# **Crop Load Density Affects 'York' Apple Juice and Hard Cider Quality**

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Abstract. To assess the impact crop load has on hard cider chemistry, 'York' apple (Malus  $\times$  domestica Borkh.) trees were hand thinned to three different crop loads: low [two apples per cm<sup>2</sup> branch cross-sectional area (BCSA)], medium (four apples per BCSA), and high (six apples per BCSA). Higher crop loads produced smaller, less acidic fruit that were slightly more mature. In juice made from fruit from these treatments, the total polyphenol content did not differ at harvest, but, after fermentation, the medium crop load had 27% and the high crop load had 37% greater total polyphenol content than the low crop load treatment had 18% and 22% greater than the medium and high crop load, respectively. YAN concentrations in juice from the medium and high crop load treatments were similar. Our results provide apple growers and hard cider producers with a better understanding of how apple crop load impacts YAN concentrations in juice and total polyphenol concentrations in juice and cider.

Hard cider is an alcoholic beverage produced from fermented apple juice or apple juice concentrate. Domestic cider consumption has increased more than 850% in the last 5 years and there are now over 550 cider producers in the United States (TTB, 2007-14; Brown, 2016). The vast majority of cider produced in the United States is made from apple cultivars that were originally planted for fresh or processing markets (Peck and Miles, 2015). Culinary apples do not have all of the fruit quality characteristics desired by cider producers, but many of the desired cider apple cultivars have not been documented as being widely planted in the United States (Miles et al., 2015; Peck, 2012; U.S. Apple Association, 2015). In the United States, where the production of traditional hard cider apple cultivars has lagged behind the increase in cider sales, methods to increase cider quality from existing apple cultivars are needed.

Fruit quality attributes that are important for culinary apple production include low incidence of damage and decay, fruit shape and size (typically measured by fruit mass), peel color, flesh firmness, soluble solid concentration (SSC), titratable acidity (TA) and pH, and flavor (La Belle, 1981). Along with the starch pattern index (SPI) and internal ethylene concentration (IEC), fruit quality factors are often measured to gauge harvest maturity (Watkins, 2003). For cider production, fruit quality attributes also include polyphenol and YAN concentrations in the fruit, and juice yield, while cosmetic attributes such as color, shape, and size are much less important (Lea, 1996).

Apple orchard management practices that focus on fruit quality characteristics that are desirable for cider production are needed. Specifically, most apples commercially grown in the United States have low YAN and polyphenol concentrations (Thompson-Witrick et al., 2014). While both exogenous nitrogen and polyphenols (i.e., "enological tannins") may be added to increase their concentration in cider, the sensory impact of addition of these products to cider warrants further investigation. For example, the addition of commercially available exogenous tannins to red wine has been shown to increase the measured total polyphenol concentration, but they did not always lead to improvement in sensory character (Harbertson et al., 2012). As such, increasing endogenous polyphenol concentration in fruit remains the generally preferred approach to achieve desired sensory characteristics for wine and cider.

In European wine grape (*Vitis vinifera* L.) production, measurable improvements in fruit quality have been achieved through

adjusting the relationship between fruit yield and vegetative growth, often referred to as crop load. Grape cluster crop load has been shown to impact secondary metabolism in grape berries, which can in turn impact wine chemistry, aroma, and flavor. For example, SSC was greater in 'Chambourcin' grapes that were from vines with reduced fruit clusters (Dami et al., 2006). Similarly, lower crop loads for 'Sauvignon blanc' grapevines resulted in wine that had more favorable sensory scores (Naor et al., 2002). A study of 'Shiraz' grapevines under five training systems in the Barossa Valley of Australia demonstrated that grape berry anthocyanin and polyphenol concentrations decreased with increasing crop load (Wolf et al., 2003). However, a point is reached when continuing to decrease crop load results in decreased yield and increased production cost, but no further increase in wine quality (Berkey et al., 2011; Bravdo et al., 1985; Preszler et al., 2010). Although horticultural practices for apples and grapes are quite different, wine grape growers exert a tremendous amount of effort optimizing fruit quality to make their crop more desirable to their buyers. For these reasons, crop load targets are often specified in vineyard management with the goal of maintaining optimal fruit quality for winemaking (Wolf, 2008).

With the increased utilization of apples for cider production, it is necessary to more fully understand how orchard management decisions, such as crop load density, impact cider quality. The development of crop load management practices can be used by orchard managers to improve cider produced from culinary apples. The goal of this project was to assess the impact of three different crop load densities on fruit and cider quality.

## **Materials and Methods**

Field treatments were conducted in a 14year-old 'York Imperial (Ramey)'/'M.9' orchard at the Alson H. Smith, Jr. Agricultural Research and Extension Center (Winchester, VA) in 2014. 'York' apples are primarily used for processing into products such as juice, vinegar, and applesauce. On 16 June ( $\approx$ 50 d after full bloom), five single-tree replications of each of the three crop load treatments were implemented by hand thinning apples to the specified crop load density level on three branches per tree. The low crop load treatment was thinned to two apples per cm<sup>2</sup> BCSA, the medium crop load was thinned to four apples per BCSA, and high crop load was thinned to six apples per BCSA. The rest of the tree was thinned to about the same crop load density by visually assessing the crop load on the three branches and replicating that spacing. Fruit was only sampled from the three branches that were hand thinned to the precise number of fruit per BCSA. The experiment was blocked based on a visual assessment of whole-tree crop load before the implementation of the treatments. Treatments were randomly assigned

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to trees with similar crop load levels. The orchard was not irrigated or fertilized during the course of this experiment and weed, insect, and disease management was executed according to regional recommendations (Pfeiffer et al., 2014).

An initial harvest was conducted on 29 Sept. to assess the relative maturity of the treatments using a pooled 10-fruit subsample from the treated branches on each tree which were analyzed for IEC, SPI, fruit firmness, SSC, and TA as described in Thompson-Witrick et al. (2014). Briefly, apples were weighed and visually assessed for percent red blush (0-100%). Flesh firmness was measured on the same samples, after removing part of the peel at two locations along the equator of each apple, using a Fruit Texture Analyzer penetrometer [Güss Manufacturing (Pty) Ltd., Strand, South Africa] fitted with an 11.1-mm-diameter Effegi tip. The SPI was determined by staining the stem side of an equatorial cross section of the apples with iodine solution (0.22% w/v iodine, 0.88% w/v potassium iodine) and rating patterns against a chart, where 1 = 100% staining and 8 = 0% staining (Blanpied and Silsby, 1992). IEC was measured on a 1-mL sample removed from the core cavity of the apple using a gas chromatograph (Agilent 7890; Wilmington, DE) equipped with a flame ion detector. The calyx half of each apple was juiced in a Champion Juicer (Lodi, CA) and SSC was measured using a digital PAL-1 refractometer (Atago U.S.A., Inc., Bellevue, WA) and reported as percent Brix. TA was measured by titrating a 5-mL juice aliquot against a 0.1 N NaOH solution to an endpoint of pH 8.1 with an autotitrator (848 TitrinoPlus, Herisau, CH). A separate 50 mL juice subsample was frozen (-80 °C) and shipped to the Enology and Fermentation Laboratory in the HABB1 Building at Virginia Tech (Blacksburg, VA) for total polyphenol analysis as described below.

On 10 Oct., an additional 10 apples per tree were analyzed for the same parameters as mentioned above. The remaining apples from the treated branches were also harvested and transported to the Enology and Fermentation Laboratory and stored at 4 °C for 1 week before processing. All other apples from each sample tree were harvested, counted (as were fruit that dropped prematurely), and reported as crop load on a square centimeter trunk cross-sectional area (TCSA) basis.

On 17 Oct., apples from four of the five field replications were cleaned in a rod and reel washer and ground into a pulp using a hammer mill (RH HM100; Herbold Meckesheim USA, Smithfield, RI). There was not enough fruit from the fifth replication to produce a sufficient volume of juice for fermentation. As the apple pulp was produced, it was layered evenly onto a custombuilt rack and cloth press and pressed until juice no longer ran from the racks. Between each treatment, the hammer mill and press were disassembled and thoroughly rinsed with water. Juice was collected in foodgrade plastic buckets, covered, and held at 4 °C for 4 d to allow particulate matter to settle before further processing. Samples for YAN analysis, described below, were taken at this time. No clarification agents or other adjuvants were added to the juice after pressing. On 21 Oct., samples were removed from refrigeration and warmed to 16 °C over a 3-h period. From each sample, 5.7 L of juice was decanted off the gross lees into 11.6-L carboys. Potassium metabisulfite was added at a rate of 33 mg $\cdot$ L<sup>-1</sup> to each carboy as an antioxidant and antimicrobial agent. Commercial yeast (Saccharomyces cerevisiae × bayanus strain EC1118 Prise de Mousse) (Scott Laboratories, Petaluma, CA) was rehydrated in warm, de-ionized water and pitched at a rate of 238 mg·L<sup>-1</sup> into each carboy. Go-Ferm® rehydration nutrient (Scott Laboratories) was added at a rate of 301 mg $\cdot$ L<sup>-1</sup> juice to the rehydration water before addition of the yeast. Each carboy was stopped with a bung and air lock and placed into a refrigerator at 16 °C. During fermentation, SSC and temperature were measured daily with a portable Density Meter (Anton Paar USA, Ashland, VA). Fermaid K yeast nutrient (Scott Laboratories) was added at 238 mg·L<sup>-1</sup> during fermentation, at 1/3 sugar depletion, per the manufacturer's recommendation. Nine days after fermentation began (30 Oct.), the headspace of each carboy was sparged with nitrogen gas to displace oxygen and minimize cider oxidation. When the SSC for all samples had reached a stable value (4 Nov.), the fully fermented ciders were decanted into 3.8-L carboys. Potassium metabisulfite was added at a rate of 33 mg  $L^{-1}$  to further minimize oxidation. Subsamples for polyphenol analysis were taken from each sample and frozen at -80 °C until the time of analysis.

Total polyphenol concentration of juice and cider samples was determined using the Folin–Ciocalteu (FC) assay as described by Waterhouse (2002). A standard curve was prepared using gallic acid (Sigma Aldrich, St. Louis, MO) and results are reported in gallic acid equivalents (GAE). Absorbance was measured at 765 nm on a spectrophotometer (Genesys 10S ultraviolet-VIS; Thermo Scientific, Madison, WI). Samples were run in triplicate.

YAN was quantified using two commercially available assay kits for determination of 1) free amino nitrogen (K-PANOPA kit; Megazyme, Wicklow, Ireland) and 2) ammonium ion (K-AMIAR rapid ammonia determination kit; Megazyme). Total YAN was determined by summing the primary amino nitrogen value and the nitrogen contribution of the ammonium ion to obtain a total YAN value. Juice samples were analyzed for YAN immediately after pressing and were centrifuged at 1096  $g_n$  for 5 min before analysis. Analyses were performed in triplicate. SSC was determined using a DMA 35 handheld digital density meter (Anton Paar USA). Ethanol concentration and residual sugar concentration in cider samples were determined by Foss WineScan FT 120 (Eden Prarie, MN). A repeated measures analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) was used to analyze fermentation rate to reflect changes in treatment and control values over time. All data were subjected to ANOVA and post hoc mean separation was performed by Tukey's HSD using SAS (version 9.2; SAS Institute Cary, NC).

#### **Results and Discussion**

Whole tree crop load was 42% to 51% greater than the targeted crop load on the hand-thinned treatments, but treatments were different from each other in a progression that matched the imposed treatments on the handthinned branches (Table 1). Only fruit from the precisely hand-thinned branches were used for fruit and cider chemical analysis. At both harvests, apples from the low crop load treatment on the hand-thinned branches were the largest in diameter and the greatest in mass, while apples from the high crop load treatment were the smallest in diameter and had the least mass (Tables 2 and 3). On a whole tree basis, apples from the low crop load treatment also had the greatest mass, while apples from the high crop load treatment had the least mass (Table 1). The relationship between low crop load and large fruit size is well known in apple production and is one of the reasons for a considerable body of research aimed at identifying the ideal crop load for different cultivars and orchard designs (Dennis, 2000; Embree et al., 2007; Marini et al., 2002; Robinson and Watkins, 2003). In addition, greater crop loads will inhibit flower bud initiation and result in low flower density and yields in the following season. 'York' apple trees are prone to biennial bearing when over cropped, so commercial growers typically strive for a crop load of between four and six fruit/ TCSA (Byers and Carbaugh, 2002; Miller and Tworkoski, 2010). There were no differences

Table 1. Crop load, mean fruit weight, and preharvest drops of 'York'/'M.9' apple trees with low [two fruit per branch cross-sectional area (BCSA)], medium (four fruit per BCSA), and high (six fruit per BCSA) crop loads in Winchester, VA, when harvested on 10 Oct.

Crop load	Whole tree crop load (fruit per TCSA)	Fruit wt (g)	Preharvest drop (%)
Low	$4.8 \pm 0.3 \ c^{z}$	190 ± 19 a	$6.0 \pm 0.0$
Medium	$7.9\pm0.5$ b	$148 \pm 15 \text{ b}$	$4.8\pm0.2$
High	$11.8 \pm 0.4 \text{ a}$	123 ± 13 c	$4.7\pm0.2$

<sup>z</sup>Mean separation within column by Tukey's honestly significant difference test at  $P \le 0.05$ ; values are mean  $\pm$  se (n = 5).

TCSA = Trunk cross-sectional area.

Table 2. Fruit diameter, mean fruit weight, red peel color, flesh firmness, starch pattern index (SPI), internal ethylene concentration (IEC), soluble solid concentration (SSC), pH, and titratable acidity (TA) of 'York'/'M.9' apples with low [two fruit per branch cross-sectional area (BCSA)], medium (four fruit per BCSA), and high (six fruit per BCSA) crop loads in Winchester, VA, when measured at harvest 1 (29 Sept.).

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Crop load	Diam (mm)	Fruit wt (g)	Color (%)	Flesh firmness (N)	SPI (1-8)	IEC ( $\mu L \cdot L^{-1}$ )	SSC (°Brix)	pH	$TA(g \cdot L^{-1})$
Low	$79.2 \pm 2 a^{y}$	196 ± 14 a	94 ± 3 ab	$98 \pm 9$ b	$1.4 \pm 0.1$ a	<lod<sup>z</lod<sup>	$11.1 \pm 0.1$	$3.37 \pm 0.01$ ab	5.9 ± a
Medium	$73.0 \pm 2$ b	$151 \pm 9 b$	96 ± 2 a	$102 \pm 8 \text{ ab}$	$1.3 \pm 0.1 \text{ ab}$	<lod< td=""><td><math>11.0 \pm 0.2</math></td><td><math display="block">3.36\pm0.01\ b</math></td><td><math>5.0 \pm b</math></td></lod<>	$11.0 \pm 0.2$	$3.36\pm0.01\ b$	$5.0 \pm b$
High	$69.2 \pm 2 c$	$128 \pm 10 \text{ c}$	$93\pm3~b$	$104 \pm 7 a$	$1.2\pm0.1$ b	<lod< td=""><td><math display="block">11.0\pm0.2</math></td><td><math display="block">3.38\pm0.01\ a</math></td><td><math>4.5 \pm c</math></td></lod<>	$11.0\pm0.2$	$3.38\pm0.01\ a$	$4.5 \pm c$
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<sup>z</sup>LOD = Limit of detection.

<sup>y</sup>Mean separation within columns by Tukey's honestly significant difference test at  $P \le 0.05$ ; values are mean  $\pm$  sE (n = 5).

Table 3. Fruit diameter, mean fruit weight, red peel color, flesh firmness, starch pattern index (SPI), internal ethylene concentration (IEC), soluble solid concentration (SSC), pH, and titratable acidity (TA) of 'York'/'M.9' apples with low [two fruit per branch cross-sectional area (BCSA)], medium (four fruit per BCSA), and high (six fruit per BCSA) crop loads in Winchester, VA, when measured at harvest 2 (10 Oct).

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Crop load	Diam (mm)	Fruit wt (g)	Color (%)	Flesh firmness (N)	SPI (1-8)	IEC ( $\mu L \cdot L^{-1}$ )	SSC (°Brix)	pH	$TA(g \cdot L^{-1})$
Low	$81.1 \pm 2 a^{z}$	209 ± 13 a	$98 \pm 1$	$89 \pm 3 b$	$1.9 \pm 0.3 \text{ b}$	$1.8 \pm 1$	$12.5 \pm 0.2$ a	$3.44 \pm 0.01 \text{ b}$	$5.4 \pm 0.2$ a
Medium	$74.4 \pm 2 \ b$	$159 \pm 10$ b	$97 \pm 1$	93 ± 4 a	$2.0\pm0.4$ b	$5.1 \pm 9$	$12.1 \pm 0.2$ b	$3.53 \pm 0.01$ a	$4.5\pm0.1\ b$
High	$71.6 \pm 2$ c	$145 \pm 10 \text{ c}$	$98 \pm 1$	$92 \pm 3$ a	$3.1\pm0.5~a$	$14.7 \pm 23$	$12.0\pm0.1\ b$	$3.52\pm0.01~a$	$3.8\pm0.1\ c$
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<sup>2</sup>Mean separation within column by Tukey's honestly significant difference test at  $P \le 0.05$ ; values are mean  $\pm$  se (n = 5).

Table 4. Total polyphenol concentration expressed as gallic acid equivalents (GAE) of fresh and fermented apple juice from 'York'/'M.9' apple trees with low [two fruit per branch cross-sectional area (BCSA)], medium (four fruit per BCSA), and high (six fruit per BCSA) crop loads in Winchester, VA.

	Total polyphenols (mg·L <sup>-1</sup> GAE)				
Crop load	Harvest 1 juice	Harvest 2 juice	Fermented cider		
Low	$209 \pm 30^{z}$	$225 \pm 20$	215 ± 14 a		
Medium	$216 \pm 15$	$245 \pm 13$	$294 \pm 11 \text{ b}$		
High	$227 \pm 21$	$218 \pm 22$	$340 \pm 10 \text{ c}$		

<sup>z</sup>Mean separation within column by Tukey's honestly significant difference test at  $P \le 0.05$ ; values are mean  $\pm$  se (n = 5 for harvest 1 and 2; n = 4 for the fermented cider).

in the amount of premature fruit drop among treatments (Table 1).

The medium crop load treatment had a greater red peel color than the high crop load treatment at the first harvest, but no differences at the second harvest were detected when all treatments had, on average, greater than 97% red coloration (Tables 2 and 3). Fruit firmness was lower in the low crop load treatment than the high crop load at harvest 1, and lower than both the medium and high crop load treatments at harvest 2. The SPI was higher (lower starch content) in the low crop treatment than the high crop load treatment at harvest 1, but the high crop load treatment had the highest SPI rating at harvest 2. There was no difference in IEC among treatments at either harvest, but numerically, fruit from the high crop load treatment had six times greater IEC at harvest 2. There was no difference in SSC at harvest 1, but at harvest 2, fruit from the low crop load treatment had the highest SSC. The high crop load treatment had greater pH values than the medium crop load treatment at harvest 1, and greater than the low crop load treatment at harvest 2. At both harvests, the low crop load treatment had the greatest TA and the high crop load treatment had the lowest TA.

When all other biological and environmental conditions are the same, higher crop loads are often reported to delay fruit maturity and result in fruit with lower SSC, greater TA, greater flesh firmness, and less red coloration (De Salvador et al., 2006; Serra et al., 2016; Wünshe et al., 2005). It is unclear why our treatments did not follow that trend; however, a similar result has been shown in 'Honeycrisp' apples (Robinson and Watkins, 2003). We detected no differences in SPI, IEC, or SSC in fruit from the first harvest, indicating that there was no maturity difference at that time (Table 2). However, at the second harvest, the high crop load treatment had the greatest SPI suggesting that it was the treatment with the most advanced maturity, although SSC and IEC were similar among the treatments (Table 3). A visual assessment of the percentage of return bloom was made in the following spring. All treatments had a very low return bloom relative to other trees in that orchard, but there were no statistical differences among the treatments.

Total polyphenol concentrations of juice samples derived from whole fruit ranged between 209 and 245 mg·L<sup>-1</sup> GAE, which is similar to the concentration range found by Thompson-Witrick et al. (2014) using apples harvested in 2013 from the same trees (1 year before this study). There was no difference in total polyphenol concentration among crop load treatments at either harvest, but after fermentation, cider from the high crop load treatment had 63% greater total polyphenol concentration (Table 4). Awad et al. (2001) found that greater crop loads decreased sugars, acids, and flesh firmness of 'Jonagold', but not of 'Elstar' apples, suggesting that genotypic differences may exist. However, crop load made little or no difference for most individual polyphenol compounds [measured by high-performance liquid chromatography (HPLC)] in the peel of either cultivar. Using whole fruit samples, Stopar et al. (2002) found that when 'Jonagold' crop

load increased by 80%, the concentration of total polyphenols (measured by FC) decreased by 30%, as did red peel color, sugars, and flesh firmness. The study also analyzed individual polyphenols such as catechin and epicatechin (measured by HPLC) which showed a decrease of 178% and 71%, respectively, between their lowest (30 fruits per tree) and highest crop load (157 fruits per tree) treatments. Another study using whole fruit samples of 'Fuji', 'Gala', and 'Golden Delicious' found inconsistent total polyphenol concentrations (measured by FC) trends and no significant differences among three crop load densities (Unuk et al., 2006). None of these studies analyzed juice or fermented cider. Thus, our results may be due to different conditions such as oxidation during processing, increasing ethanol concentration and other biochemical processes of yeast metabolism during fermentation. Polyphenol composition in apple juice and cider may be different from that of peel or flesh tissue that is immediately frozen (Renard et al., 2011). Thus, it is reasonable to expect different results when juice or cider processed to approximate commercial cider making conditions is analyzed, rather than analyzing peel or flesh that is handled to minimize oxidation and reflect polyphenol concentration and composition of intact apple fruit tissues.

Fermented cider from the high crop load treatment had the greatest total polyphenol concentration and the low crop load had the lowest total polyphenol concentration (Table 4). Polyphenol compounds are endogenous to all apples, but are found in various concentrations based on the cultivar and specific tissue being analyzed. In a separate study conducted in Virginia, it was shown that the peel of 'York' apples contained more than five times the total polyphenols of the flesh on a wet weight basis, specifically catechins, quercetin derivatives, and phloretin derivatives (Thompson-Witrick et al., 2014). It should be noted, however, that peel tissue accounts for less than 10% of the total apple mass (Peck et al., 2006). In addition, given the lack of specificity of the FC assay method, it is not possible to know which polyphenol compounds were different among the treatments in our experiment. However, other reports suggest that procyanidins and (+)-catechin in the pulp and dihydrochalcones such as quercetin and phloretin in the peel, as well as the degree of polyphenol polymerization can be used to identify ripe from unripe fruit (Alonso-Salces et al., 2005).

The juice used for polyphenol analysis at the two harvests was made from a 10-fruit sample, while the cider for each experimental unit was made from about equal volumes of fruit in order to fill the fermentation vessels with the same amount of juice for each treatment. When processing the same volume of fruit into juice for cider, smaller fruit sizes would likely have a greater skin to flesh ratio, which in turn could increase the total phenolic content of the cider. This may explain why we did not find differences in polyphenol content in the juice, but we did find greater polyphenol content as the crop load treatments increased and the fruit size correspondingly decreased. In addition, fermentation of unclarified juice with some remaining solids could have allowed for continued extraction of polyphenols from suspended solids, with increased extraction efficiency as the ethanol concentration of the matrix increased during fermentation. The samples pressed from smaller fruit (in this case, high crop load lots) would contain a higher proportion of peel in the solids, and thus extraction of polyphenols from solids during fermentation could reasonably be expected to yield higher total polyphenols in lots where the solids consisted of a higher proportion of the polyphenol-rich peel (i.e., the high crop load treatment).

Further research should include a determination of individual polyphenol compounds, specifically flavan-3-ols and procyanidins in both juice and cider, to provide insight as to whether the crop load treatments influence the composition of sensorially important polyphenols. Apple polyphenol composition may change during ripening (Renard et al.,



Fig. 1. Yeast assimilable nitrogen of juice from 'York'/'M.9' apple trees with low [two fruit per branch cross-sectional area (BCSA)], medium (four fruit per BCSA), and high (six fruit per BCSA) crop loads in Winchester, VA. Mean separation by Tukey's honestly significant difference test; error bars represent sE (n = 4).

2007). Thus, it is possible that the polyphenol composition (not only the concentration) in juice and cider from the high crop load treatment was different from that of the juice and cider from the low crop load treatment. Polyphenols present in fruit from the high crop load treatment may have been less susceptible to oxidation during processing, or more easily extracted under conditions of increasing ethanol concentration, as is the case with phloretin (Li et al., 2011). However, the FC method measures total polyphenols and to conclusively determine this hypothesis would require a quantification of individual polyphenols, an analysis that was not performed for this study.

The juice from the low crop load treatment in the present study had greater YAN concentration than either the medium or high crop load treatments (Fig. 1). After a carbon source (i.e., sugar), nitrogen is the most important nutrient required by yeast. Yeast use nitrogen to build cell mass and produce enzymes required for metabolism (Bell and Henschke, 2005). YAN refers to nitrogen forms available for yeast metabolism and includes ammonium ions as well as free amino acids. When YAN concentration in juice is insufficient, fermentation performance can be less than ideal, resulting in the production of undesirable off-aromas such as hydrogen sulfide, and/or incomplete fermentation (Bisson and Butzke, 2000; Bohlscheid et al., 2011). The minimum

YAN concentration required to complete fermentation depends on multiple factors including osmotic stress (due to high initial sugar concentration), yeast strain, and the presence of other nutrients including biotin and pantothenic acid (Bell and Henschke, 2005; Bohlscheid et al., 2011). General recommendations for minimum YAN for winemaking range from 140 to 350 mg  $\cdot$ L<sup>-1</sup>, and all the treatments would have been below this threshold (Bisson and Butzke, 2000; Mendes-Ferreira et al., 2004). The establishment of a target YAN value for cider fermentation remains a topic of current research. The greater YAN concentration in the low crop load treatment did not coincide with a significant fermentation duration when analyzed by comparing maximum fermentation rate (the slope of the curve during the logarithmic phase of the fermentation) or by repeated measures (Fig. 2). There were no differences among treatments for total alcohol, residual sugar, or pH in the fermented cider (Table 5). However, consistent with the juice data, the low crop load treatment had the highest TA in the fermented cider and the high crop load treatment had the lowest TA.

## Conclusion

Our study suggests that apple juice and cider quality can be altered by crop load management. Management strategies for chemical thinning should take into account



Fig. 2. Fermentation monitoring of juice from 'York'/'M.9' apple trees with low [two fruit per branch cross-sectional area (BCSA)], medium (four fruit per BCSA), and high (six fruit per BCSA) crop loads in Winchester, VA, for 15 d, reported as observed soluble solids concentration on each day for each treatment. Plotted values represent means and error bars represent set (n = 4).

Table 5. Total alcohol, residual sugar (RS), pH, and titratable acidity (TA) of fermented cider from 'York'/ 'M.9' apple trees with low [two fruit per branch cross-sectional area (BCSA)], medium (four fruit per BCSA), and high (six fruit per BCSA) crop loads in Winchester, VA.

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Crop load	Total alcohol (%)	RS (%)	pН	TA $(g \cdot L^{-1})$
Low	$6.4 \pm 0.1^{z}$	$< 1.0 \pm 0$	$3.53 \pm 0.03$	$5.7 \pm 0.5 \text{ a}$
Medium	$6.4 \pm 0.2$	$< 1.0 \pm 0$	$3.52 \pm 0.02$	$5.0 \pm 0.2$ b
High	$6.3 \pm 0.1$	$< 1.0 \pm 0$	$3.58\pm0.03$	$4.5\pm0.2\ c$

<sup>z</sup>Mean separation within column by Tukey's honestly significant difference test at  $P \le 0.05$ ; values are mean  $\pm$  se (n = 4).

the resulting fruit quality, especially YAN concentration in juice prefermentation and total polyphenol concentration and TA in fermented cider. Cider makers should be especially aware of the potential for YAN deficiency in fruit from orchards with a high crop load. YAN deficiency can be rectified through the addition of commercially available YAN supplements when warranted. Notwithstanding, the inherent genetics of apple cultivars will likely have the greatest impact on cider quality, including polyphenol concentration. To make ciders with considerably higher polyphenol concentrations, producers will need to source high polyphenol bittersweet or bittersharp cultivars.

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