


Article

Pruning and In-Season Canopy Manipulation Affects *MidSouth* Juice and Wine Phenolic Content

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Abstract: Low total soluble solids and high titratable acidity limit *MidSouth* use as a varietal red wine grape. While canopy management practices were reported not to have enough of an effect on these primary metabolites, they could potentially improve *MidSouth* secondary metabolites, broadening its potential as a wine grape. Two studies assessed the effects of different canopy management treatments on monomeric anthocyanin pigments and total phenolic content in *MidSouth* juice and wine. The first study compared early pruning, early pruning with leaf removal, normal pruning with leaf removal, and normal pruning. Early pruning with leaf removal showed higher total phenolics in juice and wine in 2021 but lower levels in 2020. The second study evaluated leaf removal, shoot thinning, or neither leaf removal nor shoot thinning. Leaf removal resulted in higher anthocyanins and total phenolics in 2021 juice, while shoot thinning increased total phenolics in 2021 juice and both anthocyanins and phenolics in 2021 wine. Shoot thinning demonstrated the most consistent improvement in phenolic content. *MidSouth* grapes can produce a range of wine phenolic content, depending on canopy management and postharvest treatment. Further investigation is needed to understand yearly variations and optimize *MidSouth* for regional red wine production.

Keywords: anthocyanins; canopy management; grape composition; interspecific hybrid; red wine; wine composition



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1. Introduction

Wine grapes (*Vitis* spp.) provide a significant source of phenolic compounds [1,2]. The amount and variety of these phenolic compounds shape resultant wine color, taste, bitterness, astringency, mouthfeel, and ageability [2,3]. Additionally, these compounds offer health benefits, with the potential to protect against cancer, cardiovascular issues, and neurodegenerative conditions [1,2]. Phenolic compounds can be extracted from various parts of grapes, including skins, pulp, juice, seeds, and rachises. Typically, the phenolic content is quantified as milligrams of gallic acid equivalents per liter (mg GAE/L). This content can vary widely, ranging from 114 to 5615 mg GAE/L, depending on factors such as grape variety, ripeness, geographical origin, vineyard management practices, and fermentation conditions [1–3].

Anthocyanins, which are pigmented phenolic compounds, determine the color of red grape juices and wines, and they are important to assessing the quality of these products [1,2]. While monoglucosidic malvidin is the most abundant anthocyanin in most *V. vinifera* grapes and is often used in determining monomeric anthocyanin content, that is not necessarily true for other *Vitis* species or even for all cultivars within the same species [1,2]. Thus, an alternate method for calculating monomeric anthocyanin pigment involves cyanidin-3-glucoside, the most prevalent anthocyanin in nature, serving as the standard [4,5]. As grapes ripen, their anthocyanin levels rise, with concentrations ranging from 15 to 700 mg/L in red grape juice or resultant wine [2,5].

Few wine grapes are well suited for growth in Mississippi, where relatively high temperature, humidity, and rainfall occur year-round [6–8]. However, *MidSouth* is an interspecific hybrid bunch grape that has increased in popularity as a potential red wine grape for the region due to its relatively low maintenance requirements [9]. Despite limitations of reportedly low total soluble solids (TSS) (<20 °Brix) and total anthocyanins (5.5 mg/100 g or 55 mg/L) and high titratable acidity (TA) (>10 g/L), these grapes have an interesting and distinct raspberry flavor and can produce juice that is rich in stilbenes [6,9–14].

Canopy management practices, such as different pruning timing, leaf removal, and/or shoot thinning, could potentially modify the limiting composition of *MidSouth* to more desirable levels. Earlier pruning timing, for example, could lead to a more favorable harvest time that avoids the usual hot and humid conditions of an early August harvest [6,9,15]. Additionally, enhanced photosynthesis as a result of improved light and space availability from defoliation may increase fruit TSS [15–20], which is often linked with an increase in anthocyanins and total phenolics [17,19,21,22]. Shoot thinning, which reduces crop yield and crowding within the canopy, may improve fruit quality by better distributing reserves to remaining vegetative and fruit sinks. Thus, this could lead to higher anthocyanin and total phenolic content in vines that have fewer clusters [16]. However, the results of these practices are contingent upon vine site, cultivar, and phenological stage at which these practices are performed [16–26].

Williams et al. [15] attempted to improve the TSS and TA content of *MidSouth* grapes through these canopy management practices, reporting that the labor required outweighed the benefits. While the effects of these treatments on yield components and primary metabolites were reported, their impacts on secondary metabolites before and after fermentation remain unexplored. These studies aim to investigate the effects of pruning timing, leaf removal, and shoot thinning on the total phenolic content and monomeric anthocyanin pigment in *MidSouth* fruit and wine, as well as better determine the potential of *MidSouth* for red wine production in the region.

2. Materials and Methods

2.1. Experimental Design

As previously discussed by Williams et al. [15], two studies were carried out at the Mississippi Agricultural and Forestry Experiment Station (MAFES) McNeill Research Unit in McNeill, MS (latitude 30°64' N, longitude 89°62' W; elevation 22 m asl; USDA hardiness zone 9a). A single row of *MidSouth* vines was used for both studies. In the first study, comprising 48 vines, four treatments were randomly assigned to vines within blocks, resulting in a randomized complete block design (RCBD) of four blocks with three subsamples of each treatment per block. These treatments included early pruning (T1.1) for 12 vines, early pruning plus pre-bloom leaf removal (T1.2) for 12 vines, normal pruning plus post-fruit-set leaf removal (T1.3) for 12 vines, and normal pruning as a control (T1.4) for 12 vines. In the second study, 60 vines, split into four blocks, were randomly assigned to three treatments, creating a RCBD with five subsamples of each treatment per block. These treatments consisted of post-fruit-set leaf removal (T2.1) for 20 vines, post-fruit-set shoot thinning (T2.2) for 20 vines, and a control group without leaf removal or shoot thinning (T2.3) for 20 vines.

2.2. Vine Management and Harvest

Previously published work by Williams et al. [15] further discusses the canopy manipulation treatments, general vine management, and harvest methods of these studies.

2.3. Postharvest

Immediately following harvest, the grapes underwent different treatments in each year, as shown in Figure 1. Subsamples of treatments within each block were combined, destemmed by hand, and pressed using a bladder press (Hydro Press 180L, Speidel, Ofterdingen, Germany), followed by adding the grape skins, pulp, and seeds back into

the juice in 2020. Alternatively, in 2021, the grapes were destemmed and crushed using a stainless-steel motorized crusher-destemmer (ENO 10, Enoitalia, Florence, Italy). After pressing or crushing, 100 mL aliquots were taken from each must (juice, pulp, skins, and seeds) and frozen at $-20\text{ }^{\circ}\text{C}$ for subsequent analysis. To prevent the growth of wild yeasts and bacteria, potassium metabisulfite was added to the must soon after pressing or crushing. In 2020, a concentration of 100 ppm of SO_2 was added to the must based on weight [4], later resulting in a stuck fermentation, reaching an average alcohol by volume of only 4.1%. Consequently, in 2021, the amount of SO_2 added was determined based on the measured pH levels and volume of the must [27,28]. The must was then transported in plastic 18.9 L buckets to the MAFES Experimental Seafood Processing Lab in Pascagoula, MS, for further wine preparation.

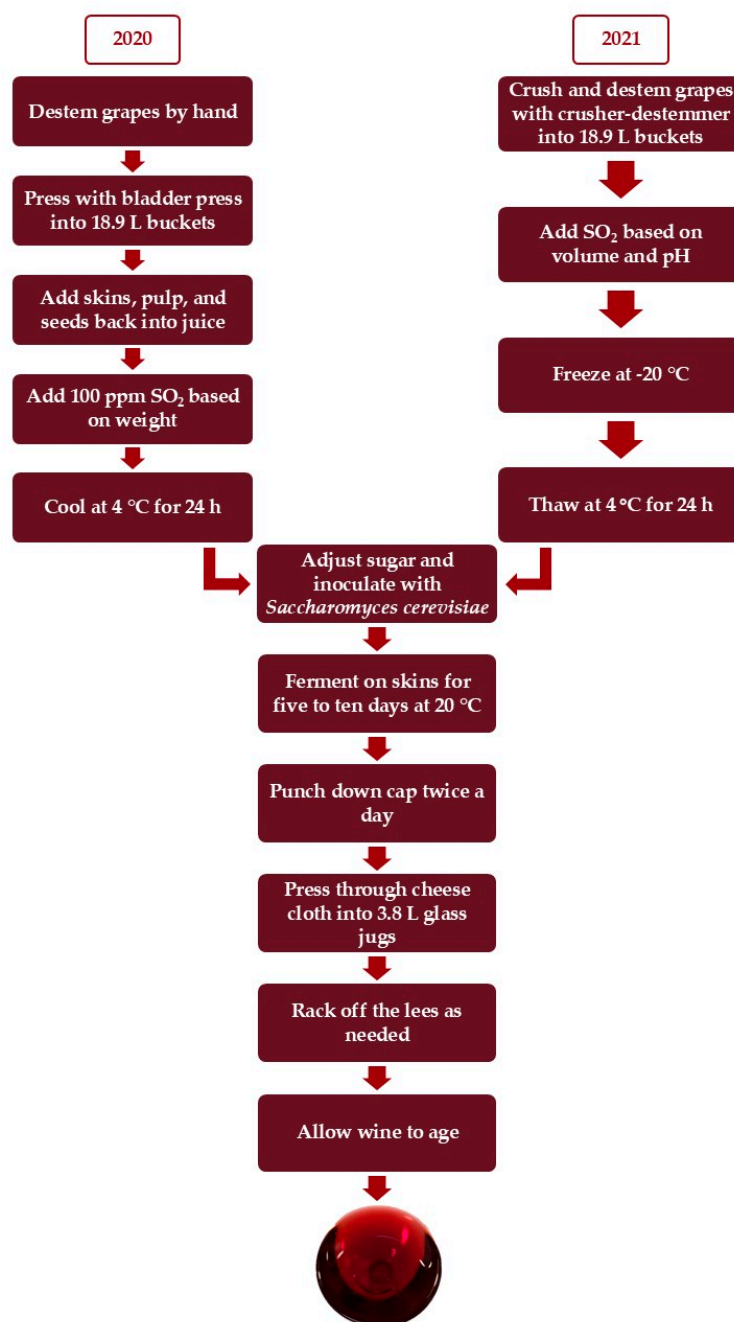


Figure 1. Flow diagram of the treatment of *MidSouth* grapes after harvesting in 2020 and 2021.

2.4. Winemaking

In 2020, the must underwent cooling at 4 °C for 24 h before the additions of sugar (target °Brix of 22), pectic enzyme, yeast nutrient, and *Saccharomyces cerevisiae* K1-V1116 (Lalvin, Lallemand Inc., Montreal, QC, Canada). Conversely, in 2021, the must was frozen at −20 °C for two months, followed by thawing at 4 °C for 24 h prior to these additions. Fermentations in both 2020 and 2021 occurred with the skins for five to ten days at room temperature (~20 °C), with twice-daily punch-downs to break up the cap of solids that formed on top. Subsequently, the must was strained through cheese cloth and transferred to 3.8 L glass jugs for secondary fermentation. Towards the end of fermentation, the wine underwent racking off the lees using a racking cane into a sanitized container, which was repeated three times before allowing the wine to age [3,4,27,28].

2.5. Phenolic Composition

Samples of both juice and wine underwent analysis for monomeric anthocyanin pigment. Must samples that were collected and frozen at harvest were thawed for 24 h before analysis, and wine was assessed two months after the start of fermentation. The anthocyanin measurement utilized a UV-Visible spectrophotometer (Evolution 60S, Thermo Scientific, Waltham, MA, USA) following the pH differential method. Absorbance measurements were read at wavelengths of 510 and 700 nm, and the pigment was quantified as milligrams of cyanidin-3-O-glucoside equivalents per liter using the following equation:

$$\text{Total Anthocyanins (mg/L)} = \frac{A \times MW \times DF \times 10^3}{\epsilon \times 1}$$

where A represents $(A_{510} - A_{700})$ pH 1.0 – $(A_{510} - A_{700})$ pH 4.5, MW (molecular weight) is 449.2 g/mol, DF (dilution factor) is 10, 1 signifies the pathlength in cm, and ϵ (molar extinction coefficient) is 26,900 L/(mol × cm) [4,5].

Total phenolic content was also measured in both juice and wine. This determination employed the Folin–Ciocalteu assay with the use of a microplate reader (FlexStation 3, Molecular Devices, LLC., San Jose, CA, USA). A standard curve was generated using known gallic acid solution dilutions. Absorbance measurements were read at 765 nm, and results were expressed as milligrams of gallic acid equivalents per liter of juice or wine (mg of GAE/L) [4].

2.6. Statistical Analysis

The general linear model (PROC GLM) in SAS statistical software (ver. 9.4; SAS Institute, Cary, NC, USA) was used to perform an analysis of variance of the data. Means were separated using Tukey's studentized range (Honestly Significant Difference) test at $\alpha \leq 0.05$. The analysis included variables such as treatment, block, and their interactions for each individual study. Due to the distinct preparations involved, the juice and wine content from each year were analyzed separately.

3. Results

3.1. Study 1

Total phenolic content of both 2020 and 2021 juice and wine and the monomeric anthocyanin pigment of 2020 wine were affected by treatment (Tables 1 and 2). Monomeric anthocyanin pigment was significantly affected only in 2020 wine, with both normal pruning treatments (T1.3, T1.4) significantly higher than early pruning (T1.1, T1.2) (Table 1). Total phenolic content was lowest in early pruning treatments (T1.1, T1.2) in 2020 juice and wine (Table 1). In 2021, early pruned vines (T1.1, T1.2) had the highest total phenolic content in the juice, but leaf removal vines (T1.2, T1.3) had the highest content in the wine (Table 2). In most cases, early pruning without defoliation (T1.1) did not differ from early pruning with defoliation (T1.2), and normal pruning with defoliation (T1.3) did not differ from normal pruning without defoliation (T1.4). Additionally, in 2020, the total phenolic

content decreased during the fermentation of juice to wine, but, in 2021, the total phenolic content increased during fermentation.

Table 1. Average *MidSouth* monomeric anthocyanin pigment and total phenolic content from juice and wine for each treatment in study 1 in McNeill, MS (2020).

Treatment ³	Juice		Wine	
	Monomeric Anthocyanin Pigment (mg/L) ¹	Total Phenolic Content (mg of GAE/L) ²	Monomeric Anthocyanin Pigment (mg/L)	Total Phenolic Content (mg of GAE/L)
1.1	62.6	493.3 b ⁴	31.7 b	108.2 b
1.2	71.0	510.2 b	37.2 b	115.5 b
1.3	66.8	562.3 ab	105.6 a	204.0 a
1.4	100.2	656.3 a	125.3 a	212.1 a
Treatment Significance	ns ⁵	*	***	***

¹ Monomeric anthocyanin pigment expressed as cyanidin-3-O-glucoside. ² GAE, gallic acid equivalent. ³ Treatment 1.1, Early Pruning; 1.2, Early Pruning + Leaf Removal; 1.3, Normal Pruning + Leaf Removal; 1.4, Normal Pruning (control). ⁴ Different lowercase letters following the means within columns indicate significant differences between treatments. ⁵ * and *** mean significantly different at $p \leq 0.05$ and 0.001, respectively, and ns means no significant difference.

Table 2. Average *MidSouth* monomeric anthocyanin pigment and total phenolic content from juice and wine for each treatment in study 1 in McNeill, MS (2021).

Treatment ³	Juice		Wine	
	Monomeric Anthocyanin Pigment (mg/L) ¹	Total Phenolic Content (mg of GAE/L) ²	Monomeric Anthocyanin Pigment (mg/L)	Total Phenolic Content (mg of GAE/L)
1.1	78.9	922.8 a ⁴	179.5	1177.7 b
1.2	133.6	896.3 a	196.2	1344.5 a
1.3	100.2	731.8 b	208.8	1297.5 a
1.4	91.8	672.5 b	187.9	1164.0 b
Treatment Significance	ns ⁵	***	ns	***

¹ Monomeric anthocyanin pigment expressed as cyanidin-3-O-glucoside. ² GAE, gallic acid equivalent. ³ Treatment 1.1, Early Pruning; 1.2, Early Pruning + Leaf Removal; 1.3, Normal Pruning + Leaf Removal; 1.4, Normal Pruning (control). ⁴ Different lowercase letters following the means within columns indicate significant differences between treatments. ⁵ *** means significantly different at $p \leq 0.001$ and ns means no significant difference.

3.2. Study 2

The total phenolic content of the wine was the only variable that differed by treatment in 2020, with shoot-thinned vines (T2.2) having higher content than leaf removal vines (T2.1) (Table 3). Both monomeric anthocyanin pigment and total phenolic content were significantly affected in 2021 (Table 4). Leaf removal vines (T2.1) exhibited the highest anthocyanin content in juice, and both leaf removal (T2.1) and shoot-thinned (T2.2) vines exhibited the highest total phenolic content in juice (Table 4). However, shoot-thinned vines (T2.2) had the highest levels of monomeric anthocyanin pigment and total phenolic content in the wine (Table 4). Notably, the only significant difference between leaf (T2.1) and shoot thinning (T2.2) treatments was in 2020 wine phenolic content, implying that, in most cases, both treatments contributed to improving color and antioxidants in *MidSouth*. Again, a

decrease was observed in total phenolic content during juice-to-wine fermentation in 2020 (Table 3). In contrast, total phenolic content increased during fermentation in 2021 (Table 4).

Table 3. Average *MidSouth* monomeric anthocyanin pigment and total phenolic content from juice and wine for each treatment in study 2 in McNeill, MS (2020).

Treatment ³	Juice		Wine	
	Monomeric Anthocyanin Pigment (mg/L) ¹	Total Phenolic Content (mg of GAE/L) ²	Monomeric Anthocyanin Pigment (mg/L)	Total Phenolic Content (mg of GAE/L)
2.1	112.7	504.4	105.6	190.7 b ⁴
2.2	121.1	504.4	127.7	227.1 a
2.3	91.8	488.4	112.1	199.1 ab
Treatment Significance	ns ⁵	ns	ns	*

¹ Monomeric anthocyanin pigment expressed as cyanidin-3-O-glucoside. ² GAE, gallic acid equivalent. ³ Treatment 2.1, Leaf Removal; 2.2, Shoot Thinning; 2.3, Control (no leaf removal or shoot thinning). ⁴ Different lowercase letters following the means within columns indicate significant differences between treatments. ⁵ * means significantly different at $p \leq 0.05$ and ns means no significant difference.

Table 4. Average *MidSouth* monomeric anthocyanin pigment and total phenolic content from juice and wine for each treatment in study 2 in McNeill, MS (2021).

Treatment ³	Juice		Wine	
	Monomeric Anthocyanin Pigment (mg/L) ¹	Total Phenolic Content (mg of GAE/L) ²	Monomeric Anthocyanin Pigment (mg/L)	Total Phenolic Content (mg of GAE/L)
2.1	154.5 a ⁴	646.9 a	225.5 ab	1301.9 ab
2.2	129.4 ab	617.9 a	271.4 a	1395.0 a
2.3	79.3 b	551.1 b	204.6 b	1213.0 b
Treatment Significance	* ⁵	***	*	*

¹ Monomeric anthocyanin pigment expressed as cyanidin-3-O-glucoside. ² GAE, gallic acid equivalent. ³ Treatment 2.1, Leaf Removal; 2.2, Shoot Thinning; 2.3, Control (no leaf removal or shoot thinning). ⁴ Different lowercase letters following the means within columns indicate significant differences between treatments. ⁵ * and *** mean significantly different at $p \leq 0.05$ and 0.001 , respectively, and ns means no significant difference.

4. Discussion

Both studies display variations in total phenolic content and monomeric anthocyanin pigment in both juice and wine as a result of different canopy management treatments. However, identifying the most successful treatment in improving color and antioxidants in *MidSouth* grapes and wine is challenging due to year-to-year differences.

As previously discussed by Williams et al. [15], annual weather differences may have influenced differences in grape composition and subsequent wine quality from year to year. Higher precipitation in the period of grape maturation in 2020 compared to 2021 (data previously reported by Williams et al. [15]), for instance, likely suppressed the amount of phenolics in 2020 grapes and wine. Findings by Ramos et al. [29] support this view, highlighting the negative impact that higher water availability can have on phenolic content in grapes. To establish a definitive trend, further testing to account for specific weather effects is necessary to better determine what effects the canopy management treatments had.

Differences in amino acid content in leaves and fruit may also account for variations by treatment. These compounds serve as precursors for important secondary metabolites in grapes, including anthocyanins and other phenolics [30]. In *MidSouth*, specifically,

Jain et al. [31] reported the highest leaf amino acid content at the pre-flowering stage, while it was lowest at the young fruit stage, and levels in the fruit were of mid-range, suggesting that there is a lot of variation in values depending on phenological stage. Guan et al. [30] reported that grape cluster shading alters the amino acid composition in berries. Thus, the different timings of canopy management treatments employed in these studies likely affected these compounds, and a better understanding of the rise of these compounds in leaves, their translocation into fruit, and the timing of this process could better optimize *MidSouth* grape and wine quality.

Some differences in yearly wine content are likely attributable to differing winemaking techniques, such as berry pressing and must cold maceration in 2020 versus berry crushing and must freezing in 2021 [3,32]. In looking specifically at rosé wines, Guerrini et al. [32] reported that crushing and destemming grapes significantly increased the extraction of phenolic compounds compared to pressing. Differences in catechin content, which makes up a large portion of phenolic content, could also account for the differences in annual *MidSouth* wines, as Custodio-Mendoza et al. [33] found that catechin content is highest in red wines, followed by rosé, and then white. Additionally, while cold maceration can be successful in extracting phenolic compounds with proper SO₂ and temperature management, freezing must prior to fermentation results in greater phenolic extraction overall [32]. Furthermore, annual differences in microbe populations likely affected wine composition as fermentation took place. The increase in phenolics observed during fermentation in 2021 aligns with findings that phenolics increase during fermentation, which arises from phenolics having greater solubility in ethanol than in an aqueous juice solution [32]. Conversely, the decrease observed in 2020 may be attributed to the partially fermented wine potentially harboring acetic acid bacteria. These bacteria thrive in sugar and alcohol-rich environments, converting ethanol into acetic acid and thereby degrading phenolic compounds [34]. Further investigation into the fermentation dynamics and microbial ecology could provide insights into optimizing phenolic retention to enhance overall wine quality.

Despite differences from year to year, both 2020 and 2021 wine phenolic compositions were comparable to popular wines on the market. The anthocyanin levels in 2020 wines from both studies were similar to *Sangiovese* rosé wines [32], while total phenolic contents were comparable to *Grenache* rosé wines [35]. This indicates that the treatments employed in these studies, combined with pressing the grapes prior to fermentation, resulted in wines with phenolic profiles comparable to those of well-regarded rosé wines [36]. In 2021, the anthocyanin levels were comparable to standard store-bought red wines as reported by Lee et al. [5], while the highest levels were comparable to *Merlot* wines [37]. Similarly, the total phenolic content closely aligned with *Merlot* wines, while higher levels approached those of *Cabernet Sauvignon* [38]. These comparisons suggest that depending on what canopy management practices are employed in combination with grape processing techniques, wine phenolic characteristics ranging from low to high can be achieved in *MidSouth*.

5. Conclusions

The inconsistencies observed between years prevent the recommendation of early pruning, with or without leaf removal (T1.1, T1.2), for *MidSouth* until further research accounting for additional influencing variables is conducted. The 2021 results revealed that normal pruned leaf removal treatments (T1.3, T2.1) had some impact on *MidSouth* juice and wine phenolics compared to control vines (T1.4, T2.3); however, the shoot thinning treatment (T2.2) showed a slightly more pronounced effect by increasing total phenolics in the juice and both anthocyanins and total phenolics in the wine. *MidSouth* demonstrates versatility and is capable of producing wines ranging from low to high phenolic content, depending on vine canopy management and grape processing methods. However, to fully optimize *MidSouth* grapes for varietal red wine production in the region, further investigation into the specific mechanisms behind the variations observed and their effects on wine composition is essential.

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