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

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Abstract. **The recent growth in the U.S. hard-cider industry has increased the demand for cider apples (*Malus* ×*domestica* Borkh.), but little is known about how to manage orchard soil fertility best to optimize horticultural performance and juice characteristics for these cultivars. To assess whether nitrogen fertilizer applied to the soil can improve apple juice and cider quality, calcium nitrate ($CaNO_3$) fertilizer was applied at different rates to the soil beneath '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. The trees were cropped in their third and fourth leaf. At the end of the first growing season of the experiment, the greatest fertilizer rate increased tree trunk cross-sectional area (TCSA) by 82% relative to the control, but this difference did not persist through to the end of the study. Yield and crop load were unaffected by the nitrogen fertilization treatments. Increasing the nitrogen fertilizer rate correlated positively with more advanced harvest maturity in 'Golden Russet' fruit, which resulted in greater soluble solid concentration (SSC). Fruit from the greatest fertilizer rate treatment had an average starch pattern index (SPI) that was 1 U greater than in the control, and an SSC that was 3% greater than the control. The fertilizer treatments did not affect juice pH, titratable acidity (TA), or total polyphenol concentrations. Yeast assimilable nitrogen (YAN) concentrations were increased by nitrogen fertilization for both cultivars in both harvest years. The greatest fertilizer treatment increased juice primary amino nitrogen by 103% relative to the control. Greater nitrogen fertilization rates correlated positively with less hydrogen sulfide production during the fermentation of 'Golden Russet' juice from the first, but not the second, harvest. During the first year, cumulative hydrogen sulfide production for the 'Golden Russet' control treatment was 29.6 $\mu g \cdot L^{-1}$ compared with the 'Golden Russet' high treatment, which cumulatively produced 0.1 $\mu g \cdot L^{-1}$. Greater maximum fermentation rates and shorter fermentation durations correlated positively with increased fertilization rate for both cultivars after the second harvest. High treatment fermentations had maximum fermentation rates 110% greater, and fermentation durations 30% shorter than the control. Other horticultural and juice-quality parameters were not affected negatively by the $CaNO_3$ treatments. 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.**

The rapid growth of the hard-cider (henceforth referred to as cider) industry within the United States during the past decade offers an opportunity for apple [*Malus* ×*domestica* (Borkh.)] growers in the United States to expand and diversify production, and potentially to 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 horticultural management practices impact economic returns, fruit quality, and the sensory attributes of the finished ciders to remain competitive and continue improving the quality of ciders available in the U.S. 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 (Boudreau et al., 2017a; Lea and Beech, 1978; Santos et al., 2016; Wargo et al., 2003). There are currently no standard nitrogen fertilization recommendations for cider apple orchards in the United States, 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 impact fruit quality and flower bud cold hardiness negatively (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-fleshed and processing apple cultivars (Stiles and Reid, 1991). Insufficient nitrogen can reduce fruit size, yield, and SSCs in fruit (Fallahi, 1997; Lea and Beech, 1978). Greater nitrogen content of apple trees has been correlated with decreased red and increased green coloration of fruit peels, less firm flesh, and reduced storage capacity (Fallahi, 1997; Raese et al., 2007; Wargo et al., 2003, 2004). Because cider fruit is in limited supply in the United States, it is typically milled and pressed within several weeks after picking; thus, peel color, flesh firmness, and long-term storage capabilities are less important fruit-quality characteristics than they are for fresh-market apples.

Within the context of producing apples for cider, increasing fruit nitrogen concentration may be beneficial for alcoholic fermentation. YAN, which is defined as the nitrogen sources metabolized by *Saccharomyces cerevisiae* yeast during fermentation, is often a limiting factor for cider fermentation rates (Bell and Henschke, 2005; Boudreau et al.,

2017b). YAN is composed of free amino nitrogen (FAN), ammonia (NH_3) ions, and some short oligopeptides (Bell and Henschke, 2005). 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). H_2S is an intermediary compound formed from the reduction of sulfate or sulfite to synthesize the sulfur-containing amino acids cysteine and methionine; excess H_2S frequently permeates the yeast cell membrane in low YAN fermentations when sufficient amino acid precursors are not available to sequester sulfide (Jiranek et al., 1995; Ono et al., 1999; Ugliano et al., 2011). Currently, there are no cider-specific guidelines for apple juice YAN concentration, but several authors have reported that apple juice is often YAN deficient based on white wine grape (*Vitis vinifera* L.) industry standards (Boudreau et al., 2018; Peck et al., 2016). Winemaking recommendations often cite 140 $mg \cdot L^{-1}$ YAN as the minimum concentration and a range between 200 and 350 $mg \cdot L^{-1}$ YAN (depending on initial sugar concentration, yeast strain, and wine style) for the successful completion of most fermentations (Bell and Henschke, 2005; Torrea et al., 2011; Ugliano et al., 2011). Apples typically have YAN concentrations of less than 100 $mg \cdot L^{-1}$. For example, a survey of 12 cultivars grown in Virginia over two growing seasons found a mean YAN concentration of 59 $mg \cdot L^{-1}$ (Boudreau et al., 2018).

Increased nitrogen fertilization of vineyards has increased YAN concentration successfully in wine grape juice (Moss, 2016; Neilsen et al., 2010). Commercial cider producers often add exogenous YAN supplements such as diammonium phosphate or autolyzed yeast cells to counter nitrogen deficiencies. However, it is possible that nitrogen fertilizers applied in the orchard can 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, multiyear studies of nitrogen fertilization in orchards are useful to elucidate the ramifications for different management strategies (Cheng et al., 2004).

Over the course of three growing seasons, different rates of $CaNO_3$ fertilizer were applied each spring to 'Golden Russet' and 'Medaille d'Or' apple trees. These cultivars were selected because of their popularity in cider production. The study began when trees were in their second year after planting and continued through their fourth year. Fruit was harvested in the third and fourth years of the study. 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 H_2S production during fermentation.

Materials and Methods

Orchard site. This study was conducted between Spring 2016 and harvest 2018 at the Cornell University Agricultural Experiment Station research orchard in Ithaca, NY (lat. 42.445111, long. –76.459564). The 'Golden Russet' and 'Medaille d'Or' apple trees were grafted onto 'Geneva.30®' rootstock and planted in Spring 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% 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. 'Geneva Russet' was spaced farther apart than 'Medaille d'Or' to accommodate the semitip bearing habit and vigorous growth of 'Golden Russet'. The trees were trained as a tall spindle and were managed uniformly with standard pruning practices and conventional pest control management for the region (Agnello et al., 2019; Robinson et al., 2011). No fruit were produced in 2016. A light crop on the trees in 2017 did not necessitate fruit thinning for either cultivar. In 2018, fruit were hand-thinned to six fruit per TCSA for both cultivars between 2 and 10 July; this crop load was selected because it is a common crop load target for culinary apple cultivars in the region. Trees did not receive supplemental irrigation during the study. For the growing seasons of 2016, 2017, and 2018 (for the months of April through October), the orchard received 45.7, 81.2, and 66.5 cm of rainfall, respectively. Full bloom was observed on 10 May 2017 and 16 May 2018 for 'Golden Russet', and on 18 May 2017 and 25 May 2018 for 'Medaille d'Or'.

Experimental design. Four nitrogen fertilizer treatments (control, low, medium, and high) were established in Spring 2016 by applying different rates of $CaNO_3$ granular fertilizer containing 15.5% nitrogen by weight (YaraLiva Tropicote, North East Lincolnshire, UK). The treatments were reapplied to the same trees each year. Control-, low-, medium-, and high-treatment trees received an equivalent of 0, 28, 56, and 112 $kg \cdot ha^{-1}$ nitrogen each year, respectively. $CaNO_3$ 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 Apr. 2016, 18 Apr. 2017, and 18 Apr. 2018, when the trees were about at half-inch green of bud development (Agnello et al., 2019). The second application was applied about 4 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. Blocks were designated by geographic position within the orchard. 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. TCSA was measured in Spring 2016 when trees were still dormant, and then in the fall of each year after the trees defoliated. TCSA was calculated from trunk circumference measured 30 cm above the graft union. Leader growth was measured in 2016 and 2017 at the same time as TCSA. Ten exposed, undamaged leaves from shoot midsections were taken from both sides of each tree between 1 and 2 m above the soil level in mid August in each year of the study, and were submitted to the Cornell Nutrient Analysis Laboratory (Ithaca, NY) for total nitrogen analysis by combustion per manufacturer protocols (VarioMax CNS; Elementar Analysensysteme GmbH, Langenselbold, Germany). In 2017 and 2018, flower clusters were counted on each tree when they were at full bloom.

'Golden Russet' harvest occurred on 24 Oct. 2017 and 30 Oct. 2018. 'Medaille d'Or' was harvested on 25 Sept. 2017 and 20 Sept. 2018. All fruit from every experimental tree were counted and weighed. A 10-fruit subsample was used to measure mass, SPI, flesh firmness, and chlorophyll a content. For 'Golden Russet' apples, russeting was approximated visually as the area of the fruit peel with visible russeting. For 'Medaille d'Or' apples, peel blush was assessed visually as the area of the fruit peel with red coloration. The SPI was rated on a 1- to 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 an SPI of 6 (≈60% starch degradation) to harvest fruit when most of the starch had hydrolyzed to sugar and before significant preharvest fruit drop. Flesh firmness was measured on the sun- and the shade-exposed sides of each fruit along the equator with a penetrometer (GS Fruit Texture Analyzer; Güss, Strand, South Africa) fitted with an 11.1-mm-diameter tip. Chlorophyll a content

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was measured on a 0- to 3-point index using a Turoni 53500 DA meter (Forli, Italy) on the sun and the shade side of each apple along the equator.

Juice chemistry. Twenty apples from each experimental unit (10 apples/tree) were milled and pressed in a Norwalk 280 juicer (Bentonville, AR) to make a single composite juice sample. The SSC, pH, TA, YAN (primary amino nitrogen and ammonium ion), and total polyphenol concentrations (using the Folin Ciocalteu assay) were measured for each juice sample. The SSC was measured with an Atago PAL-1 digital refractometer (Tokyo, Japan) and is reported as °Brix. Juice pH and TA were measured with an automatic titrator (Unitrode pH meter, 778 sample processor, and 800 Dosino dosing device; Metrohm, Herisau, Switzerland). A 5-mL juice sample was titrated against a 0.1 M NaOH solution to an endpoint of pH 8.2 and is expressed in grams per liter of malic acid equivalents. The Megazyme Primary Amino Acid (PAN) and Ammonia (Rapid) spectrophotometric assay kits (Bray, Ireland) were used to measure YAN in 96-well microplates according to manufacturer specifications and were read on a Molecular Devices Spectramax 384 Plus spectrophotometer at λ 340 nm (San Jose, CA). The PAN assay kit measures the concentrations of PAN of free amino acids by a reaction of amino nitrogen in juice samples with N-acetyl-L-cysteine and o-phthaldialdehyde, which 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 340 nm 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 3500 g_n for 10 min, filtered through a polytetrafluoroethylene 0.22-µm membrane filter (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, γ-aminobutyric acid, and L-asparagine (Sigma-Aldrich, St. Louis, MO) dissolved in 0.1 N hydrogen chloride. 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 ultraviolet 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 runtime of 10 min 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% high-performance liquid chromatography-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).

Fermentation. 'Golden Russet' in 2017 and 2018, and 'Medaille d'Or' in 2018 were used for fermentation studies (there was insufficient 'Medaille d'Or' fruit in 2017). Juice was racked off sediment after settling at 2 °C overnight. Two 200-mL subsamples 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 $mg \cdot L^{-1}$ free sulfur dioxide) was added to each flask 24 h before yeast was inoculated.

Five grams of UCD-522 yeast was rehydrated in 100 mL 40 °C water for 20 min, and then 100 mL of a conglomerated juice sample was added over 5 min per manufacturer instructions (Scott Laboratories, Petaluma, CA). Two milliliters 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, NJ) inserted into a single-hole stopper. The detector tubes contain lead acetate, which reacts with H_2S to form gray lead sulfide. 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 h. Flasks were kept at 18 °C and weighed every 24 h to track fermentation rates. Flasks were stirred for 5 min at 120 rpm on an orbital shaker twice a day to keep yeast in suspension. When the fermentation rate fell to less than 0.2 g carbon dioxide (CO_2)/d, fermentations were considered completed. Cider samples were then racked off the fine lees and stored at –80 °C.

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 related stoichiometrically 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 was immediately placed tightly 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 dissolved completely.

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 were performed to normalize data before analysis, but data are presented untransformed. To describe differences better among treatments within an individual year of the study, or of an individual cultivar, a more simplified linear mixed-effect model was used to compare data and report P and R^2 values directly in the text of the Results section. For treatment responses of both cultivars within an individual year, treatment was included as a fixed effect, and cultivar, treatment × cultivar, block, and experimental unit were included as random variables. For an individual cultivar, treatment was included as a fixed effect, and year, treatment × year, block, experimental unit, and block × year were included as random variables. For an individual cultivar within a single year, treatment was included as a fixed effect, and block and experimental unit were included as random variables. Data were analyzed using JMP Pro version 14 (SAS Institute, Cary, NC).

Results

Fruit and tree characteristics. There was a positive correlation between nitrogen fertilization rate and an increase in tree size (as measured by TCSA) for both cultivars in 2016 ($P = 0.023$, $R^2 = 0.41$), but this trend did not continue throughout the study (Table 1). The high fertilizer treatment increased TCSA 82% more than the control in 2016. However, by the end of the second year of the study, there was no significant difference in TCSA among treatments for either cultivar. There was no statistically significant relationship between fertilization rate and leader growth. In 2018, increased leaf nitrogen concentration correlated positively with fertilizer application rate for both cultivars ($P < 0.001$, $R^2 = 0.55$), but not in 2016 or 2017. There was a positive correlation between fertilization rate and bloom number throughout the study. However, there were no differences in yield or crop load for either cultivar during this study.

Greater fruit starch degradation at harvest correlated positively with greater nitrogen

Table 1. Growth, leaf nitrogen concentration, flower number, and fruit yield of 'Golden Russet' and 'Medaille d'Or' apple trees with no, low (28 kg·ha^{-1}), medium (56 kg·ha^{-1}), and high (112 kg·ha^{-1}) calcium nitrate fertilizer application rates in Ithaca, NY. Values are means ± SE.

Cultivar	Yr	Treatment	TCSA increase (%)	TCSA (cm^2)	Leader growth (cm)	Leaf nitrogen (%)	Flowers per tree	Yield (kg)	Crop load (yield/cm^2 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 ± 0.0
		Low	97 ± 6	13.9 ± 0.5	79.6 ± 8.9	1.8 ± 0.1	133 ± 19	7.5 ± 0.4	0.54 ± 0.0
		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 ± 0.1
	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 ± 0.0
		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 ± 0.1
		Low	73 ± 5	8.6 ± 0.5	51.6 ± 2.3	2.1 ± 0.1	32 ± 13	2.3 ± 0.1	0.26 ± 0.0
		Medium	85 ± 5	9.0 ± 0.4	49.9 ± 4.4	1.6 ± 0.1	15 ± 4	2.5 ± 0.2	0.28 ± 0.0
		High	75 ± 8	9.0 ± 0.3	50.4 ± 4.4	1.8 ± 0.2	19 ± 5	2.1 ± 0.6	0.22 ± 0.1
	2018	Control	72 ± 13	11.7 ± 1.3	—	1.7 ± 0.1	180 ± 33	5.2 ± 1.7	0.38 ± 0.1
		Low	65 ± 4	12.7 ± 0.4	—	2.0 ± 0.1	258 ± 45	6.3 ± 0.9	0.50 ± 0.1
		Medium	52 ± 3	13.2 ± 0.8	—	2.0 ± 0.1	312 ± 54	5.9 ± 0.6	0.44 ± 0.0
		High	50 ± 4	13.1 ± 0.5	—	2.1 ± 0.1	361 ± 26	6.8 ± 0.5	0.52 ± 0.1
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

TCSA = trunk cross-sectional area.

fertilizer rates in 'Golden Russet' (P < 0.001, R^2 = 0.49), but not with 'Medaille d'Or'. Fruit flesh firmness correlated negatively with fertilization rate for both cultivars; fruit flesh firmness in the high treatment was 9% less than the control (Table 2). 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 between increased fertilization rate and chlorophyll a content with 'Golden Russet' (P = 0.001, R^2 = 0.62). There was no correlation between fertilization rate and peel russeting in 'Golden Russet' (P = 0.373) and peel blush in 'Medaille d'Or' (P = 0.268).

Juice chemistry. There was a positive correlation between fertilization and SSC over the entire course of the study for both cultivars (Table 3). These differences were small overall; the greatest difference was found in 2018, when there was a 4% greater SSC in 'Golden Russet' juice from the high treatment than the control. There were no differences in juice pH, TA, or total polyphenol concentration among treatments for either cultivar in either harvest year (Table 3). Across all treatments, mean total polyphenol concentration for both cultivars was 25% greater in 2017 than in 2018.

Increasing nitrogen fertilization rate correlated positively with greater PAN concentrations for both cultivars in both years (Figs. 1 and 2). Across all treatments, PAN concentration was greater in 2017 than in 2018. In 2017, high-treatment 'Golden Russet' and 'Medaille d'Or' juice had 49% and 124% more PAN than the control, respectively. In 2018, high-treatment 'Golden Russet' and 'Medaille d'Or' juice had 92% and 144% more PAN than the control, respectively. NH_3 concentrations were not different among treatments for 'Golden Russet'; but, among all treatments and years, NH_3 constituted less than 5% of YAN. Differences in 'Golden Russet' YAN were therefore mostly attributed to increases in PAN, and not NH_3. For 'Medaille d'Or', there was an unidentified interference with the spectrophotometric NH_3 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 this cultivar has a high level of pectin that interfered with this assay.

Amino acid profiles. 'Golden Russet' and 'Medaille d'Or' had distinctively different amino acid concentrations and compositions (Tables 4–7). 'Golden Russet' PAN was comprised predominantly of asparagine and aspartic acid (Tables 4 and 5). In 2017, there were no statistical differences in the proportions of asparagine and aspartic acid among treatments in 'Golden Russet' (Table 5). For example,

Table 2. Fruit maturity and quality measurements of apples from 'Golden Russet' and 'Medaille d'Or' trees with no, low (28 kg·ha^{-1}), medium (56 kg·ha^{-1}), and high (112 kg·ha^{-1}) calcium nitrate fertilizer application rates in Ithaca, NY. Values are means ± SE.

Cultivar	Yr	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 ± 1.9	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	0.40 ± 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	0.40 ± 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	—

Table 3. Juice chemistry of apples from 'Golden Russet' and 'Medaille d'Or' trees with no, low (28 kg·ha^{-1}), medium (56 kg·ha^{-1}), and high (112 kg·ha^{-1}) calcium nitrate fertilizer application rates in Ithaca, NY. Values are means ± SE.

Cultivar	Yr	Treatment	Soluble solid concn (°Brix)	pH	Titratable acidity (g malic acid/L)	Total polyphenols (GAE/L)	Primary amino nitrogen (mg·L^{-1})	Ammonia (mg·L^{-1})	Yeast assimilable nitrogen (mg·L^{-1})
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	0.850	0.910	0.635	0.801	0.472	—	—
		Treatment × year	0.568	0.347	0.810	0.940	0.072	0.628	0.991

GAE = gallic acid equivalent.

asparagine comprised 66% and 73% of the control- and high-treatment PAN, respectively; and aspartic acid comprised 27% and 21% of the control- and high-treatment PAN, respectively. In 2018, aspartic acid constituted a greater proportion of PAN than asparagine in the control-treatment 'Golden Russet' juice, whereas control-treatment PAN was only 36% asparagine and 52% aspartic acid. The ratio of asparagine to aspartic acid in high-treatment 'Golden Russet' juice was similar between 2017 and 2018; in 2018, juice from the high treatment was 60% asparagine and 31% aspartic acid. In 2018, there was a positive correlation between increasing fertilizer rate with a greater proportion of PAN as asparagine, and a negative correlation with a lower proportion of PAN as aspartic acid. However, because of the greater overall PAN concentrations in treatments receiving more nitrogen fertilizer, there was a positive correlation between increasing fertilizer rate and greater aspartic acid concentrations. The remaining predominant amino acids in 'Golden Russet' juice were glutamic acid, glutamine, and serine, each of which comprised less than 4% of total PAN for all treatments (Fig. 3).

Asparagine was the principal amino acid in 'Medaille d'Or' juice, and the proportion of PAN as asparagine correlated positively with increasing fertilizer rate (Table 6). Asparagine constituted 54% of the control-treatment 'Medaille d'Or' juice, and 77% of the PAN from the high-treatment juice (Table 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 fertilizer rate with a lower proportion of aspartic acid as PAN, but a positive correlation of fertilizer rate and greater aspartic acid concentrations. Aspartic acid constituted 13% of the control-treatment 'Medaille d'Or' juice and 8% of the high-treatment juice. The proportion of glutamic acid, glutamine, and serine also correlated negatively with increasing fertilizer rate. Glutamic acid, glutamine, and serine constituted 10%, 7%, and 6% of the control-treatment PAN, respectively; and 4% each of the high-treatment PAN, respectively (Fig. 4).

Fermentation characteristics and cider chemistry. There was a positive correlation

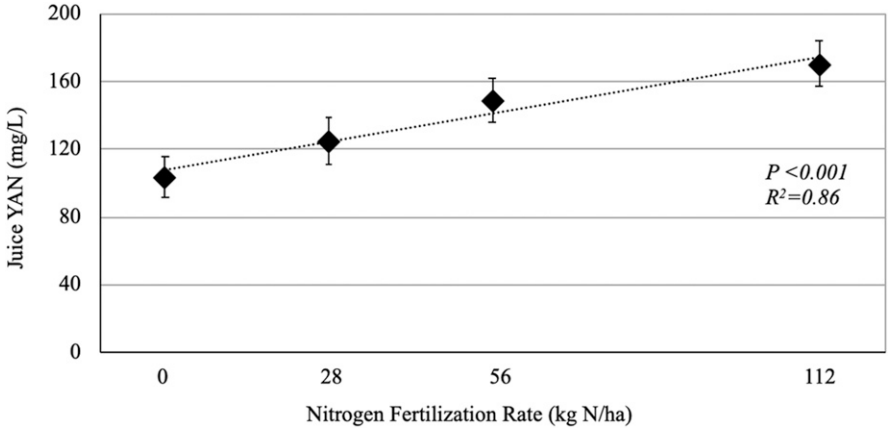

Fig. 1. Yeast assimilable nitrogen (YAN) concentration in 'Golden Russet' apple juice from trees with no, low (28 kg·ha^{-1}), medium (56 kg·ha^{-1}), and high (112 kg·ha^{-1}) calcium nitrate fertilizer application rates in Ithaca, NY. Values are means ± SE (n = 4 per year in 2017 and in 2018).

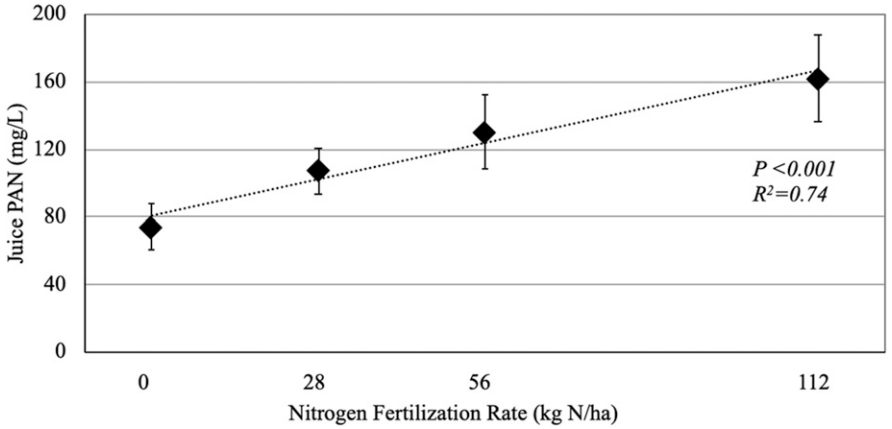

Fig. 2. Primary amino nitrogen (PAN) concentrations in 'Medaille d'Or' apple juice from trees with no, low (28 kg·ha^{-1}), medium (56 kg·ha^{-1}), and high (112 kg·ha^{-1}) calcium nitrate fertilizer application rates in Ithaca, NY. Values are means ± SE (n = 4 per year in 2017 and in 2018).

between increasing fertilizer rate and maximum fermentation rate, and an inverse correlation between fertilizer rate and fermentation duration (Table 8). In 2018, for the high 'Golden Russet' treatment, maximum fermentation rate was 131% greater than the control, and the high 'Medaille d'Or' treatment maximum fermentation rate was 89% greater than the control. In 2018, the high 'Golden Russet' treatment fermentation durations were 28% shorter than the control; and in 2018, the high 'Medaille d'Or' treatment fermentation durations were 32% shorter than the control.

In 2017, residual sugar concentrations were less than 0.3 g·L^{-1} for all treatments and did not appear to be correlated to the fertilizer treatments. In 2018, residual sugar concentration and fertilization rate correlated inversely for the 'Golden Russet' ciders (P = 0.004, R^2 = 0.59). Ciders made from the control treatment contained 0.84 g reducing sugars/L, whereas the high-treatment ciders contained 0.08 g reducing sugars/L.

There was an inverse correlation between H_2S production and fertilizer rate in 2017 (P = 0.049, R^2 = 0.10). Mean H_2S production for the control 'Golden Russet' treatment was 29.6 µg·L^{-1} compared with the high 'Golden Russet' treatment, which had 0.1 µg·L^{-1}. In 2018, H_2S production was not correlated with either 'Golden Russet' or 'Medaille d'Or' fermentation rates. In addition, residual H_2S was not detected in the finished ciders from either cultivar in either year (data not shown).

Discussion

In other studies, increasing nitrogen fertilizer rate has been shown to increase apple tree growth, fruit size, and yield in both mature and young nitrogen-limited apple orchards (Cheng and Fuchigami, 2002; Lea and Beech, 1978; Wargo et al., 2003; Xia et al., 2009). However, while trees receiving more nitrogen fertilizer were larger during the first year of our study, these initial tree size increases did not persist through to the following 2 years. In addition, nitrogen fertilizer did not affect crop yield, crop load, or fruit mass. Other studies investigating nitrogen fertilization on young apple tree growth in similar silt loam soils to those in our 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). Although 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 our study displayed symptoms of nitrogen deficiency, even though the leaf nitrogen concentrations in the high-treatment trees were still less than 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. During 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 $CaNO_3$ granular fertilizer applied in our 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 correlated negatively with nitrogen fertilization rate, and 'Golden Russet' trees receiving more nitrogen fertilizers had greater starch degradation (as measured by the SPI), suggesting the treatments advanced fruit maturity. This observation has been observed in other studies, as well (Raese et al., 2007; Wargo et al., 2003, 2004). Others have attributed the advanced ripening of apples receiving more nitrogen fertilizer to increased ethylene synthesis and cellular respiration (Fallahi, 1997). The greater SSCs 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 fruit. Because cider makers often press fruit after allowing all 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 multiyear 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), 3-year-old 'Dabinett' apple trees were transplanted into pots containing sand and were then either nitrogen fertilized or left unfertilized as a control for a single growing season. In their study, juice polyphenol content from the control trees was 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 with 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 affected total polyphenol development or concentrations in apples.

The study by Lea and Beech (1978) has been used to support the recommendation

Table 4. Juice amino acid concentrations from apples from 'Golden Russet' trees with no, low (28 kg·ha^{-1}), medium (56 kg·ha^{-1}), and high (112 kg·ha^{-1}) calcium nitrate fertilizer application rates in Ithaca, NY. Values are means ± SE.

Cultivar	Yr	Treatment	Asparagine (mg·L^{-1})	Aspartic acid (mg·L^{-1})	Glutamic acid (mg·L^{-1})	Glutamine (mg·L^{-1})	Serine (mg·L^{-1})	Threonine (mg·L^{-1})	Methionine (mg·L^{-1})	Other (mg·L^{-1})
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 5. Proportion of juice amino acids constituting free amino nitrogen from apples from 'Golden Russet' trees with no, low (28 kg·ha^{-1}), medium (56 kg·ha^{-1}), and high (112 kg·ha^{-1}) calcium nitrate fertilizer application rates in Ithaca, NY. Values are means ± SE.

Cultivar	Yr	Treatment	Asparagine (%)	Aspartic acid (%)	Glutamic acid (%)	Glutamine (%)	Serine (%)	Threonine (%)	Methionine (%)	Other (%)
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

that cider apple growers should limit nitrogen fertilization 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 because of the bitter and astringent characteristics they impart to finished ciders (Delage et al., 1991; Lea and 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 (Barbehenn and Constabel, 2011; Robbins et al., 1987). Lea and Beech (1978) hypothesized that under nitrogen deficient conditions, the trees prioritized increased tannin synthesis to deter herbivory more effectively 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 support 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 (Awad et al., 2000; Feng et al., 2014). 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.

Although primary juice chemistry and polyphenol concentrations were unaffected 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 FAN—that is, in an organic form. In fact, less than 5% of YAN was in the form of NH_3 among all treatments and both years for 'Golden Russet'. Other studies have also found NH_3 to constitute a small proportion of YAN in apples (Boudreau et al., 2018). In addition, in a related study, foliar nitrogen fertilization with urea was also found to increase apple YAN concentrations predominantly as FAN (Karl et al., 2020).

In both cultivars, asparagine was the most abundant amino acid, and it 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 FAN in trees receiving more nitrogen

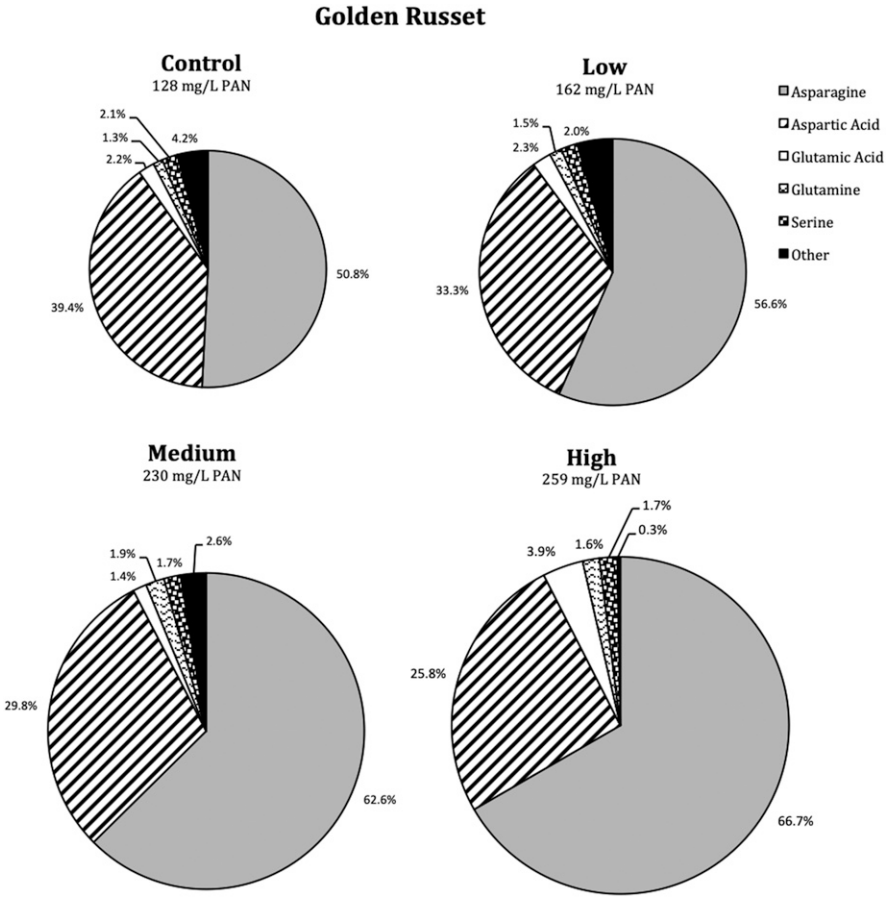

Fig. 3. Total free amino nitrogen (FAN) concentration and proportions of amino acids constituting FAN of apple juice from 'Golden Russet' trees with no, low (28 kg·ha^{-1}), medium (56 kg·ha^{-1}), and high (112 kg·ha^{-1}) calcium nitrate fertilizer application rates in Ithaca, NY. Pie chart sizes are to scale of primary amino nitrogen (PAN) concentration among different treatments. Data represent a combined model from both 2017 and 2018.

fertilizer as well (Cheng et al., 2004). More than 95% of 'Golden Russet' FAN and 85% of 'Medaille d'Or' FAN was asparagine, aspartic acid, glutamic acid, glutamine, and serine. These amino acids have been reported to be metabolized preferentially 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 used and preferred forms by *S. cerevisiae*.

Although specific target YAN concentrations have not been established for cider fermentation, apple juice YAN is typically less than the 140-mg·L^{-1} minimum threshold that is often cited as a recommendation for the wine industry (Bell and Henschke, 2005; Boudreau et al., 2017a; Peck et al., 2016). With the exception of the 'Medaille d'Or' control treatment, 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 as a result of 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 'Golden Russet' treatment, none of the treatments or cultivars met this minimum YAN target. In commercial cider production, exogenous nitrogen supplements would likely be recommended for all 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 a partial budget investigating the value of foliar urea applications on increasing juice YAN in the study by Karl et al. (2020), the increased concentrations of YAN in high-treatment juice from 'Golden Russet' compared with the control in 2018 would save $883/ha when supplemented with Fermaid O™. As a result of the lower costs of the other YAN supplements in the model, Fermaid K™ and diammonium phosphate would save $166 and $15/ha, respectively (these

Table 6. Juice amino acid concentrations from apples from 'Medaille d'Or' trees with no, low (28 kg·ha^{-1}), medium (56 kg·ha^{-1}), and high (112 kg·ha^{-1}) calcium nitrate fertilizer application rates in Ithaca, NY. Values are means ± SE.

Cultivar	Yr	Treatment	Asparagine (mg·L^{-1})	Aspartic acid (mg·L^{-1})	Glutamic acid (mg·L^{-1})	Glutamine (mg·L^{-1})	Serine (mg·L^{-1})	Threonine (mg·L^{-1})	Other (mg·L^{-1})
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	1,275.9 ± 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	1,478.0 ± 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 7. Proportion of juice amino acids constituting free amino nitrogen from apples from 'Medaille d'Or' trees with no, low (28 kg·ha^{-1}), medium (56 kg·ha^{-1}), and high (112 kg·ha^{-1}) calcium nitrate fertilizer application rates in Ithaca, NY. Values are means ± SE.

Cultivar	Yr	Treatment	Asparagine (%)	Aspartic acid (%)	Glutamic acid (%)	Glutamine (%)	Serine (%)	Threonine (%)	Other (%)
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

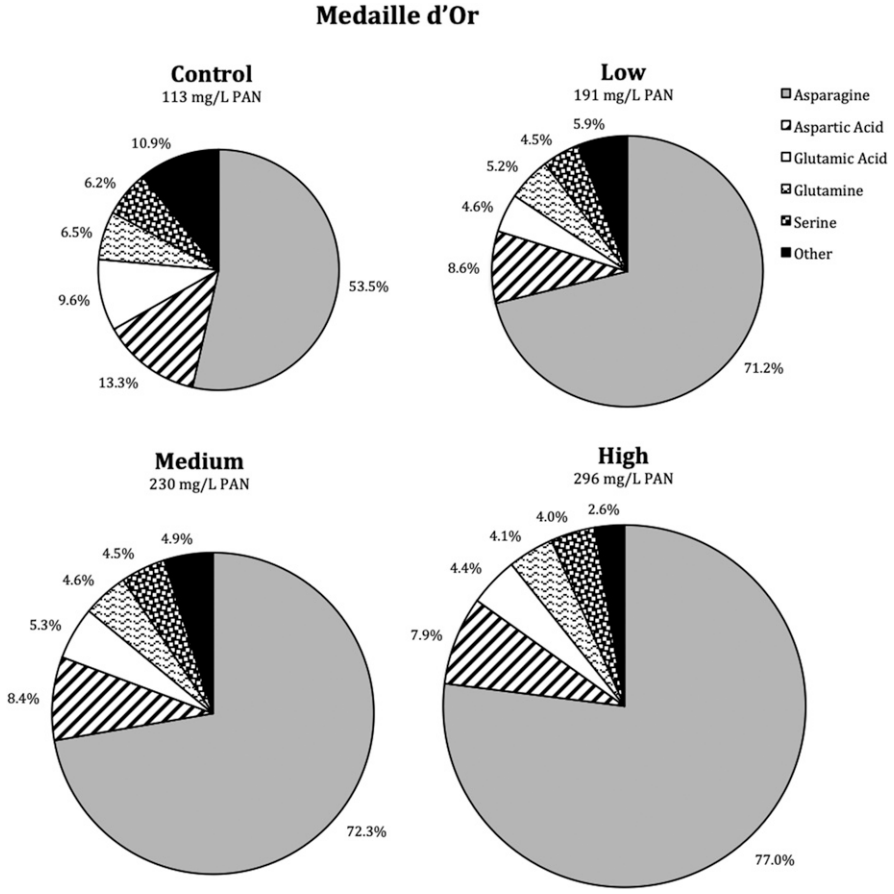

Fig. 4. Total free amino nitrogen (FAN) concentration and proportions of amino acids constituting FAN of apple juice from 'Medaille d'Or' trees with no, low (28 kg·ha^{-1}), medium (56 kg·ha^{-1}), and high (112 kg·ha^{-1}) calcium nitrate fertilizer application rates in Ithaca, NY. Pie chart sizes are to scale of primary amino nitrogen (PAN) concentration among different treatments. Data represent a combined model from both 2017 and 2018.

values do not factor in the costs of $CaNO_3$ and its application). However, of the supplements in the model, Fermaid O™ is the only exogenous supplement comprised mainly of FAN, and thus most similarly resembles the composition of apple juice YAN.

Karl et al. (2020) investigated the efficacy of using foliar urea applications to increase YAN in 'Red Spy' apple trees. In both that study and ours, nearly all increases in YAN were in the form of FAN, and, more specifically, asparagine. Overall, the magnitude of increased YAN was much greater from the foliar urea applications in the fall than $CaNO_3$ soil applications in the spring. For example, after five urea applications (cumulatively 21.5 kg N/ha), YAN increased by an average of 319% in comparison with the control. By comparison, after 3 years of repeated $CaNO_3$ applications (112 kg N/ha/year), YAN in the high 'Golden Russet' treatment was only 88% greater than the control. Dong et al. (2005) compared urea uptake by apple tree roots and leaves in 'Fuji'/'M.9' trees 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 drainage 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 documented previously 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. H_2S production is a common problem in the cider industry and is frequently attributed to deficient YAN (Jiranek et al., 1995; Ugliano et al., 2011). Although not statistically different, mean H_2S production rates were greater in treatments receiving more nitrogen fertilizer than in those with less in 2018. H_2S 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 (Boudreau et al., 2017a; Ugliano et al., 2009). However, no residual H_2S was detectible in any of the finished ciders in this study. H_2S is sparged from wines and ciders by CO_2, and H_2S production early during fermentation, such as was observed in our study, can result in little or no H_2S in the beverage at the end of fermentation (Ugliano et al., 2009). In addition, although not measured in our study, greater YAN concentrations have also been shown to increase concentrations of volatile aromatic compounds such as acetate and ethyl esters during wine fermentation (Santos et al., 2015; Tahim and Mansfield, 2019; Torrea et al., 2011). In future studies, sensory evaluation of ciders from trees receiving different rates of nitrogen fertilization could help elucidate consumer detection thresholds and preferences.

Table 8. Fermentation characteristics from apple juice 'Golden Russet' and 'Medaille d'Or' trees with no, low (28 kg·ha^{-1}), medium (56 kg·ha^{-1}), and high (112 kg·ha^{-1}) calcium nitrate fertilizer application rates in Ithaca, NY. Values are means ± SE.

Cultivar	Yr	Treatment	Hydrogen sulfide production (μg·L^{-1})	Maximum fermentation rate (mg sugar/L/h)	Fermentation duration (d)	Residual sugar (g·L^{-1})
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	14.0 ± 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

Conclusion

For both apple cultivars tested in this experiment, greater $CaNO_3$ application rates increased juice YAN in the form of FAN, 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 production were affected only minimally 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 our study, increased nitrogen fertilization had positive impacts on fruit quality from a cidermaking perspective, but had no negative impacts on tree physiology or fruit quality. Further research investigating how nitrogen fertilizer applications greater than the levels applied in our study might affect tree physiology and cider fruit quality would be valuable to help determine target nitrogen levels for cider orchard trees and to establish fertilization recommendations for the cider industry.

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