sp. hybrids found hybrid wines to contain a greater than fourfold lower tannin concentration that those made from *V. vinifera*; this difference was most strongly correlated with protein and pectin content of cell walls, not tannin concentrations within grapes (Springer and Sacks, 2014). Relatedly, gelatin fining agents added to cider can substantially reduce cider procyanidin concentrations and total flavanol concentrations can be reduced by nearly a third (Hubert et al., 2007). The addition of pectinase enzymes to milled pulp can help aid in juice extraction by increasing yield by as much as 10% (Lea and Timberlake, 1978); these enzymes have not been found to impact procyanidin concentrations (Hubert et al., 2007; Lea and Timberlake, 1978).

Research Areas of Interest to Improve Management of Apple Orchards for Cider Production

Given the demand of cider-specific apple cultivars within the United States and the lack of information on how management influences the physiological development of compounds important for fruit and juice quality in cider production, the goal of the research in this dissertation is to elucidate how management can enhance these qualities. Two main areas of interest were identified: the influence of nitrogen fertilization on tree physiology and YAN concentrations, and when tannins are synthesized in apples and what factors influence their synthesis. Experiments were carried out using both soil and foliar applied nitrogen fertilizers to investigate how nitrogen influences tree growth and physiology, juice quality, and the concentrations and composition of YAN; juice from these experiments were fermented to track fermentation rate and the production of H_2S. In order to investigate when polyphenols are produced in cider apples, and the influence of carbohydrate availability, light, and location within the tree canopy on fruit and juice polyphenol concentrations, five separate experiments were conducted.

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CHAPTER TWO

Foliar Urea Applications Increase Yeast Assimilable Nitrogen Concentration and Alcoholic Fermentation Rate in 'Red Spy' Apples Used for Cider Production

Abstract

Yeast assimilable nitrogen (YAN) is a limiting nutritional component for *Saccharomyces cerevisiae* yeast during alcohol fermentations. In apple (*Malus ×domestica* Borkh.) juice used to make cider, endogenous YAN concentrations are often below the recommended thresholds to completely utilize all of the fermentable sugar and minimize the production of off-flavors. Cider producers may supplement fermentations with exogenous nitrogen to increase YAN. Urea, commonly applied to apple orchards to increase fruit size and yields, was tested for its ability to increase endogenous apple juice YAN. Starting six weeks before harvest in 2017 and 2018, a 1% urea solution was applied to apple cv. 'Red Spy' trees one, three, or five times to create Low, Medium, and High rate treatments, respectively. Different trees were used in each year of the study. Fermentation rate, hydrogen sulfide (H_2S) production during fermentation, and residual H_2S concentrations after fermentation were measured. The relative cost of orchard urea applications was compared to the cost of supplementing with three commercially available exogenous YAN supplements using a partial budget. Relative to the Control, the High treatment increased YAN by 229% and 408% in 2017 and 2018, respectively. Over 90% of the YAN in all juice samples was composed of primary amino nitrogen (PAN). The majority of the PAN among all treatments was asparagine, however relatively more PAN was composed of asparagine as urea applications increased. Aspartic acid and then glutamic acid were the second and third most abundant amino acids in all treatments, respectively, but comprised less of the total PAN as the

number of urea applications increased. Soluble solid concentration, pH, titratable acidity, and total polyphenol concentration were not different among treatments. There was a positive correlation between increased urea application rate with maximum fermentation rate and shorter fermentation duration. An increase in the number of urea applications was also correlated with greater H_2S production during fermentation in 2017, but not in 2018. No residual H_2S was found in the cider from any treatment. Increasing the number of urea applications was less expensive than supplementing YAN with Fermaid O™ to the juice. There were no cost savings when Fermaid K™ was used as an exogenous nitrogen source and foliar urea applications were found to be more expensive than supplementing juice with diammonium phosphate. This study demonstrated that foliar urea applications can effectively increase YAN concentrations in cider apples while not negatively impacting other juice quality attributes.

Introduction

Production of hard cider (fermented apple juice) in the United States has grown from 23.1 million liters in 2007 to 173.4 million liters in 2017, making cider the fastest growing beverage sector in the country during this period (Alcohol and Tobacco Tax and Trade Bureau, 2008; Alcohol and Tobacco Tax and Trade Bureau, 2018). The growth of the cider industry offers apple producers an opportunity to expand and diversify their businesses into a niche commodity with a high price premium (Peck and Knickerbocker, 2018). In order to ensure the success of these new enterprises, additional research on how to increase cider quality through orchard management is needed. In particular, there has been minimal research on how pre-harvest nitrogen fertilizer management in apple orchards might impact economic returns, fruit quality, fermentation dynamics, and the sensory attributes of the finished product.

Nitrogen is a macronutrient that is essential for plant metabolism and its role in fresh-market apple production is well studied (Wargo et al., 2003; Lea and Beech, 1978; Santos et al., 2016); Cheng and Fuchigami, 2002; Merwin and Stiles, 1994). From budbreak to just after bloom, apple trees utilize stored nitrogen reserves to support vegetative growth, flowering, and fruitset (Cheng and Raba, 2009). For the remainder of the growing season, apple trees acquire nitrogen from the soil, or in managed systems from nitrogen applied to the leaf surface (referred to as foliar applications). Nitrogen deficient orchards are found in many regions of the world and it is a common practice to fertilize apple trees through both ground and foliar means (Merwin and Stiles, 1994). Foliar nitrogen applications in the fall ensure sufficient reserves for the following spring. Furthermore, Dong et al. (2005) found that nitrogen uptake through leaves is more efficient than through roots during the fall.

When nitrogen is limited in apple trees, there is often a decrease in fruit size, yield, and soluble solid concentrations (Xia et al., 2009). Conversely, excessive nitrogen fertilization in mature orchards can lead to decreased fruit quality by reducing flesh firmness and red peel color (Fallahi et al., 1997; Wang and Cheng, 2011). As a diagnostic tool, orchard managers test mature leaves for total nitrogen concentration once terminal shoot growth has subsided. A target total leaf nitrogen content between 2.2 and 2.4% is recommended for mature hard apples and processing cultivars (Stiles and Reid, 1991). Currently, there are no standard nitrogen fertilization guidelines for cider apple production, so cider apple orchard managers typically follow recommendations for fresh-market or processing apples.

Increasing fruit nitrogen concentration may be beneficial for cider fermentation. Specifically, yeast assimilable nitrogen (YAN) is comprised of the nitrogen compounds that are metabolized by *Saccharomyces cerevisiae* yeast for cell division and growth during alcoholic

fermentation of fruit juice (Bell and Henschke, 2005). Yeast assimilable nitrogen is composed of primary amino nitrogen (PAN), ammonia ions, and some short oligopeptides (Bell and Henschke, 2005). Unlike grapes that contain a variable, but large proportion of YAN as ammonia, the vast majority of YAN in apple fruit is composed of PAN (Bell and Henschke, 2005; Ma et al., 2018). Furthermore, although composition of amino acids vary by apple cultivar, asparagine is the most abundant amino acid in the fruit (Ma et al., 2018). However, ammonia is preferentially utilized and more rapidly metabolized by *S. cerevisiae* than most organic nitrogen sources (Crépin et al., 2012). There are no studies that specify the target YAN concentration for the cider industry; however, recent studies have shown that apple juice is often deficient in YAN according to standards developed for the wine industry (Ma et al., 2018; Peck, et al., 2016; Tahim and Mansfield, 2019).

Deficiencies in YAN result in slow or incomplete fermentations (Bell and Henschke, 2005); (Vilanova et al., 2007). The composition of YAN can impact chemical and sensory attributes of finished ciders and wines (Boudreau et al., 2017; Herraiz and Ough, 1993). In particular, low YAN is known to increase the production of hydrogen sulfide (H_2S), a malodorous reduced sulfur compound. Additionally, while some volatile aromatics in cider are derived from compounds produced in the fruit, most are secondary metabolites produced by yeast during alcoholic fermentation (Xu et al., 2007). In particular, fusel alcohols and acetate esters can be formed from the byproducts of amino acid metabolism (Sumby et al., 2010). Sufficient YAN is also critical for catalyzing the metabolic pathways of fatty acids in yeast that produce ethyl esters as a byproduct (Saerens et al., 2010). Increasing YAN concentration in wine and cider fermentations has been found to increase both ethyl and acetate ester concentrations,

which contribute a large proportion of the fruity aroma of finished wines and ciders (Santos et al., 2016; Garde-Cerdán and Ancín-Azpilicueta, 2008; Tahim and Mansfield, 2019).

Proprietary exogenous nitrogen supplements are commercially available and commonly added to fruit juice fermentations to address YAN deficiencies. Diammonium phosphate (DAP) is probably the most commonly used. However, many wine and cider producers prefer adding yeast nutrient supplements with greater PAN than ammonia concentrations in order to limit initial alcoholic fermentation rates, temperature increases from increased metabolic rates that can stress yeast cells, and changes to sensory character imparted by high ammonia additions (Charoenchai et al., 1998; Tahim and Mansfield, 2019). These high PAN supplements are commonly composed of inactivated dry yeast cells and contain minerals, sterols, and vitamins important for yeast metabolism (Ángeles et al., 2009).

Pre-harvest foliar nitrogen applications in vineyards have been found to be effective means of increasing grape YAN concentrations. For example, Moss (2016) found a cumulative application of 30 kg/ha of nitrogen applied as urea to foliage was capable of more than doubling the YAN concentration in *Vitis vinifera* cv 'Sauvignon blanc' and 'Petit Manseng' must. These foliar applications were also more effective in increasing must YAN than a 60 kg/ha ground application of nitrogen as calcium nitrate. Foliar urea applications may therefore also be an effective means of increasing apple fruit YAN for cider production. Additionally, if orchard nitrogen fertilization is capable of increasing apple YAN, it is more likely to be in PAN form than ammonia, which would be preferred for subsequent fermentation by most cider makers. While the addition of urea is forbidden as a nitrogen supplement directly to alcoholic fermentations due to the formation of ethyl carbamate, urea is metabolized to ammonia and

carbon dioxide by urease enzymes in plant tissue and is a common and safe fertilization practice (Butzke and Bisson, 1997; Witte, 2011).

In order to investigate the feasibility of orchard foliar fertilization for increasing apple juice YAN, 'Red Spy' apple trees located in Geneva, NY were provided with low, medium, or high rates of foliar urea applications during the six weeks before harvest. The experiment was repeated on different trees within the same orchard over two years. The goal of the study was to determine if foliar nitrogen applications in apple orchards can cost effectively improve cider apple juice quality. We hypothesized that foliar urea applications would increase juice YAN and fermentation rates, and decrease H_2S production during fermentation of the juice, without altering other important fruit and juice attributes such as total polyphenol, soluble solid, and titratable acid concentrations.

Materials and Methods

Orchard Site

This study was conducted in 2017 and 2018 at a Cornell University Agricultural Experiment Station research orchard in Lansing, NY (42.573875, -76.596111) on Ovid silt loam soils with a 0 to 6° slope (Soil Survey Staff, 2014). The trees, *Malus ×domestica* Borkh cv. 'Red Spy' grafted onto 'Budagovsky 9' dwarfing rootstock were trained as a vertical axis. The orchard was planted in 2012 with 1.2 m between trees and 3.7 m between rows. Over the course of the experiment, the trees were uniformly managed using standard pest control and pruning practices for the region (Agnello et al., 2019).

Experimental Design

Four foliar nitrogen treatments were applied starting six weeks prior to harvest in both years of the study. The study was a randomized complete block design, with six replications. Different trees within the orchard were used in each field season, and at a minimum, two buffer trees were located between treatment trees. Urea granular fertilizer dissolved in water at a concentration of 10 g/L was applied to treatment trees using a Solo 451 backpack mist blower (Newport News, VA) at an equivalent rate of 935 L water/ha. The number of urea applications established Low (1 application), Medium (3 applications), High (5 applications), and Control (0 applications) treatments as per the schedule listed in Table 2.1. The 2017 foliar applications were made on 24 Aug, 31 Aug, 7 Sept, 14 Sept, and 21 Sept; and the 2018 applications were made on 23 Aug, 30 Aug, 6 Sept, 13 Sept, and 20 Sept.

Table 2.1 Treatment timing of foliar urea applications on cv. 'Red Spy' apple trees grown in Lansing, NY.

Treatment	Weeks Before Harvest				
	6	5	4	3	2
Control					
Low			×		
Medium		×	×	×	
High	×	×	×	×	×

Harvest and Fruit Measurements

Harvest occurred on 5 Oct 2017 and 5 Oct 2018. All fruit from each sample tree was harvested and weighed. Ten-fruit subsamples were measured for mass, percent peel blush, starch pattern index (SPI), flesh firmness, and chlorophyll a content. Peel blush was visually approximated as the area of the fruit peel with red coloration. Starch pattern index was rated on a 0-8 point scale, with 1 = 0% starch degradation and 8 = 100% starch degradation (Blanpied and

Silsby, 1992). The SPI was also used to determine harvest date, with a target value of 6. Flesh firmness was measured on both the sun and the shade exposed side of each fruit along the equator after removing the peel with a penetrometer (Güss GS Fruit Texture Analyzer, Strand, South Africa) fitted with an 11.1 mm tip. Chlorophyll a content was measured on a 0-3 point index with a Turoni 53500 DA meter (Forli, Italy) on the sun and the shade side of each apple along the equator. Ten exposed, non-damaged leaves from shoot mid sections were taken from both sides of each tree between 1-2 m in height the day before harvest (in both 2017 and 2018) and submitted to the Cornell Nutrient Analysis Laboratory for combustion analysis of total nitrogen as per standard protocols (VarioMax CNS, Elementar Analysensysteme GmbH, Langenselbold, Germany). Trunk cross sectional area (TCSA) was calculated by measuring trunk circumference at 30 cm above the graft union and recorded each fall after the trees defoliated.

Milling, Juicing, and Fermentation

Ten fruit from each experimental unit were milled and pressed in a Norwalk 280 juicer (Bentonville, AR) to make a juice sample. After settling at 2 °C overnight, juice was racked off gross lees and aliquoted into two 200 ml samples in 250 ml Erlenmeyer flasks. Potassium metabisulfite solution (100µL of a 17.5% w/v to yield 50 mg/L of free SO_2) was added to each flask and stirred 24 hours before yeast was inoculated.

To rehydrate yeast for inoculation, 5 g of *S. cerevisiae* UCD-522 yeast was rehydrated in 100 ml 40 °C water for 20 minutes and then 100 ml of a juice sample added over five minutes. Two ml of rehydrated yeast solution was then added to each fermentation flask. Each flask was fitted with a Kitigawa 120SB H_2S detector tube (Pompton Lakes, New Jersey) that was inserted

into a single-hole stopper. The detector tubes contain lead acetate, which reacts with H_2S to form gray-colored lead sulfide. This method, originally reported by (Ugliano and Henschke, 2010), has been used to monitor H_2S during cider fermentations by Boudreau et al. (2017). Color change in the H_2S detector tubes were recorded every 24 hours. Flasks were kept at 18 °C and weighed every 24 hours to track fermentation rates by calculating the mass of sugar metabolized from mass loss of CO_2. Maximum fermentation rate was determined by calculating the slope of the fermentation curve during the exponential phase of yeast growth. Flasks were stirred for 5 minutes at 120 rpm on an orbital shaker twice a day to keep yeast in suspension. Once fermentation rate fell below 0.2 g CO_2/day, fermentations were considered completed. Cider samples were then racked off the fine lees and stored at -80 °C.

Juice Chemistry

Soluble solid concentration, pH, titratable acidity (TA), YAN (PAN and ammonium ion), and total polyphenol concentrations via the Folin Ciocalteu assay were measured in each juice sample. Soluble solid concentration was measured with an Atago PAL-1 digital refractometer (Tokyo, Japan) and reported as °Brix. Juice pH and TA were measured with an automatic titrator [Metrohm Unitrode pH meter, 778 sample processor, and 800 Dosino dosing device (Herisau, Switzerland)]. A 5 mL juice sample was titrated against a 0.1 M NaOH solution to an endpoint of pH 8.2 and expressed as g/L of malic acid equivalents. The Megazyme Primary Amino Nitrogen and Ammonia (Rapid) spectrophotometric assay kits (Bray, Ireland) were used to measure YAN in 96 well microplates according to manufacturer specifications and read on a Molecular Devices Spectramax 384 Plus spectrophotometer at λ 340nm (San Jose, CA). The PAN assay kit measures free amino acids by a reaction of amino nitrogen in juice samples with

N-acetyl-L-cysteine and o-phthaldialdehyde that forms isoindole derivatives that are measured by an increase in absorbance at λ 340 nm. The Ammonia Ion kit functions via the reaction of NH_3 with NADPH and 2-Oxoglutarate to yield $NADP^+$, L-glutamic acid, and water; the increase in absorption of $NADP^+$ at λ 340nm is stoichiometric with the amount of NH_3 in the sample. Total polyphenols were measured with the Folin Ciocalteu assay in a 96 well microplate at λ 765 nm (Singleton and Rossi, 1965). Folin Ciocalteu's phenol reagent and sodium bicarbonate were supplied by Sigma-Aldrich (St. Louis, MO).

Amino Acid Quantification and Characterization

Amino acid concentrations were quantified using a Waters Corporation AccQ-Tag Ultra Derivatization Kit on an Acquity UPLC (Milford, MA) following the protocol of Ma et al. (2018). Juice samples were centrifuged at 3,500 ×g for 10 minutes, filtered through PTFE 0.22 µm membrane filters (Micro Solv, Eatontown, NJ) and spiked with an internal standard of L-(+)-norvaline (Acros Organics, NJ) to a final concentration of 2.5 mM. A working standard was made of Waters Amino Acid Hydrolysate Standard and four stock solutions of –norvaline, L-glutamine, GABA and L-asparagine (Sigma-Aldrich, St. Louis, MO) dissolved in 0.1 N HCl. The working standard contained 0.25 mM L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine, and 0.125 mM L-cysteine.

Juice samples and standards were derivatized using an AccQ-Tag Ultra Derivatization Kit following manufacturer instructions to generate amino acid derivatives with stable UV absorbance characters. A Waters AccQ-Tag Ultra Amino Acid Analysis Column (BEH C_{18} 1.7 µm column) on a Waters H-Class UPLC/PDA system was used. Each run had a total run time of

10 minutes using the following mobile phases (A-D): A- 100%AccQ-Tag Ultra eluent A concentrate; B- 90:10 water-AccQ-Tag Ultra eluent B; C-100% HPLC-grade water; D- 100% AccQ-Tag Ultra eluent B. Amino acids were detected at λ 260 nm. Empower™ Software was used to integrate and quantify peaks using the ApexTrack function (Waters Corporation, Milford, MA).

Cider Chemistry

Residual sugars in fermented ciders were measured using a Megazyme Sucrose, D-Fructose, and D-Glucose kit in a 96 well microplate spectrophotometric method at λ 340 nm. Through enzymatic oxidation of sucrose, D-Fructose, and D-Glucose in samples, $NADP^+$ is reduced to NADPH, which increases in absorbance at 340 nm stoichiometrically with the concentration of residual sugars in the cider samples.

Residual H_2S in fermented ciders was measured using the method developed by (Jastrzembski et al., 2017). Fifteen ml of cider was placed in a 125 ml plastic bottle with a 5 cm long section of tubing attached to the top of a screw cap lid. A Gastec 4LT H_2S detector tube was inserted in the end of the tubing (Kitagawa, Japan). A single Alka Seltzer Gold™ tablet was placed in the bottle and the screw cap immediately tightly placed on the bottle. The amount of residual H_2S sparged from the CO_2 generated by the Alka Seltzer™ tablet was recorded from the detector tube after the tablet had completely dissolved.

Economic Modeling

A partial budget analysis comparing foliar urea application costs with YAN supplementation cost for cider fermentation was calculated. To do this, juice volume from each

tree was estimated from its yield, and the cost of foliar urea applications was subtracted from the value of increased juice YAN in comparison to Control juice YAN in each year. The value of each treatment was expressed as $/ha of trees relative to the cost of using Fermaid O™, Fermaid K™, or diammonium phosphate (DAP) nitrogen supplements. Economic model assumptions are listed in Table 2.2. Fermaid O™ is a USDA organically certified blend of yeast autolysates that contains 40 mg N/g. Fermaid K™ is a blend of yeast autolysates and DAP with a YAN concentration of 100 mg N/g. Both Fermaid products are commercially available yeast nutrient supplements produced by Lallemand Inc. (Montreal, Canada) and commonly used in the cider industry. Diammonium phosphate contains 180 mg N/g. Juice extraction efficiency was estimated at 0.60 L/Kg fruit using a Lancman 6-Easy-Feed elevator-grinder (Vransko, Slovenia) and a rack and cloth press (Oesco 81.3 cm model no. 7876, Conway, MA) located at the Cornell University Agricultural Experiment Station research orchard in Ithaca, NY.

Table 2. Partial budget model variables and parameters used to compare foliar urea applications and exogenous nitrogen fermentation supplementation costs.

Description	Value	Source
Foliar urea applications	$37.69/ha	Farris et al. (2013)
Urea	$0.56/kg	Nutrien
Fermaid O™	$33.80/kg	Scott Labs
Fermaid K™	$15.70/kg	Scott Labs
Diammonium phosphate	$2.60/kg	BSG Wine

Statistical Analysis

Data were compared using a linear mixed effects model with the number of foliar urea applications as a continuous response variable. Differences were considered significant at $P \leq 0.05$. The number of treatment applications, year, and treatment × year were included in the model as fixed effects; block, and block × year were included within the model as random

variables. A logit transformation of blush, petiole nitrogen, and amino acid proportion percentage data was performed prior to analysis but presented as untransformed data. Data were analyzed using JMP Pro version 14 (SAS Institute, Cary, NC).

Results

Fruit and Tree Characteristics

Yield and crop load were unaffected by urea application treatment, but overall, they were lower in 2017 than 2018 (Table 2.3). Fruit receiving more foliar urea applications had less red coloring and greater chlorophyll a content than those receiving fewer urea applications. Fruit from the High treatment had 10% less red coloration and 18% greater chlorophyll a content than fruit from the Control treatment. There were no differences in fruit mass, firmness, or starch pattern index among treatments. Overall, there was a positive correlation of increased urea applications with greater leaf nitrogen concentrations. Trees in the High treatment had 5% greater leaf nitrogen concentrations than the Control.

Juice Chemistry

There were no treatment differences in the soluble solid concentration, pH, titratable acidity, or polyphenol concentration in the juice (Table 2.4). Yeast assimilable nitrogen concentrations were greater in fruit from treatments receiving more urea applications than those receiving fewer applications. Juice from the High treatment had 229% and 408% higher YAN concentrations than the Control in 2017 and 2018, respectively (Figure 2.1). Overall, YAN concentrations in 2017 were 47% greater than those in 2018. The majority of YAN in all treatments was comprised of PAN. Primary amino nitrogen constituted 95% of YAN in 2017 and

92% in 2018. More urea applications increased both PAN and NH_3 concentrations in fruit, but the majority of increases in YAN among treatments, were comprised of PAN. Even though NH_3 concentrations increased with more urea applications, proportionally less juice YAN was comprised of NH_3 than PAN ($P<0.001$, $R^2=0.49$).

Amino Acid Profiles

Asparagine was the most abundant amino acid found in the apple juice from this experiment (Table 2.5). Asparagine also constituted a greater proportion of PAN in treatments receiving more urea applications than those receiving fewer (Figure 2.2). Asparagine constituted 73.8% of the PAN in High treatment juice and 53.2% of Control juice (Table 2.6). Aspartic acid and glutamic acid had the second and third greatest proportions of amino acids among juice all treatments, respectively, and were more abundant in treatments receiving more urea applications. However, aspartic acid and glutamic acid proportionally constituted less of PAN in treatments receiving more urea applications than those receiving fewer. Aspartic acid and glutamic acid constituted 13.8% and 10.1% of Control treatment juice PAN, but only 5.8% and 3.8% of High treatment juice PAN, respectfully.

Glutamine was the fourth most abundant amino acid within the juice, but there was no difference in the proportion of glutamine among treatments, and it collectively contributed 10.7% of PAN across all treatments and years. The remainder of the PAN within the apple juice samples were serine, alanine, and histidine, which all contributed relatively little to PAN. Cysteine and methionine concentrations were generally below the limit of detection, with the exception of a single High treatment replicate in 2017 that contained 1.7 mg/L of methionine.

Table 2.3 Tree and fruit measurements of cv. 'Red Spy' apple trees grown in Lansing, NY with different numbers of foliar urea applications. Values are means ± standard error (n=6 per year). P-values are from "Combined" data from both years of the study.

Year	Treatment	Yield (kg/tree)	Crop Load (kg/TCSA[Z])	Mass (g)	Peel Blush (%)	Starch Pattern Index (1-8)	Firmness (N)	Chlorophyll a Index	Leaf Nitrogen (%)
2017	Control	16.7±2.1	1.2±0.2	347.4±14.9	84.0±1.3	6.8±0.2	62.8±1.8	1.02±0.26	2.2±0.1
	Low	15.1±1.2	1.0±0.1	367.2±18.1	79.5±1.7	6.9±0.2	57.9±1.9	1.08±0.09	2.3±0.1
	Medium	18.5±1.3	1.1±0.2	368.0±15.7	78.2±1.8	6.5±0.2	63.8±1.6	1.22±0.06	2.3±0.1
	High	18.1±2.7	1.0±0.1	357.4±12.1	75.0±1.6	6.9±0.3	59.0±2.8	1.13±0.09	2.3±0.1
2018	Control	20.7 ±1.3	1.3±0.1	223.0±19.3	79.0±1.7	6.0±0.2	59.0±4.1	1.16±0.05	2.2±0.0
	Low	19.8±1.5	1.3±0.1	230.8±17.1	81.0±1.6	5.7±0.4	58.3±1.4	1.2±0.07	2.2±0.0
	Medium	21.9±1.9	1.3±0.1	239.1±8.1	74.0±2.0	6.0±0.3	59.7±5.9	1.36±0.07	2.3±0.1
	High	20.5±1.4	1.3±0.1	223.0±8.1	72.0±2.0	6.3±0.1	60.8±9.6	1.53±0.07	2.4±0.0
Combined	Control	18.7±1.2	1.3±0.1	285.2±22.1	81.5±1.3	6.4±0.2	60.9±1.8	1.09±0.06	2.2±0.0
	Low	17.5±1.2	1.1±0.1	299.0±22.1	80.3±1.3	6.3±0.2	58.2±1.8	1.14±0.06	2.2±0.0
	Medium	20.2±1.2	1.2±0.1	303.5±21.2	76.1±1.6	6.3±0.2	61.8±2.1	1.29±0.05	2.3±0.1
	High	19.3±0.9	1.2±0.1	290.2±21.4	73.5±1.8	6.6±0.2	59.9±3.3	1.33±0.08	2.3±0.0
P-value	Treatment	*0.265*	*0.607*	*0.798*	*0.002*	*0.28*	*0.623*	*<0.001*	*0.013*
	Year	*0.010*	*0.011*	*<0.001*	*0.099*	*0.003*	*0.370*	*0.038*	*0.058*
	Treatment × Year	*0.482*	*0.463*	*0.838*	*0.868*	*0.251*	*0.818*	*0.066*	*0.231*

[Z]TCSA=trunk cross sectional area

Table 2.4 Juice chemistry from cv. 'Red Spy' apples from trees grown in Lansing, NY receiving different numbers of foliar urea applications. Values are means ± standard error (n=6 per year). P-values are from "Combined" data from both years of the study.

Year	Treatment	Soluble Solid Concentration (°Brix)	pH	Titratable Acidity (g malic acid/L)	Primary Amino Nitrogen (mg/L)	Ammonia (mg/L)	Yeast Assimilable Nitrogen (mg/L)	Total Polyphenols (g GAE[Z]/L)
2017	Control	14.1±0.4	3.50±0.04	6.8±0.3	27.5±3.5	1.9±1.3	30.7±3.3	0.71±0.10
	Low	13.9±0.4	3.52±0.03	6.3±0.2	35.8±2.7	1.7±0.2	37.5±2.8	0.66±0.07
	Medium	14.1±0.3	3.49±0.02	6.9±0.2	52.5±6.5	2.2±0.3	54.7±6.6	0.84±0.01
	High	14.6±0.4	3.54±0.03	7.0±0.3	96.6±10.1	4.4±0.7	101.0±10.5	0.71±0.08
2018	Control	12.8±0.1	3.41±0.02	7.0±0.2	13.8±1.1	1.9±0.4	15.7±1	0.52±0.02
	Low	13.0±0.3	3.42±0.02	7.1±0.3	33.3±6.5	2.2±0.3	35.5±6.6	0.62±0.04
	Medium	13.0±0.1	3.41±0.01	7.2±0.1	33.3±6.5	2.2±0.3	35.5±6.6	0.52±0.03
	High	12.8±0.2	3.43±0.01	7.2±0.1	76.8±8.7	2.9±0.1	79.7±8.7	0.52±0.03
Combined	Control	13.5±0.3	3.46±0.02	6.9±0.2	20.7±2.7	1.9±0.3	23.2±2.8	0.62±0.06
	Low	13.5±0.3	3.47±0.02	6.7±0.2	27.7±2.7	1.8±0.3	29.5±2.8	0.64±0.06
	Medium	13.5±0.2	3.45±0.02	7.1±0.1	42.9±5.7	2.2±0.2	45.1±5.8	0.68±0.05
	High	13.7±0.3	3.49±0.02	7.1±0.1	86.7±7.0	3.7±0.4	90.4±7.3	0.62±0.04
P-value	Treatment	0.402	0.194	0.156	<0.001	*<0.001*	*<0.001*	*0.348*
	Year	0.007	<0.001	0.027	0.006	*0.443*	*0.008*	*0.003*
	Treatment × Year	0.200	0.620	0.588	0.636	*0.040*	*0.610*	*0.966*

[Z]GAE=gallic acid equivalent

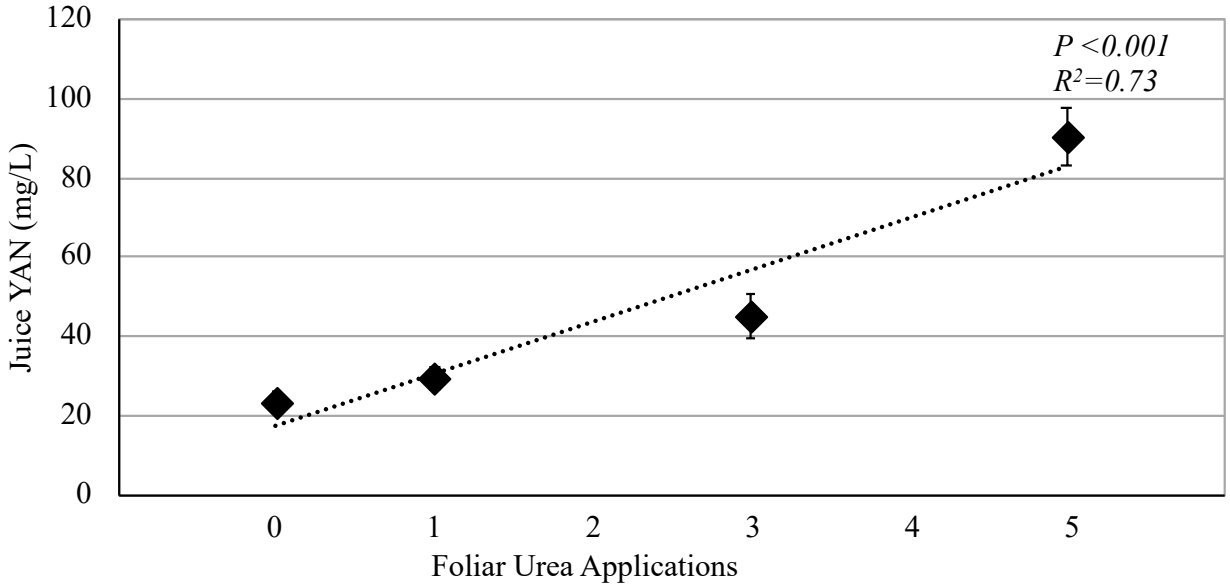

Figure 2.1. Yeast assimilable nitrogen (YAN) concentrations in cv. 'Red Spy' apple juice from trees grown in Lansing, NY receiving different numbers of foliar urea applications in 2017 and 2018. Values are means ± standard error (n=6 per year).

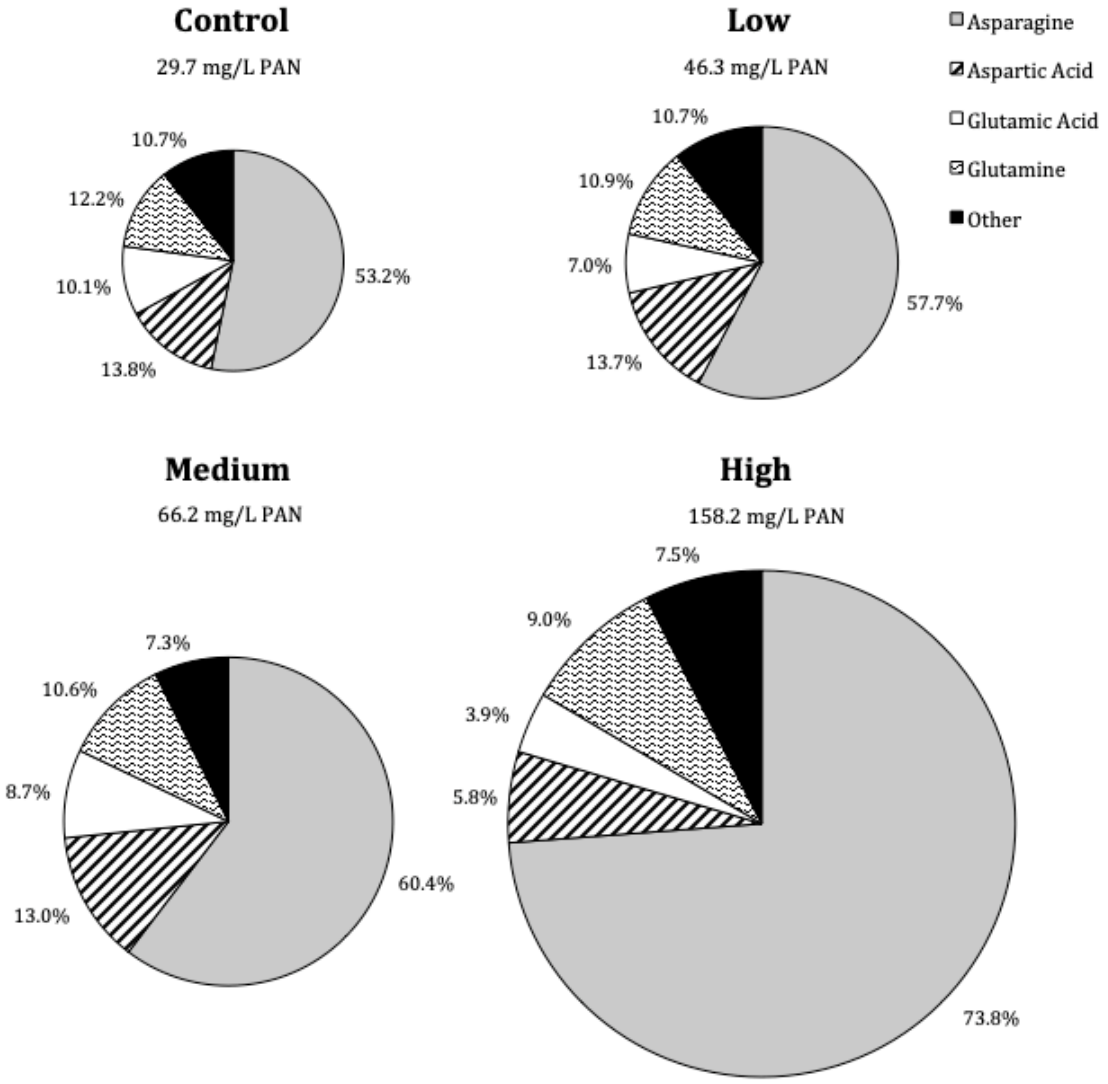

Figure 2.2 Total concentrations of primary amino nitrogen (PAN) and proportions of amino acids constituting PAN of apple juice from cv. 'Red Spy' apple trees grown in Lansing, NY receiving different numbers of foliar urea applications. Pie chart sizes are to scale of PAN concentration among the treatments. Data represent a "Combined" mean from both years of the study.

Fermentation Characteristics and Cider Chemistry

Apple juice from trees receiving more foliar urea applications had greater maximum fermentation rates, and finished fermenting sooner than those receiving fewer applications (Table 2.7). The maximum fermentation rate for the High treatment juice was 147% and 374% greater than the Control in 2017 and 2018, respectively. Fermentation for the High treatment juice were also 39% and 45% shorter in duration than the Control. Maximum fermentation rates in 2017 were greater than those in 2018, and fermentation durations were shorter. More H_2S was produced during fermentations in 2017 than 2018; H_2S synthesis was more than 23-fold greater in 2017 than 2018. There was a positive correlation between foliar urea applications and H_2S synthesis during fermentation; High treatment fermentations produced 142% more H_2S than the Control. However, there were no measurable quantities of residual H_2S in ciders from any treatment in either year (data not shown).

Ciders made from apples receiving more foliar urea applications also contained the lowest residual sugar concentrations. Fermentations from the High and Medium treatments had less residual sugar than the Control (Table 2.7). The High treatment had no measurable residual sugars after fermentation in either year. The Medium treatment had no measurable residual sugars in 2017. Overall, residual sugar concentrations were higher in 2018 than in 2017. In 2018, Control and Low had 2.3 and 2.2 g/L of residual sugar, respectively.

Economic Model

Increases in YAN from foliar urea applications more than offset the application cost when supplementing juice with Fermaid O™, resulting in a negative correlation between foliar urea applications and exogenous nitrogen supplementation costs (Figure 2.3). When supplementing with Fermaid O™, the High treatment would have been $1,246 and $1,271 less expensive per ha in comparison to the Control in 2017 and 2018, respectively (Table 2.8). The number of urea applications

was not correlated with the cost of fermentation supplementation when using Fermaid K™. Increases in YAN were disproportionally greater in High than in Low and Medium trees per urea application, resulting in Low and Medium having a smaller net gain in YAN per urea application than High. This resulted in a small net mean positive return for High when supplementing with Fermaid K™ ($54.80/ha), but a small mean negative return for Low and Medium ($-21.50 and $-41.46, respectively). Due to the very low cost of DAP, gains in YAN did not offset application costs, and there was a positive correlation of foliar urea applications with the cost of supplementing nitrogen. Sensitivity analysis for these economic models found that these conclusions would be maintained even when increasing the target YAN concentrations up to 300 ppm.

Table 2.5 Amino acid concentrations from cv. 'Red Spy' apples from trees grown in Lansing, NY receiving different numbers of foliar urea applications. Values are means ± standard error (n=6 per year). P-values are from "Combined" data from both years of the study.

Year	Treatment	Asparagine	Aspartic Acid	Glutamic Acid	Glutamine	Serine	Alanine	Histidine	Other
2017	Control	115.3±28.9	50.0±1.6	38.6±6.2	12.7±2.4	5.0±0.5	2.5±0.3	3.0±0.2	1.1±0.2
	Low	173.8±30.2	76.4±5.7	31.9±1.2	18.6±2.4	5.7±0.4	3.3±0.2	3.0±0.1	1.1±0.1
	Medium	235.8±33.6	98.1±9.3	69.2±11.5	26.6±8.0	8.0±2.3	7.9±1.5	1.7±0.8	1.2±0.3
	High	644.8±95.9	123.7±11.8	78.4±14	69.9±3.6	37.4±7.9	23.1±3.9	3.8±0.3	4.8±0.4
2018	Control	48.9±11.8	25.4±1.2	20.6±1.3	16.9±2.9	4.7±0.3	1.3±0.3	2.9±0.2	0.8±0.1
	Low	49.7±11.8	29.8±4.8	22.9±1.8	20.8±2.4	5.9±1.0	1.6±0.4	3.1±0.3	1.4±0.2
	Medium	93.7±26.0	48.4±5.3	31.9±0.6	28.3±1.1	7.7±0.6	2.9±0.4	2.7±0.2	1.1±0.2
	High	523.4±98.6	71.5±8.6	45.2±6.4	37.8±1.7	16.6±1.9	8.8±1.2	3.3±0.1	2.2±0.3
Combined	Control	82.1±17.9	37.7±5.4	29.6±4.1	14.8±1.9	4.9±0.3	1.9±0.3	3.0±0.2	1.0±0.2
	Low	119.2±27.5	57.6±8.7	27.2±1.8	18.3±1.8	6.2±0.6	2.8±0.5	3.1±0.1	1.4±0.2
	Medium	165.1±30.7	70.2±9.1	48.2±7.0	28.2±3.6	9.1±1.0	5.3±0.9	2.5±0.3	1.3±0.2
	High	581.7±68.2	90.7±10.0	63.3±8.6	52.3±9.2	23.1±3.1	15.8±2.8	3.4±0.2	3.6±0.4
P-value	Treatment	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.324*	*<0.001*
	Year	*0.002*	*0.005*	*0.012*	*0.332*	*0.096*	*0.032*	*0.258*	*0.118*
	Treatment × Year	*0.660*	*0.181*	*<0.001*	*0.022*	*0.010*	*0.007*	*0.995*	*0.002*

Table 2.6 Proportion of amino acids constituting primary amino nitrogen from cv. 'Red Spy' apples from trees grown in Lansing, NY receiving different numbers of foliar urea applications. Values are means ± standard error (n=6 per year). P-values are from "Combined" data from both years of the study.

Year	Treatment	Asparagine (%)	Aspartic Acid (%)	Glutamic Acid (%)	Glutamine (%)	Serine (%)	Alanine (%)	Histidine (%)	Other (%)
2017	Control	59.5±4.6	14.3±1.6	10.2±1.2	7.1±1.3	2.0±0.4	2.5±0.1	2.4±0.3	3.5±0.7
	Low	69.4±2.9	13.6±1.2	5.4±0.8	5.2±0.8	1.3±0.1	3.9±0.1	1.3±0.2	2.0±0.3
	Medium	69.1±2.5	13.1±0.8	7.4±1.3	6.3±1.4	1.7±0.3	7.1±0.2	0.8±0.4	1.5±0.4
	High	70.8±3.9	6.8±1.1	4.1±0.8	10.6±3.8	2.4±0.5	1.9±0.3	0.6±0.1	2.8±0.5
2018	Control	47.0±5.9	13.4±1.0	9.9±1.0	17.2±3.7	3.2±0.3	0.9±0.2	4.0±0.4	4.4±1.0
	Low	41.5±5.5	13.9±0.9	10.5±1.5	18.6±2.1	3.6±0.5	1.0±0.2	4.4±1.2	6.4±0.6
	Medium	50.4±5.4	14.5±1.0	9.3±1.5	16.3±2.2	3.0±0.3	1.3±0.1	2.2±0.2	3.0±0.2
	High	78.9±4.1	5.9±0.	3.6±0.8	6.5±1.8	1.8±0.3	1.0±0.1	0.8±0.3	1.8±0.1
Combined	Control	53.2±4.0	13.8±0.9	10.1±0.7	12.2±2.4	2.6±0.3	1.0±0.1	3.2±0.4	3.9±0.6
	Low	57.7±4.7	13.7±0.8	7.0±1.0	10.9±2.3	2.3±0.4	0.9±0.1	2.9±0.8	4.2±0.7
	Medium	60.4±4.0	13±0.8	8.7±0.9	10.6±2.0	2.2±0.3	1.5±0.3	1.3±0.3	2.2±0.3
	High	73.8±2.9	5.8±0.7	3.9±0.5	8.6±1.8	2.2±0.4	1.8±0.4	0.7±0.12	2.2±0.3
P-value	Treatment	*<0.001*	*<0.001*	*<0.001*	*0.113*	*0.209*	*0.004*	*<0.001*	*0.004*
	Year	*0.005*	*0.934*	*0.158*	*0.010*	*0.007*	*0.041*	*0.008*	*0.014*
	Treatment × Year	*0.013*	*0.627*	*0.414*	*0.003*	*0.005*	*0.018*	*0.064*	*0.034*

Table 2.7 Fermentation characteristics of cv. 'Red Spy' apple juice fermented with UCD 522 yeast from tree receiving different numbers of foliar urea applications. Values are means ± standard error (n=6 per year). P-values are from "Combined" data from both years of the study.

Year	Treatment	Hydrogen Sulfide Production (µg/L)	Maximum Fermentation Rate (mg sugar/L/hr)	Fermentation Duration (days)	Residual Sugar (g/L)
2017	Control	40.1±20.3	215±94	17.8±0.9	1.5±0.9
	Low	58.1±16.3	278±104	16.3±1.0	0.1±0.0
	Medium	114.5±20.2	366±75	13.1±0.8	0.0±0.0
	High	95.3±23.3	531±63	10.8±0.6	0.0±0.0
2018	Control	0.0±0.0	31±4	27.7±1.5	2.3±0.6
	Low	4.6±3.9	31±4	26.6±0.7	2.2±0.7
	Medium	6.3±4.9	63±14	24.3±2.0	0.7±0.5
	High	1.8±1.1	147±17	15.3±1.9	0.0±0.0
Combined	Control	20.1±11.4	123±30	22.8±1.6	1.9±0.5
	Low	31.4±11.4	155±30	21.5±1.6	1.1±0.5
	Medium	60.4±19.1	215±48	18.7±2.0	0.4±0.3
	High	48.6±17.9	339±61	13.1±1.2	0.0±0.0
P-value	Treatment	*0.030*	*<0.001*	*<0.001*	<0.001
	Year	*<0.001*	*<0.001*	*<0.001*	0.077
	Treatment × Year	*0.037*	*<0.001*	*0.050*	0.131

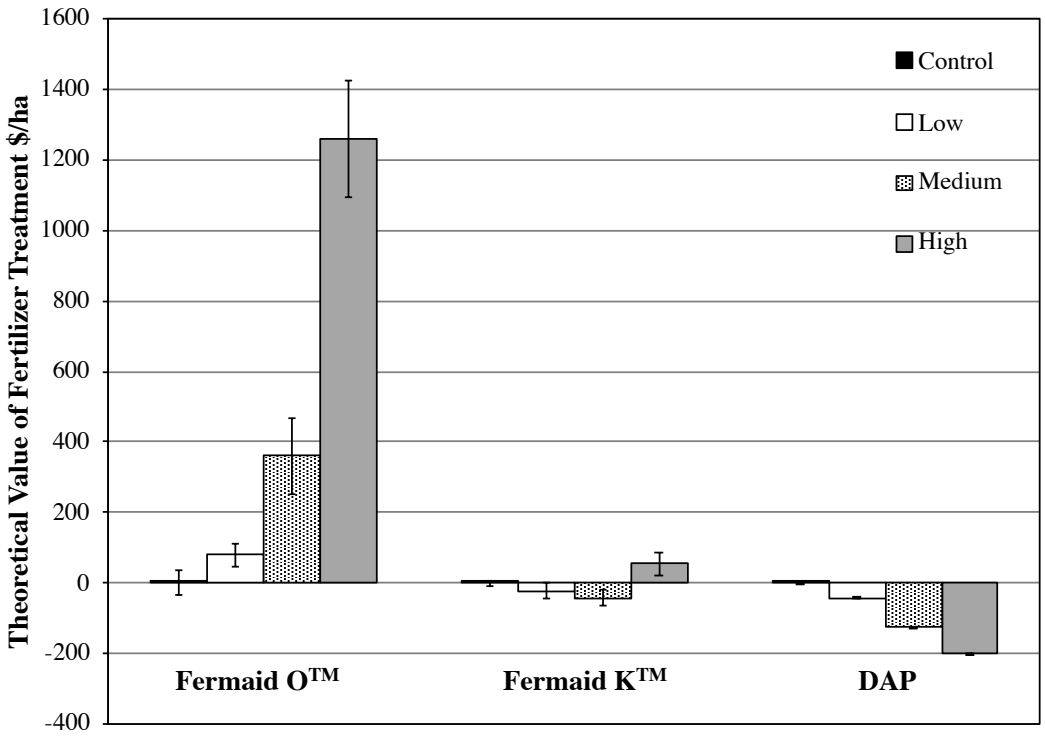

Figure 2.3 The theoretical cost of applying foliar urea to cv. 'Red Spy' apple trees grown in Lansing, NY versus adding exogenous yeast assimilable nitrogen in the form of Fermaid O™, Fermaid K™, or diammonium phosphate (DAP). Values are means ± standard error (n=6 per year).

Table 2.8 Economic modeling of foliar urea applications of cv. 'Red Spy' apple trees increasing juice yeast assimilable nitrogen (YAN) and offsetting exogenous YAN supplements of three common fermentation aids. Values are means ± standard error (n=6 per year). P-values are from "Combined" data from both years of the study.

Year	Treatment	Fermaid O™ ($/ha)	Fermaid K™ ($/ha)	Diammonium Phosphate ($/ha)
2017	Control	3.69±69.55	0.69±13.09	0.06±1.15
	Low	66.95±55.59	-23.81±25.63	-43.02±2.24
	Medium	370.96±133.91	-39.42±25.38	-126.25±2.21
	High	1245.93±250.25	52.45±47.11	-200.09±4.12
2018	Control	0.47±25.77	0.09±4.85	0.01±0.42
	Low	91.51±45.75	-19.19±8.61	-42.61±0.75
	Medium	349.31±186.12	-43.50±35.03	-126.61±3.07
	High	1270.91±237.87	57.15±44.78	-199.67±3.92
Combined	Control	2.08±35.36	0.39±6.66	0.03±0.58
	Low	79.22±34.52	-21.50±22.51	-42.82±1.97
	Medium	360.13±109.36	-41.46±20.58	-126.43±1.80
	High	1258.42±164.64	54.80±30.99	-199.88±2.71
P-value	Treatment	*0.002*	*0.184*	*<0.001*
	Year	*<0.001*	*0.129*	*<0.001*
	Treatment × Year	*<0.001*	*0.010*	*<0.001*

Discussion

For commercial production of fresh-market apples, modest nitrogen fertilization rates are recommended to maintain sufficient nitrogen status for photosynthesis and fruit cell division and expansion. However, excessive fertilization rates can negatively impact fruit quality and bud cold hardiness (Stiles and Reid, 1991). Excess application of nitrogen has been correlated with decreased red and increased green coloration in apples, lower flesh firmness, and reduced fruit quality after long-term storage (Wargo et al., 2004; Wargo et al., 2003; Raese et al., 2007; Fallahi, 1997). While fruit receiving more urea applications in our study had less red coloration and more chlorophyll, these attributes are not important quality characteristics for cider apples.

Decreased flesh firmness was not observed in our study and would only be considered to be an important attribute for cider apples if they were going to be stored for long periods. Leaf nitrogen concentrations among all treatments were within current recommended concentrations for hard and processing apple trees (Stiles and Reid, 1991). It is not clear from this study if reaching higher nitrogen statuses of trees from further fertilization would have negative implications on tree physiology, or fruit quality from a cidermaking perspective.

A previously published study investigating nitrogen fertilization of apple trees for cider production was conducted by Lea and Beech (1978). In their study, three-year-old cv. Dabinett trees were transplanted into pots filled with sand and then either fertilized with nitrogen or left unfertilized for a single growing season. Unfertilized trees had 17% reduced total polyphenol concentration compared to fertilized trees, as well as a 35% decrease in fruit yield. This study is often referenced as evidence that nitrogen fertilization limits polyphenol production in apples, but the greater decrease in yield than polyphenol content illustrates that mean total fruit polyphenol production per tree was higher in the fertilized trees. In other words, the polyphenols were more diluted in a larger crop, but the total fruit polyphenol production per tree was similar or greater in fertilized trees. Our study found that foliar urea applications did not affect total polyphenol concentrations by total juice volume or total polyphenols on a per tree basis. Furthermore, phenological assessment of procyanidins, the polyphenols that contribute the majority of astringency and bitterness to hard ciders, has shown nearly all of these polyphenols are produced during the cell division phase of fruit growth (Renard et al., 2007). Even if nitrogen fertilization were to inhibit polyphenol production, application in the six weeks prior to harvest would occur after procyanidin production occurs and would thus not be expected to greatly influence these sensorily important compounds.

While foliar nitrogen applications were made in the form of urea, an ammonia-based fertilizer, nearly all of the increases in YAN in juice were attributable to gains in organic forms of nitrogen (PAN or amino acids), rather than inorganic forms (ammonium ions). In grapes, cultivar and season have been found to better predictors of YAN composition than the form of nitrogen fertilizer, as well (Garde-Cerdán et al., 2017). The proportional increase of asparagine to other major amino acids in our study was similar to (Sugimoto et al., 2011), who found concentrations of aspartic acid, glutamine, and glutamic acid to decrease earlier in fruit maturation of cv. 'Jonagold' apples than asparagine. The timing of urea applications in our study corresponded with a period of fruit development when Sugimoto et al. (2011) found asparagine metabolism was not active, but other predominant amino acids were actively metabolized during this period of fruit development. Cheng et al. (2004) also found that with higher rates of nitrogen fertilization, an increasing proportion of both free amino acids and proteins in tree tissue were composed of asparagine.

The principal amino acids in cv. 'Red Spy' juice from this study have been reported to be preferentially metabolized by *S. cerevisiae* yeast. These amino acids are selectively utilized because they require fewer intermediary steps to donate nitrogen in *de novo* amino acid synthesis than the non-preferred amino acids (Waterhouse et al., 2016). Asparagine, aspartic acid, glutamic acid, glutamine, serine, and alanine all fall under this preferential class. Histidine, of which there was less than 4 mg/L among all treatments and years, was the only amino acid that constituted more than 1% of PAN from juice samples in this study that is considered to be in the non-preferred class of amino nitrogen. Nearly all of the increases in YAN from urea applications were therefore in forms preferentially metabolized by yeast during fermentation.

Faster and more complete fermentation of reducing sugars was another benefit of higher YAN concentration achieved through foliar urea application. The correlation between increasing YAN and increased fermentation rate, and the propensity for incomplete fermentations in low YAN grape juices are well established (Bell and Henschke, 2005). Greater YAN concentrations have been correlated with increased concentrations of volatile aromatic compounds including fusel alcohols and acetate esters derived from amino acid metabolism, as well as ethyl esters, primarily produced from yeast fatty acid metabolism (Torrea et al., 2011; Santos et al., 2015). Supplementation with amino acids versus ammonia yields differences volatile aromatic profile differences in grape-based wines, but the causal mechanisms have not been well described (Torrea et al., 2011), and whether these changes are positive or negative in terms of sensory characteristics remains an area of current research (Tahim and Mansfield, 2019). Additionally, research in the relationship between concentrations and forms of YAN on fermentation kinetics and sensory properties has primarily been performed on grape wine and not hard apple cider. Volatile aromatic compounds that can be increased using foliar urea applications should be a target of future research.

The increase in juice YAN as PAN resulting from foliar urea applications is noteworthy. Of the supplements evaluated in the economic model in our study, exogenous Fermaid O™ supplementation would yield a pre-fermentation juice most comparable to the juice obtained through the foliar application of urea. The economic model demonstrated foliar urea applications to be less costly than Fermaid O™, thus the strategy evaluated in this study has the potential to be a cost-effective means to increase apple juice PAN prior to harvest. The partial budget model demonstrated the economic viability of foliar urea applications offsetting the cost of Fermaid K™, a slightly less expensive form of exogenous nitrogen than Fermaid O™. Adding exogenous

DAP to juice was less expensive than making foliar urea applications to increase juice YAN due to the low cost of DAP. Because of the large differences in the cost of each nitrogen supplement, the cost of nitrogen additions varied more with the selection of supplement, than with foliar urea applications. Furthermore, in our discussions with cider producers, many have stated a preference for using organic forms of nitrogen (such as the proprietary Fermaid products) over inorganic nitrogen in the form of DAP.

Overall, more urea applications were correlated with greater H_2S production. While this is the opposite of what we hypothesized would occur, this finding is not without precedent. For instance, while H_2S production is more commonly associated with insufficient YAN, cases of greater YAN concentrations increasing H_2S production have been observed in other studies (Boudreau et al., 2017; Jiranek, et al., 1995; Ugliano et al., 2009). However, H_2S synthesis was more strongly correlated with year than treatment, with fermentations in 2017 having much greater H_2S synthesis rates than in 2018. Hydrogen sulfide is an intermediary compound formed from the reduction of sulfate or sulfite in order to synthesize the sulfur containing amino acids cysteine and methionine (Ono et al., 1999). Other nutrients deficiencies, such as thiamin and pantothenic acid can also lead to an accumulation of H_2S (Wainwright, 1971; Wang et al., 2003). It is not clear what caused H_2S production during our experiment, but all treatments had lower YAN concentrations than the 140 ppm minimum that is commonly recommended for wine production (Bell and Henschke, 2005). Additional exogenous nitrogen and mineral/nutrient additions would be advised under commercial settings, which may have reduced or eliminated H_2S production. However, because H_2S is naturally sparged with CO_2 during cider fermentation, the timing of H_2S synthesis is critical in determining how much residual H_2S will remain in the finished product (Ugliano et al., 2009).

In our study, H_2S synthesis was measured early in the fermentation and no residual H_2S was found in the finished ciders. The sparging of CO_2 and daily stirring of ciders to keep yeast in suspension may have resulted in the removal of H_2S during fermentation. The method to measure H_2S in this paper relies on mass transfer of CO_2 to pass through the detector tubes for measurement. It is therefore not sensitive to quantify H_2S production over smaller time periods within the fermentation due to differences in mass transfer resulting from different fermentation rates. Additionally, wines containing H_2S are known to undergo reactions to form mercaptans and other complex sulfide compounds with negative sensory attributes that were not measured in this study (Waterhouse et al., 2016); however, cider samples were frozen after finishing fermentation to minimize the opportunity for these reactions to occur. Measurement of head space gas for concentrations of H_2S and other reduced sulfur compounds via gas chromatography and mass spectrometry could have been performed throughout fermentation to more accurately measure the timing of synthesis and concentrations of these compounds throughout fermentation (Mendes-Ferreira et al., 2010). However, the method utilized in the current paper has been found to be an effective means for measuring differences in total H_2S synthesis among treatments. Anecdotally, sulfurous aromas were not smelled in finished ciders from this experiment by the author. Sensory evaluation of finished ciders would be beneficial in future studies to provide insight into sensory differences beyond H_2S production that may be imparted by foliar urea application compared to additions of exogenous nitrogen. Hydrogen sulfide production during cider fermentation is a poorly understood and complex topic and remains to be a major challenge for cider producers.

Conclusion

Our study demonstrated that foliar applications of urea are effective at increasing YAN in apple juice, which can have positive benefits for cider fermentation. Additionally, nearly all of the increases in YAN were in the form of PAN, with asparagine constituting the majority of amino nitrogen. These increases in YAN resulted in faster and more complete fermentations of reducing sugars. The increases in PAN resulting from these urea applications were an economically advantageous alternative to exogenous PAN additions. While commercial apple growers in the United States are not typically compensated for making additional foliar nutrient applications to help increase juice YAN, a vertically integrated orchard and cidery could find these applications as beneficial for simultaneously fertilizing the orchard and improving the quality of the crop for cider production.

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CHAPTER THREE

Soil Nitrogen Fertilization Increases Yeast Assimilable Nitrogen Concentrations in 'Golden Russet' and 'Medaille d'Or' Apples Used for Cider Production

Abstract

The recent growth in the United States hard cider industry has increased the demand for cider apple (*Malus ×domestica* Borkh.) cultivars, but little is known about how to best manage soil fertility to optimize horticultural performance and juice quality. In order to assess if increased nitrogen fertilization can improve apple juice and cider quality, calcium nitrate fertilizer was applied at different rates to the soil beneath cv. 'Golden Russet' and 'Medaille d'Or' trees over the course of three growing seasons. The experiment started when the trees were in their second leaf and ran for three years. The trees were cropped in their third and fourth leaf. At the end of the first growing season of the experiment, the highest fertilizer rate increased tree size by 82% relative to the control, but these differences did not persist through to the end of the study. Yield and crop load were not affected by the nitrogen fertilization treatments. Increasing nitrogen fertilizer rate was positively correlated with more advanced harvest maturity in Golden Russet fruit, which resulted in greater soluble solid concentrations. The fertilizer treatments did not affect juice pH, titratable acidity, or total polyphenol concentrations. Yeast assimilable nitrogen concentrations were increased by nitrogen fertilization for both cultivars in both harvest years. The highest fertilizer treatment increased juice primary amino nitrogen by 103% relative to the Control. Greater nitrogen fertilization rates were positively correlated with less hydrogen sulfide production during the fermentation of Golden Russet juice from the first, but not the second harvest. Greater maximum fermentation rates and shorter fermentation durations were

positively correlated with increased fertilization rate for both cultivars after the second harvest. In orchards producing apples specifically for the hard cider industry, nitrogen fertilizer could increase juice YAN, thus reducing the need for exogenous additions during cider production. Other horticultural and juice quality parameters were not negatively affected by these treatments.

Introduction

The rapid growth of the cider industry within the United States over the past decade offers an opportunity for apple [*Malus ×domestica* (Borkh.)] growers in the United States to expand and diversify production, and potentially increase their profitability with the cultivation of heirloom and high tannin cultivars specifically for cider production (Peck and Knickerbocker, 2018; Peck et al., 2013). Many high acid and high tannin concentration cultivars sought after by cider producers for the sensory attributes of their juice are not available in sufficient quantities to meet demand within the United States (Pashow, 2018). However, many of these cultivars possess horticultural traits that pose challenges to their production in commercial orchards, such as biennial bearing and vigorous vegetative growth (Merwin et al., 2008). Growers need information on how management practices impact economic returns, fruit quality, and attributes of finished ciders in order to remain competitive and continue improving the quality of ciders in the US market.

Nitrogen fertilization is a little studied area of cider apple orchard management that potentially impacts productivity, fruit tannin concentrations, fermentation kinetics, and cider sensory attributes (Wargo et al., 2003; Lea and Beech, 1978; Boudreau et al., 2017a; Santos et al., 2016). There are currently no standard nitrogen fertilization recommendations for cider apple orchards in the U.S., so growers typically follow guidelines for fresh market or processing apple

orchards. Sufficient nitrogen for tree growth, photosynthesis, and fruit development is essential, but excessive nitrogen fertilization can negatively impact fruit quality and bud cold hardiness (Raese et al., 2007). Moderate nitrogen fertilization rates, with target total leaf nitrogen content between 2.2 and 2.4%, are therefore recommended for hard and processing apple cultivars (Stiles and Reid, 1991). Insufficient nitrogen can reduce fruit size, yield, and soluble solid concentrations in fruit (Fallahi, 1997; Lea and Beech, 1978). Higher nitrogen content of apple trees have been correlated with decreased red and increased green coloration of fruit peels, less firm flesh, and reduced storage capacity (Wargo et al., 2003; Wargo et al., 2004; Raese et al., 2007; Fallahi, 1997). Cider fruit is typically milled and pressed shortly after picking, thus peel color, flesh firmness, and storage duration are less important fruit quality characteristics.

Within the context of producing apples for cider, increasing fruit nitrogen concentration may be beneficial for alcoholic fermentation. Yeast assimilable nitrogen (YAN), which are defined as the nitrogen sources metabolized by *Saccharomyces cerevisiae* yeast during fermentation, is typically a limiting factor for cider production (Bell and Henschke, 2005; Boudreau et al., 2017b). Deficient YAN concentrations can result in incomplete and/or slow fermentations that produce inferior quality products (Bell and Henschke, 2005). Low YAN concentrations are also known to increase the production of hydrogen sulfide (H_2S) during fermentation, a common defect in ciders because it smells like cabbage or rotten eggs (Boudreau et al., 2017b; Jiranek et al., 1995). Currently, there are no cider-specific guidelines for apple juice YAN concentration, but several authors have reported that apple juice is typically YAN deficient based on wine grape industry standards (Boudreau et al., 2018; Peck et al., 2016). Current winemaking recommendations often cite 140 mg/L YAN as the minimum concentration and a range between 200 and 350 mg/L YAN (depending on initial sugar concentration, yeast

strain, and wine style) for the successful completion of most fermentations (Bell and Henschke, 2005; Ugliano et al., 2011; Torrea et al., 2011). Apples typically have YAN concentrations under 100 mg/L. For example, a survey of 12 cultivars grown in Virginia over two growing seasons found a mean YAN concentration of 59 mg/L (Boudreau et al., 2018).

Increased nitrogen fertilization of vineyards have successfully increased YAN concentration in grape wine must (Moss, 2016; Neilsen et al., 2010). Commercial cider producers often add exogenous YAN supplements to eliminate nitrogen deficiencies, such as diammonium phosphate or autolyzed yeast cells. However, it is possible that nitrogen fertilizers applied in the orchard may improve tree growth and yield, and simultaneously reduce the need for exogenous YAN additions. Because apple trees are perennial crops that rely on nitrogen reserves from the previous growing year to support initial growth and fruit set, multi-year studies of nitrogen fertilization in orchards are useful to elucidate ramifications for different management strategies (Cheng et al., 2004).

Over the course of three growing seasons, different rates of calcium nitrate fertilizer were applied each spring to ‘Golden Russet’ and ‘Medaille d’Or’ apple trees. The study started when trees were in their second year after planting and continued through their fourth year. Fruit was harvested in years three and four. The overall goal of this research was to assess the impact of nitrogen fertilization on tree growth, yield, fruit and juice quality, and fermentation characteristics. We hypothesized that increased nitrogen fertilization would increase tree growth, juice YAN concentrations, and fermentation rates, and reduce hydrogen sulfide production during fermentation.

Materials and Methods

Orchard Site

This study was conducted between spring of 2016 and harvest of 2018 at the Cornell University Agricultural Experiment Station research orchard in Ithaca, NY (42.445111, -76.459564). The apple trees cvs. 'Golden Russet' and 'Medaille d'Or' grafted onto 'Geneva.30®' rootstock and trained as a tall spindle were planted in the Spring of 2015. Trees were planted in a north-south row orientation with 3.7 m between rows in Hudson and Collamer silt loam soils on 2 to 6 percent slopes (Soil Survey Staff, 2014). The Golden Russet trees were spaced at 1.7 m between trees; Medaille d'Or trees were spaced at 1.2 m between trees. The trees were trained as a tall-spindle and uniformly managed with standard pruning practices and pest control management for the region (Agnello et al., 2019). No fruit was produced in 2016. A light crop on the trees in 2017 did not necessitate fruit thinning for either cultivar. In 2018, fruit were thinned to 6 fruit/trunk cross sectional area (TCSA) for both cultivars between 2-10 July.

Experimental Design

Four nitrogen fertilizer treatments (Control, Low, Medium, and High) were established in the spring of 2016 by applying different rates of calcium nitrate ($CaNO_3$) granular fertilizer. The treatments were reapplied to the same trees each year. Low, Medium, and High treatment trees received an equivalent of 28, 56, and 112 kg/ha of nitrogen each year. Calcium nitrate was applied in two equal aliquots by spreading the granules in a ring around each tree starting 15 cm and extending to 40 cm away from the trunk. The first annual applications occurred on 19 April 2016, 18 April 2017, and 18 April 2018, when the trees were approximately at half inch green of

bud development (Agnello et al., 2019). The second application was applied approximately four weeks later on 16 May in each year of the study.

The study was designed as a randomized complete block, with four double-tree replications per treatment. Each cultivar had a complete set of treatments and replicates. There was at least one buffer tree between each experimental unit.

Tree, Fruit, and Harvest Measurements

Medaille d'Or was harvested on 25 Sept 2017 and 20 Sept 2018. Golden Russet harvest occurred on 24 Oct 2017 and 30 Oct 2018. All fruit from every experimental tree was counted and weighed. A ten-fruit subsample was used to measure mass, starch pattern index (SPI), flesh firmness, and chlorophyll a content. For Medaille d'Or apples, peel blush was visually assessed as the area of the fruit peel with red coloration. For Golden Russet apples, russeting was visually approximated as the area of the fruit peel with visible russeting. Starch pattern index was rated on a 0-8 point scale, with 1= 0% starch degradation and 8=100% starch degradation (Blanpied and Silsby, 1992). Harvest was based on when a subsample of fruit from the buffer trees had a SPI of 6, with approximately 60% starch degradation. Flesh firmness was measured on the sun and the shade exposed side of each fruit along the equator with a penetrometer (Güss GS Fruit Texture Analyzer, Strand, South Africa) fitted with an 11.1 mm diameter tip. Chlorophyll a content was measured on a 0-3 point index using a Turoni 53500 DA meter (Forli, Italy) on the sun and the shade side of each apple along the equator.

Ten exposed, non-damaged leaves from shoot mid-sections were taken from both sides each tree between 1-2 m above the soil level in mid-August in each year of the study and submitted to the Cornell Nutrient Analysis Laboratory (Ithaca, NY) for total nitrogen analysis by

combustion as per manufacturer protocols (VarioMax CNS, Elementar Analysensysteme GmbH, Langenselbold, Germany). Trunk cross-sectional area (TCSA) was measured in Spring 2016 when trees were still dormant, and then in the fall of each year after the trees defoliated. Trunk cross-sectional area was calculated from trunk circumference measured 30 cm above the graft union.

Milling, Juicing, and Fermentation

Twenty apples from each experimental unit (ten apples per tree) were milled and pressed in a Norwalk 280 juicer (Bentonville, AR) to make a single composite juice sample. Golden Russet in 2017 and 2018 and Medaille d'Or in 2018 were used for fermentation studies. (There was insufficient Medaille d'Or yields in 2017.) Juice was racked off the gross lees after settling at 2 °C overnight. Two 200 ml sub-samples for each experimental unit were aliquoted into 250 ml Erlenmeyer flasks. Potassium metabisulfite (100 µL of a 17.5% w/v solution to yield 50 mg/L of free SO_2) was added to each flask 24 hours before yeast was inoculated.

Five grams of UCD-522 yeast was rehydrated in 100 ml 40 °C water for 20 minutes and then 100 ml of a conglomerated juice sample was added over five minutes. Two ml of rehydrated yeast solution was then added to each fermentation flask. Each flask was fitted with a Kitigawa 120SB H_2S detector tube (Pompton Lakes, New Jersey) inserted into a single-hole stopper. The detector tubes contain lead acetate, which reacts with H_2S to form gray-colored lead sulphide. This method was originally reported by Ugliano and Henschke (2010) and has been used to monitor H_2S during cider fermentations by Boudreau et al. (2017a). Color change in the H_2S detector tubes were recorded every 24 hours. Flasks were kept at 18 °C and weighed every 24 hours to track fermentation rates. Flasks were stirred for 5 minutes at 120 rpm on an orbital

shaker twice a day to keep yeast in suspension. Once fermentation rate fell below 0.2 g CO_2/day, fermentations were considered completed. Cider samples were then racked off the fine lees and stored at -80 °C.

Juice Chemistry

Soluble solid concentration (SSC), pH, titratable acidity (TA), YAN (PAN and ammonium ion), and total polyphenol concentrations (using the Folin Ciocalteu assay) were measured for each juice sample. Soluble solid concentration was measured with an Atago PAL-1 digital refractometer (Tokyo, Japan) and reported as °Brix. Juice pH and TA were measured with an automatic titrator [Metrohm Unitrode pH meter, 778 sample processor, and 800 Dosino dosing device (Herisau, Switzerland)]. A 5 mL juice sample was titrated against a 0.1 M NaOH solution to an endpoint of pH 8.2 and expressed as g/L of malic acid equivalents. The Megazyme Primary Amino Acid and Ammonia (Rapid) spectrophotometric assay kits (Bray, Ireland) were used to measure YAN in 96 well microplates according to manufacturer specifications and read on a Molecular Devices Spectramax 384 Plus spectrophotometer at λ 340nm (San Jose, CA). The PAN assay kit measures free amino acids by a reaction of amino nitrogen in juice samples with N-acetyl-L-cysteine and o-phthaldialdehyde that forms isoindole derivatives that are measured by an increase in absorbance at λ 340 nm. The Ammonia Ion kit functions via the reaction of NH_3 with NADPH and 2-oxoglutarate to yield $NADP^+$, L-glutamic acid, and water; the increase in absorption of $NADP^+$ at λ 340nm is stoichiometric with the amount of NH_3 in the sample. Total polyphenols were measured with the Folin Ciocalteu assay in a 96 well microplate at λ 765 nm (Singleton & Rossi, 1965). Folin Ciocalteu's phenol reagent and sodium bicarbonate were supplied by Sigma-Aldrich (St. Louis, MO).

Amino Acid Quantification and Characterization

Amino acid concentrations were quantified using a Waters Corporation AccQ-Tag Ultra Derivatization Kit on an Acquity UPLC (Milford, MA) following the protocol of Ma et al. (2018). Juice samples were centrifuged at 3,500 ×g for 10 minutes, filtered through a PTFE 0.22 µm membrane filters (Micro Solv, Eatontown, NJ) and spiked with an internal standard of L-(+)-norvaline (Acros Organics, NJ) to a final concentration of 2.5 mM. A working standard was made of Waters Amino Acid Hydrolysate Standard and four stock solutions of –norvaline, L-glutamine, GABA and L-asparagine (Sigma-Aldrich, St. Louis, MO) dissolved in 0.1 N HCl. The working standard contained 0.25 mM L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine, and 0.125 mM L-cysteine.

Juice samples and standards were derivatized using the AccQ-Tag Ultra Derivatization Kit following manufacturer instructions to generate amino acid derivatives with stable UV absorbance characteristics. A Waters AccQ-Tag Ultra Amino Acid Analysis Column (BEH C_{18} 1.7 µm column) on a Waters H-Class UPLC/PDA system was used. Each run had a total run time of 10 minutes using the following mobile phases (A-D): A) 100% AccQ-Tag Ultra eluent A concentrate; B) 90:10 water-AccQ-Tag Ultra eluent B; C) 100% HPLC-grade water; and D) 100% AccQ-Tag Ultra eluent B. Amino acids were detected at λ 260 nm. Empower™ Software was used to integrate and quantify peaks using the ApexTrack function (Waters Corporation, Milford, MA).

Cider Chemistry

Residual sugars in fermented ciders were measured using a Megazyme Sucrose, D-Fructose, and D-Glucose kit in a 96 well microplate spectrophotometric method at λ 340 nm. Through enzymatic oxidation of sucrose, D-fructose, and D-glucose in samples, $NADP^+$ is reduced to NADPH, which is stoichiometrically related to the concentration of residual sugars.

Residual H_2S in fermented ciders was measured using the method developed by Jastrzembski et al. (2017). Fifteen milliliters of cider was placed in a 125 ml plastic bottle with a 5-cm long section of tubing attached to the top of a screw cap lid. A Gastec 4LT H_2S detector tube was inserted in the end of the tubing (Kanagawa, Japan). A single Alka Seltzer Gold™ (Dr. Miles Medicine, Elkhart, IN) tablet was placed in the bottle and the screw cap immediately tightly placed on the bottle. The amount of residual H_2S sparged from the CO_2 generated by the Alka Seltzer™ tablet was recorded from the detector tube after the tablet had completely dissolved.

Statistical Analysis

Data were compared using a linear mixed effects model. Treatments were considered significant at $P \leq 0.05$. Treatment (rate of nitrogen fertilization), cultivar, year, treatment × cultivar, and treatment × year were included as fixed effects. Block, experimental unit, and block × year were included within the model as random variables. A logit transformation of blush, russet, petiole nitrogen, and amino acid proportion percentage data was performed prior to analysis, but data are presented untransformed. Data were analyzed using JMP Pro version 14 (SAS Institute, Cary, NC).

Results

Fruit and Tree Characteristics

There was a positive correlation of nitrogen fertilization rate and an increase in tree size for both cultivars (as measure by TCSA) in 2016 ($P=0.023$, $R^2=0.41$), but not throughout the study. The highest fertilizer rate increased TCSA 82% more than the Control in 2016. However, by the end of the second year of the study there was no difference in TCSA among treatments for either cultivar (Table 3.1). There was no relationship of fertilization rate and leader growth. For both cultivars, increased leaf nitrogen concentration was positively correlated with fertilizer application rate in 2018 for both cultivars ($P<0.001$, $R^2=0.55$), but not in 2016 or 2017. There was a positive correlation of fertilization rate and bloom number throughout the study. However, there were no differences in yield or crop load for either cultivar during this study (Table 3.1).

Greater fruit starch degradation at harvest was positively correlated with greater nitrogen fertilizer rates in Golden Russet but not with Medaille d'Or (Table 3.2). Fruit flesh firmness was negatively correlated with fertilization rate for both cultivars; High fruit flesh firmness was 9% less than the Control. Chlorophyll a content was not influenced by treatment in either cultivar in 2017 or in Medaille d'Or in 2018. In 2018, there was a positive correlation of increased fertilization rate and chlorophyll a content with Golden Russet ($P=0.001$, $R^2=0.62$). There was no correlation of fertilization rate and peel russeting in Golden Russet ($P=0.373$) and peel blush in Medaille d'Or ($P=0.268$).

Table 3.1. Measurements of cv. 'Golden Russet' and 'Medaille d'Or' trees with different calcium nitrate fertilizer application rates. Values are means ± standard error.

Cultivar	Year	Treatment	TCSA[Z] increase (%)	TCSA (cm^2)	Leader growth (cm)	Leaf nitrogen (%)	Blooms	Yield (kg)	Crop load (yield/ TCSA)
Golden Russet	2016	Control	178±15	6.7±0.2	104.3±4.8	2.2±0.0	-	-	-
		Low	223±16	7.9±0.5	125.5±6.3	2.4±0.1	-	-	-
		Medium	272±51	6.5±0.5	122.5±9.8	2.3±0.1	-	-	-
		High	298±84	7.2±0.4	121.4±9.8	2.1±0.1	-	-	-
	2017	Control	97±5	13.1±0.5	80.3±7.1	1.8±0.1	33±4	5.1±0.3	0.39±0.02
		Low	97±6	13.9±0.5	79.6±8.9	1.8±0.1	133±19	7.5±0.41	0.54±0.03
		Medium	90±8	12.1±0.5	78.6±5.9	1.8±0.2	102±19	6.1±1.2	0.49±0.1
		High	80±4	12.6±0.8	81.6±4.2	2.0±0.0	95±15	5.6±0.4	0.45±0.06
	2018	Control	49±1	19.3±0.6	-	1.7±0.1	236±32	16.5±0.8	1.02±0.0
		Low	51±7	20.9±0.9	-	1.7±0.0	277±10	17.0±0.9	1.03±0.0
		Medium	53±4	20.0±1.1	-	1.9±0.1	286±30	16.5±1.3	1.02±0.01
		High	56±7	20.3±0.9	-	2.0±0.1	291±39	15.2±1.1	1.02±0.0
Medaille d'Or	2016	Control	121±21	3.9±0.4	57.9±11.3	2.2±0.1	-	-	-
		Low	144±5	4.9±0.3	78.6±4.3	2.3±0.0	-	-	-
		Medium	172±8	5.5±0.1	84.6±2.2	2.1±0.2	-	-	-
		High	172±9	5.1±0.3	76.0±5.3	2.1±0.1	-	-	-
	2017	Control	74±15	7.1±1.2	40.4±6.4	1.8±0.1	19±8	1.6±0.4	0.26±0.10
		Low	73±5	8.6±0.5	51.6±2.3	2.1±0.1	32±13	2.3±0.1	0.26±0.02
		Medium	85±5	9.0±0.4	49.9±4.4	1.6±0.1	15±4	2.5±0.2	0.28±0.02
		High	75±8	9.0±0.3	50.4±4.4	1.8±0.2	19±5	2.1±0.6	0.22±0.06
	2018	Control	72±13	11.7±1.3	-	1.7±0.1	180±33	5.2±1.7	0.38±0.12
		Low	65±4	12.7±0.4	-	2.0±0.1	258±45	6.3±0.85	0.5±0.07
		Medium	52±3	13.2±0.8	-	2.0±0.1	312±54	5.9±0.62	0.44±0.03
		High	50±4	13.1±0.5	-	2.1±0.1	361±26	6.8±0.47	0.52±0.05
P-value	Treatment		0.151	0.296	0.115	0.246	0.025	0.936	0.407
	Cultivar		<0.001	<0.001	<0.001	0.691	0.010	<0.001	<0.001
	Year		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Treatment × Cultivar		0.600	0.232	0.708	0.596	0.891	0.110	0.641
	Treatment × Year		0.047	0.898	0.289	<0.001	0.013	0.897	0.584

[Z]Trunk Cross-Sectional Area

Table 3.2. Measurements of cv. 'Golden Russet' and 'Medaille d'Or' trees with different calcium nitrate fertilizer application rates. Values are means ± standard error.

Cultivar	Year	Treatment	Mass (g)	Starch pattern index (1-8)	Firmness (N)	Chlorophyll a index	Peel russet/blush (%)
Golden Russet	2017	Control	169.9±5.2	5.3±0.2	90.0±2.5	0.63±0.05	92±3
		Low	172.7±4.8	5.9±0.2	87.8±19	0.48±0.04	97±2
		Medium	173.1±3.9	6.0±0.2	86.8±2.2	0.53±0.04	94±2
		High	167.0±4.5	6.8±0.2	78.6±1.8	0.47±0.02	96±1
	2018	Control	172.4±5.0	6.6±0.1	90.3±2.4	0.25±0.03	95±2
		Low	170.8±3.8	6.9±0.1	84.4±1.8	0.28±0.02	96±2
		Medium	183.4±7.3	7.1±0.1	85.5±1.6	0.31±0.02	95±2
		High	167.5±5.9	7.3±0.3	85.5±1.9	0.38±0.02	96±2
Medaille d'Or	2017	Control	88.3±4.8	7.5±0.1	79.6±3.3	0.37±0.03	16±4
		Low	91.4±3.8	7.8±0.1	76.0±2.4	0.28±0.03	14±4
		Medium	86.3±2.5	7.7±0.1	70.8±2.2	0.31±0.03	13±2
		High	94.0±7.6	7.6±0.1	65.6±3.6	0.28±0.03	11±3
	2018	Control	93.9±5.8	5.6±0.8	77.4±2.3	0.4±0.07	9±1
		Low	89.5±7.0	5.9±0.1	73.6±0.5	0.36±0.05	8±3
		Medium	86.6±3.1	5.4±0.5	78.2±3.1	0.4±0.04	11±3
		High	84.7±5.0	5.6±0.4	76.0±3.2	0.42±0.06	7±2
P-value		Treatment	0.877	0.160	0.003	0.860	-
		Cultivar	<0.001	0.005	<0.001	0.020	-
		Year	0.950	0.075	0.691	0.107	-
		Treatment × Cultivar	0.307	0.139	0.200	0.865	-
		Treatment × Year	0.116	0.326	0.031	0.141	-

Juice Chemistry

There was a positive correlation of fertilization and soluble solid concentration over the course of the entire study (Table 3.3). These differences were small overall; the greatest difference found in the study was 4% greater soluble solid concentration of High treatment juice than the Control in 2018 Golden Russet. There were no differences in juice pH, titratable acidity, or total polyphenol concentration among treatments for either cultivar in either harvest for this

study (Table 3.3). Total polyphenol concentrations among both cultivars were 25% greater in 2017 than 2018.

Fertilization rate was positively correlated with greater primary amino nitrogen (PAN) concentrations for both cultivars in both years within this study (Figures 3.1, 3.2). Overall, PAN concentrations were greater in 2017 than in 2018. High treatment juice from Golden Russet and Medaille d'Or trees in 2017 had 49% and 124% more PAN than the Control, respectively. In 2018, Golden Russet and Medaille d'Or High treatment juice had 92% and 144% more PAN than the Control, respectively. Ammonia concentrations were not different among treatments for Golden Russet, but among all treatments and years, ammonia constituted less than 5% of YAN for Golden Russet. Differences in Golden Russet YAN were therefore mostly attributed to PAN increasing, and not ammonia. For Medaille d'Or, there was an unidentified interference with the spectrophotometric ammonia method. Additional centrifuging, filtering, diluting in 2.5 pH buffer, and adding polyvinylpolypyrrolidone with additional centrifugation were tried individually and in combination, but the assay still failed to produce consistent results. We speculate that this cultivar has a high level of pectin that interfered with this assay.

Table 3.3. Juice chemistry of cv. 'Golden Russet' and 'Medaille d'Or' apples with different calcium nitrate fertilizer application rates. Values are means ± standard error.

Cultivar	Year	Treatment	Soluble solid concentration (°Brix)	pH	Titratable acidity (g malic acid/L)	Total polyphenols (GAE[Z]/L)	Primary amino acids (mg/L)	Ammonia (mg/L)	Yeast assimilable nitrogen (mg/L)
Golden Russet	2017	Control	22.2±0.3	3.61±0.03	8.9±0.2	1.75±0.05	153.3±16.2	4.4±0.1	158.5±10.3
		Low	22.2±0.1	3.53±0.03	8.9±0.4	1.54±0.08	174.0±18.8	4.0±0.2	180.5±16.4
		Medium	22.7±0.4	3.53±0.04	9.1±0.3	1.58±0.10	202.8±8.1	4.1±0.4	204.8±7.6
		High	22.5±0.4	3.59±0.02	8.5±0.2	1.66±0.03	228.0±12.5	4.3±0.3	226.8±10.0
	2018	Control	18.9±0.2	3.48±0.02	8.1±0.3	0.92±0.07	72.5±3.6	3.2±0.4	75.7±3.6
		Low	19.0±0.1	3.50±0.02	7.7±0.2	0.90±0.07	93.5±8.0	2.4±0.1	95.9±8.0
		Medium	19.2±0.2	3.50±0.01	7.7±0.2	0.86±0.04	117.0±14.3	3.2±0.7	120.2±14.4
		High	19.6±0.2	3.51±0.02	7.9±0.2	0.96±0.03	139.2±14.4	3.3±0.6	142.5±13.8
Medaille d'Or	2017	Control	20.0±1.2	4.22±0.07	4.4±0.5	6.35±0.32	94.9±17.0	-	-
		Low	20.6±0.4	4.19±0.02	4.1±0.1	6.58±0.02	140.3±5.7	-	-
		Medium	22.1±0.6	4.15±0.03	4.4±0.2	6.61±0.07	181.9±11.9	-	-
		High	21.4±0.9	4.30±0.08	4.2±0.3	6.51±0.09	211.8±34.0	-	-
	2018	Control	16.4±0.3	4.06±0.04	5.2±0.2	4.54±0.21	46.1±5.1	-	-
		Low	16.6±0.7	4.07±0.02	4.6±0.2	5.60±0.81	74.1±7.5	-	-
		Medium	16.1±0.6	3.97±0.01	4.8±0.4	5.44±0.26	78.7±18.0	-	-
		High	16.4±0.7	4.04±0.04	4.8±0.2	5.01±0.57	112.4±16.0	-	-
P-value		Treatment	0.049	0.588	0.236	0.815	<0.001	0.651	<0.001
		Cultivar	<0.001	<0.001	<0.001	<0.001	0.201	-	-
		Year	<0.001	<0.001	0.243	<0.001	<0.001	0.001	<0.001
		Treatment × Cultivar	0.85	0.91	0.635	0.801	0.472	-	-
		Treatment × Year	0.568	0.347	0.81	0.94	0.072	0.628	0.991

[Z]Gallic Acid Equivalent

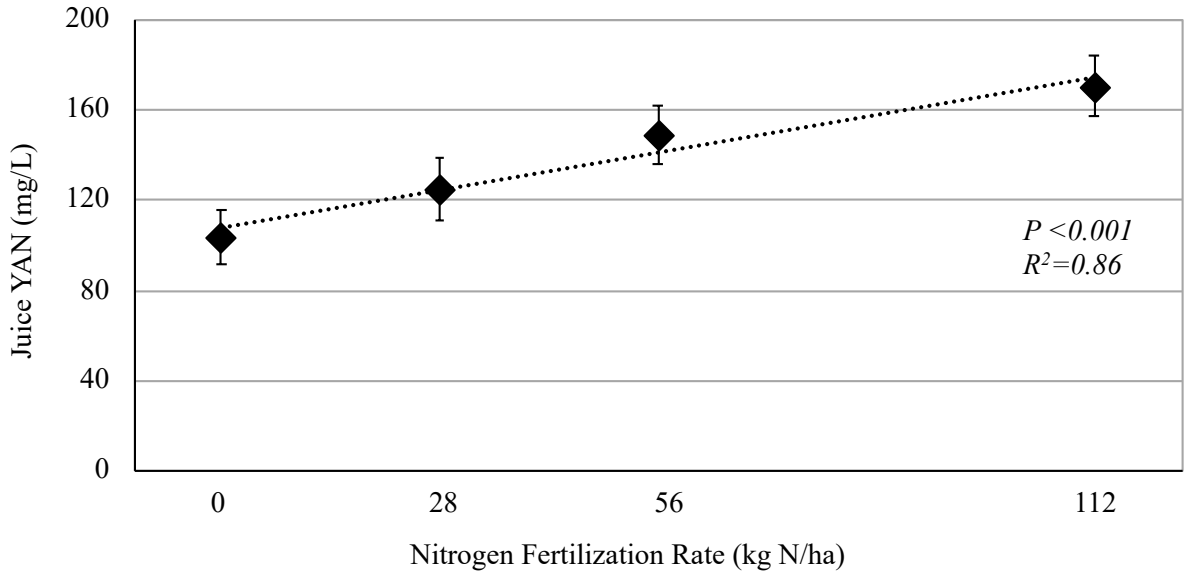

Figure 3.1. Concentrations of yeast assimilable nitrogen (YAN) in cv. 'Golden Russet' apple juice from trees grown in Ithaca, NY receiving different rates of calcium nitrate fertilizer in 2017 and 2018. Values are means ± standard error (n=4 per year).

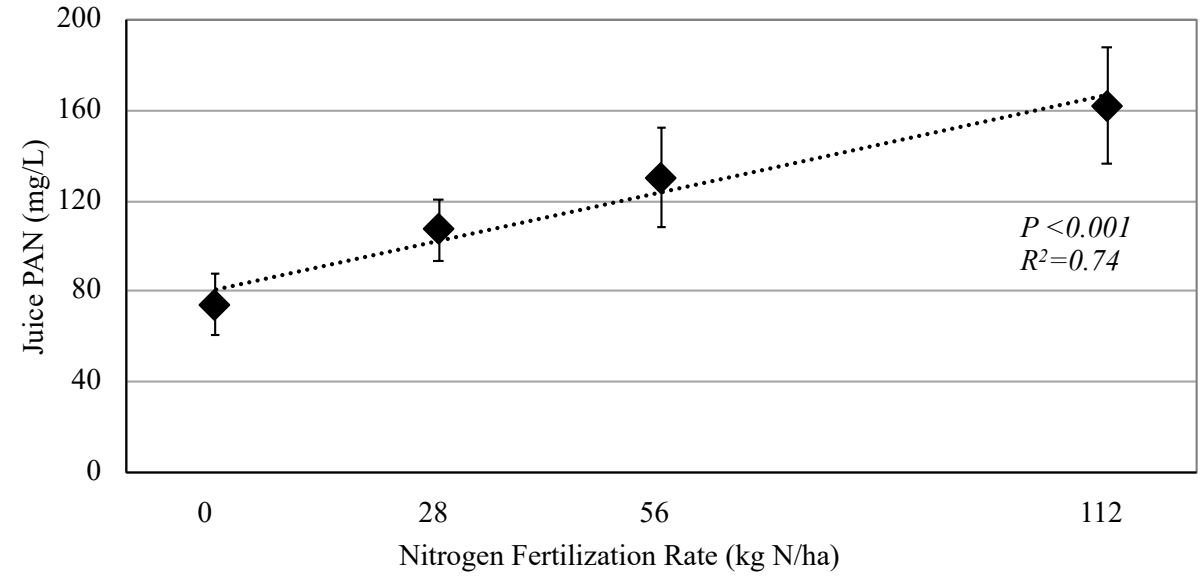

Figure 3.2. Primary amino nitrogen (PAN) concentrations in cv. 'Medaille d'Or' apple juice from trees grown in Ithaca, NY receiving different rates of calcium nitrate fertilizer in 2017 and 2018. Values are means ± standard error (n=4 per year).

Amino Acid Profiles

Golden Russet and Medaille d'Or had distinctively different amino acid profiles. Golden Russet PAN was predominantly comprised of asparagine and aspartic acid (Table 3.4). In 2017, there were no statistical differences in the proportions of asparagine and aspartic acid among treatments in Golden Russet (Table 3.5). For example, asparagine comprised 66% and 73% of Control and High treatment PAN, respectively, and aspartic acid comprised 27% and 21% of Control and High treatment PAN, respectively. In 2018, aspartic acid constituted a larger proportion of PAN than asparagine in Golden Russet Control juice; Control PAN was only 36% asparagine, and 52% aspartic acid. The ratio of asparagine to aspartic acid in Golden Russet High treatment juice was similar between 2017 and 2018; in 2018 High juice was 60% asparagine and 31% aspartic acid. There was a positive correlation of fertilization rate with a greater proportion of PAN as asparagine and a negative correlation with a lower proportion of PAN as aspartic acid in 2018. However, due to greater overall PAN concentrations in treatments receiving more fertilizer, there was a positive correlation of fertilization rate and greater aspartic acid concentrations. The remaining predominant amino acids in Golden Russet were glutamic acid, glutamine, and serine, which each comprised less than 4% of total PAN for all treatments (Figure 3.3).

Asparagine was the principal amino acid in Medaille d'Or juice, and the proportion of PAN as asparagine was positively correlated with fertilization rate (Table 3.6). Asparagine constituted 54% of MD Control juice, and 77% of High juice PAN (Table 3.7). Aspartic acid was the second most predominant amino acid in Medaille d'Or juice. As with Golden Russet juice, there was a negative correlation of fertilization rate with a lower proportion of aspartic acid as PAN, but a positive correlation of fertilization rate and greater aspartic acid concentrations.

Aspartic acid constituted 13% of Medaille d'Or Control juice, and 8% of High juice. The proportion of glutamic acid, glutamine, and serine were also negatively correlated with fertilization rate. Glutamic acid, glutamine, and serine constituted 10%, 7%, and 6% of Control PAN, respectively, and each 4%, of High PAN, respectively (Figure 3.4).

Fermentation Characteristics and Cider Chemistry

There was a positive correlation between fertilization rate and increased maximum fermentation rate and an inverse correlation between fertilizer rate and fermentation (Table 3.8). In 2018, High Golden Russet maximum fermentation rate was 131% greater than Control and Medaille d'Or High maximum fermentation rate was 89% greater than the Control. In 2018, Golden Russet High fermentation durations were 28% faster than the Control, and in 2018 MD High fermentation durations were 32% faster than the Control.

In 2017, residual sugar concentrations were under 0.3 g/L for all treatments and there was no correlation of fertilization rate. In 2018, there was an inverse correlation between residual sugar concentration and fertilization rate in Golden Russet ciders ($P=0.004$, $R^2=0.59$). Ciders made from the Control treatment fruit contained 0.84 g/L reducing sugars, while High ciders had contained 0.08 g/L reducing sugars.

There was an inverse correlation of H_2S production and fertilization rate in 2017 ($P=0.049$, $R^2=0.10$). Mean Golden Russet Control H_2S production was 29.6 µg/L, while mean Golden Russet High H_2S production was 0.1 µg/L. Hydrogen sulfide production was not correlated with either Golden Russet or Medaille d'Or fermentation rates in 2018. Additionally, residual H_2S was not detected in the finished ciders from either year or cultivar (data not shown).

Table 3.4. Juice amino acid concentrations of cv. 'Golden Russet' apples with different calcium nitrate fertilizer application rates. Values are means ± standard error.

Cultivar	Year	Treatment	Asparagine (mg/L)	Aspartic Acid (mg/L)	Glutamic Acid (mg/L)	Glutamine (mg/L)	Serine (mg/L)	Threonine (mg/L)	Methionine (mg/L)	Other (mg/L)
Golden Russet	2017	Control	563.9±127.2	438.2±44.4	29.4±1.5	5.5±0.7	25.7±4.6	13.7±1.8	2.8±0.5	16.0±0.7
		Low	647.2±158.9	424.0±61.9	32.0±2.4	5.6±1.2	24.9±3.2	13.4±1.6	2.4±0.2	22.8±0.3
		Medium	974.2±69.6	525.9±55.2	34.2±1.4	7.9±1.5	36.2±4.1	19.3±2.5	3.8±1.1	22.6±1.2
		High	965.3±53.5	564.2±39.4	32.7±2.5	11.6±1.4	36.8±3.2	17.7±1.5	3.1±0.2	26.0±1.8
	2018	Control	131.2±5.0	386.3±18.5	23.0±1.9	8.1±0.6	13.6±1.0	4.5±0.2	3.3±0.2	20.7±1.2
		Low	286.0±79.1	508.2±43.9	36.1±8.4	15.9±6.4	20.3±2.0	6.8±1.0	5.8±0.8	35.5±5.1
		Medium	461.9±124.7	644.6±66.7	30.9±12.9	27.5±7.0	22.8±4.6	5.1±1.9	4.6±1.8	28.4±8.0
		High	698.6±168.3	649.7±71.4	60.9±11.0	31.5±7.7	32.0±7.2	6.6±1.9	3.0±1.1	20.7±3.0
P-value		Treatment	<0.001	<0.001	0.003	0.001	<0.001	0.062	0.795	0.573
		Year	<0.001	0.130	0.197	<0.001	0.002	<0.001	0.077	0.136
		Treatment × Year	0.492	0.274	0.010	0.052	0.494	0.302	0.387	0.115

Table 3.5. Proportion of juice amino acids constituting primary amino nitrogen (%) of cv. 'Golden Russet' apples with different calcium nitrate fertilizer application rates. Values are means ± standard error.

Cultivar	Year	Treatment	Asparagine (mg/L)	Aspartic Acid (mg/L)	Glutamic Acid (mg/L)	Glutamine (mg/L)	Serine (mg/L)	Threonine (mg/L)	Methionine (mg/L)	Other (mg/L)
Golden Russet	2017	Control	66.1±0.3	27.0±0.2	1.7±0.2	0.7±0.2	1.9±0.1	0.9±0.1	0.2±0.0	1.5±0.2
		Low	68.9±0.3	23.9±1.7	1.8±0.4	0.6±0.1	1.8±0.2	0.9±0.1	0.1±0.0	2.0±0.4
		Medium	74.4±1.2	19.9±1.1	1.2±0.1	0.5±0.1	1.7±0.1	0.8±0.1	0.1±0.0	1.3±0.2
		High	72.9±1.4	21.2±1.2	1.1±0.1	0.8±0.1	1.7±0.1	0.7±0.0	0.1±0.0	1.4±0.1
	2018	Control	35.6±0.2	51.8±0.5	2.8±0.3	2.0±0.1	2.3±0.1	0.7±0.0	0.4±0.0	4.5±0.3
		Low	44.3±5.2	42.7±3.8	2.8±0.6	2.5±0.9	2.1±0.1	0.6±0.1	0.4±0.0	4.5±0.4
		Medium	50.9±8.3	39.7±7.6	1.5±0.4	3.2±1.0	1.7±0.4	0.3±0.1	0.2±0.1	2.5±0.5
		High	60.4±4.5	30.5±3.6	2.9±0.8	2.5±0.5	1.7±0.2	0.3±0.0	0.1±0.0	1.6±0.5
P-value		Treatment	<0.001	<0.001	0.372	0.537	0.028	<0.001	<0.001	<0.001
		Year	<0.001	<0.001	0.002	<0.001	0.145	<0.001	<0.001	<0.001
		Treatment × Year	0.021	0.024	0.473	0.756	0.204	<0.001	0.236	<0.001

Table 3.6. Juice amino acid concentrations of cv. 'Medaille d'Or' apples with different calcium nitrate fertilizer application rates. Values are means ± standard error.

Cultivar	Year	Treatment	Asparagine (mg/L)	Aspartic Acid (mg/L)	Glutamic Acid (mg/L)	Glutamine (mg/L)	Serine (mg/L)	Threonine (mg/L)	Other (mg/L)
Medaille d'Or	2017	Control	373.6±120.6	121.4±18.3	89.2±5.2	39.5±9.8	56.2±6.7	24.0±5.0	71.6±4.7
		Low	752.9±91.0	147.7±10.7	73.7±17.2	48.8±15.6	75.6±5.2	21.3±3.5	63.8±13.9
		Medium	1275.9±132.6	169.2±17.5	85.4±8.1	52.4±3.4	107.5±7.6	22.6±1.7	77.9±6.3
		High	1478.0±449.6	202.6±12.3	93.4±11.2	48.9±7.6	101.4±8.8	26.0±2.2	58.3±8.4
	2018	Control	220.7±37.3	145.7±6.6	114.0±5.3	33.3±3.6	41.4±2.3	11.6±3.3	26.0±3.4
		Low	549.4±89.7	146.5±15.1	98.0±7.4	51.2±3.5	52.2±7.1	13.1±1.6	36.9±5.8
		Medium	558.7±184.9	161.2±19.0	120.4±4.1	49.9±7.5	50.0±3.6	11.7±1.3	31.4±1.8
		High	751.1±103.1	190.7±18.0	126.8±6.3	59.3±7.2	62.1±11.2	17.4±1.9	37.5±4.3
P-value		Treatment	<0.001	<0.001	0.056	0.011	<0.001	0.204	0.979
		Year	0.003	0.817	0.001	0.997	<0.001	<0.001	<0.001
		Treatment × Year	0.037	0.208	0.580	0.823	0.060	0.597	0.278

Table 3.7. Proportion of juice amino acids constituting primary amino nitrogen (%) of cv. 'Medaille d'Or' apples with different calcium nitrate fertilizer application rates. Values are means ± standard error.

Cultivar	Year	Treatment	Asparagine (mg/L)	Aspartic Acid (mg/L)	Glutamic Acid (mg/L)	Glutamine (mg/L)	Serine (mg/L)	Threonine (mg/L)	Other (mg/L)
Medaille d'Or	2017	Control	56.0±7.4	10.5±1.6	7.6±1.8	6.1±1.4	6.3±0.8	2.7±2.3	10.8±2.3
		Low	73.3±2.5	7.5±1.2	3.3±0.7	4.2±1.0	4.8±0.5	1.2±1.5	5.8±1.5
		Medium	79.9±2.0	5.4±0.6	2.5±0.3	3.1±0.4	4.3±0.3	0.8±0.6	4.1±0.6
		High	82.2±3.5	4.7±1.2	2.2±0.9	3.0±0.8	4.0±0.5	0.9±0.6	3.0±0.6
	2018	Control	51.0±2.8	17.1±1.2	12.2±1.3	7.1±0.3	6.2±0.5	1.5±0.3	5.0±0.1
		Low	69.1±3.3	9.7±1.5	6.0±1.1	6.2±0.7	4.2±0.2	0.9±0.1	3.9±0.4
		Medium	64.6±6.9	11.5±2.2	8.1±1.9	6.2±0.8	4.7±1.1	1.0±0.3	3.8±0.8
		High	71.8±2.8	9.4±1.0	5.7±0.8	5.2±0.4	3.9±0.7	1.0±0.2	3.1±0.3
P-value		Treatment	<0.001	0.002	0.003	0.017	0.003	0.011	<0.001
		Year	0.036	0.012	0.002	0.002	0.811	0.340	0.024
		Treatment × Year	0.192	0.854	0.721	0.489	0.382	0.059	0.011

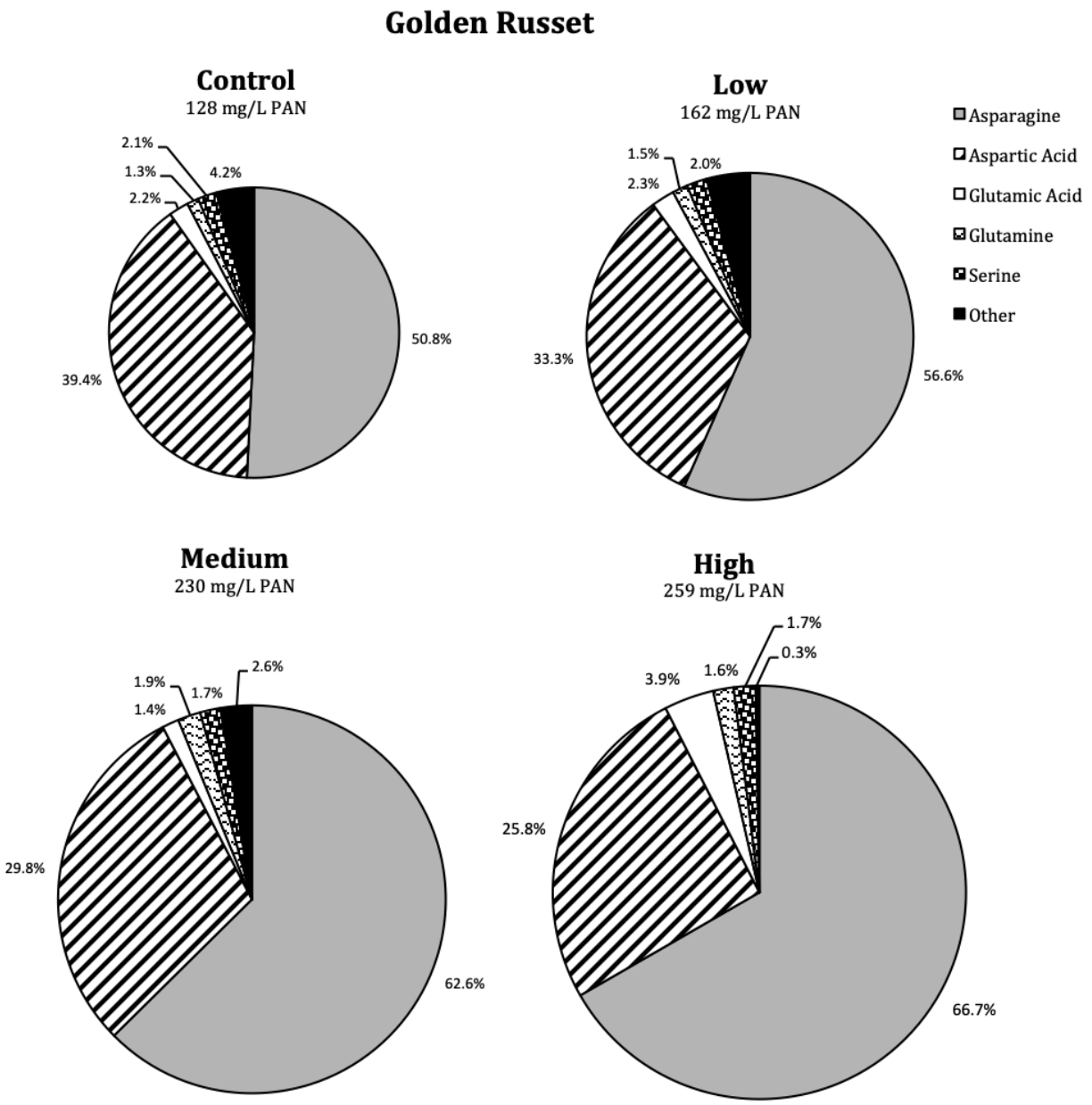

Figure 3.3. Total concentrations of primary amino nitrogen (PAN) and proportions of amino acids constituting PAN of juice from cv. 'Golden Russet' apple trees receiving different rates of calcium nitrate fertilizer quantified via UPLC. Pie chart sizes are to scale of PAN concentration among different treatments. Data represent a combined model from both harvests of the study.

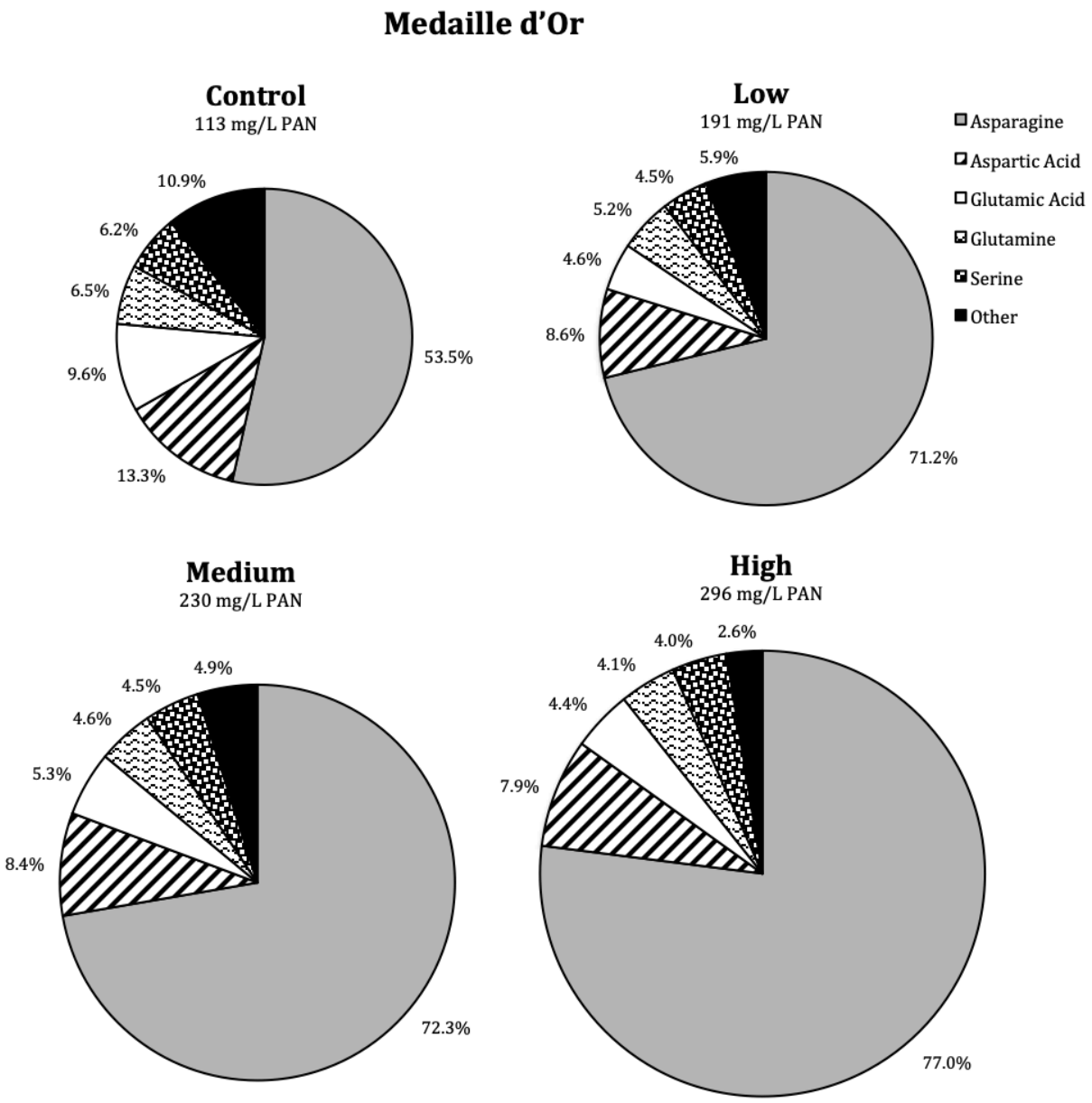

Figure 3.4. Total concentrations of primary amino nitrogen (PAN) and proportions of amino acids constituting PAN of juice from cv. 'Medaille d'Or' apple trees receiving different rates of calcium nitrate fertilizer quantified via UPLC. Pie chart sizes are to scale of PAN concentration among different treatments. Data represent a combined model from both harvests of the study.

Table 3.8. Fermentation characteristics of cv. 'Golden Russet' and 'Medaille d'Or' juice fermented with UCD 522 yeast from tree receiving different rates of calcium nitrate fertilizer. Values are means ± standard error. Lowercase letters indicate a separation of treatments by a Tukey HSD test at a 5% significance level within each cultivar for each year.

Cultivar	Year	Treatment	Hydrogen sulfide production (μg/L)	Maximum fermentation rate (mg sugar/L/hr)	Fermentation duration (days)	Residual sugar (g/L)
Golden Russet	2017	Control	29.6±13.2	480±40	13.6±1.3	0.18±0.11
		Low	4.3±3.1	480±20	14.1±1.3	0.24±0.12
		Medium	6.1±5.5	510±60	14±1.09	0.22±0.12
		High	0.1±0.0	540±30	13.6±0.6	0.11±0.03
Golden Russet	2018	Control	3.0±0.8	282±14	19.9±0.8	0.84±0.33
		Low	1.9±1.3	383±32	17.4±0.9	0.38±0.21
		Medium	3.2±1.5	441±54	15.8±0.8	0.12±0.08
		High	5.8±1.1	652±37	14.3±1.3	0.08±0.06
Medaille d'Or	2018	Control	1.1±0.9	299±30	14.0±1.0	0.30±0.06
		Low	12.1±4.6	426±30	12.5±0.9	0.15±0.06
		Medium	9.9±4.0	414±57	11.8±1.1	0.25±0.11
		High	17.4±5.6	553±24	9.5±0.3	0.11±0.07
P-value		Treatment	0.303	<0.001	<0.001	0.306
		Cultivar	0.058	0.634	0.002	0.336
		Year	0.103	0.014	<0.001	0.062
		Treatment × Cultivar	0.175	0.035	0.489	0.017
		Treatment × Year	0.030	<0.001	<0.001	0.009

Discussion

Nitrogen fertilization has been shown to increase apple tree growth, fruit size, and yield under nitrogen limiting conditions in other studies (Wargo et al., 2003; Cheng and Fuchigami, 2002; Xia et al., 2009; Lea and Beech, 1978). However, while trees receiving more nitrogen fertilizer were larger during the first year of our study, these initial tree size increases did not persist in the following two years. Additionally, nitrogen fertilizer did not affect crop yield, crop load, or fruit mass. Other studies investigating nitrogen fertilization on apple tree growth in similar silt loam soils to those in this current study also found that trees receiving no additional

nitrogen fertilizer were not nitrogen deficient, and growth was not promoted from receiving additional nitrogen fertilizer (Thompson and Peck, 2017). While there was a positive correlation of nitrogen fertilization rate with increasing leaf nitrogen concentration in the final year of the study, none of the trees in this study displayed symptoms of nitrogen deficiency, even though the leaf nitrogen concentrations in the High treatment trees were still below the 2.2% minimum recommended concentration for processing apple trees (Stiles and Reid, 1991). This is perhaps related to poorly drained soils in this orchard. In the early spring, when fertilizer treatments were applied, standing water was sometimes observed. This may have limited nutrient uptake by the roots (Olien, 1989). Furthermore, some of the calcium nitrate granular fertilizer applied in this study may have leached from the field site, further reducing nitrogen uptake by the roots. Nonetheless, enough nitrogen was taken up by the trees to impact juice quality and fermentation dynamics.

Flesh firmness was negatively correlated with nitrogen fertilization rate, and Golden Russet trees receiving more nitrogen fertilizers had greater starch degradation (as measured by SPI), suggesting that the treatments advanced fruit maturity. This observation has been observed in other studies as well (Wargo et al., 2003; Wargo et al., 2004; Raese et al., 2007). Others have attributed the advanced ripening of apples receiving more nitrogen fertilizer to increased ethylene synthesis and cellular respiration (Fallahi, 1997). The greater soluble solid concentrations (SSC) in juice from trees receiving higher nitrogen fertilizer rates were likely from more starch degraded to soluble sugars, than evidence of more carbohydrates in the fruits. Since cider makers often press fruit after allowing all of the starch to be degraded into soluble, fermentable sugars, the SSC and SPI differences we found may not be particularly important to commercial operations.

To our knowledge, this is the first multi-year field study that investigates the impact of ground-applied nitrogen fertilizer on polyphenol concentration in cider apples. In a study by Lea and Beech (1978) three year old cv. 'Dabinett' apple trees were transplanted into pots containing sand and then either nitrogen fertilized or left unfertilized as a control for a single growing season. In this study, juice polyphenol content from the control trees were 17% greater than juice from trees receiving nitrogen fertilizer; however, yield was reduced by 35%. When taking into account the increased polyphenol content and reduced yield, total polyphenol production per tree was reduced in the control compared to the fertilized trees by 25%. This suggests that total polyphenol production on a per-tree basis was actually greater in fertilized trees, but more diluted on a per-apple basis in the larger crop. In our study, over the course of three field seasons, there was no indication that nitrogen fertilization impacted total polyphenol development or concentrations in apples.

The study by Lea and Beech (1978) has been used to support the recommendation that cider apple growers should limit nitrogen fertilization in order to improve juice quality. Tannins, an important class of polyphenols in cider apples, are water soluble phenolic polymers that have the ability to precipitate proteins (Bate-Smith, 1962). In apples, they are comprised of procyanidins, polymers of catechin and epicatechin flavan-3-ol subunits; and greater concentrations are highly desired by cider makers due to the bitter and astringent characteristics they impart to finished ciders (Delage et al., 1991; Lea & Arnold, 1978). Tannins are known to deter herbivory by reducing the digestibility of proteins by binding to them, and can be toxic to insects with high pH guts (Robbins et al., 1987; Barbehenn and Constabel, 2011). Lea and Beech hypothesized that under nitrogen deficient conditions, the trees prioritized increased tannin synthesis to better deter herbivory in nutrient stressed conditions. However, there have been no

studies to confirm this hypothesis and our study suggests a different physiological response to limited nitrogen availability.

Furthermore, we suggest that sufficient nitrogen concentrations during fruit development supports greater photosynthesis (and thus greater carbohydrate availability), which would promote, and not reduce, tannin synthesis. More exposed sections of an apple tree canopy and fruit from trees with a lower crop load, have been reported to have greater catechin and epicatechin concentrations than fruit from more shaded sections of the canopy or from trees with a greater crop load (Feng et al., 2014; Awad et al., 2000). In our study, fruit from both cultivars had greater polyphenol concentrations in 2017 than 2018, which correspond with a lower crop load in 2017 than 2018.

While primary juice chemistry and polyphenol concentrations were not affected by the treatments in our study, increasing nitrogen fertilizer rates did increase YAN concentration. Furthermore, in Golden Russet, the vast majority of YAN, as well as all increases in YAN associated with greater nitrogen fertilization rates, were PAN, that is, in an organic form. In fact, less than 5% of YAN was in the form of ammonia among all treatments and both years for Golden Russet. Other studies have also found ammonia to constitute a small proportion of YAN in apples (Boudreau et al., 2018). Additionally, in Chapter Two of this dissertation, foliar nitrogen fertilization was also found to increase apple YAN concentrations predominantly in the form of PAN.

In both cultivars, asparagine was the most abundant amino acid, and increased proportionally as the nitrogen rates increased. Asparagine has been found to be the most abundant amino acid in many apple cultivars (Ma et al., 2018) and to account for a greater proportion of PAN in trees receiving more nitrogen fertilizer, as well (Cheng et al., 2004). Over

95% of Golden Russet PAN and 85% of Medaille d'Or PAN was asparagine, aspartic acid, glutamic acid, glutamine, and serine. These amino acids have been reported to be preferentially metabolized by acids by *S. cerevisiae* yeast because they require fewer intermediary steps to donate nitrogen in de novo amino acid synthesis (Ljungdahl and Daignan-Fornier, 2012; Waterhouse et al., 2016). The increase in YAN predominantly in these forms has positive implications for cider making because nearly all gains in YAN were in easily utilized and preferred forms by *S. cerevisiae*.

Although specific target YAN concentrations have not been established for cider fermentation, apple juice YAN is typically below the 140 ppm minimum threshold that is often cited as a recommendation for the wine industry (Peck et al., 2016; Boudreau, et al., 2017a; Bell and Henschke, 2005). With the exception of Medaille d'Or Control, juice from both cultivars and all treatments in the first crop in the orchard met this recommended minimum concentration. However, the YAN concentration in 2017 was probably high due to the very light crop on these trees, which has been found to promote greater YAN concentrations (Peck et al., 2016). As the orchard matures, subsequent full crops on the trees would be expected to have lower YAN concentrations similar to those in the second harvest in 2018. In the second harvest, with the exception of the High treatment for Golden Russet, none of the treatments or cultivars met this minimum YAN target. In commercial cider production, exogenous nitrogen supplements would likely be recommended for all of the samples in 2018, but smaller, and less expensive, additions would be advised for juice from trees receiving more nitrogen fertilizers. Using the juice extraction rates and the costs of exogenous YAN supplements from the partial budget in Chapter Two of this dissertation, the increased concentrations of YAN in High treatment juice from Golden Russet compared to the Control in 2018 would save $883/ha when supplementing with

Fermaid O™. Due to lower costs of the other YAN supplements in the model, Fermaid K™ and diammonium phosphate would save $166 and $15/ ha, respectively. However, of the supplements in the model, Fermaid O™ is the only exogenous supplement comprised mainly of PAN, and thus most similarly resembles the composition of apple juice YAN.

Chapter Two of this dissertation investigated the efficacy of using foliar urea applications to increase YAN in a cv. 'Red Spy' orchard. In both studies, nearly all increases in YAN were in the form of PAN, and more specifically, asparagine. Overall, the magnitude of increased YAN was much greater from the foliar urea applications in the fall than calcium nitrate soil applications in the spring. For example, after five urea applications (21.5 kg N/ha total), YAN increased by an average of 319% in comparison to the Control. By comparison, after three years of repeated calcium nitrate applications (112 kg N/ha/year), YAN in the High treatment for Golden Russet was only 88% greater than the Control. Dong et al. (2005) compared urea uptake by apple tree roots and leaves in cv. Fuji/M.9 throughout a growing season and found uptake of applied nitrogen to be lowest by roots in May (11%) and greatest by leaves in September (48%). The low efficacy of nitrogen uptake by roots in the early spring, as well as the poor draining conditions discussed earlier, possibly contributed to YAN increases being much greater for foliar nitrogen applications.

Increased YAN concentrations from treatments with more nitrogen fertilizer had faster, more complete fermentation of reducing sugars, which has been previously documented in both wine and cider literature (Bell and Henschke, 2005; Boudreau et al., 2017a). In 2017, H_2S production in Golden Russet was greater in fermentations from trees receiving less nitrogen fertilizer as well. Hydrogen sulfide production is a common problem in the cider industry and frequently attributed to deficient YAN (Jiranek et al., 1995; Ugliano et al., 2011). While not

statistically different, mean H_2S production rates were greater in treatments receiving more nitrogen fertilizer than those with less in 2018. Hydrogen sulfide production during alcoholic fermentation is a poorly understood phenomenon, and greater H_2S production has been observed in fermentations with greater YAN concentrations in both wine and ciders (Ugliano et al., 2009; (Boudreau et al., 2017a). However, no residual H_2S was detectible in any of the finished ciders in this study. Hydrogen sulfide is sparged from wines and ciders by CO_2, and H_2S production early during fermentation, such as was observed in this study, can result in little or no H_2S in the beverage at the end of fermentation (Ugliano et al., 2009). Additionally, while not measured in this study, greater YAN concentrations have also been shown to increase concentrations of volatile aromatic compounds such as acetate and ethyl esters during wine fermentation (Tahim and Mansfield, 2019; Torrea et al., 2011; Santos et al., 2015). In future studies, sensory evaluation of ciders from trees receiving different rates of nitrogen fertilization could help elucidate consumer detection thresholds and preferences.

Conclusion

For both apple cultivars tested in this experiment, greater calcium nitrate application rates increased juice YAN in the form of PAN, primarily by increasing asparagine. Greater YAN concentrations increased fermentation rates, and sometimes decreased H_2S production during fermentation. Tree size, fruit yield, and other juice quality parameters for cider productions were only minimally impacted by the nitrogen fertilization treatments. Nitrogen fertilizer applications in orchards producing apples specifically for hard cider production could reduce the need for exogenous nitrogen additions after harvest. Within the range of nitrogen concentrations of trees in this study, increased nitrogen fertilization had positive impacts on fruit quality from a

cidermaking perspective, while not having negative impacts on tree physiology or fruit quality. Further research investigating how tree nitrogen statuses greater than the levels reached in this study impacts tree physiology and cider fruit quality would be valuable to help determine target nitrogen levels for cider orchard trees and establish fertilization recommendations for the cider industry.

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CHAPTER FOUR

The Timing, and Influence of Carbohydrate Availability, Light, and Fruit Location Within the Tree Canopy on Polyphenol Synthesis in Apples Used for Hard Cider Production

Abstract

Apples high in polyphenol concentration, especially catechin, epicatechin, and their condensed tannin polymers, are sought after by the hard cider industry due to the bitterness and astringency that they contribute to finished ciders. Polyphenol concentrations vary widely among cultivars and even year-to-year a cultivar within an orchard. However, the factors that influence polyphenol synthesis in cider apples are not well understood. From 2016 to 2018, five separate experiments were conducted to investigate when polyphenols are produced in cider apples, and how carbohydrate availability, light, temperature, and location within the tree canopy on fruit and juice polyphenol concentrations may affect their production. To investigate polyphenol synthesis during fruit development and the influence of reducing carbohydrate availability during the cell division phase of fruit growth, fruit were sequentially harvested at one, three, and five weeks after full bloom (WAFB), and at fruit maturity in 'Dabinett', 'Major', and 'Ellis Bitter' trees in 2018. In 'Dabinett' trees, shade cloth was either placed over whole trees, or trees were left unshaded as a control from 1-5 WAFB. Shade cloth was placed over individual branches of 'Ellis Bitter' and 'Major' branches from 1-3, 3-5, and 1-5 WAFB, or left unshaded as a control. Total polyphenol concentrations increased rapidly between one and three WAFB, were slightly lower at five WAFB and then gradually declined until harvest; fruit mass increased linearly between one WAFB and harvest. The rapid increase in polyphenol concentrations suggest that most polyphenol synthesis occurred during the first five WAFB and these compounds became

more diluted as fruit grew larger. Fruit from unshaded 'Dabinett' trees had 22%, 15%, and 32% greater total polyphenol concentrations than shaded trees at three and five WAFB, and at harvest, respectively. Juice from Control trees has 30% greater polyphenol concentrations compared with juice from shaded trees. Fruit mass was 43 and 64% greater in Control trees than shaded trees at three and five WAFB, respectively, and 11% larger at harvest. In the shaded branches in 'Major' and 'Ellis Bitter', fruit from unshaded branches had 10% greater polyphenol concentrations than shaded branches at three WAFB; at five WAFB and at harvest, the control had 11% greater total polyphenols than the 1-5 shaded treatment. Control juice had 30%, 25%, and 28% greater total polyphenol concentrations than 1-3, 3-5, and 1-5 treatment juice, respectively. To investigate the influence of limited carbohydrate availability on polyphenol synthesis later in the growing season, different opacities of shade cloth, that block 30%, 50%, and 80% of plant assimilable radiation (PAR) were placed over branches of 'Major' and 'Ellis Bitter' trees in 2016, and 'Major' and 'Harry Master's Jersey' trees in 2017, from four WAFB until harvest. Fruit from branches covered in shade cloth that blocks 80% of plant assimilable radiation (PAR) had 26% lower juice total polyphenol concentrations in 2016, but only 6% lower concentrations in 2017 than the control. Fruit mass, peel blush, soluble solid concentration, and titratable acidity were all reduced by shading. To investigate the influence of fruit location within the tree canopy, fruit were harvested from the top, exterior east facing, exterior west facing, and interior sections of tree canopies. Fruit from 'GoldRush' were sampled in 2016, 2017, and 2018; fruit from 'Major' in 2016, 'Harry Master's Jersey' in 2017, and 'Somerset Redstreak' in 2018 were used in this study. Juice from fruit in the top sections of canopies had 20% greater polyphenol concentrations than juice from the east and west sections of the canopy, and 33% more than the interior section of the canopy. Fruit from the interior sections of canopies were smaller, had less red coloration,

and lower soluble solid concentration than fruit from exposed regions of canopies. To determine if the development of polyphenols in cider apples is dependent fruit light exposure, fruit from ‘Ellis Bitter’ and ‘GoldRush’ in 2016, and ‘Major’, ‘Ellis Bitter’, and ‘GoldRush’ in 2017 were covered with opaque paper bags at three WAFB until harvest. There were no differences in total polyphenol concentrations between control and bagged fruit juice in ‘Ellis Bitter’. Juice from bagged ‘Major’ fruit were 27% higher in total polyphenols than that of the control. ‘GoldRush’, control fruit juice had 61% higher total polyphenol concentrations. These investigations provide evidence that the majority of polyphenol synthesis in apple fruit primarily occurs early in fruit development. I hypothesize that during this time period, which coincides with the cell division phase of fruit growth, there is a source sink relationship between carbohydrate availability and polyphenol synthesis. Carbohydrate availability after the early fruit growth phase had a limited effect on polyphenol concentrations at harvest. Additionally, there may be cultivar specific interactions between light exposure and fruit polyphenol synthesis.

Introduction

Even though catechin, epicatechin, and their procyanidin polymers are the most abundant polyphenols in apple fruit and the most important polyphenol class for cider organoleptic properties, relatively little is known about the mechanisms and timing of their synthesis and polymerization (Guyot et al., 2002; Lu and Foo, 1997; Treutter, 2000; Lea and Timberlake, 1974). The flavan-3-ol monomers, catechin and epicatechin, polymerize with catechin initiators and epicatechin elongation units to form procyanidins, also known as condensed tannins (Delage et al., 1991). These compounds defend plant tissue from herbivory by forming peroxides and quinone free radicals in insect digestive tracks with a high pH, or by reducing plant tissue forage

quality by binding to proteins, making them undigestible (Barbehenn and Constabel, 2011; Robbins et al., 1987).

In ciders, flavan-3-ols provide both bitter flavors and astringent mouthfeel. Catechin and epicatechin monomers and polymers under four subunits long have bitter flavors, while longer units do not have a taste or aroma, but provide astringency (Lea and Arnold, 1978). Condensed tannins provide astringency to red wine and cider by binding with and precipitating salivary proteins out of solution. This decreases the lubricating properties of saliva, resulting in the perception of "dryness" by increasing the coefficient of friction in the mouth (Prinz and Lucas, 2000).

All apple cultivars produce catechins, epicatechins, and their polymers in the peel and flesh, but they are typically in very low concentrations in commercial fresh eating and processing cultivars (Thompson-Witrick et al., 2014; Zhang et al., 2010). Flavan-3-ol concentrations are typically under 100 mg/L in apple juice from fresh eating and processing varieties (Kahle et al., 2005). Tannic apples have been selected for hard cider because they possess flavan-3-ol concentrations many fold greater than fresh eating apples. They frequently have flavan-3-ol concentrations that exceed 2 g/L (Guyot et al., 2003). Juice from these cider-specific cultivars is typically blended with juice from other cultivars that provide target levels of sugar, acidity, and tannins in traditional artisanal ciders (Lea and Drilleau, 2003).

While the cider industry in the United States has grown over ten fold since 2005, cider makers report that the supply of high tannin cider-specific cultivars does not meet demand (Brager and Crompton, 2017; Becot et al., 2016). A survey of Virginia cider makers found that two-thirds were willing to pay 50% or more for high tannin cider apples than for culinary varieties, and an economic analysis of New York cider orchards found that under a number of

operational models and horticultural practices, that growing cider-specific apple cultivars can be profitable (Peck et al., 2013; Peck and Knickerbocker, 2018). However, tannic cider cultivars have not been grown in any substantial quantity in the United States, and many of these cultivars possess horticultural traits that pose challenges to their production in commercial orchards (Merwin et al., 2008).

Among the challenges in growing high tannin cider cultivars is a fluctuation in tannin concentrations among growing seasons. This is confounded by a lack of understanding for how and when these compounds are produced, and how management practices may increase catechin, epicatechin, and condensed tannin concentrations in cider apples. A survey of 'Dabinett' and 'Tremlett's Bitter' juice tannin concentrations over a ten-year period in Long Ashton, UK revealed that tannin concentrations fluctuated ±50% among years (Lea, unpublished data). A study of high tannin cider apples grown in coastal and eastern Washington found tannin juice concentrations varied ±25% among the four years of the study (Alexander et al., 2016). In fact, the year effect was a more significant factor in determining tannin concentrations, than geographic region.

Analysis of polyphenol profiles in French cider apples at different stages of development suggests that most flavan-3-ol synthesis in cider apples occurs during the first six weeks of fruit development, during the cell division phase of fruit growth (Renard et al., 2007). Studies of other apple cultivars have also found that the majority of polyphenol synthesis in apple fruit occurs during the early stages of fruit development (Zhang et al., 2010; Ju et al., 1995; Henry-Kirk et al., 2012). While some polyphenols, such as anthocyanins, flavonols, and hydroxycinnamic acids, are influenced by light exposure to fruit, flavan-3-ol synthesis does not appear to be as sensitive to light exposure (Chen et al., 2012; Takos et al., 2006; Ju et al., 1997; Awad et al.,

2000). Additionally, while some of the enzymes involved in flavan-3-ol metabolic pathway are light sensitive, their concentrations have not been found to be rate limiting for the synthesis of flavonoids and anthocyanidins during the 60 days after full bloom, when most flavan-3-ol synthesis occurs (Ju et al., 1997; Ju et al., 1995; Lister et al., 1996a). For example, adjusting crop load 50 days after full bloom has been found not to influence total polyphenol concentrations in apples (Peck et al., 2016). Additionally, varying the harvest date by four weeks at the end of fruit development was not found to influence total polyphenol concentration in apples (Ewing et al., 2019).

In order to investigate when polyphenols are produced in cider apples, and the influence of carbohydrate availability, light, temperature, and location within the tree canopy on fruit and juice polyphenol concentrations, five separate experiments were conducted between 2016 and 2018. To investigate polyphenol synthesis during fruit development and the influence of reducing carbohydrate availability during the cell division phase of fruit growth, fruit were sequentially harvested in 'Dabinett', 'Major', and 'Ellis Bitter' trees in 2018. In 'Dabinett' trees shade cloth was either placed over whole trees, or trees were left unshaded as a control from 1-5 weeks after full bloom (WAFB). Shade cloth was placed over individual branches of 'Ellis Bitter' and 'Major' branches from 1-3, 3-5, and 1-5 WAFB, or left unshaded as a control. Increased shading duration was hypothesized to lower total polyphenol concentrations. To investigate the influence of limited carbohydrate availability on polyphenol synthesis later in the growing season different opacities of shade cloth, that block 30%, 50%, and 80% of plant assimilable radiation (PAR) were placed over branches of 'Major' and 'Ellis Bitter' trees in 2016, and 'Major' and 'Harry Master's Jersey' trees in 2017, from four WAFB until harvest. Fruit from branches with increased shading opacity was hypothesized to have lower total

polyphenol concentrations. To investigate the influence of fruit location within the tree canopy, fruit were harvested from the top, exterior east facing, exterior west facing, and interior sections of tree canopies. Fruit from 'GoldRush' were sampled in 2016, 2017, and 2018; fruit from 'Major' in 2016, ;Harry Master's Jersey' in 2017, and 'Somerset Redstreak' in 2018 were used in this study. Fruit from more exposed sections of the tree canopy were hypothesized to have greater polyphenol concentrations. To determine if the development of polyphenols in cider apples is dependent fruit light exposure, fruit from 'Ellis Bitter' and 'GoldRush' in 2016, and 'Major', 'Ellis Bitter', and 'GoldRush' in 2017 were covered with opaque paper bags at three WAFB until harvest. Bagging was not hypothesized to influence total polyphenol concentrations.

Materials and Methods

Orchard Site

These studies were conducted in 2016, 2017, and 2018 at a Cornell University Agricultural Experiment Station research orchard in Lansing, NY (42.570056, -76.594836) on Hudson-Cayuga silt loam soils with between a 2 and 20º slope (Soil Survey Staff, 2014). The trees, *Malus ×domestica* Borkh., were trained as a vertical axis and managed using standard pest control and pruning practices for the region (Agnello et al., 2019). Tree rows were aligned in a north/south orientation. The cultivars 'Ellis Bitter', 'Major', 'Dabinett', and 'Harry Master's Jersey' used in the Early Branch Shading, Early Tree Shading, and Late Branch Shading experiments were grafted onto 'Malling 9' (M.9) rootstock, and planted in 2003 with 1.8 m between trees and 3.7 m between rows. The cultivars 'Major' and 'Harry Master's Jersey' used in the Fruit Location study and 'Major' and 'Ellis Bitter' in the Fruit Bagging study were grafted onto 'Geneva® 30' (G.30) rootstock and planted in 2003 with 2 m between trees and 3.7 m

between rows; the cultivar ‘Somerset Redstreak’ used in the same study was grafted onto ‘M.9’ rootstock, planted in 2003 with 1.8 m between trees and 3.7 m between rows. The ‘GoldRush’ trees used in the “Fruit Location” and “Fruit Bagging” study were grafted on ‘M.7’ rootstock and planted in 1992 with 2.4 m between trees and 4.6 m between rows.

Experiment Design “Early Tree Shading”

One week after full bloom (WAFB) in the spring of 2018, the fruit on 12 ‘Dabinett’ trees were thinned to a single king bloom per flowering cluster. Six of the trees were randomly chosen and covered with black polyethylene plastic woven shade cloth that blocked 60% of PAR for four weeks, between the periods of one and five WAFB. The other six trees were left as a control. Ten fruit samples were collected between 1-2 m in height at weeks one, three, and five after full bloom, and at harvest. An additional ten fruit from each control tree were also harvested at weeks seven, nine, and 12 after harvest. Full bloom was observed on 23 May 2018, and control and shade treatments established on 30 May. Plant assimilable radiation (PAR) was measured within each treatment three weeks after full bloom, as described below.

Experiment Design “Early Branch Shading”

This study was a randomized complete block design, with seven ‘Ellis Bitter’ and eight ‘Major’ single-tree blocks. In the spring of 2018, four branches per tree between 1-2 m in height were thinned to 10 fruit per cm^2 branch cross-section area one WAFB. One of four shading treatments was then randomly assigned to each of these branches; each tree contained all four treatments. Black polyethylene plastic woven shade cloth that blocks 60% PAR was draped and fastened onto branches for the period of 1-3, 3-5, and 1-5 WAFB (Figure 4.1). There was also an

unshaded control. Fruit samples were collected at one, three, and five WAFB. Ten fruit per experimental unit were harvested at the end of one WAFB, and four fruit per experimental unit were harvested at the end of three and five WAFB, and at harvest. Additionally, four fruit samples from non-treatment branches between 1-2 m in height from each tree were sampled at seven, nine, and 12 WAFB to serve as reference points for polyphenol concentrations and fruit growth through the season. Full bloom was observed on 'Major' on 19 May 2018 and shading treatments started on 26 May. Full bloom was observed on 'Ellis Bitter' on May 21 2018 and shading treatments started on May 28. Canopy exposure PAR availability was measured within treatments during weeks two and four after full bloom.

Figure 4.1 Shading periods and fruit sampling times of different treatments on cv. 'Ellis Bitter' and 'Major' apple tree branches grown in Lansing, NY in 2018.

Experiment Design "Late Branch Shading"

In the spring of 2016 and 2017 four galvanized steel wire cubic enclosures measuring 30.5 cm on each side were fastened to the ends of branches in the top 2 m of the tree canopies. This study was a randomized complete block design with shade enclosures installed in eight 'Ellis Bitter' and eight 'Major' trees in 2016, and eight 'Major' and eight 'Harry Master's Jersey' trees in 2017. One of four shading treatments was then randomly assigned to each of these

branches; each tree had one branch of each treatment. Four weeks after full bloom, black polyethylene plastic woven shade cloth that blocks 30%, 50%, and 80% of PAR were draped over and then affixed to the enclosures to establish Low, Medium, and High shading treatments. One enclosure was left uncovered as a control. The shade cloth was left on the enclosures until harvest. Full bloom was observed on 17 May 2016 on both 'Ellis Bitter' and 'Major' and treatments were established on 13 June 2016. Full bloom was observed on 18 May 2017 in 'Major' and 'Harry Master's Jersey'. Treatments were established on 15 June. In 2017, temperature dataloggers were placed within the enclosures in four 'Major' and four 'Harry Master's Jersey' trees when shade cloth treatments were implemented, and recorded temperature once an hour until harvest. Canopy exposure PAR availability was measured within treatments monthly until harvest. The number of leaves within each enclosure were counted after harvest.

Experimental Design "Fruit Location"

During the growing seasons of 2016, 2017, and 2018, fruit growth, quality, and maturity in different locations within apple tree canopies were characterized. The East and West portions of the canopy were defined as the exterior canopy 1-3 m above the ground on the respective east and west sides of orchard rows. The Interior was characterized as the shaded area in proximity to the main trunk 1-3 m above the ground, and the Top zone was characterized as the exposed area of the canopy in the top 1 m of the tree. This study was a randomized complete block design, with eight trees of two cultivars (n=16) used in each year. 'GoldRush' trees were used in all three years; 'Major' was used in 2016, 'Harry Master's Jersey' in 2017, and 'Somerset Redstreak' was used in 2018. Different cultivars were used based on the trees having sufficient crop load, but this also allowed testing these treatments on multiple genotypes.

Canopy exposure PAR availability was measured in the East, West, Interior, and Top portions of eight trees from two cultivars in each year of the study once per month from June through harvest. In 2017, temperature dataloggers were placed within the treatment areas in four 'GoldRush' and four 'Harry Master's Jersey' trees one WAFB, and recorded temperature once an hour until harvest. At harvest, 10 apples were harvested from the East, West, Internal, and Top treatments of each tree.

Experimental Design "Fruit Bagging"

This study was conducted in 2016 and 2017 in a randomized complete block design with five 'Major', five 'Ellis Bitter', and five 'GoldRush' trees in each year of the study; different trees were used in each year of the experiment. In 2016, 'Major' trees were discarded from the study due to tree death and drought stress. On each tree, opaque paper bags containing a red, black, and brown layer were placed around ten fruit bearing spurs in exposed positions within the tree canopy between 1-3 m above the ground three WAFB. The bags were left in place until harvest. Fruit from the closest adjacent spur to the bagged apple was harvested on the same day to serve as a control.

Early Shading Fruit Measurements, Cortex Freeze Drying, and Polyphenol Extraction

All fruit samples were weighed, and diameter measured. Week one fruit samples were too small to peel and were immersed in liquid nitrogen before being stored at -80 ºC. All other samples were peeled and cortex tissue separated from the core and immediately immersed in liquid nitrogen before being stored at -80 ºC. Week one and cortex tissue samples were then placed in a VirTis Freezemobile 6 (Gardiner, NY) until fully lyophilized. Samples were then

ground in an IKA model A11 electric grinder (Staufen, Germany) to produce a fine powder. Ground tissue (100 mg) was mixed with 4 ml solution of 70% methanol and 0.1% acetic acid to extract polyphenols from tissue samples. The tissue and methanol solution were obscured with an opaque cover and mixed on an orbital shaker at 60 rpm for 15 hours. The solution was then centrifuged at 500 ×g for 5 minutes and the supernatant separated from the pellet.

Plant Assimilable Radiation Measurements

Canopy exposure PAR availability was measured in all studies, except for Fruit Bagging, with a Decagon LP80 ceptometer (Pullman, WA). Measurements were taken between 1200-1400 hr in full sun conditions. The ceptometer consists of an 84 cm light probe containing 80 photosensors placed in the tree canopy and an external photosensor positioned in full sun exposure. Ten simultaneous PAR measurements were taken in rapid succession and averaged to provide a ratio between PAR concentrations in full sun and within different treatments in the tree canopy.

For the Early Tree Shading study, the photosensor was held 1 m away from the trunk and 1.7 m above the ground parallel to the tree row on the east and west side of the tree row; east and west side values were averaged to calculate a single canopy exposure PAR availability value per experimental unit. For the Fruit Location study, the photosensor was held 1 m away from the trunk and 1.7 meters above the ground parallel to the tree row on the east and west side of rows for East and West measurements, respectively. The photosensor was held 1.7 m from the ground and against the trunk for Interior measurements, and approximately 1 m below the top of the leader for Top measurements. For the Early Branch Shading and Late Branch Shading studies, only the terminal 10.5 cm of the 84 cm long light probe containing ten photosensors were

activated in order to characterize the PAR environment within the targeted treatment area. This section of the probe was placed above the foliage under the shade cloth and held level to take PAR measurements.

Gas Exchange Measurements

A LI-COR lixt 6400 portable photosynthesis gas exchange system (Lincoln, NE) was used to measure differences in photosynthesis rates on the control and shaded 'Dabinett' trees from the Early Tree Shading experiment on 20 June 2018. Gas exchange rates were measured on three healthy exposed leaves on both the east and west sides of the canopy. The CO_2 flow rate was set to 400 µmol/s stomatal ratio to 0.5, and stomatal leaf area to 2 cm^2. A 2:1 ratio of blue to red light photons were emitted from the instrument lamp when taking measurements. To simulate PAR availability under control and treatment shade light conditions, 1,500 µmol/m^2/s of photons were emitted while taking measurements on control treatment leaves, and 500 µmol µmol/m^2/s of photons were emitted while taking measurements on shade treatment leaves.

Temperature Datalogging

The temperature dataloggers deployed during the 2017 field season in the Late Branch and Fruit Location studies, Thermocron DS1921G-F5# (iButton Link LLC, Whitewater, WI), were hung inside a piece of PVC pipe to prevent contact of the datalogger with direct sunlight. Growing degree days (GDD) base 10 °C was calculated for 4-6 WAFB, and from four WAFB until harvest for each datalogger in the Early Branch experiment. Growing degree days (GDD) base 10 °C was calculated for 1-6 WAFB and from one WAFB until harvest in the Fruit Location study.

Harvest, Fruit Measurements, and Juicing

Harvest dates can be found in table 4.1. In the Early Branch Shading experiment four fruit were harvested from each experimental unit on sampling dates to provide sufficient fruit throughout the study; in all other studies ten-fruit samples were harvested. In the Late Branch Shading experiment, total yield was measured from each experimental unit before a ten-fruit subsample was taken to measure fruit and juice attributes. Fruit were measured for mass, percent peel blush, starch pattern index (SPI), and flesh firmness. Peel blush was visually approximated as the area of the fruit peel with red coloration, except for GoldRush, which was graded on a greenness scale of 1 (yellow) to 5 (green) using the Cornell McIntosh apple color chart (Simons, 1948). Starch pattern index (SPI) was rated on a 0-8 point scale, with 1=0% starch degradation and 8=100% starch degradation (Blanpied and Silsby, 1992). The SPI was also used to determine harvest date, with a target value of at least 6, with approximately 60% starch degradation. For the Late Branch Shading and Fruit Location studies, flesh firmness, after removing the peel, was measured on both the sun and the shade exposed side of each fruit along the equator with a penetrometer (Güss GS Fruit Texture Analyzer, Strand, South Africa) fitted with an 11.1 mm tip. During the 2017 and 2018 harvests for all experiments, chlorophyll a content was measured on a 0-3 point index with a Turoni 53500 DA meter (Forli, Italy) on the sun and the shade side of each apple along the equator.

Fruit from each experimental unit were milled and pressed with a Norwalk 280 juicer (Bentonville, AR) to make a juice sample. Soluble solid concentration, pH, titratable acidity (TA), and total polyphenol concentrations (via the Folin Ciocalteu assay) were measured for each juice sample. Soluble solid concentration was measured with an Atago PAL-1 digital refractometer (Tokyo, Japan) and reported as °Brix. Juice pH and TA were measured with an

automatic titrator [Metrohm Unitrode pH meter, 778 sample processor, and 800 Dosino dosing device (Herisau, Switzerland)]. A 5 mL juice sample was titrated against a 0.1 M NaOH solution to an endpoint of pH 8.2 and expressed as g/L of malic acid equivalents.

Total polyphenols were measured with the Folin Ciocalteu assay in a 96 well microplate at λ 765 nm as described in Singleton and Rossi (1965). Folin Ciocalteu's phenol reagent, sodium bicarbonate, and gallic acid were supplied by Sigma-Aldrich (St. Louis, MO). For the assay, eight standards ranging from zero to three g/L of gallic acid were analyzed on each plate to generate a standard curve. On 96 well microplates, 34.9 µL of deionized water and 1.5 µL of juice, tissue extract, or gallic acid standard were mixed in individual wells. Mixed into each well was 90.9 µL of 0.2 N Folin Ciocalteu reagent. The water, sample, and Folin Ciocalteu reagent were left to incubate between six and eight minutes before 72.6 µL of a 7% weight/volume solution of sodium carbonate was mixed into each well. One hour after the addition of the sodium carbonate solution, the absorbance at λ 765 nm was read on a Molecular Devices (San Jose, CA) SpectraMax Plus 284 spectrophotometer. Total polyphenol concentrations, measured as gallic acid equivalents, were calculated from the standard curve generated from each plate.

Table 4.1. Harvest dates of experiments in different cultivars and years within this study.

Experiment	Year	Cultivar	Harvest Date
Early Tree	2018	Dabinett	10 October
Early Branch	2018	Ellis Bitter	30 August
		Major	3 September
Late Branch	2016	Major	1 September
		Ellis Bitter	31 August
	2017	Major	5 September
		Harry Master's Jersey	21 September
Fruit Location	2016	GoldRush	1 November
		Major	1 September
	2017	GoldRush	1 November
		Harry Master's Jersey	15 September
	2018	GoldRush	30 October
		Somerset Redstreak	18 September
Fruit Bagging	2016	GoldRush	1 November
		Major	1 September
		Ellis Bitter	31 August
	2017	GoldRush	1 November
		Major	5 September
		Ellis Bitter	3 September

Statistical Analysis

Data in this chapter were compared using a linear mixed effects model. Treatments were considered significant at $P \leq 0.05$. The Tukey HSD method was used for post-hoc mean separation testing in the Early Branch Shading, Fruit Location, and Fruit Bagging experiments. In the Late Branch Shading experiment, degree of shade cloth opacity was included as a continuous variable in statistical analysis. Treatment, cultivar, year, treatment × cultivar, and treatment × year were included as fixed effects. Block was included within the model as a random variable. A logit transformation of peel blush and canopy exposure PAR percentage data

was performed prior to analysis, but presented as untransformed data. Data were analyzed using JMP Pro version 14 (SAS Institute, Cary, NC).

Results

Light Environment and Photosynthesis Rates in Early Shading Experiments

When shade cloth was placed over trees in the Early Tree Shading experiment, canopy exposure of plant assimilable radiation (PAR) was reduced by 71% and net photosynthetic rate was reduced by 38% in comparison with the Control (Table 4.2). Two weeks after full bloom (WAFB) of the Early Branch Shading experiment, unshaded treatments (Control and 3-5 WAFB) had 62% more PAR exposure than the 1-3 and 1-5 WAFB treatments. At four WAFB, unshaded treatments (Control and 1-3 WAFB) had 69% more exposure of PAR than shaded treatments (3-5 and 1-5 WAFB) (Table 4.3).

Table 4.2 Percentage of plant assimilable radiation (PAR) permeating the canopy and shadecloth, and photosynthesis rate of Control leaves exposed to 1,500 μmol/m^2/s of photons and Shade leaves exposed to 500 μmol/m^2/s of photons in cv. 'Dabinett' apple trees grown in Lansing, NY. Treatment trees were shaded during weeks 1-5 after full bloom. Values are means ± standard error (n=6).

Treatment	Canopy Exposure PAR (%)	Photosynthesis Rate (μmol CO_2 /m^2/s)
Control	47.9±3.9	20.9±0.5
Shade	13.8±1.1	12.9±0.5
P-value	<0.001	<0.001

Table 4.3 Percentage of plant assimilable radiation (PAR) permeating the canopy and shadecloth two and four weeks after full bloom (WAFB) from cv 'Ellis Bitter' and 'Major' apple trees grown in Lansing, NY. Branches on individual trees were subjected to different shading treatments during weeks 1-5 after full bloom. Values are means ± standard error ('Ellis Bitter' n=7, 'Major' n=8). Different lowercase letters indicate a separation of treatments by the Tukey HSD method at a 5% significance level.

Cultivar	Treatment	Canopy Exposure PAR Availability (%)	
		Week 2	Week 4
Ellis Bitter	Control	59.1±3.7 a	65.6±2.5 a
Major		53.1±6.0	58.0±5.3
Ellis Bitter	1-3 WAFB	22.6±2.0 b	62.5±3.1 a
Major		20.3±1.4	54.5±4.7
Ellis Bitter	3-5 WAFB	51.7±4.1 a	20.7±1.7 b
Major		49.6±2.8	18.7±1.7
Ellis Bitter	1-5 WAFB	20.1±1.6 b	18.0±2.6 b
Major		17.6±2.1	18.3±1.3
P-value	Treatment	<0.001	<0.001
	Cultivar	0.677	0.077
	Treatment × Cultivar	0.904	0.263

Fruit Development and Total Polyphenol Concentrations in Early Branch and Tree Shading Experiments

Total polyphenol concentrations increased rapidly between one and three WAFB and then gradually decreased in concentration until harvest (Figures 4.2 and 4.3, Tables 4.4 and 4.5). In the Early Tree Shading experiment, between one and three WAFB, Control fruit increased in total polyphenol concentrations by 86%; Early Branch Shading Control fruit increased in total polyphenol concentrations by 141% during the same period. At five WAFB, Early Tree Control fruit total polyphenol concentrations were 15% lower than at three WAFB, and Early Branch Control fruit total polyphenol concentrations were reduced by 30%. By harvest at 20 WAFB, Early Tree Control fruit total polyphenol concentrations were reduced by 89% from their

concentration at five WAFB; Early Branch Control fruit total polyphenol concentrations were reduced by 77% at harvest, which was at 14 WAFB.

Fruit mass increased rapidly between one and three WAFB. Early Tree Control fruit had a 16-fold increase in mass during this period and Early Branch Control fruit increased in size 19-fold (Tables 4.6 and 4.7). Fruit mass in Control fruit increased between three and five WAFB by 157% and 215% in the Early Tree and Early Branch shading experiments, respectively. Between five WAFB and harvest at 20 WAFB, fruit mass increased by 400% in the Early Tree Shading Experiment. Fruit mass increased by 542% in Early Branch Control fruit between 5 WAFB and harvest at 14 WAFB.

In both the Early Tree and Early Branch Shading experiments, at one WAFB (before shading treatments were implemented) there was no difference among treatments in fruit total polyphenol concentrations (Tables 4.4 and 4.5). At three WAFB, Control fruit had 22% greater total polyphenol concentrations than the Shade treatment in the Early Tree Shading experiment. In the Early Branch Shading experiment, unshaded treatments (Control and 3-5) had 10% greater total polyphenol concentrations than shaded treatments (1-3 and 1-5). At five WAFB in the Early Tree Shading experiment, total polyphenol concentrations were 15% greater in the Control than Shade trees. In the Early Branch Shading experiment, Control treatment fruit had total polyphenol concentrations that were 11% greater than in the 1-5 treatment at five WAFB; there were no statistical differences between the Control and the 1-3 or 1-5 treatments. At harvest, Control fruit had 32% greater total polyphenol concentrations than Shade fruit, and Control juice had 30% greater total polyphenols than Shade juice in the Early Tree Shading Experiment. At harvest in the Early Branch Shading experiment, Control fruit had 11% greater total polyphenol concentrations than the 1-5 treatment; there were no differences among the Control, 1-3, or 3-5

treatments. Control juice had 30%, 25%, and 28% greater total polyphenol concentrations than 1-3, 3-5, and 1-5 treatment juice, respectively.

There were no differences in fruit mass among treatments in the Early Tree and Branch Shading experiments at one WAFB. At three WAFB, Control fruit were 43% larger than Shade treatment fruit in the Early Tree experiment. Unshaded fruit (Control and 3-5) were 17% larger than shaded treatments (1-3 and 1-5) in the Early Branch experiment. At five WAFB, Control fruit were 64% larger than Shade fruit in the Early Tree experiment. Control fruit were 20%, 28%, and 32% larger than 1-3, 3-5, and 1-5 treatment fruit in the Early Branch experiment, respectively. At harvest, Control fruit were 11% larger than Shade fruit in the Early Tree Shading experiment. There were no statistical differences in fruit mass in the Early Branch Shading experiment at harvest.

Increased fruit diameter correlated with increased fruit mass during fruit growth and maturation (Tables 4.8 and 4.9). There were no differences in fruit diameter among treatments at one WAFB in either Early Tree or Branch Shading experiments. At three WAFB Control fruit were 14% wider than Shade fruit in the Early Tree Shading experiment. In the Early Branch experiment 3-5 treatment fruit were 11% and 10% wider than 1-3 and 1-5 treatment fruit, respectively. At five WAFB Control fruit were 20% wider than Shade fruit. In the Early Branch Shading experiment, Control fruit were 8%, 10%, and 11% wider than 1-3, 3-5, and 1-5 treatment fruit, respectively. Fruit diameter at harvest was not different among the treatments in either experiment.

Table 4.4 Total polyphenol concentrations (gallic acid equivalents) of dried fruit cortex tissue at different stages of development and juice from cv 'Dabinett' apple trees grown in Lansing, NY. Trees were either shaded or un-shaded during weeks 1-5 after full bloom. Values are means ± standard error (n=6).

	Total Polyphenols Flesh (mg/g)							Juice (g/L)
Treatment	Week 1	Week 3	Week 5	Week 7	Week 9	Week 12	Week 20 (Harvest)	Week 20 (Harvest)
Control	60.90±1.92	113.30±2.82	96.28±2.02	51.44±3.75	39.54±0.71	27.41±0.94	11.02±0.3	1.28±0.1
Shade	66.3.0±2.17	93.00±2.62	83.71±1.63	-	-	-	8.36±0.45	0.99±0.07
P-value	0.150	0.002	0.003	-	-	-	0.005	0.047

Table 4.5 Total polyphenol concentrations (gallic acid equivalents) of dried fruit cortex tissue at different stages of development and juice from cv 'Ellis Bitter' and 'Major' apple trees grown in Lansing, NY. Branches on individual trees were subjected to different shading treatments during in the five weeks after full bloom (WAFB). Values are means ± standard error ('Ellis Bitter' n=7, 'Major' n=8). Different lowercase letters indicate a mean separation among treatments by the Tukey HSD method at a 5% significance level.

		Total Polyphenols Flesh (mg/g)							Juice (g/L)
Cultivar	Treatment	Week 1	Week 3	Week 5	Week 7	Week 9	Week 12	Week 14 (Harvest)	Week 14 (Harvest)
Ellis Bitter	Control	62.48±4.29	125.12±2.52 a	80.00±3.70 a	61.79±1.63	45.77±0.83	30.86±1.8	22.14±0.98 a	0.76±0.05 a
Major		45.9±2.80	136.38±1.73	102.29±1.44	55.36±1.19	39.51±0.6	25.24±0.57	19.75±0.88	1.07±0.09
Ellis Bitter	1-3 WAFB	63.89±4.67	115.09±3.46 b	73.8±1.42 ab	-	-	-	22.57±0.73 ab	0.66±0.04 b
Major		43.42±2.87	127.88±5.69	101.62±3.46				19.25±0.55	0.75±0.07
Ellis Bitter	3-5 WAFB	66.7±3.72	126.89±2.17 a	73.21±1.07 ab	-	-	-	20.03±0.69 ab	0.65±0.07 b
Major		39.74±3.13	138.72±2.67	100.30±2.14				19.8±0.98	0.82±0.06
Ellis Bitter	1-5 WAFB	56.63±4.73	114.01±1.99 b	71.72±2.80 b	-	-	-	20.29±0.54 b	0.59±0.08 b
Major		40.73±2.66	124.34±3.72	93.18±2.72				17.34±0.95	0.83±0.15
P-value	Treatment	0.126	<0.001	0.007	-	-	-	0.030	0.012
	Cultivar	0.004	0.004	<0.001	-	-	-	0.005	0.034
	Treatment × Cultivar	0.124	0.974	0.423	-	-	-	0.214	0.470

Table 4.6 Mass of fruit at different stages of development from 'Dabinett' apple trees grown in Lansing, NY. Trees were either shaded or un-shaded during weeks 1-5 after full bloom. Values are means ± standard error (n=6).

	Mass (g)						
Treatment	Week 1	Week 3	Week 5	Week 7	Week 9	Week 12	Week 20 (Harvest)
Control	0.29±0.02	4.94±0.22	12.7±0.19	15.83±0.55	29.19±0.9	42±0.94	63.46±2.25
Shade	0.3±0.02	3.47±0.24	7.78±0.23				56.84±1.65
P-value	0.457	0.001	<0.001	-	-	-	0.039

Table 4.7 Mass of fruit at different stages of development from 'Ellis Bitter' and 'Major' apple trees grown in Lansing, NY. Branches on individual trees were subjected to different shading treatments during weeks 1-5 after full bloom. Values are means ± standard error ('Ellis Bitter' n=7, 'Major' n=8). Different lowercase letters indicate a separation of treatments by the Tukey HSD method at a 5% significance level.

		Mass (g)						
Cultivar	Treatment	Week 1	Week 3	Week 5	Week 7	Week 9	Week 12	Week 14 (Harvest)
Ellis Bitter	Control	0.30±0.03	6.21±0.55 ab	16.73±0.62 a	19.66±0.69	33.32±1.2	48.81±1.85	93.25±3.16
Major		0.12±0.01	2.16±0.28	9.58±0.45	14.07±0.32	21.78±1.07	46.78±1.04	76.27±2.95
Ellis Bitter	1-3	0.28±0.02	4.91±0.49 b	13.77±1.26 b	-	-	-	89.71±4.32
Major		0.12±0.01	2.04±0.19	8.18±0.53				75.2±2.39
Ellis Bitter	3-5	0.31±0.02	6.23±0.27 a	13.21±0.77 b	-	-	-	95.25±4.76
Major		1.3±0.01	2.54±0.25	7.33±0.42				65.6±3.52
Ellis Bitter	1-5	0.29±0.03	5.2±0.19 ab	12.73±1.05 b	-	-	-	86.74±4.88
Major		0.13±0.01	2.03±0.23	7.26±0.56				72.92±2.2
P-value	Treatment	0.551	0.001	<0.001	-	-	-	0.518
	Cultivar	<0.001	<0.001	<0.001	-	-	-	<0.001
	Treatment × Cultivar	0.679	0.212	0.575	-	-	-	0.114

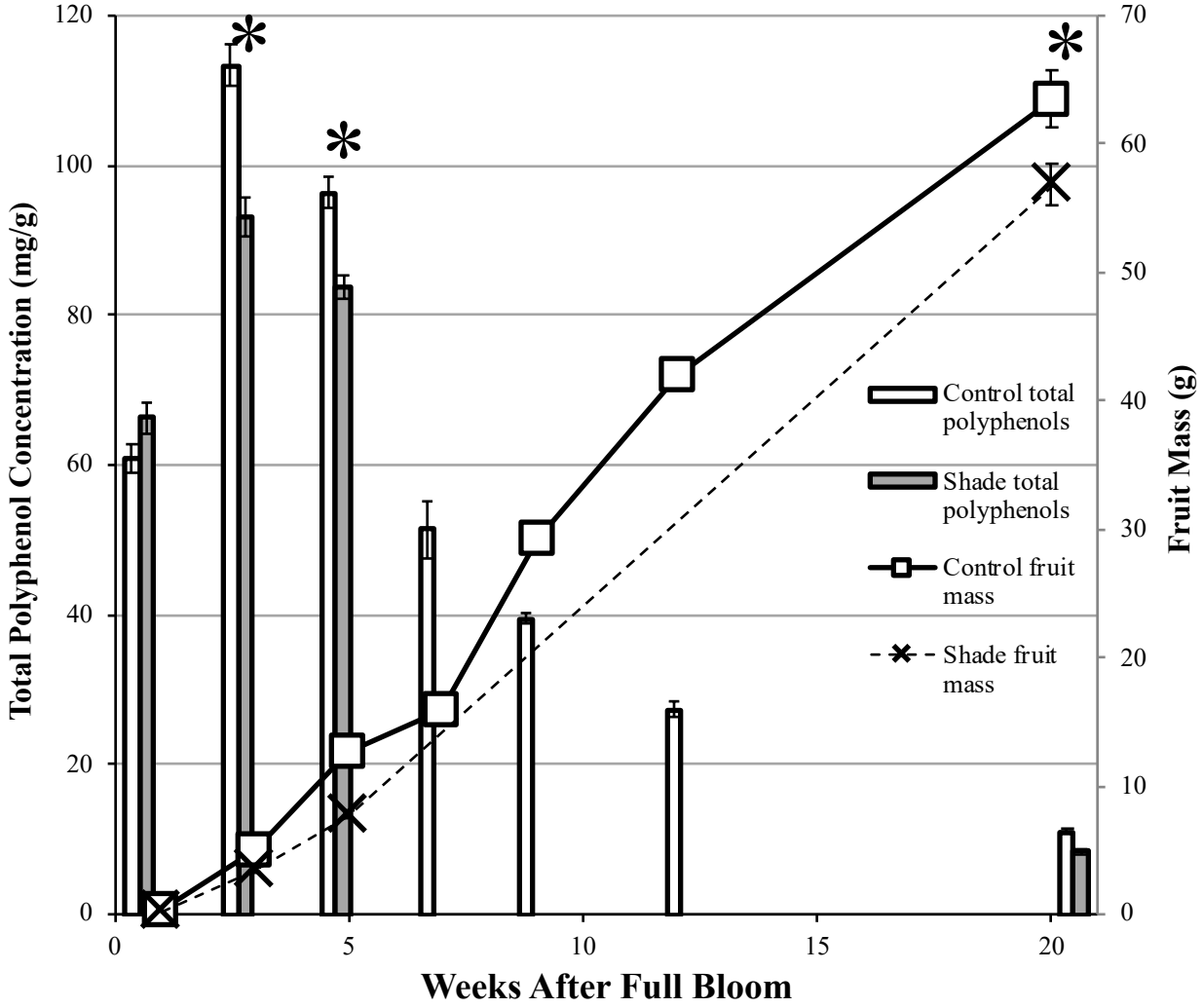

Figure 4.2 Total polyphenol concentrations (gallic acid equivalents) of dried fruit cortex tissue and fruit mass at different stages of development of 'Dabinett' apple trees grown in Lansing, NY. Trees were either shaded or un-shaded during weeks 1-5 after full bloom. Values are means ± standard error (n=6). Asterisks represent statistically significantly differences for both total polyphenol concentrations and mass between treatments in a categorical mixed effects model at a 5% significance level.

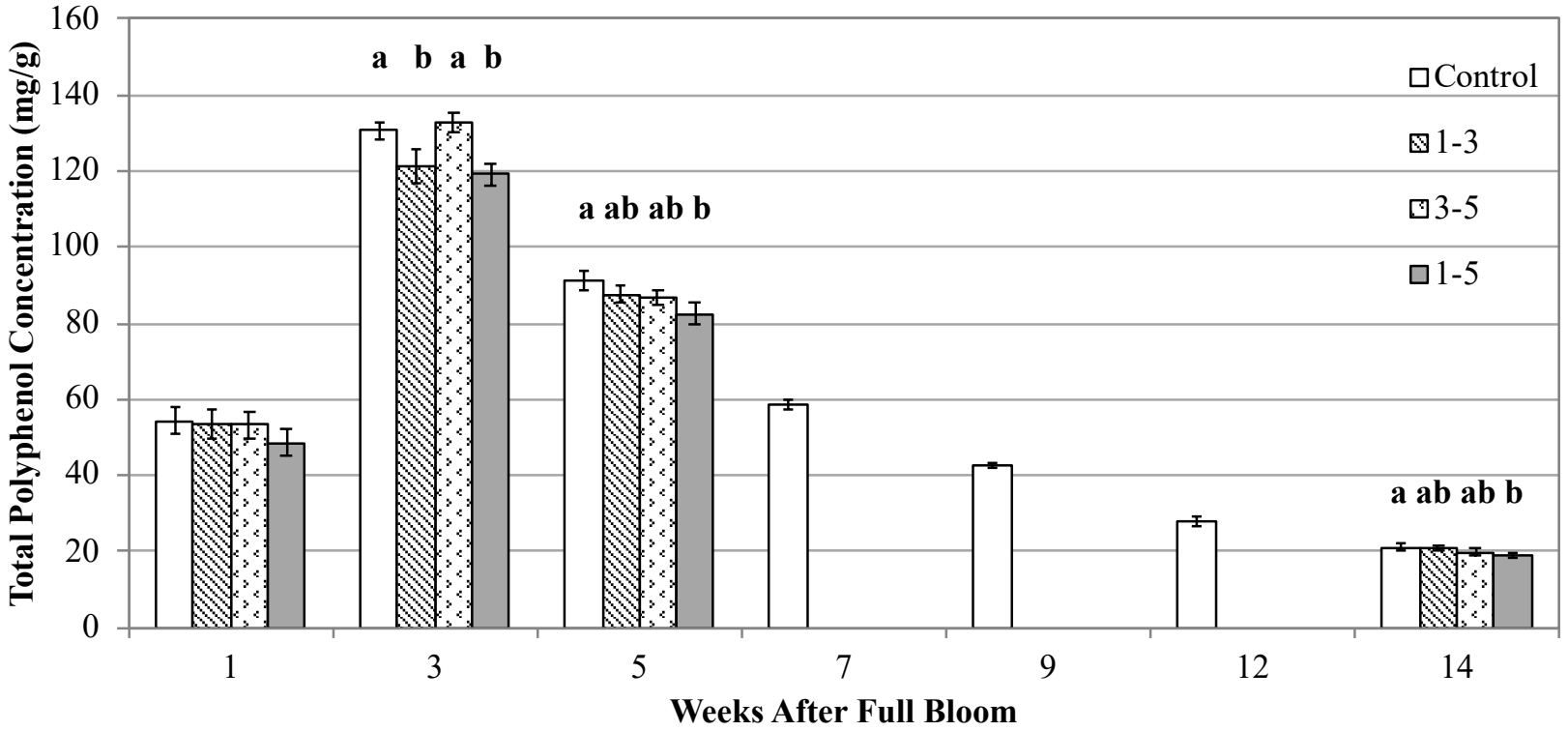

Figure 4.2 Total polyphenol concentrations (gallic acid equivalents) of dried fruit cortex tissue and fruit mass at different stages of development of 'Ellis Bitter' and 'Major' apple trees grown in Lansing, NY. Branches on individual trees were subjected to different shading treatments during weeks 1-5 after flowing. Values are means ± standard error (n= 7 'Ellis Bitter' + 8 'Major'=15). Different lowercase letters indicate a separation of treatments by the Tukey HSD method at a 5% significance level.

Table 4.8 Diameter of fruit at different stages of development from 'Dabinett' apple trees grown in Lansing, NY. Trees were either shaded or un-shaded during weeks 1-5 after full bloom. Values are means ± standard error (n=6).

	Diameter (mm)						
Treatment	Week 1	Week 3	Week 5	Week 7	Week 9	Week 12	Week 20 (Harvest)
Control	6.02±0.15	20.33±0.34	29.47±0.19	31.66±0.38	39.67±0.45	45.96±0.39	51.24±0.73
Shade	6.20±0.21	17.92±0.43	24.54±0.29				49.66±0.5
P-value	0.516	0.001	<0.001	-	-	-	0.102

Table 4.9 Diameter of fruit at different stages of development from 'Ellis Bitter' and 'Major' apple trees grown in Lansing, NY. Branches on individual trees were subjected to different shading treatments during the five weeks after full bloom (WAFB). Values are means ± standard error ('Ellis Bitter' n=7, 'Major' n=8). Different lowercase letters indicate a separation of treatments by the Tukey HSD method at a 5% significance level.

		Diameter (mm)						
Cultivar	Treatment	Week 1	Week 3	Week 5	Week 7	Week 9	Week 12	Week 14 (Harvest)
Ellis Bitter	Control	5.91±0.40	22.23±0.81 ab	32.73±0.63 a	34.84±0.57	41.42±0.49	48.64±0.65	61.37±0.69
Major		3.68±0.25	14.52±0.72	26.53±0.46	30.78±0.27	36.09±0.67	47.85±0.44	56.54±0.76
Ellis Bitter	1-3 WAFB	5.76±0.27	20.09±1.02 b	30.21±1.16 b	-	-	-	61.31±1.17
Major		3.77±0.21	14.39±0.54	25.07±0.68				55.84±0.71
Ellis Bitter	3-5 WAFB	6.13±0.26	22.54±0.38 a	30.02±0.64 b	-	-	-	61.28±0.95
Major		3.80±0.22	15.82±0.52	24.00±0.48				53.00±0.94
Ellis Bitter	1-5 WAFB	5.96±0.37	5.96±0.37 b	29.51±0.88 b	-	-	-	60.31±1.58
Major		3.89±0.57	3.89±0.57	23.83±0.64				55.23±0.53
P-value	Treatment	0.541	0.009	<0.001	-	-	-	0.240
	Cultivar	<0.001	<0.001	<0.001	-	-	-	<0.001
	Treatment × Cultivar	0.706	0.451	0.861	-	-	-	0.253

Fruit and Juice Characteristics in Early Branch and Tree Shading Experiments at Harvest

At harvest, there were no differences in fruit starch pattern index, peel blush, or chlorophyll a content in both the Early Tree and Branch Shading experiments. There were also no differences in juice soluble solid concentration, pH, or titratable acidity (Tables 4.10 and 4.11).

Table 4.10 Fruit and juice characteristics of 'Dabinett' apple trees grown in Lansing, NY. Trees were either shaded or un-shaded during weeks 1-5 after full bloom. Values are means ± standard error (n=6).

Treatment	Starch Pattern Index (1-8)	Peel Blush (%)	Chlorophyll a Index	Soluble Solids (°Brix)	pH	Titratable Acidity (g malic acid/L)
Control	4.57±0.18	72±2	1.67±0.05	11.0±0.3	4.61±0.10	1.06±0.04
Shade	4.47±0.21	75±2	1.63±0.04	10.4±0.2	4.72±0.10	1.1±0.03
P-value	0.725	0.236	0.6334	0.126	0.082	0.478

Table 4.11 Fruit and juice characteristics of 'Ellis Bitter' and 'Major' apple trees grown in Lansing, NY. Branches on individual trees were subjected to different shading treatments during the five weeks after full bloom (WAFB). Values are means ± standard error ('Ellis Bitter' n=7, 'Major' n=8). Different lowercase letters indicate a separation of treatments by the Tukey HSD method at a 5% significance level.

Cultivar	Treatment	Starch Pattern Index (1-8)	Peel Blush (%)	Chlorophyll a Index	Soluble Solids (°Brix)	pH	Titratable Acidity (g malic acid/L)
Ellis Bitter	Control	6.9±0.4	55±2	1.08±0.04	9.4±0.2	4.36±0.02	1.79±0.04
Major		2.9±0.2	48±3	1.05±0.11	10.5±0.3	4.32±0.03	2.22±0.17
Ellis Bitter	1-3 WAFB	7±0.2	53±2	1.23±0.05	9.0±0.2	4.36±0.03	1.81±0.04
Major		2.9±0.2	42±4	1.16±0.08	9.8±0.2	4.31±0.02	2.22±0.04
Ellis Bitter	3-5 WAFB	6.6±0.28	50±2	1.26±0.06	9.1±0.3	4.38±0.03	1.82±0.04
Major		2.3±0.2	44±4	1.09±0.1	9.7±0.2	4.31±0.02	2.28±0.1
Ellis Bitter	1-5 WAFB	7.2±0.14	48±3	1.32±0.05	8.2±0.3	4.39±0.02	1.76±0.08
Major		2.9±0.2	44±3	1.03±0.11	10.3±0.5	4.34±0.02	2.28±0.11
P-value	Treatment	0.072	0.133	0.115	0.088	0.217	0.951
	Cultivar	<0.001	0.006	0.170	0.001	0.113	<0.001
	Treatment × Cultivar	0.802	0.306	0.069	0.163	0.812	0.884

Light Environment in Late Branch Shading Experiment

There was a linear relationship between treatment shade cloth opacity and canopy exposure of PAR within Late Branch Shading experiment enclosures (Figure 4.3). In the Control treatment, canopy exposure of PAR was 90.7%; Low, Medium, and High treatment branches were exposed to 59%, 46%, and 18% of PAR, respectively.

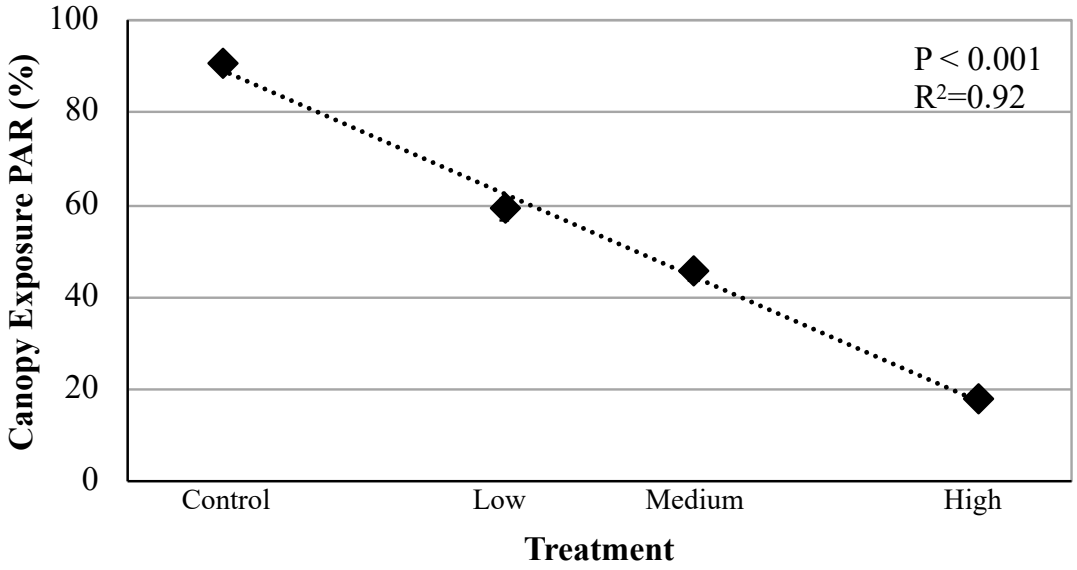

Figure 4.3 Percentage of plant assimilable radiation (PAR) permeating the canopy and shadecloth of 'Major', 'Ellis Bitter', and 'Harry Master's Jersey' apple trees grown in Lansing, NY. Branches on individual trees were subjected to different shading opacities from week four after full bloom until harvest. Values are means ± standard error (n= 8 'Major' 2016, 8 'Ellis Bitter' 2016, 8 'Major' 2017, and 8 'Harry Master's Jersey' 2017=32).

Table 4.12 Percentage of plant assimilable radiation (PAR) permeating the canopy and shadecloth in June, July, and August of 'Major', 'Ellis Bitter', and 'Harry Master's Jersey' apple trees grown in Lansing, NY in 2016 and 2017. Branches on individual trees were subjected to different shading opacities from four weeks after full bloom until harvest. Values are means ± standard error (n= 8 'Major' 2016, 8 'Ellis Bitter' 2016, 8 'Major' 2017, and 8 'Harry Master's Jersey' 2017=32).

Year	Cultivar	Treatment	Canopy Exposure PAR (%)		
			June	July	August
2016	Major	Control	91.5±2.2	90.9±3.2	89.5±3.2
2016	Ellis Bitter		88.8±3.6	86.2±4.9	85.4±4.9
2017	Major		92.7±1.4	90.4±1.9	91±2.2
2017	Harry Master's Jersey		95.3±1	92.9±1.6	94.4±1.9
2016	Major	Low	52.9±3.9	53.5±4.7	53.6±6.4
2016	Ellis Bitter		48.8±4.1	51.8±6.8	43.3±5.9
2017	Major		50.8±1.3	52.2±4.8	58.9±6.3
2017	Harry Master's Jersey		52.2±3.2	60.3±4.4	52.6±2.3
2016	Major	Medium	35.3±3.5	38.5±5.5	35.7±4.8
2016	Ellis Bitter		36.9±3.8	42.7±3.2	33.2±4.7
2017	Major		39.7±1.5	33.8±3.2	30±2.9
2017	Harry Master's Jersey		38.6±1.8	38.4±4.1	36.5±1.9
2016	Major	High	15.5±2.2	13.4±2.5	11.3±2.5
2016	Ellis Bitter		18.1±1.6	16.6±2.5	12.4±3.8
2017	Major		15.4±1.8	9.7±2.1	11.8±1.6
2017	Harry Master's Jersey		17.6±2.1	15.6±2.6	13.8±2
P-value	Treatment		<0.001	<0.001	<0.001
	Cultivar		0.845	0.330	0.435
	Year		0.726	0.467	0.898
	Treatment × Cultivar		0.570	0.436	0.683
	Treatment × Year		0.952	0.578	0.658

Enclosure Temperature in Late Branch Shading Experiment

The presence of shade cloth reduced temperatures within enclosures. From 4-6 WAFB Low, Medium, and High treatments had 9%, 9%, and 8% fewer growing degree days base 10 °C than the Control (Table 4.13). From four WAFB until harvest, Low, Medium, and High treatments had 9%, 8%, and 5% fewer growing degree days base 10 °C than the Control. The

presence or absence of shade cloth had an influence on temperature, but shadecloth opacity did not influence the accumulation of growing degree days.

Table 4.13 Growing degree days base 10 °C inside shading enclosures of 'Major' and 'Harry Master's Jersey' apple trees grown in Lansing, NY in 2017. Branches on individual trees were subjected to different shading opacities from four weeks after full bloom until harvest. Values are means ± standard error (n= 4 'Major' and 4 'Harry Master's Jersey'=8).

Cultivar	Treatment	Growing Degree Days Base 10 °C	
		4-6 Weeks After Full Bloom	4 Weeks After Full Bloom Until Harvest
Major	Control	140.4±7.7	764.0±37.4
Harry Master's Jersey		136.5±5.0	826.4±30.3
Major	Low	128.0±2.4	700.7±13.4
Harry Master's Jersey		123.1±3.1	741.1±28.3
Major	Medium	124.6±3.1	699.4±21.3
Harry Master's Jersey		126.6±3.1	759.5±22.0
Major	High	122.2±5.0	697.2±30.8
Harry Master's Jersey		132.4±3.4	813.1±20.8
P-value	Treatment	0.024	0.026
	Cultivar	0.813	0.025
	Treatment × Cultivar	0.101	0.453

Fruit and Juice Characteristics in Late Branch Shading Experiment

When shading increased, fruit mass and yield decreased in the Late Branch Shading experiment (Table 4.14). Fruit mass in Low, Medium, and High treatment branches were 8%, 10%, and 7% smaller than the Control, respectively. Yield per branch was 8%, 17%, and 21% less in Low, Medium, and High treatments than the Control, respectively. However, there was no relationship between shading treatment and the ratio of yield to leaf number. Peel blush was also reduced by increased shading; High treatment fruit had 29% less peel blush than the Control

(Figure 4.3). There was no relationship between shading intensity and starch pattern index, fruit firmness, or chlorophyll a content. Soluble solid concentration in juice was reduced with increased shading opacity; Low, Medium, and High treatment juice had 7%, 8%, and 12% lower soluble solid concentrations than the Control, respectively (Table 4.15). High treatment juice also had 5% lower titratable acidity than the Control, but Low and Medium treatment juice had similar titratable acidity concentrations to the Control. Greater shading intensity also resulted in lower total polyphenol concentrations in the juice. Low, Medium, and High treatment juice had 8%, 10%, and 14% lower total polyphenol concentrations than the Control, respectively. However, total differences in polyphenols were higher in 2016 than 2017. High treatment juice was 26% lower in total polyphenols than the Control in 2016, but only 6% lower in 2017.

Table 4.14 Harvest and fruit characteristics of 'Major', 'Ellis Bitter', and 'Harry Master's Jersey' apple trees grown in Lansing, NY in 2016 and 2017. Branches on individual trees were subjected to different shading opacities from four weeks after full bloom until harvest. Values are means ± standard error (n= 8 'Major' 2016, 8 'Ellis Bitter' 2016, 8 'Major' 2017, and 8 'Harry Master's Jersey' 2017=32).

Year	Cultivar	Treatment	Mass (g)	Yield (g)	Yield (g) /Leaf Count	Peel Blush (%)	Starch Pattern Index (1-8)	Firmness (N)	Chlorophyll a Index
2016	Major	Control	59.8±4.0	437±64	7.3±1.3	48.6±5.4	4.5±0.5	63.6±4.0	-
2016	Ellis Bitter		51.3±1.6	728±62	10.5±1.5	19.4±2.6	7.4±0.1	74.1±1.1	-
2017	Major		66.2±5.1	785±103	10.3±1.6	50.0±6.2	5.5±0.3	73.2±3.5	1.05±0.09
2017	Harry Master's Jersey		68.1±3.5	771±77	9.1±0.6	99.6±8.1	6.8±0.3	74.2±2.6	0.36±0.04
2016	Major	Low	53.3±5.3	387±110	4.5±1.2	36.1±9.6	5.1±0.7	71.9±2.1	-
2016	Ellis Bitter		53.6±3.3	766±125	7.5±1.1	29.1±4.8	7.1±0.3	74.4±1.8	-
2017	Major		60.0±3.9	645±61	8.9±1.0	27.8±6.1	5.6±0.6	72.2±3.1	1.23±0.09
2017	Harry Master's Jersey		58.5±2.2	692±63	8.8±1.3	92±10.9	6.7±0.2	77.1±2.9	0.42±0.05
2016	Major	Medium	55.9±3.0	499±153	6.4±1.9	42.1±8.5	5.6±0.8	69.4±1.6	-
2016	Ellis Bitter		51.1±2.4	673±78	12.2±2	38.5±3.5	7.2±0.1	74.1±1.9	-
2017	Major		58.2±4.6	634±63	7.6±1.0	20.4±5.5	5.5±0.4	71.7±2.6	1.22±0.08
2017	Harry Master's Jersey		55.1±2.2	461±71	6.1±0.7	53.8±3.7	6.4±0.4	71.8±3.1	0.57±0.11
2016	Major	High	57.8±2.7	381±91	7.2±2.2	21.2±7.5	5.7±0.7	71.2±1.7	-
2016	Ellis Bitter		58.0±2.3	738±157	11.5±1.8	8.7±2.6	6.8±0.1	71.6±1.2	-
2017	Major		56.3±2.8	545±58	8.1±1.5	21.9±3.1	6.3±0.5	70.7±2.2	1.1±0.11
2017	Harry Master's Jersey		56.1±3.4	485±32	6.9±0.5	71.1±4.4	6.6±0.3	69.2±2.4	0.48±0.08
P-value	Treatment		0.022	0.009	0.485	<0.001	0.760	0.131	0.230
	Cultivar		0.310	0.004	0.018	<0.001	<0.001	0.226	<0.001
	Year		0.340	0.010	0.088	0.172	0.323	0.262	-
	Treatment × Cultivar		0.054	0.581	0.678	0.093	0.017	0.064	0.491
	Treatment × Year		0.304	0.443	0.310	0.130	0.442	0.298	-

Table 4.15 Juice characteristics of 'Major', 'Ellis Bitter', and 'Harry Master's Jersey' apple trees grown in Lansing, NY. Branches on individual trees were subjected to different shading opacities from four weeks after full bloom until harvest. Values are means ± standard error (n= 8 'Major' 2016, 8 'Ellis Bitter' 2016, 8 'Major' 2017, and 8 'Harry Master's Jersey' 2017=32).

Year	Cultivar	Treatment	Soluble Solid Concentration (°Brix)	pH	Titratable Acidity (g malic acid/L)	Total Polyphenols (g GAE/L)
2016	Major	Control	14.1±0.6	4.53±0.04	2.5±0.1	1.58±0.21
2016	Ellis Bitter		11.9±0.3	4.57±0.02	2±0.1	1.04±0.11
2017	Major		11.2±0.3	4.48±0.02	2±0.1	1.26±0.08
2017	Harry Master's Jersey		12.5±0.2	4.73±0.03	1.6±0.1	2.24±0.06
2016	Major	Low	12.6±0.5	4.56±0.03	2.5±0.1	1.24±0.13
2016	Ellis Bitter		11.1±0.2	4.63±0.02	2.1±0	1.01±0.11
2017	Major		10.6±0.2	4.53±0.03	2.1±0	1.18±0.06
2017	Harry Master's Jersey		12±0.1	4.8±0.03	1.6±0	2.17±0.11
2016	Major	Medium	13.1±0.5	4.57±0.03	2.5±0.1	1.18±0.14
2016	Ellis Bitter		10.6±0.3	4.64±0.01	2±0.1	0.92±0.09
2017	Major		10.7±0.3	4.54±0.03	2±0	1.21±0.09
2017	Harry Master's Jersey		11.5±0.1	4.79±0.02	1.6±0	2.19±0.09
2016	Major	High	11.8±0.3	4.75±0.04	2.2±0.1	0.99±0.05
2016	Ellis Bitter		10.3±0.3	4.7±0.01	1.9±0.1	0.89±0.04
2017	Major		10.5±0.2	4.58±0.03	2±0	1.29±0.05
2017	Harry Master's Jersey		11.3±0.1	4.83±0.02	1.6±0.1	2.07±0.08
P-value		Treatment	<0.001	<0.001	0.021	0.003
		Cultivar	<0.001	<0.001	<0.001	<0.001
		Year	<0.001	0.010	<0.001	0.862
		Treatment × Cultivar	0.348	0.132	0.126	0.032
		Treatment × Year	0.010	0.010	0.009	<0.001

[Z]GAE=gallic acid equivalents

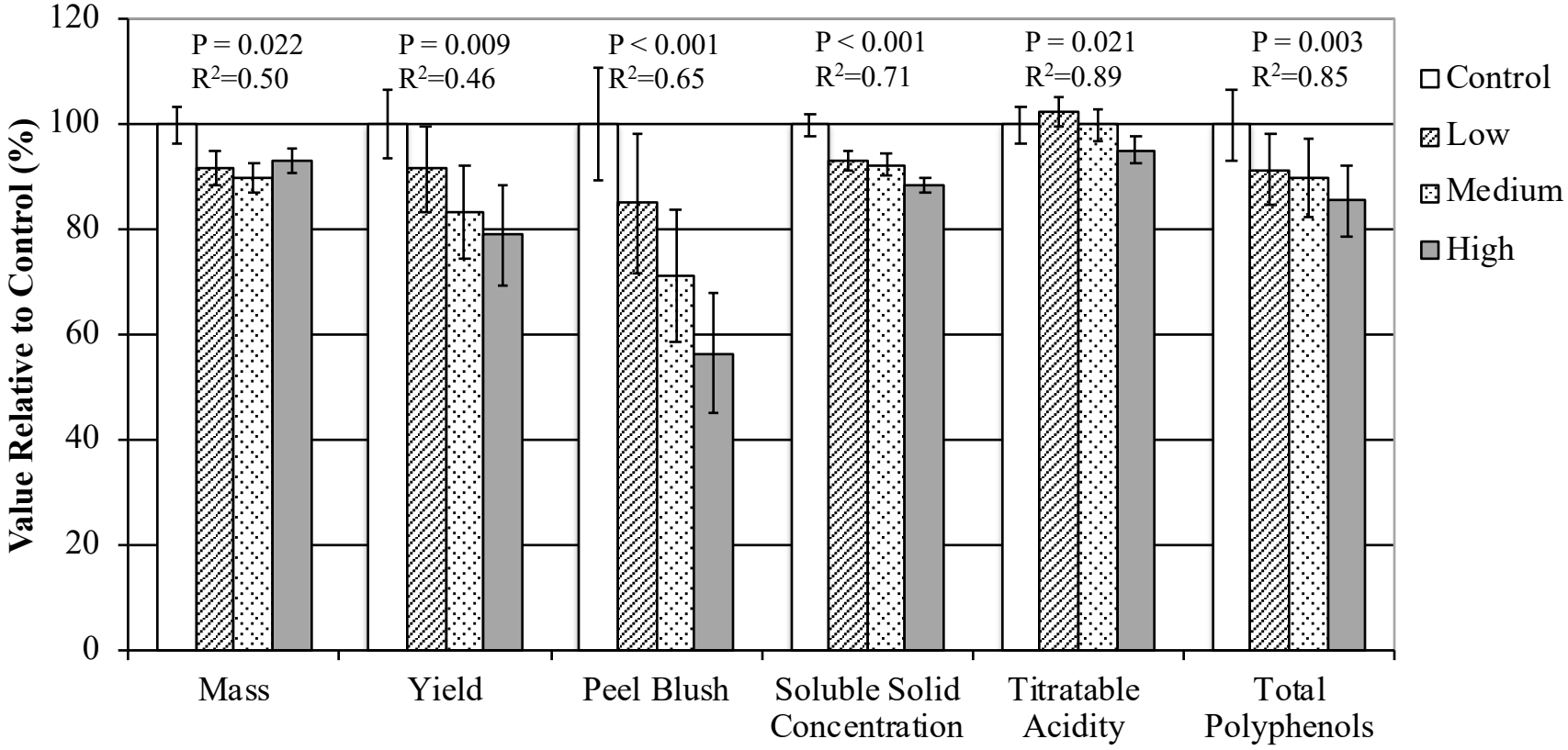

Figure 4.3 Mean fruit and juice characteristics from 'Major' (n=8 for 2016 and 2017), 'Ellis Bitter' (n=8), and 'Harry Master's Jersey' (n=8) apple trees grown in Lansing, NY in 2016 and 2017. Branches on individual trees were subjected to different shading opacities from four weeks after full bloom until harvest. Values are means relative to the Control treatment ± standard error.

Light Environment in Fruit Location Experiment

Canopy exposure of PAR was greatest in the Top sections of tree canopies in the Fruit Location experiment (Table 4.16). On average, canopy exposure of PAR was 68% in the Top sections of trees. East and West sections of trees had similar exposure to PAR to one another, receiving on average 48% and 42% of PAR to these sections of the tree canopy, respectively. The East and West sections of tree canopies received, on average 35% less PAR than the Top sections of trees. The Interior sections had the least exposure to PAR in the study; on average only 8% of PAR reached the Interior section of the canopy, 89% less than the Top sections of trees and 83% less than the East and West sections of tree canopies. Relative differences in canopy exposure of PAR within treatments were similar to one another throughout the growing season.

Table 4.16 Percentage of plant assimilable radiation (PAR) permeating the canopy in different regions of 'GoldRush', 'Major', 'Harry Master's Jersey', and 'Somerset Redstreak' apple trees grown in Lansing, NY in 2016, 2017, and 2018. Values are means ± standard error (n= 8×3 years in 'GoldRush', 8 'Major' in 2016, 8 'Harry Master's Jersey' in 2017, and 8 'Somerset Redstreak'in 2018=32). Different lowercase letters indicate a separation of treatments by the Tukey HSD method at a 5% significance level.

Cultivar	Treatment	Canopy Exposure PAR (%)				
		June	July	August	September	October
GoldRush	East	38.1±2.5	57.3±2.1	50.3±2.7	60.7±3.2	56.6±2.1
Major		61.8±4.2	55.0±6.6	52.5±3.5	-	-
Harry Master's Jersey		49.0±6.4 b	66.8±4.0 b	65.5±2.9 b	48.5±2.3 b	- b
Somerset Redstreak		53.0±3.3	53.7±3.3	73.7±2.2	69.4±1.9	-
GoldRush	West	27.5±4.3	40.9±5.7	41.4±6.0	46.8±6.3	41.1±5.8
Major		58.0±5.5	49.9±4.3	63.5±2.7	-	-
Harry Master's Jersey		47.1±3.2 b	62.2±2.2 b	65.1±4.6 b	56.4±3.3 b	- b
Somerset Redstreak		60.1±3.2	51.9±2.9	73.1±3.9	67.3±7.3	-
GoldRush	Interior	4.0±0.9	3.6±0.5	4.5±0.6	9.6±1.8	8.9±2.4
Major		11.7±3.8	8.6±4.2	12.3±3.0	-	-
Harry Master's Jersey		9.0±1.8.0 c	11.4±3.1 c	6.5±1.6 c	5.4±1.0 c	- c
Somerset Redstreak		16.1±1.1	12.4±2.2	23.0±3.1	19.7±1.5	-
GoldRush	Top	71.5±2.1	76.7±1.8	77.2±2.2	83.8±2.0	80.7±2.2
Major		76.8±4.3	65.8±4.1	76.0±4.4	-	-
Harry Master's Jersey		71.6±2.4 a	82.8±1.9 a	84.5±2.6 a	82.5±2.6 a	- a
Somerset Redstreak		87.6±4.3	72.8±2.7	87.8±2.2	84.7±0.9	-
P-value	Treatment	<0.001	<0.001	<0.001	<0.001	<0.001
	Cultivar	<0.001	0.053	<0.001	0.533	-
	Year	0.151	0.003	<0.001	<0.001	<0.001
	Treatment × Cultivar	0.111	0.050	<0.001	0.127	-
	Treatment × Year	0.158	0.002	<0.001	0.003	0.266

Temperature in Fruit Location Experiment

The Top and West sections of tree canopies accumulated more growing degree days (GDD) than the Interior sections of tree canopies from 1-6 WAFB; Top and West tree sections had 4% more growing degree days base 10 °C during this period (Table 4.17). There was no difference in GGD accumulation among East tree sections and other parts of the tree canopy from 1-6 WAFB. From one WAFB until harvest Top and West sections of tree canopies accumulated more growing degree days than the East and Interior sections. Top sections had 6% and 9% more growing degree days base 10 °C than East and Interior tree sections, respectively. West tree sections had 7% and 10% more growing degree days base 10 °C than East and Interior tree sections, respectively.

Table 4.17 Growing degree days base 10 °C in different regions of 'GoldRush', and 'Harry Master's Jersey' apple trees grown in Lansing, NY in 2017. Values are means ± standard error (n= 4 'GoldRush', and 4 'Harry Master's Jersey' =8). Different lowercase letters indicate a separation of treatments by the Tukey HSD method at a 5% significance level.

Cultivar	Treatment	Growing Degree Days base 10 °C			
		1-6 Weeks After Full Bloom		1 Week After Full Bloom Until Harvest	
GoldRush	East	328.8±2.3	ab	1334.4±9.2	b
Harry Master's Jersey		333.7±8.2		976.0±20.0	
GoldRush	West	337.9±5.7	a	1400.8±22.7	a
Harry Master's Jersey		353.5±7.9		1075.7±30.0	
GoldRush	Interior	323.3±6.8	b	1287.1±17.8	b
Harry Master's Jersey		322.6±4.4		939.7±13.2	
GoldRush	Top	330.8±5.0	a	1362.0±22.5	a
Harry Master's Jersey		359.3±3.0		1096.2±21.0	
P-value	Treatment	0.004		<0.001	
	Cultivar	0.009		<0.001	
	Treatment × Cultivar	0.124		0.182	

Fruit and Juice Characteristics in Fruit Location Experiment

Fruit from the Top, East, and West sections of tree canopies were larger than fruit from the Interior section; Interior fruit were 7% smaller than Top fruit and 6% smaller than fruit from the East and West sections of tree canopies (Figure 4.4). There was 72% greater peel blush on Top fruit than Interior fruit, and 53% greater peel blush on East and West fruit than Interior fruit. On a green scale color index for 'GoldRush' fruit, Interior fruit were 0.9 units greener than fruit from the Top section of the canopy, and 0.6 and 0.7 units greener than East and West fruit, respectively (Table 4.18). East section fruit were 0.3 units greener than Top fruit. Chlorophyll a content in fruit peels was 37% greater in Top fruit than Interior fruit, and 33% greater in East and

West fruit than Interior fruit. Interior fruit were also less ripe than Top and West fruit; Top, East and West fruit were 0.4, 0.3, and 0.6 starch pattern index (SPI) units higher than the Interior, respectively. Soluble solid concentrations were greatest in the Top section of trees; Top juice was 3% higher in soluble solids than West juice and 12% higher than Interior juice (Table 4.19). East and West juice was 9% higher in soluble solids than Interior Juice. Interior juice was 0.1 pH units more alkaline than Top, East, and West section juice. Total polyphenol concentration was greatest in Top juice and lowest in Interior Juice. Top juice had 20% more total polyphenols than East and West juice, and 33% more total polyphenols than Interior juice. East and West juice had 11% more total polyphenols than Interior juice.

Table 4.18 Fruit characteristics of apples from different regions of 'GoldRush', 'Major', 'Harry Master's Jersey', and 'Somerset Redstreak' apple tree canopies grown in Lansing, NY in 2016, 2017, and 2018. Values are means ± standard error (n= 8×3 years in 'GoldRush', 8 'Major' in 2016, 8 'Harry Master's Jersey' in 2017, and 8 'Somerset Redstreak' in 2018=32). Different lowercase letters indicate a separation of treatments by the Tukey HSD method at a 5% significance level.

Cultivar	Treatment	Mass (g)	Peel Blush (%)	Green Scale (1-5)	Chlorophyll a Index	Starch Pattern Index (1-8)	Firmness (N)
GoldRush	East	186.9±9.5	-	2.6±0.1	0.41±0.07	4.3±0.2	71.7±3.8
Major		63.3±3.4	46.6±3.3	-	-	3.3±0.2	77.6±2.0
Harry Master's Jersey		68.6±2.6 ^{a}	68.1±11.5 ^{a}	- ^{b}	1.37±0.15 ^{a}	3.0±0.2 ^{a}	101.8±1.6
Somerset Redstreak		67.5±3.4	64.3±3.6	-	1.14±0.05	4.9±0.3	74.8±2.0
GoldRush	West	187.4±10.5	-	2.7±0.1	0.33±0.06	4.8±0.2	69.4±3.4
Major		63.7±2.5	33.8±2.5	-	-	3.3±0.3	76±1.4
Harry Master's Jersey		70.1±2.2 ^{a}	72.8±4.5 ^{a}	- ^{bc}	1.28±0.08 ^{a}	3.1±0.2 ^{a}	96.1±2.0
Somerset Redstreak		62.9±3.4	61.3±2.3	-	1.15±0.05	5.1±0.2	76.7±0.8
GoldRush	Interior	175.9±7.5	-	3.2±0.1	0.54±0.09	4.3±0.3	70.5±3.7
Major		59.0±1.9	5.8±1.4	-	-	2.7±0.2	67.7±9.9
Harry Master's Jersey		69.0±3.7 ^{b}	68.4±9.1 ^{b}	- ^{a}	1.41±0.09 ^{b}	2.8±0.2 ^{b}	101.4±0.8
Somerset Redstreak		58.4±2.1	37.9±5.3	-	1.49±0.03	4.0±0.3	78.7±1.2
GoldRush	Top	190.9±11.2	-	2.3±0.2	0.34±0.06	4.4±0.2	71.4±3.7
Major		62.1±2.0	58.4±4.3	-	-	3.2±0.3	77.0±1.3
Harry Master's Jersey		70.3±2.0 ^{a}	70.1±4.5 ^{a}	- ^{c}	1.37±0.09 ^{a}	3.0±0.2 ^{a}	101.0±1.8
Somerset Redstreak		62.8±2.6	63.5±5.3	-	1.14±0.05	5.2±0.3	76.7±1.3
P-value	Treatment	0.013	<0.001	<0.001	<0.001	<0.001	0.072
	Cultivar	<0.001	<0.001	-	<0.001	<0.001	<0.001
	Year	<0.001	<0.001	<0.001	0.277	<0.001	<0.001
	Treatment × Cultivar	<0.001	0.001	-	0.027	<0.001	<0.001
	Treatment × Year	<0.001	0.001	0.120	0.015	0.008	0.026

Table 4.19 Juice characteristics of apples from different regions of 'GoldRush', 'Major', 'Harry Master's Jersey', and 'Somerset Redstreak' apple tree canopies grown in Lansing, NY in 2016, 2017, and 2018. Values are means ± standard error (n= 8×3 years in 'GoldRush', 8 'Major' in 2016, 8 'Harry Master's Jersey' in 2017, and 8 'Somerset Redstreak' in 2018=32). Different lowercase letters indicate a separation of treatments by the Tukey HSD method at a 5% significance level.

Cultivar	Treatment	Soluble Solid Concentration (°Brix)	pH	Titratable Acidity (g malic acid/L)	Total Polyphenols (g GAE/L)
GoldRush	East	13.8±0.2	3.4±0.0	9.0±0.2	0.5±0.0
Major		15.1±0.3	4.4±0.0	2.7±0.2	1.8±0.1
Harry Master's Jersey		11.3±0.2 ab	4.6±0.0 b	1.8±0.0	1.6±0.1 b
Somerset Redstreak		10.5±0.2	4.4±0.0	1.7±0.0	1.5±0.1
GoldRush	West	13.3±0.2	3.4±0.0	8.4±0.2	0.4±0.0
Major		14.3±0.4	4.4±0.0	3.0±0.1	1.6±0.1
Harry Master's Jersey		11.5±0.3 b	4.5±0.0 b	2.0±0.1	1.8±0.1 b
Somerset Redstreak		10.8±0.2	4.4±0.0	1.6±0.0	1.5±0.1
GoldRush	Interior	12.8±0.2	3.5±0.0	8.3±0.2	0.4±0
Major		12.9±0.4	4.4±0.0	2.9±0.1	1.2±0.1
Harry Master's Jersey		10.2±0.3 c	4.6±0.0 a	2.1±0.0	1.4±0.1 c
Somerset Redstreak		9.7±0.1	4.4±0.0	1.9±0.0	1.6±0.1
GoldRush	Top	14.3±0.2	3.4±0.0	8.9±0.2	0.5±0.0
Major		15.1±0.5	4.4±0.0	2.8±0.1	1.9±0.1
Harry Master's Jersey		11.5±0.3 a	4.5±0.0 b	1.9±0.0	1.9±0.1 a
Somerset Redstreak		10.9±0.2	4.4±0.0	1.6±0.0	1.8±0.1
P-value	Treatment	<0.001	<0.001	0.828	<0.001
	Cultivar	<0.001	<0.001	<0.001	<0.001
	Year	<0.001	0.006	<0.001	0.132
	Treatment × Cultivar	0.058	0.100	<0.001	<0.001
	Treatment × Year	<0.001	0.165	<0.001	0.876

[Z]GAE=gallic acid equivalents

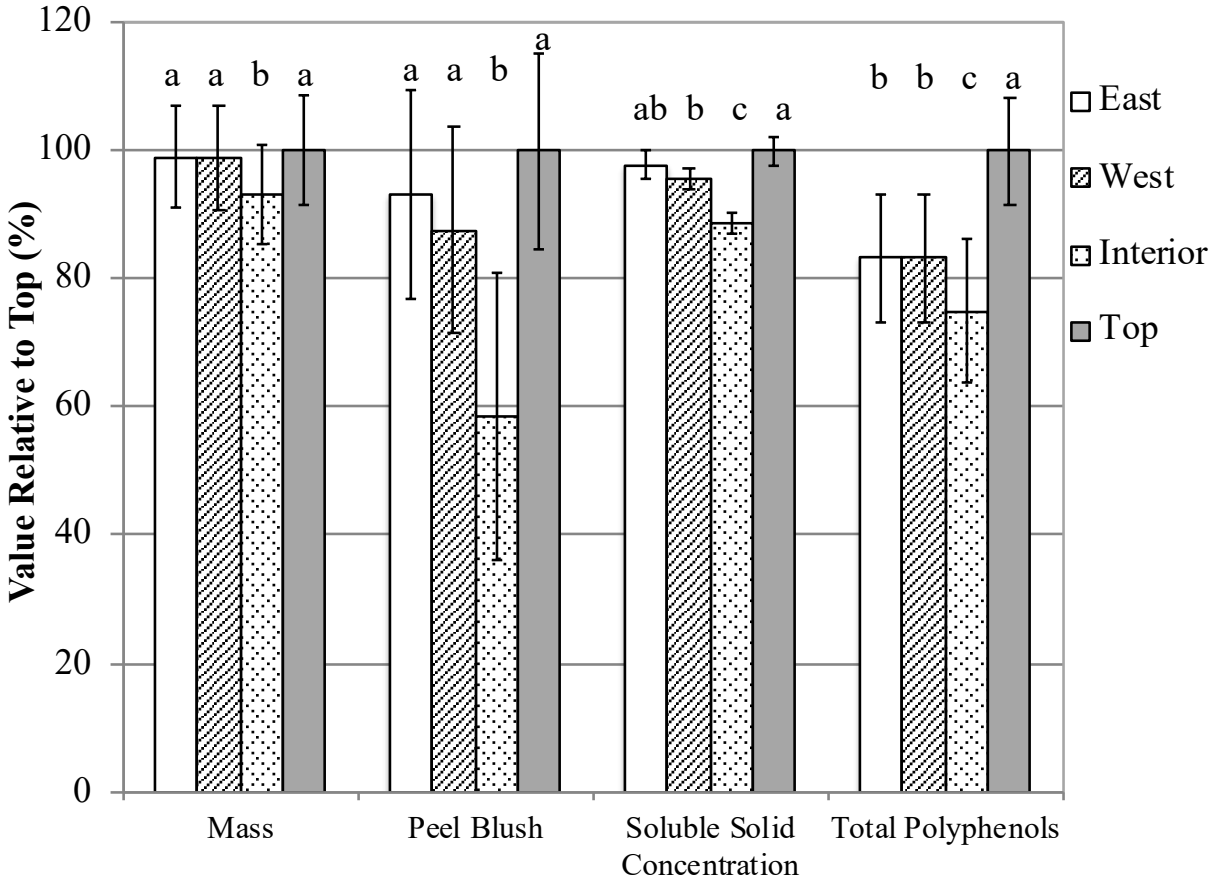

Figure 4.4 Fruit and juice characteristics of apples from different regions of 'GoldRush', 'Major', 'Harry Master's Jersey', and 'Somerset Redstreak' apple tree canopies grown in Lansing, NY in 2016, 2017, and 2018. Values are mean values relative to the Top treatment ± standard error (n= 8×3 years in GoldRush, 8 'Major' in 2016, 8 'Harry Master's Jersey' in 2017, and 8 'Somerset Redstreak' in 2018=32). Different lowercase letters indicate a separation of treatments by the Tukey HSD method at a 5% significance level.

Fruit and Juice Characteristics from the Fruit Bagging Experiment

Fruit mass was smaller in the bagged fruit than the Control for 'Major' and 'Ellis Bitter'; bagged fruit were 26% and 25% smaller than the Control, in 'Major' and 'Ellis Bitter' respectively ($P<0.001$). There was no difference between 'GoldRush' bagged and Control fruit mass ($P=0.996$). In 'Major' and 'Ellis Bitter' bagged fruit had less than 1% peel blush coloration, while Control fruit had 50% or greater peel blush coloration (Table 4.20). In 'GoldRush' bagged apples, fruit peels were very light yellow, all scoring 1.0 on the Green Scale, while Control fruit

had a mean Green Scale score of 3.0. Control fruit had greater chlorophyll a content than bagged fruit in all cultivars, as well. Bagged fruit also had less starch at harvest in ‘Major’ and ‘Ellis Bitter’ ($P<0.001$). ‘Major’ and ‘Ellis Bitter’ bagged fruit scored 1.2 and 0.4 starch pattern index units higher than the Control, respectively. There was no difference in starch index pattern for the ‘GoldRush’ apples ($P=0.505$). There was no difference in flesh firmness between treatments.

Soluble solid concentrations were greater in Control than bagged fruit for all cultivars (Table 4.21). Juice pH was lower in Control juice than in bagged fruit juice. In ‘GoldRush’, titratable acidity was 23% greater in Control juice than bagged fruit juice. In ‘Major’ and ‘Ellis Bitter’, was not statistically different in titratable acidity ($P=0.190$). Overall, there was no statistical differences in total polyphenol concentrations among treatments. However, in ‘GoldRush’ total polyphenol concentrations were 61% greater in Control juice than bagedg fruit juice ($P<0.001$). In ‘Major’, total polyphenol concentrations were 27% greater in bagged fruit juice than the Control ($P=0.026$). In ‘Ellis Bitter’, there was no statistically significant difference between treatments ($P=0.796$).

Table 4.20. Fruit characteristics of bagged (Bag) and un-bagged (Control) apples from 'Major', 'Ellis Bitter', and 'GoldRush' apple trees grown in Lansing, NY in 2016, and 2017. Bag apples had an opaque paper bag placed over fruit at three weeks after full bloom until harvest. Values are means ± standard error (n= 5 'Ellis Bitter and 5 'GoldRush' in 2016 and 5 'Major', 5 'Ellis Bitter and 5 'GoldRush' in 2016 2017=25).

Cultivar	Treatment	Mass (g)	Peel Blush (%)	Green Scale (1-5)	Chlorophyll a Index	Starch Pattern Index (1-8)	Firmness (N)
Major	Control	89.3±7.6	69.6±4.6	-	0.46±0.12	6.2±0.2	65.4±1.5
Ellis Bitter		67.0±3.1	50.8±1.9	-	1.30±0.09	7.0±0.1	73.3±2.2
GoldRush		175.6±0.3	-	3.0±0.2	0.64±0.06	3.9±0.1	84.1±1.1
Major	Bag	66.4±6.2	0.4±0.1	-	0.02±0.01	7.7±0.2	58.4±1.4
Ellis Bitter		50.1±3.3	0.1±0.0	-	1.14±0.06	7.4±0.2	74.7±3.0
GoldRush		175.5±20.6	-	1.0±0.0	0.23±0.04	4.0±0.1	85.2±0.7
P-value	Treatment	<0.001	<0.001	<0.001	<0.001	<0.001	0.098
	Cultivar	<0.001	<0.001	-	<0.001	<0.001	<0.001
	Year	<0.001	0.337	0.926	-	0.015	<0.001
	Treatment × Cultivar	0.006	<0.001	-	0.121	<0.001	0.005
	Treatment × Year	0.823	0.301	0.926	-	0.488	0.490

Table 4.21. Juice characteristics of bagged (Bag) and unbagged (Control) apples from 'Major', 'Ellis Bitter', and 'GoldRush' apple trees grown in Lansing, NY in 2016, and 2017. Bag apples had an opaque paper bag placed over fruit at three weeks after full bloom until harvest. Values are means ± standard error (n= 5 'Ellis Bitter and 5 'GoldRush' in 2016 and 5 'Major', 5 'Ellis Bitter and 5 'GoldRush' in 2016 2017=25).

Cultivar	Treatment	Soluble Solid Concentration (°Brix)	pH	Titratable Acidity (g malic acid/L)	Total Polyphenols (g GAE/L)
Major	Control	13.1±0.4	4.44±0.01	2.2±0.1	1.39±0.19
Ellis Bitter		10.6±0.3	4.44±0.02	2.0±0.0	0.95±0.08
GoldRush		14.0±0.3	3.38±0.02	9.4±0.3	0.37±0.02
Major	Bag	12.4±0.6	4.49±0.05	2.3±0.1	1.77±0.21
Ellis Bitter		10.4±0.4	4.47±0.02	2.1±0.0	0.97±0.05
GoldRush		12.9±0.2	3.49±0.01	7.6±0.2	0.23±0.02
P-value	Treatment	<0.001	<0.001	<0.001	0.159
	Cultivar	<0.001	<0.001	<0.001	<0.001
	Year	0.160	0.257	0.926	0.955
	Treatment × Cultivar	0.015	0.008	<0.001	<0.001
	Treatment × Year	0.322	0.306	0.521	0.064

[Z]GAE=gallic acid equivalents

Discussion

The rapid accumulation of polyphenols in fruit during the first five weeks after full bloom (WAFB) and then their gradual decline in concentrations during the rest of fruit growth and development in the Early Tree and Branch Shading experiments suggests that most of the synthesis of these compounds occurs during the cell division phase of fruit development. Similarly, Renard et al. (2007) found that nearly all synthesis of flavan-3-ol (catechin, epicatechin, and their procyanidin polymers) synthesis occurred during the first six WAFB. Moderate production of these compounds continued during the rest of fruit development, but phenolics also become more diluted as fruit cells take up water and enlarge. However, there is

evidence of some cultivar-specific variation in this pattern. Renard et al. (2007) showed greater procyanidin development for ‘Kermerrien’ compared with ‘Avrolles’ after the cell division phase of fruit development. Zhang et al. (2010) found epicatechin concentrations to increase exponentially during the cell division phase of fruit growth, and to then steadily accumulate until harvest in ‘Honeycrisp’.

Renard et al. (2007) also found that at five weeks after full bloom the vast majority of cortex tissue polyphenols in high tannin cider apples were flavan-3-ols, followed by caffeoylquinic acid and dihydrochalcones. Depending on cultivar, flavan-3-ols were between 13.8 and 3.9 times more abundant than other classes of polyphenols. In cortex tissue of cider apples at harvest, Guyot et al. (1998) found a similar result with procyanidins and flavan-3-ol monomers constituting the vast majority of polyphenols. This suggests that the majority of polyphenols synthesized during the cell division phase in the experiments within this chapter were catechins, epicatechins, and their procyanidin polymers. After cell division, flavan-3-ol monomers polymerize and increase in mean polymer length for the remainder of the growing season (Renard et al., 2007; Zhang et al., 2010).

Measurement of polyphenols in this dissertation did not measure concentrations of specific compounds. Polyphenol characterization via high performance liquid chromatography would have elucidated exactly which polyphenols were synthesized during our study and recording ratios of fresh to dry weight of tissue samples would have allowed the quantification of total polyphenols on a per fruit basis. Additionally, the Folin Ciocalteu assay used in this study has some limitations. It quantifies polyphenols from a redox reaction of reducing compounds and the Folin Ciocalteu reagent. Reducing compounds include polyphenols, but other compounds in fruit, juice, and cider, such as ascorbic acid, sulfur dioxide, and reducing sugars can interfere

with the assay by inadvertently being quantified as polyphenols (Everette et al., 2010). While the Folin Ciocalteu measures non-polyphenol compounds, it has low variability, a relatively wide working range, and is cost and time effective. The Folin Ciocalteu assay has also been found to be more accurate than the Lowenthal permanganate assay, another common polyphenol assay for fruit products (Ma et al., 2019). Given these characteristics, the Folin Ciocalteu assay is a useful tool in measuring relative differences in polyphenol concentrations among samples from the same cultivar, such as in this research.

Enzyme concentrations in the metabolic pathway for polyphenol synthesis are greatest in young fruit, including phenylalanine ammonia-lyase, chalcone-synthase, and dihydroflavonol reductase, which are all involved in the metabolic pathway for anthocyanidin and flavan-3-ol synthesis (Ju et al., 1997; Ju et al., 1995). The periods of high enzyme concentrations during fruit cell division correspond with the periods of high polyphenol synthesis in fresh eating apple cultivars, flavan-3-ol synthesis in cider apples, and polyphenol synthesis in the Early Tree and Branch Shading experiments (Renard et al. 2007, Ju et al., 1997, Ju et al., 1995; Lister et al., 1996a; Henry-Kirk et al., 2012; Zhang et al., 2010). These enzymes are light dependent in apple peels for anthocyanin accumulation, but are not found to be rate limiting for the synthesis of flavonoids and anthocyanidins during the 60 days after full bloom (Ju et al., 1997; Ju et al., 1995; Lister et al., 1996a).

There is cultivar dependent variation in the influence of light exposure on fruit and the synthesis of flavan-3-ols in apple tissue. Chen et al. (2012) found fruit bagging to decrease concentrations in flavan-3-ol concentrations in ‘Red Delicious’ and ‘Golden Delicious’ fruit cortex tissue while not having any impact on flavan-3-ol synthesis in ‘Royal Gala’. Additionally, concentrations of flavan-3-ols have not been found to be affected by light exposure in fruit peels

of ‘Elstar’ and ‘Jonagold’ apples (Awad et al., 2000). There currently is no explanation for the cultivar specific differences in the influence of fruit light exposure on flavan-3-ol synthesis. However, the synthesis of flavan-3-ols are much less light sensitive than other classes of polyphenols. Fruit bagging of ‘Cripps’ Red’ apples reduced transcription factors for anthocyanin and flavonol synthesis by 40- and 70-fold, respectively, but transcription factors for flavan-3-ol synthesis declined only two to four fold (Takos et al., 2006). While anthocyanins were not detectible in the fruit peel of bagged ‘Cripps’ Red’ apples, and flavonol concentrations were reduced in bagged fruit in comparison to the control, there was no difference in condensed tannins among treatments.

The similar total polyphenol concentrations between bagged and Control ‘Ellis Bitter’ juice, and greater total polyphenol concentrations of bagged than Control juice in ‘Major’ in the Fruit Bagging experiment suggests that flavan-3-ol and procyanidin synthesis is not regulated by light exposure to fruit in these cultivars. In ‘Major’ fruit, the greater fruit mass of Control than bagged fruit suggest that total polyphenol content on a per fruit basis were similar between treatments, and that greater fruit mass in the Control lead to lower total polyphenol concentrations in juice. The greater total polyphenol concentrations in Control than bagged fruit in ‘GoldRush’ suggests that light exposure does influence polyphenol synthesis in the cortex of this cultivar. ‘GoldRush’ is a fresh eating apple, low in total polyphenols in comparison to high-tannin cider cultivars, and frequently included in hard cider blends for acidity (Thompson-Witrick et al., 2014). Juice from similar cultivars have greater proportions of hydroxycinnamic acids and dihydrochalcones than flavan-3-ols (Kahle et al., 2005; Guyot et al., 2003). Fruit bagging has been shown to lower hydroxycinnamic acid concentrations in the peel and flesh of apples (Chen et al., 2012). From our data, we cannot specify which polyphenols were produced

in lower concentrations due to fruit bagging in ‘GoldRush’. However, because light exposed directly on the fruit did not reduce polyphenol concentrations in the Fruit Bagging study in ‘Major’ and ‘Ellis Bitter’ this suggests that differences in total polyphenol concentrations in Early and Late Shading studies resulted from reduced carbohydrate availability, and not a direct impact of light on enzyme activity.

Carbohydrate availability during fruit development appears to be a controlling factor for polyphenol synthesis in apple. This was shown when branches shaded for only two weeks during the cell division phase had lower total juice polyphenol concentrations at harvest in comparison to the unshaded control the Early Branch Shading experiment. Not only were total polyphenol concentrations reduced via shading whole trees and branches during this period, but these treatments also resulted in smaller fruit size. During the cell division stage of fruit growth, fruit cell number is dependent on carbohydrate availability for cell growth and division (Lakso et al., 1995). Limiting carbohydrate availability during this period, either from reduced net photosynthesis or competition with other sinks (such as fruit), reduces cell number and growth, and ultimately maximum fruit size (Lakso et al., 1989; Grappadelli et al., 1994; Bepete and Lakso, 1998). Fruit are dependent on localized carbohydrate resources from spur leaves between one and three weeks after full bloom; shading of branches reduces fruit size and delays the export of carbohydrates from extension shoots to developing fruit (Grappadelli et al., 1994).

Even though shading intensities and duration were greater in the Late than Early Branch Shading experiments, polyphenol concentrations were reduced more by the earlier reduction in carbohydrate availability. As extension shoot leaves mature, fruit receive a greater proportion of carbohydrates from them rather than spur leaves. Thus, initiating shading four WAFB in the Late Branch Shading experiment was towards the end of the primary period of polyphenol synthesis

in young fruit (Grappadelli et al., 1994; Renard et al., 2007; Ju et al., 1995). Furthermore, shading starting at four WAFB did not consistently impact polyphenol concentrations in the Late Branch Shading study, with a greater reduction of total polyphenol concentrations in 2016 than 2017. Adjusting carbohydrate availability to fruit via reducing crop load at four and eight WAFB has not been found to impact polyphenol concentrations in a study of 'Red Elstar' and 'Jonagold' apple trees by Awad et al. (2001). In that same study, fruit soluble solid concentration and titratable acidity were increased in trees with a lower crop load, similar to fruit with less or no shading in the Late Branch experiment having more soluble solids and titratable acidity than more heavily shaded fruit.

Differences in total polyphenol concentrations of fruit in different sections of trees in the Fruit Location study were influenced by localized differences in carbohydrate availability during fruit development, and depending on cultivar, potentially influenced by light exposure to individual fruit. Similar to the shaded regions of the canopy in the Early Tree and Branch Shading studies, Interior sections of the tree had less light available for spur leaves and extension shoots early in fruit development when most polyphenol synthesis occurs (Ju et al., 1995; Awad et al., 2001). As discussed earlier, there might be some genetic variation in the sensitivities of light exposure (and thus carbohydrate supply) on apple flavan-3-ols (Chen et al., 2012). Similar to the Fruit Location Study, fruit polyphenol concentrations, including flavan-3-ols, have been found to be greater in the exterior, sun exposed regions of the canopy than fruit from the interior (Feng et al., 2014). Additionally, similar to the Fruit Location study, Feng et al. (2014) found fruit size and soluble solid concentration to be greater in fruit from the exterior region of the canopy than the interior.

Temperature affects apple tree metabolic rates, however there are no known studies that have investigated the relationship of temperature and flavan-3-ol concentrations in apple (Calderón-Zavala et al., 2002). While the Top and West tree sections had similar growing degree accumulations to one another in the Fruit Location experiment, the fruit from Top had greater juice total polyphenol concentrations. Conversely, West tree sections had greater growing degree accumulation than East tree sections but had similar juice total polyphenol concentrations to one another. PAR exposure was a better predictor of total polyphenols than temperature. Similarly, growing degree accumulation was slightly reduced by the presence of shade cloth in the Late Branch study, however increasing shade cloth opacity did not correlate with increasingly lower growing degree accumulations. However, increasing shade cloth opacity was correlated with reducing total polyphenol concentrations. The differences in temperature observed within treatments in these experiments were not correlated with changes in total polyphenol concentrations.

Conclusion

The synthesis of flavan-3-ols and their procyanidin polymers in apple fruit are primarily produced early in fruit development during the cell division phase of fruit growth. There is a source-sink relationship between carbohydrate availability and polyphenol synthesis during this time period. Adjusting carbohydrate availability to fruit after approximately five weeks after full bloom has minimal impact on polyphenol concentrations at harvest. There may additional cultivar specific interactions between light exposure and flavan-3-ol synthesis in fruit. If growers and cidermakers wish to increase the concentrations of polyphenols in fruit for hard cider, early adjustment of crop load may offer a means to increase fruit size and polyphenol concentrations.

Additionally, pruning practices and tree training systems that provide more uniform light penetration throughout the tree canopy may result in higher polyphenol concentrations and fruit quality for cider making.

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CHAPTER FIVE

Concluding Remarks and Reflections

The rise in popularity of hard cider fortuitously coincided with my MS research in viticulture at Cornell University. In the heart of the Finger Lakes, one of the historical epicenters of heirloom apple cultivars in the United States, and at one of the leading centers of pomological research in the world, I became enamored with the history and diversity of apples that surrounded me (Bunker, 2008). I owe a debt of gratitude to Dr. Ian Merwin for his guidance on my masters committee, sparking my interest in pomology, and letting me pick the drops from orchard to let me make my first batches of hard cider. My roommate at the time, Dr. Miles Schwartz-Sax, who has worked curating the *Malus* collection at Harvard University's Arnold Arboretum, and I embarked on an ambitious amateur cider making project together in the unheated rear stairway of our apartment building. It is hard to imagine that this peripheral interest in apples during my masters and cider making hobby would lead to so much.

I am both grateful and lucky that Dr. Gregory Peck was appointed as the new professor in sustainable tree fruit production at Cornell at the end of my masters, that my masters advisor Dr. Justine Vanden Heuvel encouraged me to apply for a PhD in his lab, and that Greg then agreed to take me on to study cider apple physiology and management. My interest in plant science has always encompassed both a desire to understand how plants function and interact with their environment, and how management can improve outcomes for society and industry. The renaissance in artisanal cider production offers an opportunity to both research topics in apple physiology and management that have not received much attention and help support a new and growing segment of the apple industry in the United States.

Nitrogen management in apple orchards for fresh market and processing apples has aimed to strike a balance between providing sufficient nitrogen for plant metabolism, while avoiding overly aggressive vegetative growth and a reduction in fruit quality from decreased red coloration, less firm flesh, and reduced storage capacity (Fallahi, 1997; Lea and Beech, 1978). (Xia et al., 2009; Cheng and Raba, 2009; Wargo et al., 2003; Wargo et al., 2004; Raese et al., 2007; Fallahi, 1997). Peel color, flesh firmness, and storage capacity are not important quality parameters for cider apples, but yeast assimilable nitrogen (YAN) concentrations are typically well below recommended concentrations for the completion of alcoholic fermentation and optimum synthesis of volatile aromatic compounds by yeast (Bell and Henschke, 2005; Ugliano et al., 2011; Torrea et al., 2011; Boudreau et al., 2018). Due to the different quality parameters important for fresh market versus hard cider fruit, greater rates of nitrogen fertilization may offer benefits for cider fruit quality by increasing juice YAN concentrations.

Three years of annually applying calcium nitrate to the soil did not have an influence on tree size at the end of the experiment, but the highest rate of fertilization increased juice primary amino nitrogen (PAN) concentrations by 103% compared to the Control. Foliar applied urea was even more effective on increasing juice YAN; the highest rate of foliar fertilization increased juice YAN by 319% in comparison to the Control in just a six-week period prior to harvest. In both of these studies, other parameters of fruit and juice quality from a cider-making perspective, including total polyphenol concentrations, were not diminished. Increases in juice YAN were much greater than the small and inconsistent increases in leaf total nitrogen concentrations. Taken as a whole, these experiments suggest nitrogen fertilization has a much greater impact on fruit YAN concentrations than on leaf tissue nitrogen concentrations or promoting tree growth in apples. Economic analysis of foliar urea applications demonstrated that, in addition to benefits of

providing nitrogen to the orchard, increases in YAN from fertilizing with urea were less costly than exogenous YAN additions as Fermaid O™, and broke even with costs of exogenous YAN additions as Fermaid K™. Given these findings, higher target nitrogen statuses of apples grown for hard cider production may be cost effective means of increasing juice quality for the industry. Within both nitrogen fertilization studies, leaf nitrogen concentrations never exceeded recommended levels for hard apple cultivars and processing orchards. Further research in which nitrogen statuses of trees exceeded current guidelines for processing orchards would help elucidate the range of nitrogen concentrations in which fruit quality was improved for cider production while not having negative impacts on tree physiology or fruit quality. This would help determine target nitrogen levels for cider orchard trees and establish fertilization recommendations for the cider industry.

Continuing the study of soil applied calcium nitrate or conducting multi-year experiments of foliar urea applications on the same trees each year may help elucidate the long-term implications of greater nitrogen fertilization regiments in cider apple orchards, and on fruit quality. Additionally, throughout my research I heard from enologists, fermentation supply representatives, and cider makers that PAN provides a superior YAN source than ammonia; they contend that PAN is more slowly and efficiently utilized than ammonia, providing more steady and easier to manage fermentations with improved volatile aromatic synthesis. However, little or no scientific research has been published on this topic to support these claims. More research on the influence of PAN versus ammonia on fermentation kinetics and volatile aroma synthesis would be invaluable for both the cider and winemaking industries. However, the popularity of exogenous YAN additions that contain PAN, such as Fermaid O™ and Fermaid K™, that are many fold more expensive than ammonia containing supplements like DAP, suggests that

cidermakers would find the increases in YAN primarily in the form of PAN from nitrogen fertilization to be a high-value improvement for juice quality.

The understanding of tannin synthesis in cider apples and how management can increase their concentration is not well understood. A largely unsubstantiated claim within the cider industry is that greater rates of nitrogen fertilization reduces tannin concentrations. Both the soil and foliar nitrogen fertilization experiments in this dissertation refute this claim. Total polyphenol concentrations were not impacted in either of these studies, even after three years of repeated calcium nitrate applications in Chapter Three of this dissertation. As discussed earlier in this dissertation, the study by Lea and Beech (1978), which found fruit from potted unfertilized trees in sand culture to have 17% greater total polyphenol concentrations, and a 35% reduced yields in comparison to fertilized trees, demonstrates that total polyphenol production per tree was reduced in the unfertilized trees by 25%, and that nitrogen fertilization did not inhibit tannin synthesis.

The experiments described in Chapter Four of this dissertation demonstrate that polyphenol development mostly occurs in the first five weeks after full bloom (WAFB), and that polyphenol concentrations can be manipulated during this period. Shading of whole trees or branches, even for a two-week period during this critical stage in fruit development, reduced photosynthetic rates of leaves and carbohydrate availability to developing fruit and likely resulted in reduced total polyphenol production. The impact of this early season light manipulation on polyphenol production persisted to harvest and would likely impact cider quality. For example, shading whole trees from one to five WAFB reduced juice total polyphenol concentrations by 23% and fruit mass by 12% at harvest. The fruit bagging experiment reveals that light exposure to fruit has a variable influence on polyphenol concentrations, depending on

cultivar. However, because ‘Major’ and ‘Ellis Bitter’ polyphenol concentrations were not reduced by fruit bagging, this suggests that reduced polyphenol concentrations from shading resulted from reduced carbohydrate availability during the first five weeks after full bloom. Reduced polyphenol concentrations of fruit from the more shaded interior sections of the tree canopy in comparisons to the top or exposed sides also support this hypothesis.

If carbohydrate availability during the first five WAFB is a critical period for optimizing tannin synthesis in developing apple fruit, adjusting crop load as early as possible may help optimize tannin concentrations and fruit mass at harvest. Apple trees produce many more fruitlets than they can support and then shed a significant number in the first 30 days after flowering (Robinson et al., 2016). Most conventional apple crop thinning in the Northeastern United States occurs during the fruitlet stage of development using plant hormones such as naphthaleneacetic acid and 6-benzyladenine, and/or carbamate insecticides like carbaryl to induce fruitlet abscission (Dennis, 2000). Temperature based pollen tube growth models are in development that can accurately determine when fertilization of king blooms occur and aid in the use of flower thinning to adjust crop load (Yoder et al., 2013). Adjusting crop load via flower thinning has been shown to produce larger fruit and reduce biennial bearing compared with thinning fruitlets later in the year (Peck et al., 2016; Meland, 2009). Future studies investigating the use of flower thinning to adjust crop load in cider apples and resultant development of fruit polyphenols in trees with different crop loads would help further elucidate the relationship with carbohydrate availability and polyphenol synthesis in apples. If successful at increasing tannin concentrations, it may also offer orchard managers a tool to increase tannin concentrations in cider apples. Additionally, lower polyphenol concentrations in more shaded interior sections of tree canopies suggest that orchard managers can optimize tannin concentrations by utilizing

orchard designs with greater light exposure to fruiting spurs, such as the tall and spindle trellis systems on dwarfing or semi-dwarfing rootstocks (Robinson and Lakso, 1991).

The proposed hypothesis for improving apple fruit and juice quality for cider production by increasing YAN and tannin concentrations in this dissertation may only have a limited influence on the cider industry because currently apple growers are not typically compensated by cider makers for increasing these quality attributes in their fruit. A vertically integrated orchard and cidery may find management strategies to increase YAN and tannin concentrations in their fruit an attractive means of improving cider quality. Given the reticence of apple growers to make large long-term investments in growing cider cultivars that can only be used in a young cider industry, and a lack of incentives to maximize juice YAN and tannin concentrations, it is imperative for cider makers to shoulder some of the risk in the cultivation of cider cultivars and encourage growers to optimize fruit quality (Becot et al., 2016). Long-term contracts and rewards for meeting higher quality standards for apple growers will be important to incentivize more cider apple production and an improve cider quality in the United States. The growth and advancements in quality within the cider industry in the United States are encouraging, and I hope that the research in this dissertation expands our understanding of cider apple physiology and can play a role in helping improve cider apple production.

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