EVALUATING LEAF REMOVAL METHODS EFFECTS ON CROP YIELD, DISEASE MANAGEMENT, AND PRIMARY AND SECONDARY METABOLITES IN BUNCH WINE GRAPES

by

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(Under the Direction of Savithri Nambeesan)

ABSTRACT

Leaf removal is a vineyard canopy management technique used to manage disease and improve fruit quality. Leaf removal strategies employ, in combination, alterations in timing and extent of removal. Leaf removal methods can be optimized by climatic conditions to have the greatest positive impact on disease control, crop yield maintenance, and secondary metabolite accumulation. The research presented herein evaluated fruit zone leaf removal methods to improve fruit quality and maintain adequate crop yield in bunch grapes grown in North Georgia. Fruit zone leaf removal consistently improved disease control and improved or maintained primary and secondary metabolites, metrics that determine fruit quality. Pre-bloom leaf removal, especially to greater extents, decreased crop yield and fruit quality. Leaf removal on one canopy side of a divided canopy trellis had no effect on the crop yield or fruit quality of the separate canopy side.

INDEX WORDS: berry temperature, Cabernet franc, Carminare noir, canopy management, Chardonnay, crop yield, disease management, leaf removal, phenolics source: sink relationship, trellising system

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

European (*Vitis vinifera* L.) and hybrid bunch grapes are grown worldwide with the goal of producing economical crop yields in tandem with consumer-acceptable wine. The wine industry in Georgia has grown rapidly over the last twenty years, with close to 60 wineries now open in the state. In 2017, the Georgia wine industry generated $4.1 billion in economic activity and it continues to grow (John Dunham & Associates, 2017). There is thus greater need than ever for research and innovation to optimize the sustainable production of grapes and wines in Georgia and worldwide.

Choices such as site, cultivar, management techniques, and trellis system must be made to optimize crop quality while remaining economically viable. Climate, biotic, and abiotic pests determine the cultivars that can be grown in specific regions and the management techniques that are required to achieve desired production goals. In humid growing regions, such as those in the southeastern US, excessive grapevine canopy growth results in shaded leaves and fruit zones (Hatch et al., 2011; Hickey et al., 2016). Management strategies must be implemented to improve grape exposure to air and light because dense canopies exacerbate rot incidence and severity (English et al., 1992; Hickey et al., 2018a; Wolf et al., 1986). Trellis systems impact the vine source-sink balance, canopy exposure, and management level needed to maintain appropriate crop yields. Each consideration can be manipulated to optimize vineyard management and produce high quality fruit.

Several vineyard management practices can be used to manage rots and improve the health and value of the crop, one of which is selective fruit zone leaf removal. Fruit zone leaf removal is a commercially-implemented practice used to decrease rot incidence, increase spray penetration, and promote wine sensory impact and compound development. Optimal fruit zone leaf removal timing and magnitude could differ between cultivars and across climatically-distinct growing regions. Studies conducted in arid growing regions of the western US reported a reduction in anthocyanins in red grapes that were highly exposed on the west sides of north/south-oriented vineyard rows (Bergqvist et al., 2001; Spayd et al., 2002; Tarara et al., 2008). However, one to two leaf layers appears to be too conservative to offer as much late-season rot management relative to fruit zones void of leaf layers in the eastern US (Hickey and Wolf, 2018).

Cluster exposure to ambient conditions can improve grape quality and thus wine quality potential. For example, sun exposure decreases methoxypyrazines, ammonia-like aromatic compounds that produce vegetal and herbaceous characters, and increases norisoprinoids, which are desirable and generally associated with floral and fruity flavors (Ryona et al., 2008; Crupi et al., 2010). Cluster exposure can impact phenolic compounds such as anthocyanins and tannins which affect color and mouthfeel of wine, respectively. Tannins are generally less affected by fruit microclimate conditions relative to anthocyanins, the latter of which appear to be limited by high temperatures once a radiation threshold of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ has been achieved (Downey et al., 2006). Anthocyanins are generally improved or maintained in exposed relative to shaded fruit zones across a wide range of climates (Chorti et al., 2010; Frioni et al., 2017; Hickey et al., 2018a). However, climate and growing conditions tend to perturb the consistency with which anthocyanins are impacted by leaf removal. For example, Frioni et al., (2017) reported that leaf removal improved anthocyanins relative to shaded clusters, but only in the cooler, cloudier year and not in the relatively warmer and sunnier year. These collective studies show the leaf removal impacts secondary metabolites that are important for wine quality, but variably so across diverging climatic conditions.

Fruit zone leaf removal affects primary grape metabolites (i.e. acids, sugars), which are important for wine alcohol and acidity as well as microbial stability as related to pH. Leaf removal does not generally affect soluble solids (Brix) accumulation as crop with leaves removed maintain similar sugar levels to those with fewer or no leaves removed (Hickey and Wolf, 2018). As berries are subjected to radiant heat, the titratable acidity, a key component in harvest decisions and evaluation of ripeness, decreases with increased fruit exposure (Jackson and Lombard, 1993). This is primarily due to temperature-induced malic acid degradation (Lakso and Kliewer, 1975) and tartaric acid levels that remain stable but decrease in concentration as berry size increases (Johnson and Carroll, 1973). Similar Brix levels and lower titratable acidity in exposed relative to shaded grapes results in an increase in the Brix: TA ratio in exposed relative to shaded grapes.

Leaf removal alters the microclimate by increasing air circulation through the canopy, improving light interception in the fruit zone, and impacting berry temperatures during the day and night (Bledsoe et al., 1988; Zoecklein et al., 1992; Hunter et al., 1995; Ristic et al., 2007; Guidoni et al., 2008; VanderWeide et al., 2018). According to several studies (Bergqvist et al., 2001; Hickey and Wolf, 2018; Spayd et al., 2002; Tarara et al., 2008), average berry temperature is increased by fruit zone leaf removal, relative to no leaf removal, regardless of timing of implementation or canopy side of exposure. Only these studies have measured and reported berry temperature; still, most leaf removal studies cite berry temperature as a change-inducing factor for fruit composition and color accumulation. As berries are exposed to sunlight, radiant heating increases berry temperature above that of ambient temperature. In the southeastern US, the variable cloudiness is anticipated to moderate radiant grape heating relative to that experienced in arid, less cloudy growing regions such as those in the western US (Faust and Logan, 2018). Without efficient methods to estimate berry temperature, assumptions must be made. The development of new methods could help the authors empirically prove that the net effect of fruit zone leaf removal, including change in berry temperature, in humid, cloudy growing regions may be that rot management and fruit quality are tandemly improved.

Trellising system choice impacts vineyard management techniques, vine-productivity, and source-sink balance (Reynolds and Vanden Heuvel, 2009). Trellis systems are most often categorized as either single canopy or divided canopy. The most widely-used single canopy trellis system is the vertical-shoot-positioned (VSP) system. Consequently, vineyard machinery is best-adapted to VSP, and the majority of best management practices have been developed on/for VSP. A common benefit of a divided canopy system is increased crop yield per vine relative to a single canopy system such as the VSP. When the canopy is divided, more leaf area is exposed to sunlight relative to single canopy systems thus providing more carbon source to produce crop. Management of vines trained to divided canopy systems differs from that of single canopy systems, and the source-sink balance is essential to maintain because of the increased crop load often associated with dividing the canopy.

There are numerous approaches to fruit zone leaf removal and they primarily differ by timing, extent, and method of application (e.g. hand vs. mechanical). Removal of leaves can limit the photosynthetically active area of the vine thus reducing the carbon source and impacting the source-sink balance. The goal of research conducted as part of this dissertation was to implement leaf removal techniques and evaluate their effects on disease management, crop quality and quantity, source-sink balance, and changes in berry temperature. A primary aim was to implement leaf removal at variable timings and extents to improve canopy management practices in Georgia vineyards. The use of different cultivars and training systems intended to account for some of the environmental and cultural differences across the state and further investigate the source-sink relationship in grapevines.

A. Leaf Removal Methods

In commercial wine grape production, leaf removal is usually implemented post-fruit set and pre-bunch closure, when the berries are at least BB sized (Modified EL stage 30) (Dry and Coombe, 2004; Poni et al., 2006). Leaf removal is a time-consuming practice. Therefore, the timing and magnitude of leaf removal often cannot be implemented at an exact growth stage throughout the entire vineyard. Large vineyards that start canopy management and leaf removal after fruit set often struggle to finish before bunch closure. The result of such action is suboptimal disease control throughout the most critical disease protection period for clusters (pre-bloom through bunch closure) and failure of fruit acclimation to ambient conditions from an early development stage, which often results in increased fruit sunburn (Spayd et al., 2002). An efficient leaf removal method is thus needed to optimize rot control and limit sunburn threat.

Mechanical leaf removal is a more efficient means of exposing grape clusters relative to hand leaf removal' mechanical leaf removal is currently being adopted by growers in the southeastern US. Some mechanical leaf removers employ a potentiometer as a position sensor to track the canopy as the tractor moves along the row while others use pressurized air to clear leaf area from the fruit zone. The machine sensors ensure limited berry damage and removal of whole or parts of leaves. There have been few studies that have evaluated the effectiveness of mechanical versus traditional hand pulling in the eastern US, but mechanical leaf removal will likely rise in popularity with increasing labor shortages. Mechanical leaf removal reduces labor cost by requiring fewer workers and less time to complete the practice (Julian et al., 2008; Vierra, 2005). Mechanical leaf removal therefore permits leaf removal at a specific growth (EL) stage to elicit desired effects across several acres.

Another commercially-implemented fruit zone management practice is to remove leaves on only one side of the canopy, typically the eastern, or “morning-sun”, canopy side. Leaf removal on one side is used to avoid excessive radiant heating of grapes in the afternoon that has been shown to result in reduced anthocyanins compared to grapes with less sunlight exposure (Bergqvist et al., 2001; Spayd et al., 2002; Tarara et al., 2008). In regions where cloud cover is typical, removing leaves from both sides of the canopy can increase airflow and spray penetration without reducing anthocyanin accumulation or resulting in sun burned berries. Studies have shown that extensive fruit zone leaf removal on both sides of the canopy in comparison to no leaf removal can improve primary chemistry and increase or maintain phenolics, thus improving wine quality potential of *V. vinifera* (Frioni et al., 2017; Hickey et al., 2018a; Hickey and Wolf, 2017, 2018). Removal of leaves on the morning side is not necessarily best management in in the southeastern US as it is in consistently sunny regions.

B. Leaf Removal Timing

The growth stage at which fruit zone leaf removal is implemented can affect cluster structure, crop yields, and canopy growth. Fruit set, berry size, and cluster compactness can all be reduced when leaves are removed before bloom (Poni et al., 2006). With smaller berry size, there can be an increase in skin: berry ratio and skin: pulp ratio (Poni et al., 2009) resulting in increased soluble solids and increased anthocyanins, thus increasing the color and total phenolic index of the wine (Diago et al., 2012; Tardiguila et al., 2010, 2012). Increased color and phenolics can have an overall positive impact on wine quality potential (Poni et al., 2006, 2008, 2009; Intrieri et al., 2008; Tardaguila et al., 2008, 2010; Diago et al., 2010; Palliotti et al., 2011, 2012).

Removal of leaves after fruit set has numerous benefits. Similar to pre-bloom leaf removal, post-fruit set leaf removal increases airflow and spray penetration leading to decreased rot (Wolf et al., 1986; English et al., 1992; Hickey and Wolf, 2018). Sun exposure increases anthocyanins and carotenoids that ultimately contribute to color and aroma of wines, while also improving or maintaining primary metabolites like titratable acidity, Brix, and pH (Razungles et al., 1998). Unlike pre-bloom leaf removal, post-fruit set leaf removal does not affect the berry size, cluster architecture, or overall crop yield (Hickey and Wolf, 2018; VanderWeide et al., 2018). Since fruit set has occurred at the time of post-fruit set leaf removal, there is less effect on the source-sink balance than in pre-bloom leaf removal.

Though pre-bloom leaf removal can improve certain aspects of crop quality, removal of excessive foliage before bloom also can reduce crop quantity in comparison to post-fruit set leaf removal. Removal of basal leaves before bloom limits carbohydrates supply from these source tissues to flowering clusters, which can ultimately limit fruit set (Frioni et al., 2017; VanderWeide et al., 2018;). In some cases, where crop yield can be excessive, pre-bloom leaf removal is used to reduce yield to manage crop loads (Diago et al., 2012; Poni et al., 2006, 2009). Reduced fruit set will reduce berry number, ultimately resulting in a decrease in crop yield unless the berry number reduction is offset by increased berry weight. The reduced fruit set could improve fruit quality by decreasing cluster compactness and exposing a greater number of berries to ambient conditions in comparison to the higher fruit set with leaf removal after fruit set (Diago et al., 2012; Poni et al., 2006, 2009). A decrease in cluster compactness can improve rot management (Hed et al., 2009; Hickey and Wolf, 2018; Sabbattini and Howell, 2010). However, recent work suggests that pre-bloom leaf removal may not be as efficacious as post-fruit set leaf removal at managing rots (Hickey et al., 2018a), perhaps due to floral debris that remains within the cluster from reduced fruit set serving as an infection site for *B. cineraea*. Regardless of timing, fruit zone leaf removal can establish an open fruit zone, increase the leaf area: crop weight ratio (Poni et al., 2006), decrease berry size (Poni et al., 2006), create a favorable microclimate in the fruit zone (VanderWeide et al., 2018) and promote thicker berry skins resulting in more anthocyanins (Poni et al., 2008).

C. Leaf Removal to Improve Fruit Composition

Viticulturists have been studying canopy management practices since the 1960's (Shaulis et al., 1966). Canopies can be manipulated in numerous ways such as through training, pruning, and leaf removal; each practice can change the shape, size, and architecture of the canopy and therefore affect the sunlight penetration through the canopy and fruit zone. With basal leaf removal, much of the canopy remains within the intended training system, but fruit zone leaves are removed to increase radiation penetration to the berries. Many studies have evaluated the influence of sunlight exposure on grapes and how berry composition and wine quality are affected (Dokoozlian and Kliewer, 1996; Poni et al., 2006, 2008, 2009; Intrieri et al., 2008; Tardaguila et al., 2008, 2010; Diago et al., 2010; Palliotti et al., 2011, 2012). Grape anthocyanins have been shown to be decreased in the western US when clusters are too severely exposed (Bergqvist et al., 2001; Spayd et al., 2002; Tarara et al., 2008). A study performed in 2004 by Jeong et al., proposed that excess shade could inhibit the accumulation of anthocyanins while hormones ABA and NAA could have promoting or inhibiting effects, respectively. If ABA concentrations increase quickly the berries ripen faster than those with lower concentrations resulting in inconsistent berry quality at harvest. The concentration of ABA and anthocyanins is higher in berries exposed to 20 °C than in berries exposed to 30°C (Yamane et al., 2006). The warmer temperatures could thus be inhibiting ABA, resulting in lower anthocyanin concentrations since ABA affects the VvMYBA1 gene upstream of 17 other genes controlling the anthocyanin biosynthesis pathway. The temperature of the fruit zone increases throughout the day with increasing ambient air temperature. Climate has a great bearing on grape temperature and the hours of exposure to temperatures beyond the cited critical berry temperature threshold for grape anthocyanin accumulation which is between 30 and 35°C (Spayd et al., 2002; Tarara et al., 2008). In some areas of the US, sunlight remains high throughout the day, while in others, afternoon cloud cover often reduces radiant heating of grapes and thus maintains or improves grape anthocyanin concentrations (Hickey and Wolf, 2018).

Excessive fruit shading can result in poor aroma and flavor development (Hunter et al., 1991). This decrease in varietal character produces fruit with poorer wine quality potential (Jackson and Lombard, 1993), and an increase in undesirable aroma and flavor compounds (Kwasniewski et al., 2010; Ryona et al., 2008; Smith et al., 1988). Shaded fruit will have reduced color and sugars while retaining high TA levels (Smart et al., 1985), all undesirable attributes for making balanced red wines without oenological intervention. A previously recommended best management practice was thus to highly expose fruit to achieve fruit and wine quality (Smart and Robinson, 1991). A decade and more later, extreme fruit exposure was found to limit quality potential in red grapes (Spayd et al., 2002; Tarara et al., 2008). Yet, other studies have reported that open canopies can increase desirable compounds like monoterpenes (Reynolds et al., 1996), a compound class contributing to floral notes in wines, increase sugar accumulation (Bledsoe et al., 1988) directly involved in alcohol production, increase anthocyanin and phenol concentrations (Carbonneau, 1985), and positively affect wine sensory perception (Di Profio et al., 2011). There is obvious conflict in the literature about best fruit zone management practice, but basal leaf removal generally produces positive responses in terms of its impact on fruit metabolites associated with wine quality.

D. Leaf Removal to Manage Disease

Rot management can be improved with fruit zone leaf removal, particularly in a humid environment. *V. vinifera* and interspecific hybrid winegrapes experience numerous diseases in the southeastern US including black rot (*Guignardia bidwellii),* Botrytis bunch rot (*Botrytis cinerea*), downy mildew (*Plasmopara viticola*), powdery mildew (*Erysiphe necator*), and Phomopsis cane and leaf spot (*Phomopsis viticola)* to name a few. Dense canopies exacerbate fungal rots, which can be managed through selective basal leaf removal (Austin and Wilcox, 2011, English et al., 1989; Hed et al., 2009; Hickey et al., 2017; Hickey and Wolf, 2018; Sabbatini and Howell, 2010). As leaves are removed from the fruit zone, the canopy immediately surrounding the grape clusters becomes less dense; the result is improved rot management (English et al., 1992; VanderWeide et al., 2018). Leaf removal improves spray penetration and results in better pesticide coverage on clusters relative to shaded fruit zones (Komm and Moyer, 2015). As discussed, pre-bloom leaf removal decreases cluster compactness (Tardiguila et al., 2010, 2012) which can improve bunch rot management (Hed et al., 2009; Molitor et al., 2014; Sabbatini and Howell, 2010), though some studies have shown that pre-bloom leaf removal is not as effective as post-fruit set leaf removal (Chapter 2). More open canopies do not necessarily equate to less bunch rot diseases in humid regions where fungi thrive

(Acimovic et al., 2016). There are two primary disease issues of grape clusters that are of major threat and concern throughout eastern US vineyards: Botrytis bunch rot and sour rot.

i. Botrytis

Botrytis bunch rot is one of the primary fungal diseases affecting grapevines and other fruits, resulting in pre- and post-harvest crop loss (Romanazzi et al., 2016). Botrytis bunch rot is caused by *Botrytis cinerea*, a fungal pathogen that causes a fluffy grey rot on the fruit that leads to shriveling and blackening of the fruit until the berries harden. Under humid, wet conditions, Botrytis bunch rot thrives on damaged and young tissues. The pathogen can remain as a latent infection in berries without showing symptoms until the fruit begins to soften and accumulate sugar as it ripens (Keller et al., 2003). Latent infections can be activated by increased humidity and other environmental factors common in the eastern US. The fungal spores can then spread and directly penetrate tissues or enter wounded berries within the cluster through the entire post-veraison period. Since latent infections are the source of secondary inoculum for most bunch rot diseases, management strategies such as spraying fungicides are best done before bunch closure. Though there are fungicides that can aid in preventing infections from occurring, management techniques used to decrease moisture and humidity in the canopy and clusters are critical to disease management throughout the season. The three primary times to spray for botrytis control are early bloom, berry touch, and veraison. Botrytis bunch rot can develop resistance to multiple synthetic fungicides, making it difficult to manage in the vineyard (Calvo-Garrido et al., 2017). One successful cultural management practice to manage *Botrytis* is to use fruit zone leaf removal to encourage drying through air movement in grape clusters. Conidia can infect when in free moisture or extremely high humidity. Keeping the blooms and fruit dry will aid in decreasing rot severity. Blowing the caps off of the flowers and out of the canopy could also be helpful as to get rid of dead tissues that could accumulate and become a resting place for disease (Keller et al., 2003); this may especially be important to implement with pre-bloom leaf removal because of the hypothetical increase in floral debris that remain within the clusters when fruit set is reduced. Even with fruit zone leaf removal, bunch rot can still be prevalent (Acimovic et al., 2016).

ii. Sour rot

Sour rot is a disease complex resulting from combined pathogens, including acetic acid bacteria, yeast, and fruit flies that infects *V. vinifera* and hybrid winegrapes (Smith et al., 2014). Sour rot is normally observed as sugars accumulate and the berries ripen. Indicators can vary but mostly include the smell of acetic acid, browning skin, and oozing of the degraded berry when touched (Hall et al., 2018). Injury to the fruit, including sun scald, bird damage, insect damage, and mechanical damage open up the fruit to infection by the causal agents. *Drosophila spp.* (fruit flies) act as vectors spreading the bacteria to damaged fruit. The sour rot complex is exacerbated by many different factors including environmental, physical, and microbiological influences (Zoecklein et al., 2000).

At first, it might be difficult to distinguish Botrytis bunch rot from sour rot because they both result in degradation of fruit. Indeed, both can be found on the same cluster. Sour rot differs from other diseases in that there is no specific fungal pathogen or molds present as signs of a pathogen, whereas *B. cinerea* produces a fluffy grey fruiting bodies. Grapes experiencing sour rot begin to brown to varying degrees as their skins become thin and fragile. Sour rot depreciates fruit quality because the acetic acid production destroys the fruits flesh and gives off the smell for which sour rot was named (Guerzoni and Marchetti, 1987). Historically it has been difficult to find effective biological or chemical controls for sour rot in wine grapes (Nigro et al., 2006). However, application of Mustang Maxx (FMC Corporation, Philadelphia, PA) and other insecticides for Drosophila management and Oxidate (BioSafe Systems, Hartford, CT) for reduction in acetobacter and yeasts has been proven an effective control of sour rot as long as both antimicrobial and insecticides are used after 15 Brix till harvest (Hall et al., 2018). There has been evidence that mechanical and manual leaf removal can reduce sour rot in comparison to vines without leaf removal (Calvo-Garrido et al., 2013; Hickey et al., 2018b). Recent work has also shown that training systems can also impact sour rot incidence, with a high wire system resulting in greater sour rot levels relative to lower-wire or VSP systems (Hall et al., 2018). Thus, sour rot management is optimized by integrated pest management practices including chemical approaches in tandem with cultural practices such as training systems and fruit zone leaf removal.

E. Source-sink Balance

Trellising and canopy management methods are utilized to improve vineyard efficiency and to manipulate the source-sink balance of a grapevine. Sources are plant tissues that intercept sunlight and manufacture or store carbohydrates to be used for the health and growth of the plant. Sinks are plant tissues where the stored carbohydrates are utilized, such as the berries of a grapevine. The most effective source tissues in a grapevine are young leaves that are actively photosynthesizing to provide energy to the growing parts of the plant such as meristems and fruit. When the sources manufacture excess carbohydrates, the plant is able to grow and reproduce through development of fruit. However, when source tissues are taken away, the plant has to adapt in some way to maintain a balance between source and sink.

During pre-bloom leaf removal, source tissues are removed at a critical growth stage. Often, fruit set is reduced when extensive source tissues are removed, and in order to maintain balance, the vine aborts a portion of its future berries resulting in limited berry number per cluster and reduced crop yield relative to no leaf removal (Frioni et al., 2019; Hickey et al., 2018a). Post-fruit set leaf removal most frequently retains crop yields since fruit has set, and the leaves removed post-fruit set are no longer the most photosynthetically active; relative to pre-bloom leaf removal, the source-sink balance is not as severely interrupted in post-fruit set leaf removal.

Trellis systems can manipulate the vine source-sink relationship by altering the shape and growth pattern of the canopy and the entire vine (Reynolds and Vanden Heuvel, 2009). Divided canopy systems offer more leaf area exposed to sunlight and therefore have more active source tissues. Divided canopy systems often have more sink tissues relative to single canopy systems, because many divided systems have multiple fruit zones. Dividing the canopy has been shown to improve sunlight interception and source tissue efficiency by increasing the amount of ripened fruit per unit leaf area relative to single canopies (Kliewer and Dokoozlian, 2005). Trellis systems manipulate the shape of the vine, affecting the source-sink relationships within and impact sugar accumulation and fruit composition (Dai et al., 2009; Frioni et al., 2019). The spatial separation of divided canopies creates a source-sink dynamic more complex than that of a single canopy system.

F. Conclusion

Canopy management is an important part of overall vineyard health and productivity. Leaf removal improves efficacy of disease management inputs and overall crop quality. Canopy management methods have different effects based on timing and extent of source tissue removal. Pre-bloom leaf removal often improves fruit chemistry and composition and reduces crop yield; Crop yield reduction can be beneficial in high cropping or tight-clustered cultivars and detrimental in low cropping cultivars. Post-fruit set leaf removal benefits from the same effects on disease management and fruit composition as pre-bloom leaf removal, while retaining crop yield. Failing to manage the grapevine canopy in a subtropical climate such as that of the southeastern US results in high disease pressure and poor fruit quality. Leaf removal methods should therefore be optimized by climate, cultivar, trellis system, and winery goals.

CHAPTER 2

FRUIT ZONE LEAF REMOVAL TIMING AND EXTENT ALTERS BUNCH ROT, PRIMARY FRUIT COMPOSITION, AND CROP YIELD IN GEORGIA-GROWN CHARDONNAY (VITIS VINIFERA L.)[1]

[1] Vogel, A., R. White, C. MacCallister, C. Hickey. Submitted to *Hortscience*

Abstract. Fruit zone leaf removal is a vineyard management practice used to manage bunch rots, fruit composition, and crop yield. We were interested in evaluating fruit zone leaf removal effects on bunch rot, fruit composition, and crop yield in Chardonnay grown proximate to the geographical, southeastern extreme of *V. vinifera* production in the US. The experiment consisted of seven treatments: no leaf removal (NO); pre-bloom removal of four or six leaves (PB-4, PB-6), post-fruit set removal of four or six leaves (PFS-4, PFS-6), and pre-bloom removal of two or three leaves followed by post-fruit set removal of two or three leaves (PB-2/PFS-2, PB-3/PFS-3). While leaf removal reduced Botrytis bunch rot and sour rot when compared to NO, effects were inconsistent across the two seasons. Fruit zone leaf removal treatments reduced titratable acidity (TA) and increased soluble solids when compared to NO. PB-6 consistently reduced berry number per cluster, cluster weight, and thus crop yield relative to PFS-4. Our results show that post-fruit set fruit zone leaf removal to zero leaf layers aids in rot management, reduces TA, increases soluble solids, and maintains crop yield when compared to no leaf removal. We would therefore recommend post-fruit set leaf removal to zero leaf layers over no leaf removal if crops characterized by relatively greater soluble solids: TA ratio and reduced bunch rot are desirable for winemaking goals.

Introduction.

Climate and pests dictate the cultivars that can be sustainably grown within a region, and management practices are used to achieve production goals within those cultivars. Two goals of vineyard and winery enterprises are to produce economical crop yields and consumer-preferred wines. Cultural practices used to achieve these goals vary by growing region. In humid, subtropical growing regions, such as in the southeastern US, excessive grapevine canopy growth results in shaded leaves and fruit zones (Giese et al., 2015; Hatch et al., 2011; Hickey et al., 2016). The humidity of the southeastern US macroclimate is intensified within a shaded fruit zone microclimate. Management strategies are implemented to increase grape cluster exposure by thinning dense canopies that can otherwise exacerbate rot incidence and severity (English et al., 1989; Wolf et al., 1986; Hed et al., 2009; Hickey et al., 2018b). Fruit zone leaf removal is used to decrease rot incidence (Hed et al., 2015; Smith and Centinari, 2019), increase spray penetration (Hed and Centinari, 2018), and promote the development of desirable (Bubola et al., 2017) and reduce the presence of undesirable (Ryona et al., 2008) wine sensory impact compounds.

Fruit zone leaf removal is conventionally implemented after fruit set and before bunch closure (Poni et al., 2006). Removing leaves from only the morning-sun canopy side (e.g. the east side of north/south-oriented rows) has become standard practice in the eastern US, where current recommendation is to retain an average of one to two fruit zone leaf layers (Reynolds and Wolf 2008). In humid regions, more late-season bunch rots are observed in fruit zones with one to two leaf layers relative to fruit zones devoid of leaves (Hed et al., 2015; Bubola et al., 2017), even in rot tolerant cultivars such as Cabernet Sauvignon (Hickey and Wolf, 2018). However, questions persist regarding optimal timing and magnitude of fruit zone leaf removal across cultivars and climatically unique growing regions. Optimal leaf removal method is dictated by the radiation and temperatures experienced within a region (Spayd et al., 2002; Tarara et al., 2008).

Cluster exposure to ambient conditions can change grape metabolites and thus wine quality potential. Leaf removal affects grape soluble solids, titratable acidity (TA) and pH (Palliotti et al., 2012) which are important for wine alcohol, acidity, mouthfeel, and microbial stability. As berries are subjected to radiant heat with increased fruit exposure, TA generally decreases as a function of malic acid respiration (Lakso and Kliewer 1975; Jackson and Lombard 1993). In some regions, lower acidity may be desirable for the production of less astringent wines, as acidity in grapes and astringency in wine are positively correlated (Reynolds et al., 2006). Though best fruit zone management practice differs across climatically-distinct regions and cultivars (Spayd et al., 2002; Tarara et al., 2008; Hickey et al., 2018a; Hickey and Wolf, 2019), removal of some leaves surrounding clusters can positively impact wine quality potential by increasing or decreasing several metabolites (Crupi et al., 2010; Lee et al., 2005; Hunter et al., 1991; Jackson and Lombard 1993; Ryona et al., 2008).

Fruit set, berry size, and cluster compactness can all be reduced when leaves are removed before bloom (Poni et al., 2006). The reduced fruit set has also been associated with an increase in skin thickness, skin to pulp ratio, and phenolics (Diago et al., 2012; Poni et al., 2006 and 2009). Reduced fruit set results in a reduction in berry number per cluster, ultimately resulting in looser clusters. While a decrease in cluster compactness can improve rot management (Sabbatini and Howell 2010; Hed et al., 2009), pre-bloom leaf removal may not always result in superior rot management relative to post-fruit set leaf removal (Hickey et al., 2018b; Liggieri et al., 2018). Further, removal of excessive fruit zone foliage before bloom substantially reduces crop yield (Poni et al., 2006 and 2009; Diago et al., 2012; Hickey and Wolf, 2018).

Post-fruit set leaf removal has numerous benefits. Like pre-bloom leaf removal, post-fruit set leaf removal increases airflow and pesticide spray penetration leading to decreased rot (Wolf et al., 1986; English et al., 1989; Hickey and Wolf, 2018). The resulting fruit exposure can decrease titratable acidity, increase soluble solids, and balance pH in comparison to no leaf removal (Reynolds et al., 2007; Bavaresco et al., 2008; Bubola et al., 2017). Unlike pre-bloom leaf removal, which can drastically reduce crop yield (Sabbatini and Howell, 2010), post-fruit set leaf removal maintains crop yield (Hickey and Wolf, 2018; VanderWeide et al., 2018). Post-fruit set leaf removal may offer greater economic sustainability relative to pre-bloom leaf removal.

Best leaf removal practice should be based on previous findings, optimized for vineyard production goals, and refined for specific cultivars. Further investigation of best fruit zone management practice is required in regions in which no formal leaf removal studies have been conducted, such as in Georgia, the state with the most southeastern *V. vinifera* industry. The present study evaluated the effect of different leaf removal regimes on crop yield, rot incidence, rot severity, and primary fruit composition of Chardonnay grown in north Georgia, a humid, subtropical region. Single- and double-implementations of leaf removal over time were evaluated in our experiment for both practical application and novelty ― effects of which have not been previously documented to the authors' knowledge. We hypothesized that pre-bloom leaf removal would reduce crop yield, and that leaf removal to the greatest magnitudes would reduce bunch rot and juice titratable acidity.

Materials and Methods.

Experimental vineyard and treatments. Our experiment used Chardonnay clone 5 grafted onto C-3309 rootstocks maintained in a commercial vineyard in Dahlonega, Georgia. Vines were planted in 1999 with 2.13 m (vine) x 3.05 m (row) spacing in rows that were oriented east to west. Soil was a Haysville sandy loam (NRCS, 2018). Vines were trained onto a single canopy system with bilateral cordons and vertical shoot-positioning (VSP). Vines were spur pruned in the dormant season and were thinned to 24 shoots per vine in the springtime of both years. Herbicide applications maintained the under-trellis free of vegetation. Shoots were hedged throughout the season before falling over the top catch wire. Pest management was standard for the region and uniformly applied across treatments and blocks.

Experimental units consisted of four vines between vineyard posts. In some cases, vines were missing or infected with a systemic disease, resulting in fewer than four individual vines in an experimental unit. Visual symptoms were reminiscent of Pierce's disease, but laboratory tests were not conducted to confirm or refute these observations. In both years, the vines that were excluded at harvest were also excluded at dormant pruning; in 2018, additional vines were missing or infected at dormant pruning. A total of 10% and 7.9% of vines in the entire trial were either missing or systemically infected at harvest in 2017 and 2018, respectively, while a total of 10% and 12.1% of vines in the trial were missing or systemically infected during dormant pruning following each season. Thus, while infrequent, the missing or systemically infected vines precluded our ability to measure crop weight, cluster number, and dormant pruning weight from all four vines in every experimental unit.

Treatments were implemented in a randomized complete block design and replicated in five blocks. Treatments were uniformly implemented in the same experimental units over the 2017 and 2018 growing seasons. Treatments were maintained throughout the season by periodically removing vegetative ingress into the fruit zone. Treatments were as follows [note – all modified EL stages in treatment descriptions and below methods taken from Dry and Coombe (2004)]:

No leaf removal: NO (no leaves or lateral shoots removed in the fruit zone).

Pre-bloom leaf removal: PB-4 [removal of leaves and laterals from primary shoot basal nodes 1-4 at modified EL stage 17 (single flowers well-separated)]; PB-6 (removal of leaves and laterals from primary shoot basal nodes 1-6 at modified EL stage 17).

Post-fruit set leaf removal: PFS-4 [removal of leaves and laterals from primary shoot basal nodes 1-4 at modified EL stage 31 (pea sized berries)]; PFS-6 (removal of leaves and laterals from primary shoot basal nodes 1-6 at modified EL stage 31).

Combined pre-bloom and post-fruit set leaf removal: PB-2/PFS-2 (removal of leaves and laterals from primary shoot basal nodes opposite each cluster at modified EL stage 17 and one above the top cluster and one below the bottom cluster at modified EL stage 31); PB-3/PFS-3 (removal of leaves and laterals from primary shoot basal buds opposite each cluster and one below the bottom cluster at modified EL stage 17 and from nodes 4-6 at modified EL stage 31).

Meteorology. Temperature and rainfall data were recorded from 1 Apr to 31 Oct in 2017 and 2018 using a weather station located on the vineyard site and roughly 180 m from the experimental vineyard blocks. The weather station was comprised of a HMP35 temperature and humidity probe (Vaisala, Helsinki, Finland) and a TB4 rain gauge (Hydrological Services America, Lake Worth, FL), which were logged with a CR1000 data logger (Campbell Scientific, Logan, UT).

Dormant cane pruning weight. The weights of pruned canes were recorded on a per-vine basis using a field scale during the dormant periods between the growing seasons of 2017 and 2018 and 2018 and 2019. Dormant cane weights per vine were then expressed on a linear m of row basis using vine spacing. Dormant pruning weight was averaged within each experimental unit to maintain experimental design and statistical integrity.

Fruit zone architecture. Point quadrant analysis (PQA) data was collected at modified EL stage 35 (veraison; berry softening and sugar accumulation). A thin, metal probe was inserted through the fruit zone perpendicularly relative to the cordon and at a frequency of three repetitions per meter in each experimental unit; approximately 22 probe insertions were made through the canopies within each experimental unit. Probe insertions allowed quantification of fruit zone leaf layer number (LLN) (Smart and Robinson 1991). Photosynthetic photon flux density (PPFD) was measured by inserting an LP-80 ceptometer (Decagon Devices, Inc., Pullman, WA) into the fruit zone above, and parallel to, the cordon. Under consistent, ambient conditions on sunny days, two PPFD readings each were taken from the middle two vines in every experimental unit at the modified EL stage 35. Measurements were averages of PPFD readings taken while orienting the ceptometer in three different orientations above the cordon (45˚ north, vertical, 45˚ south). The PPFD and probe insertion data were used to generate cluster exposure flux availability (CEFA) using enhanced point quadrant analysis (EPQA version 1.6.2) (Meyers and Vanden Heuvel 2008).

Bunch rot incidence and severity and crop loss due to rot. Botrytis bunch rot severity and incidence measurements were quantified on three different occasions; once after commencement of modified EL stage 35, once at an intermediate date between veraison and modified EL stage 38 (harvest), and once again immediately before EL stage 38. On each occasion, twenty-five randomly selected clusters were evaluated for Botrytis bunch rot incidence and severity within each experimental unit. Sour rot incidence and severity were rated on the same clusters in which Botrytis bunch rot was rated, but only at EL stage 38. Incidence was calculated as the number of clusters visually diagnosed with Botrytis bunch rot or sour rot of infection divided by the total number of clusters evaluated. Severity was rated by visual estimation of the percentage of each cluster that was infected by Botrytis bunch rot or sour rot. Estimated crop loss due to rot was calculated as the quotient of (1) and (2): (1) the product of rot incidence and severity; and (2) 100.

Components of crop yield. Crop yield was measured with a field scale on a per-vine basis at EL stage 38 on 22 Aug 2017 and 29 Aug 2018. Cluster number per vine was recorded. Average cluster weight was determined as the quotient of crop weight and cluster number per vine. Immediately prior to harvest on 21 Aug 2017 and 29 Aug 2018, a random, composite berry sample of 120 berries, taken equally from both canopy sides (60 berries per side), was collected to calculate average individual berry weight. Berry number per cluster was determined as the quotient of average cluster weight and average individual berry weight. Crop yield per vine and components thereof were averaged within each experimental unit to maintain experimental design and statistical integrity.

Primary juice composition. After modified EL stage 35, composite samples of 80 berries were equally collected from both canopy sides (40 berries per side); collection dates paralleled those of pre-harvest Botrytis bunch rot ratings in order to compare rot and soluble solids development over time. The 120-berry composite sample, randomly collected from each experimental unit immediately prior to EL stage 38 (above mentioned in “components of crop yield” methods), was used for soluble solids, titratable acidity and pH analyses. The fresh berry samples were evenly hand pressed, and expressed juice was centrifuged for five minutes at 4,000 rotations per minute (RPM). One mL of centrifuged juice was then used to measure soluble solids with a PAL-1 Atago digital pocket refractometer (Atago USA Inc., Bellevue, WA). Total titratable acidity (TA) was measured on 5 mL of juice diluted with 40 mL of distilled water using an 848 Titrino Plus automated titration system (Metrohm USA, Riverview, FL) and titrating to an endpoint of pH 8.2 with a 0.1 M NaOH base. The pH was measured on undiluted juice using the pH probe on the automated titration system.

Statistical analysis. Statistical computation was performed using JMP Pro v. 13. A mixed model was used to evaluate the random block effect and fixed treatment effect using 2-way ANOVA for EPQA, rot incidence, rot severity, primary chemistry, and components of crop yield. Significance ($\alpha \leq 0.05$) was determined with Tukey's HSD for all treatment effects. All data was analyzed within the time point collected (e.g. "year" was not used as a model effect for data in tables and "date" was not used as a model effect for the pre-harvest soluble solids and estimated crop loss data set in Figure 2.2). A bivariate linear fit model was used to determine the relationship between LLN and crop loss due to Botrytis bunch rot and sour rot, the relationship between LLN and incidence and severity of Botrytis bunch rot and sour rot at harvest, and the relationship between the number of leaves removed before bloom and the change in components of yield relative to treatments in which pre-bloom leaf removal was not conducted.

Results and Discussion.

Meteorology. In 2017, 2276 growing degree days (GDD) accumulated with precipitation of 1088 mm from April 1 to October 31 (Figure 2.1). In 2018, 2407 GDD accumulated with precipitation of 1081 mm from April 1 to October 31. The greatest monthly rainfall occurred in May of each year and the greatest GDD number were accumulated in June, July, and August of both years. When considering the harvest dates of 22 Aug 2017 and 29 Aug 2018, a relatively greater amount of GDD were accumulated, and more rain fell, before harvest in 2018 relative to 2017.

Dormant cane pruning weight. Dormant cane pruning weight was unaffected by treatment (Table 2.1). Pruning weight was greater in 2017 than in 2018 in spite of greater precipitation in 2018 compared to 2017 (Figure 2.1). While speculative, the greater pruning weight in 2017 relative to

2018 may have been a function of the relatively lower crop yield in 2017 (Table 2.2) resulting in less resource competition to vegetative growth than in 2018. Smart and Robinson (1991) report that pruning weights from balanced vines are between 0.3 and 0.6 kg/m row in a single canopy system such as the low, bilateral cordon system trained to VSP employed in our study. Pruning weights in our study tended to fall within, or above, that documented range, indicating healthy canopy vegetative growth. Our pruning weight data was reflective of high vine size and was likely a function of ample storage carbohydrates (vines were almost 20 years old) combined with the vigor induced by a humid, subtropical climate. This supra-optimal vine size supports the need for research on remedial canopy management strategies such as fruit zone leaf removal.

Fruit zone architecture. All leaf removal treatments resulted in greater fruit zone porosity relative to NO, as demonstrated by the lower leaf layer number (LLN) and greater cluster exposure flux availability (CEFA) observed in leaf removal plots (Table 2.1). As leaves were removed to greater magnitudes, fruit zone LLN was reduced by greater extents. When compared to recently recommended fruit zone leaf layer numbers (Reynolds and Wolf 2008), NO resulted in greater fruit zone leaf layers, while PB-4, PFS-4, and PB-2/PFS-2 produced similar leaf layers and PB-6, PF-6, and PB-3/PFS-3 produced fewer leaf layers. Fruit zone LLN was generally inversely related to fruit zone CEFA, logically indicating that greater incident radiation reached the clusters within fruit zones characterized by fewer leaf layers. NO had a significantly lower CEFA than all other treatments (Table 2.1). PB-6 and PFS-6 resulted in 423.1% and 407.7% greater CEFA than NO. PB-4 and PFS-4 increased CEFA by 238.4% and 246.2% compared to NO, and PB-2/PFS-2 and PB-3/PFS-3 had CEFA values that were 265.4% and 376.9% greater than NO across both years.

Post-veraison estimated crop loss and sugar accumulation. Greater estimated amounts of crop loss due to Botrytis bunch rot were observed in 2017 relative to 2018 even though slightly greater rainfall was observed in 2018 (Figure 2.1). Treatment effect on estimated crop loss due to bunch rot varied over the post-veraison period (Figure 2.2A, 2.2B). In 2017, the estimated crop loss due to rot was greater in NO when compared to PFS-4 and PFS-6 on 29 Jun, when compared to PFS-4, PFS-6, and PB-6 on 18 Jul, and when compared to PFS-6 on 21 Aug. In 2018, estimated crop loss was extremely variable, leading to no significant differences in treatments across all dates. In both years, however, the estimated amount of crop lost to rot over the final month of maturation was greatest in NO. While lower soluble solids were observed in 2017 than in 2018, the rate of soluble solids accumulation was similar across treatments (Figure 2.2C, 2.2D). However, NO had significantly lower soluble solids when compared to PB-4, PB-6, PFS-6, PB-2/PFS22, and PB-3/PFS-3 at harvest in 2017, and when compared to all other treatments at harvest in 2018. Soluble solids increased by approximately four to five °Brix in the final month of maturation, which came at the expense of a considerable increase in the amount of estimated crop loss due to rot, primarily in NO. Consequently, commercially acceptable maturity may be more consistently attained without attendant crop loss due to rot when fruit zones are managed to the regionally recommended (Reynolds and Wolf 2008) average of one to two leaf layers, at minimum. Fruit zone leaf removal may therefore aid in abating rot ingress when it is desirable to delay harvest in order to reach targeted primary fruit composition values.

Bunch rot incidence and severity at harvest. Rot incidence and severity at harvest was generally reduced by the leaf removal treatments when compared NO, although results were inconsistent across seasons (Table 2.2). In 2017, Botrytis bunch rot severity was 48% to 77% greater in NO relative to all leaf removal treatments excepting PB-6. Treatments did not affect sour rot incidence or severity in 2017. In 2018, Botrytis bunch rot incidence was reduced by PFS-4 (50%), PB-2/PFS-2 (50%), PB-4 (59%), PB-6 (71%), PB-3/PFS-3 (76%), and PFS-6 (80%), while Botrytis bunch rot severity was only reduced by PFS-6 (94%), when compared to NO. In 2018, sour rot incidence was reduced by PFS-6 (63%) and PB-3/PFS-3 (49%) when compared to NO while all leaf removal treatments reduced sour rot severity by a range of 68% to 92% when compared to NO (Table 2.2). Leaf layer number (LLN) was positively, linearly related to crop loss due to Botrytis bunch rot and sour rot (Figure 2.3) and to the incidence and severity of Botrytis bunch rot and sour rot (Figure 2.4). However, these relationships tended to be stronger in 2018 than in 2017 and stronger for Botrytis bunch rot than for sour rot (Figure 2.4). These results suggest that leaf removal particularly improves rot management in wetter (2018) over drier (2017) years and perhaps controls Botrytis bunch rot more so than sour rot. Our study confirmed that leaf removal aids in bunch rot management in Georgia, and that leaf removal to relatively greater magnitudes can improve rot management. We hypothesize that inconsistent results across seasons may have been a function of lower fruit zone radiation intensity (and reduced cluster drying) experienced in the east/west-oriented rows at the experimental vineyard. Leaf removal treatments may have more consistently reduced rot compared to NO if direct radiation dried exposed clusters, as may have occurred in north/south-oriented rows.

Previous work across variable climates has reported that bunch rot is reduced with fruit zone leaf removal (Wolf et al., 1986; English et al., 1989; Hed et al., 2015; Hickey and Wolf, 2018; Smith and Centinari, 2019). A study on Cabernet franc in North Carolina demonstrated that rot is reduced by removal of six basal shoot leaves when compared to no leaf removal (Hickey et al., 2018b). Rot reduction is consistently attributed to a less dense canopy and an open fruit zone (Hed and Centinari 2018) as well as decreased cluster compactness due to decreased fruit set and/or berry size (Hed et al 2015; Hickey and Wolf, 2018; Palliotti et al., 2012; Tardaquila et al., 2010). Moreover, leaf removal opens the fruit zone to allow for greater spray penetration and better microclimate for disease management when compared to fully foliated fruit zones. A study in Pennsylvania showed that leaf removal at different timings in Grüner Veltliner can improve rot management, especially during wet years (Smith and Centinari 2019). Our findings corroborate those of Smith and Centinari (2019), that fruit zone leaf removal may afford greater rot control over fully foliated fruit zones in wetter than in drier years; such trends exemplify greater need for sound fruit zone management in humid relative to dry/arid climates.

Components of crop yield. Timing and extent of leaf removal differentially affected crop yield components, consistently across seasons (Table 2.3). Crop yield was statistically reduced by PB-6 when compared to PFS-4 in 2017 and PFS-4 and PB-2/PFS-2 in 2018. PB-6 reduced crop yield via a reduction in berry number per cluster and thus average cluster weight. In 2017, PB-6 reduced berry number per cluster by a range of 21.7% to 39.9% when compared to PFS-4, PFS-6, PB-2/PFS-2, and PB-3/PFS-3; in 2018, PB-6 reduced berry number per cluster by a range of 34.5% to 36.7% compared to PFS-4 and PFS-6. PB-4 did not reduce berry number per cluster, cluster weight, nor crop yield while PB-6 reduced each of those responses. Therefore, pre-bloom removal of six, and possibly five, basal leaves may be an approximate threshold at which fruit set and crop yield are statistically reduced in Chardonnay, at least under conditions similar to those of this field experiment. Bivariate, linear fits of the number of leaves removed before bloom and the percent change in crop yield components were investigated (Figure 2.5). Our data, which assumes a linear response, shows a negative, linear relationship between the number of leaves removed before bloom and percent reduction in berry number per cluster, cluster weight, and crop yield in 2017 and 2018 (Figure 2.5). When averaged over both seasons, it was estimated that removal of each additional basal leaf would reduce crop yield by 5.89%, cluster weight by 4.49%, and berry number per cluster by 4.64%.

Our results validate that pre-bloom leaf removal reduces crop yield (Diago et al., 2012) which has been documented to be a function of decreased fruit set (Poni et al., 2006) and thus berry number per cluster and cluster weight (Hickey and Wolf, 2018; Poni et al., 2006). Fruit composition can be improved with leaf removal at earlier phenological stages, which may be a desirable tradeoff to a crop reduction in ample-yielding cultivars such as Tempranillo, Sangiovese, and Trebbiano (Diago et al., 2012; Poni et al., 2006). With Chardonnay, a rot-prone cultivar, a decrease in berries per cluster loosens the cluster resulting in a high-quality crop due to improved sunlight, radiation, and pesticide penetration (Hed and Centinari 2018). Grapevines subjected to post fruit set leaf removal in Georgia experience similar benefits of open canopies [less rot (see Table 2.2); balanced fruit chemistry (see Table 2.4)] to those subjected to pre-bloom leaf removal. However, post fruit set leaf removal can maintain, or increase, crop yield relative to grapevines with unmanaged fruit zones or subjected to pre-bloom leaf removal — by way of reducing the amount of crop lost to fewer berries per cluster (see Table 2.3) and rot (see Figures 2.3 and 2.4).

Primary juice composition at harvest. Leaf removal treatments tended to increase juice soluble solids and decrease juice total titratable acidity (TA) at harvest when compared to NO (Table 2.4). Excepting PFS-4, all leaf removal treatments consistently increased soluble solids by a range of 3.9% to 6.2% when compared to NO in 2017, and by a range of 3.9% to 5.4% when compared to NO in 2018 (Table 2.4). In 2017, several leaf removal treatments reduced juice TA by a range of 8% to 18% when compared to NO. Juice TA was not as drastically affected in 2018, the rainier season of the two, but was reduced by PFS-6 (9%), PB-2/PFS-2 (9%), and PB-

3/PFS-3 (12%) when compared to NO. Juice pH was only modestly and inconsistently affected by treatment with NO having the lowest recorded pH values in both years. As a function of concomitant increased soluble solids and decreased TA, the soluble solids: TA ratio was consistently greater in all leaf removal treatments relative to NO. When fruit is shaded, it is not exposed to the radiant heat and will have reduced soluble solids while retaining high TA levels (Smart et al., 1985), but zero fruit zone leaf layers can increase fruit quality and wine quality potential (Smart and Robinson 1991). A greater soluble solids: TA ratio may enable an earlier harvest without having undesirable, high wine astringency due to excessive acidity (Reynolds et al., 2006). An earlier harvest date may be desirable for commercial producers because of the heightened disease pressure experienced towards the end of summer in the eastern US (as seen in Figure 2.2), which is often accompanied by hurricanes and extended rain events. Harvesting relatively early may preclude varietal character from fully developing. While measuring secondary metabolites was beyond the scope of our experiment, exposed grapes have been shown to increase favorable wine sensory impact compounds in white-berried grape cultivars (Allegro et al 2019; Reynolds et al 2007).

Conclusion. Fruit zone leaf removal can produce desirable responses such as balanced primary fruit composition, decreased incidence and severity of bunch rots, and, if practiced after fruit set or to lesser magnitudes before bloom, crop yield maintenance. Leaf removal practices should be regionally tailored because while vineyards located in humid, subtropical climates can benefit from having less than one fruit zone leaf layer, vineyards located in arid climates might require cluster shading to preserve color, acidity, and integrity under consistently high radiant heating. The goal of fruit zone management is to create a microclimate that is more conducive to optimizing fruit quality and disease management than would otherwise be attained under the macroclimate of the region. Our results illustrate that post-fruit set leaf removal to approximately one leaf layer or less can increase juice soluble solids: TA ratio and maintain disease-free crops relative to removing leaves before bloom or refraining from leaf removal. These methods may be particularly applicable in vineyards planted in humid growing regions. Since leaf removal is a labor-intensive process, future research should evaluate and compare the effects of mechanical and manual leaf removal on the effects of fruit zone architecture, crop yield, and fruit composition of multiple cultivars.

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Table 2.1 Pre-bloom and post-fruit set leaf removal effect on average fruit-zone leaf layer number (LLN) and cluster exposure flux availability (CEFA) in Chardonnay at veraison and dormant pruning weight in 2017 and 2018

		2017	
Treatment[a]	**LLN**	**CEFA**	**Pruning weight (kg/m row)**
NO	3.0 a	0.07 d	0.82
PB-4	1.2 b	0.43 c	0.80
PB-6	0.1 d	0.72 a	0.70
PFS-4	0.8 c	0.46 c	0.79
PFS-6	0.1 d	0.69 a	0.80
PB-2/PFS-2	0.8 c	0.49 bc	0.81
PB-3/PFS-3	0.1 d	0.61 ab	0.83
Significance[b]	<0.0001	<0.0001	ns
		2018	
Treatment	**LLN**	**CEFA**	**Pruning weight (kg/m row)**
NO	2.8 a	0.19 c	0.67
PB-4	1.2 b	0.44 b	0.60
PB-6	0.5 cd	0.64 a	0.54
PFS-4	1.1 bc	0.44 b	0.55
PFS-6	0.2 d	0.63 a	0.51
PB-2/PFS-2	1.1 bc	0.46 b	0.54
PB-3/PFS-3	0.3 d	0.63 a	0.59
Significance	<0.0001	<0.0001	ns

[a]NO=no leaf removal; PB-4 and PB-6= pre-bloom leaf removal four and six leaves, respectively; PFS-4 and PFS-6 = post-fruit set removal of four and six leaves, respectively. PB-2/PFS-2 and PB-3/PFS-3= pre-bloom leaf removal of two leaves with post-fruit set removal of two leaves and pre-bloom leaf removal of three leaves with post-fruit set removal of three leaves, respectively.

[b]Significance of treatment effects (p > F; ns = not significant at 0.05 level). Means in the same treatment group (columns) not sharing a letter are significantly different, and means in the same column without letters are not significantly different, at 0.05 level based on adjusted p-values using Tukey HSD.

Table 2.2 Pre-bloom and post-fruit set leaf removal effect on the incidence and severity of Botrytis bunch rot and sour rot in Chardonnay at harvest in 2017 and 2018.

		2017		
	Botrytis bunch rot		**Sour rot**	
Treatment[a]	**Incidence**	**Severity**	**Incidence**	**Severity**
NO	68.0	8.97 a	56.8	6.86
PB-4	60.8	4.70 b	56.8	4.26
PB-6	61.6	5.06 ab	61.6	3.89
PFS-4	61.6	4.70 b	60.0	3.41
PFS-6	51.2	2.02 b	48.8	2.20
PB-2/PFS-2	58.4	3.90 b	58.4	2.09
PB-3/PFS-3	62.4	3.70 b	56.8	3.16
Significance[b]	ns	0.0015	ns	ns
		2018		
	Botrytis bunch rot		**Sour rot**	
Treatment	**Incidence**	**Severity**	**Incidence**	**Severity**
NO	63.2 a	9.60 a	73.6 a	11.3 a
PB-4	25.6 bc	1.74 ab	47.2 ab	2.12 b
PB-6	18.4 bc	1.05 ab	48.0 ab	2.67 b
PFS-4	32.0 b	2.49 ab	48.0 ab	2.47 b
PFS-6	12.8 c	0.58 b	27.2 b	0.95 b
PB-2/PFS-2	32.0 b	3.11 ab	47.2 ab	3.66 b
PB-3/PFS-3	15.2 bc	1.63 ab	36.0 b	2.34 b
Significance	<0.0001	0.0454	0.0007	0.0009

[a]NO=no leaf removal; PB-4 and PB-6= pre-bloom leaf removal four and six leaves, respectively; PFS-4 and PFS-6 = post-fruit set removal of four and six leaves, respectively. PB-2/PFS-2 and PB-3/PFS-3= pre-bloom leaf removal of two leaves with post-fruit set removal of two leaves and pre-bloom leaf removal of three leaves with post-fruit set removal of three leaves, respectively.

[b]Significance of treatment effects ($p > F$; ns = not significant at 0.05 level). Means in the same treatment group (columns) not sharing a letter are significantly different, and means in the same column without letters are not significantly different, at 0.05 level based on adjusted p-values using Tukey HSD.

Table 2.3 Pre-bloom and post-fruit set leaf removal effect on components of crop yield in Chardonnay at harvest in 2017 and 2018.

2017					
Treatment[a]	**Crop weight (kg/vine)**	**Cluster number**	**Cluster weight (g)**	**Berry #/cluster**	**Berry weight (g)**
NO	4.36 ab	23.8	184.7 bc	94.5 bc	1.95
PB-4	4.19 ab	21.3	198.1 bc	96.1 bc	2.06
PB-6	3.62 b	21.1	169.9 c	84.3 c	2.02
PFS-4	5.53 a	22.8	241.2 a	117.9 a	2.04
PFS-6	4.65 ab	21.3	220.9 ab	115.2 a	1.92
PB-2/PFS-2	4.70 ab	21.4	221.8 ab	110.3 ab	2.01
PB-3/PFS-3	4.60 ab	22.8	202.3 bc	102.6 ab	1.97
Significance[b]	0.0087	ns	<0.0001	<0.0001	ns
2018					
Treatment	**Crop weight (kg/vine)**	**Cluster number**	**Cluster weight (g)**	**Berry #/cluster**	**Berry weight (g)**
NO	5.28 ab	34.5	152.7 ab	87.8 ab	1.76 a
PB-4	5.34 ab	36.7	145.6 ab	84.1 ab	1.73 ab
PB-6	3.87 b	33.1	116.3 b	69.9 b	1.66 ab
PFS-4	6.91 a	39.7	172.4 a	106.7 a	1.62 ab
PFS-6	6.08 ab	35.4	173.4 a	110.4 a	1.58 b
PB-2/PFS-2	6.29 a	38.5	163.9 ab	100.2 ab	1.63 ab
PB-3/PFS-3	4.72 ab	36.1	129.4 ab	82.1 ab	1.58 b
Significance	0.0078	ns	0.0088	0.0056	0.0069

[a]NO=no leaf removal; PB-4 and PB-6= pre-bloom leaf removal four and six leaves, respectively; PFS-4 and PFS-6 = post-fruit set removal of four and six leaves, respectively. PB-2/PFS-2 and PB-3/PFS-3= pre-bloom leaf removal of two leaves with post-fruit set removal of two leaves and pre-bloom leaf removal of three leaves with post-fruit set removal of three leaves, respectively.

[b]Significance of treatment effects (p > F; ns = not significant at 0.05 level). Means in the same treatment group (columns) not sharing a letter are significantly different, and means in the same column without letters are not significantly different, at 0.05 level based on adjusted p-values using Tukey HSD.

Table 2.4 Leaf removal effect on mean soluble solids, titratable acidity (TA), pH, and soluble solids: TA ratio in Chardonnay at harvest in 2017 and 2018.

		2017		
Treatment[a]	**Soluble solids (°Brix)**	**TA (g/L)**	**pH**	**Soluble solids: TA ratio**
NO	17.8 b	10.1 a	3.27 b	1.77 c
PB-4	18.5 a	9.30 b	3.30 ab	2.00 b
PB-6	18.9 a	8.91 bc	3.33 ab	2.13 ab
PFS-4	18.4 ab	9.02 bc	3.31 ab	2.05 b
PFS-6	18.7 a	8.50 c	3.33 ab	2.21 ab
PB-2/PFS-2	18.6 a	8.77 bc	3.31 ab	2.13 ab
PB-3/PFS-3	18.8 a	8.26 c	3.35 a	2.28 a
Significance[b]	0.0006	<0.0001	0.0096	<0.0001
		2018		
Treatment	**Soluble solids (°Brix)**	**TA (g/L)**	**pH**	**Soluble solids: TA ratio**
NO	20.4 b	8.98 a	3.36 b	2.28 b
PB-4	21.4 a	8.38 ab	3.42 ab	2.57 a
PB-6	21.3 a	8.26 ab	3.44 a	2.59 a
PFS-4	21.2 a	8.33 ab	3.41 ab	2.55 a
PFS-6	21.3 a	8.19 b	3.42 ab	2.60 a
PB-2/PFS-2	21.5 a	8.14 b	3.41 ab	2.65 a
PB-3/PFS-3	21.4 a	7.86 b	3.45 a	2.73 a
Significance	0.0016	0.0042	0.0094	0.0007

[a]NO=no leaf removal; PB-4 and PB-6= pre-bloom leaf removal four and six leaves, respectively; PFS-4 and PFS-6 = post-fruit set removal of four and six leaves, respectively. PB-2/PFS-2 and PB-3/PFS-3= pre-bloom leaf removal of two leaves with post-fruit set removal of two leaves and pre-bloom leaf removal of three leaves with post-fruit set removal of three leaves, respectively.

[b]Significance of treatment effects (p > F; ns = not significant at 0.05 level). Means in the same treatment group (columns) not sharing a letter are significantly different, and means in the same column without letters are not significantly different, at 0.05 level based on adjusted p-values using Tukey HSD.

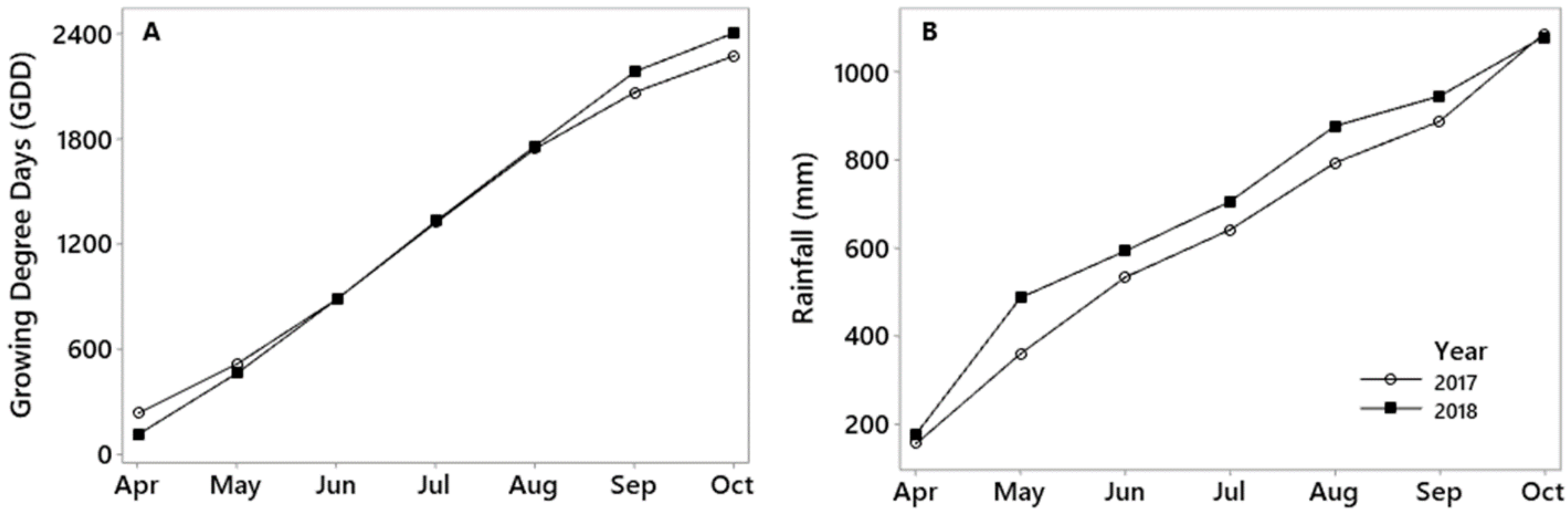


Figure 2.1 Growing degree day (A) and rainfall (B) accumulation for 2017 and 2018 at the experimental vineyard in Dahlonega, GA. Growing degree days were calculated using a base of 10˚C.

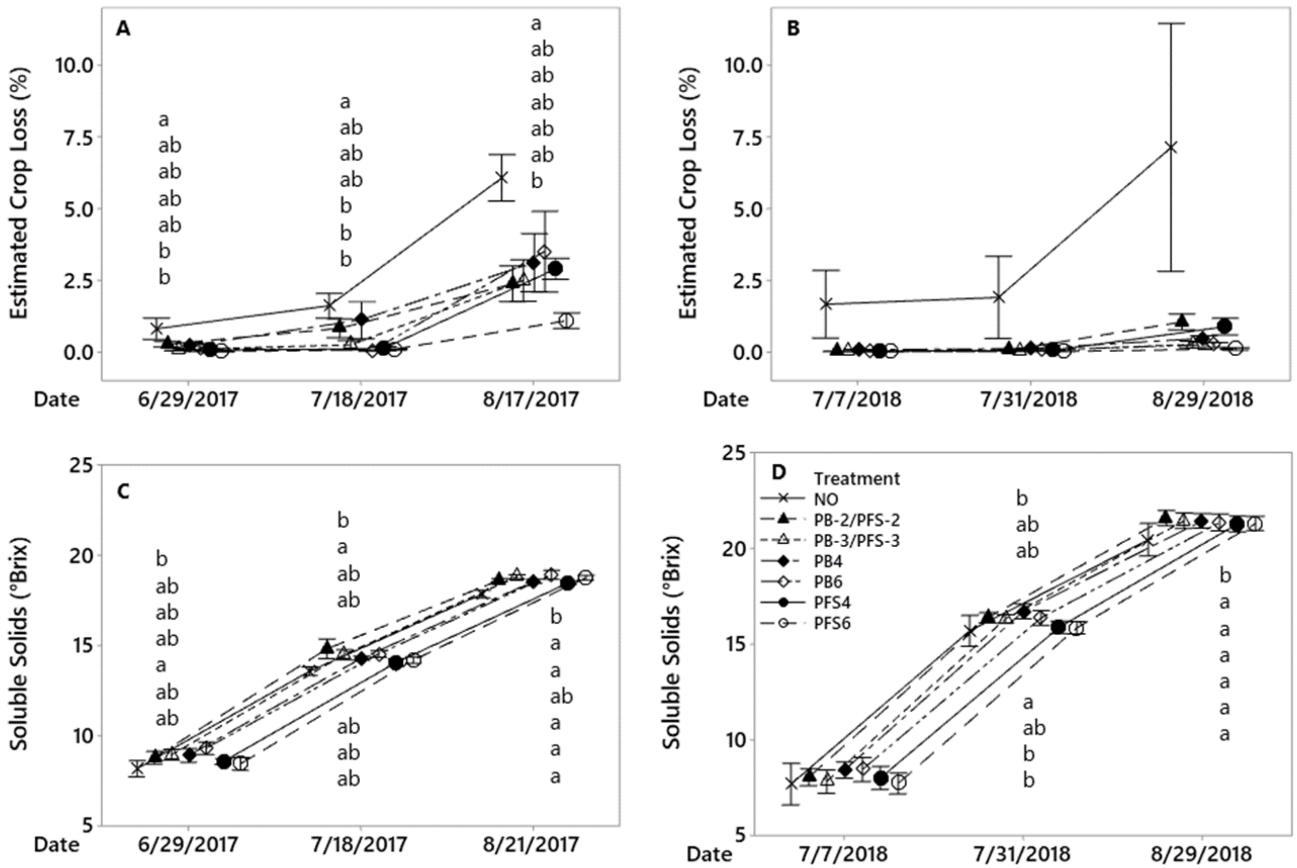
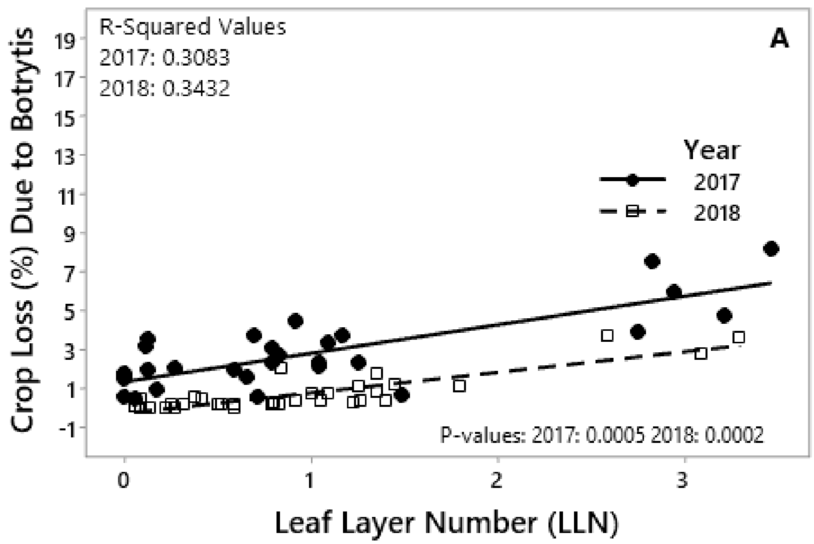


Figure 2.2 Pre-bloom and post fruit-set effect on estimated crop loss due to Botrytis bunch rot in 2017 (A) and 2018 (B) and soluble solids development over time in 2017 (C) and 2018 (D). Treatments reflect timing and level of leaf removal: no leaf removal (NO), pre-bloom leaf removal of four leaves (PB-4) and six leaves (PB-6), post-fruit set removal of four leaves (PFS-4) and six leaves (PFS-6), pre-bloom leaf removal of two leaves with post-fruit set removal of two leaves (PB-2/PFS-2) and pre-bloom leaf removal of three leaves with post-fruit set removal of three leaves (PB-3/PFS-3). Means within the same date not sharing the same letter were statistically significantly different and means in same date without letters were not statistically significantly different ($\alpha = 0.05$) Tukey's honest significant difference. Error bars indicate standard error.
(Note: Letter separators are ordered by treatment as they appear in legend.)



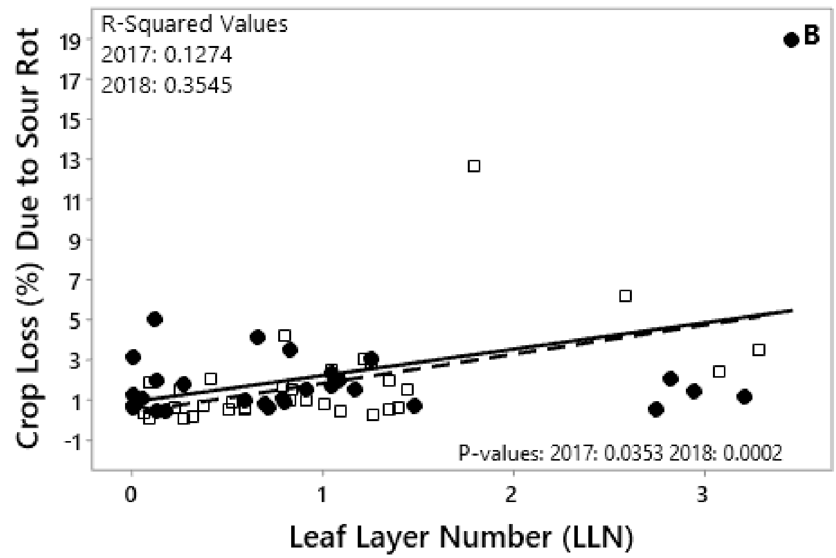


Figure 2.3 Leaf layer number (LLN) effect on total crop loss due to Botrytis bunch rot (A) and sour rot (B) at harvest 2017 and 2018.

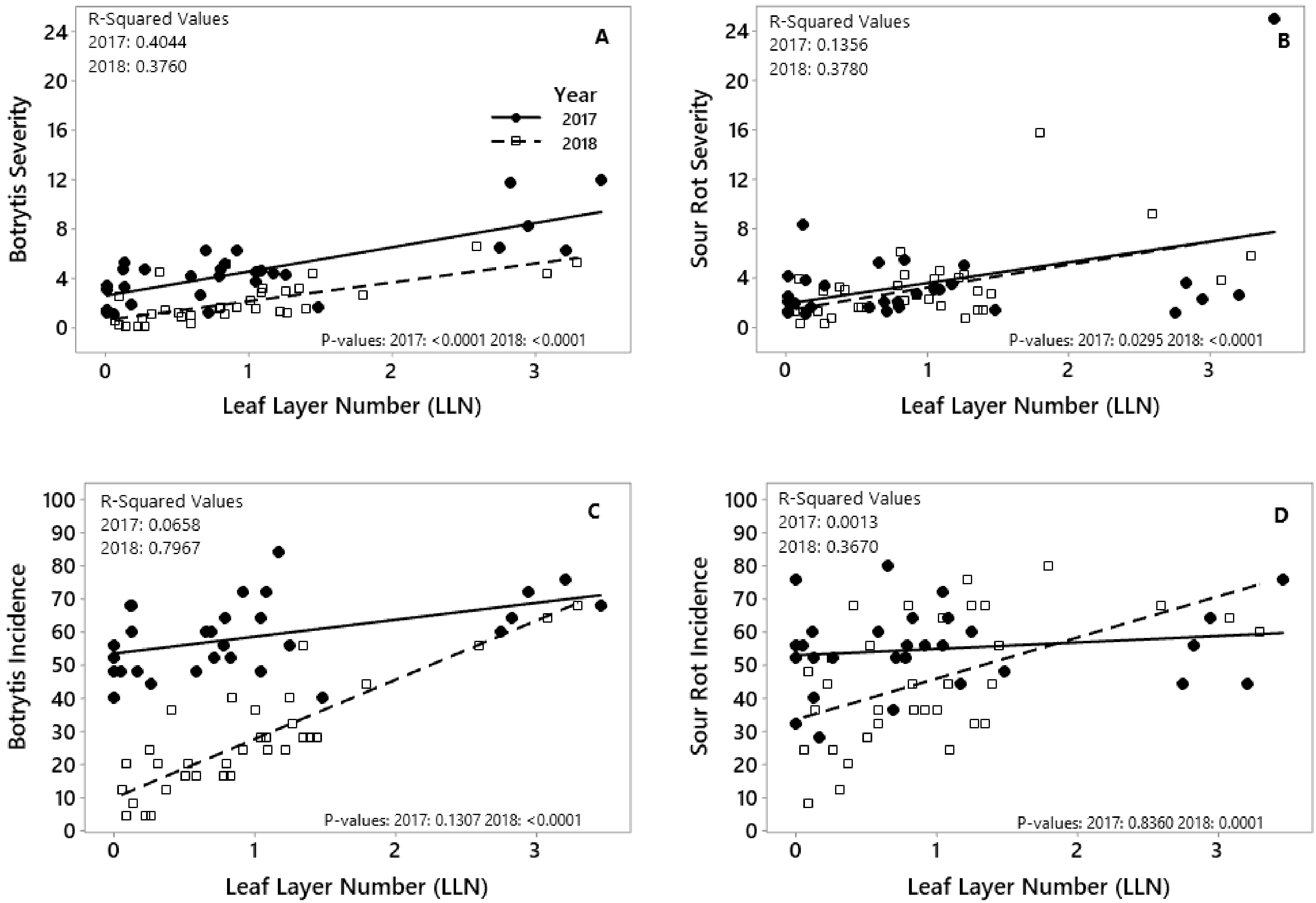


Figure 2.4 Leaf layer number (LLN) effect on Botrytis bunch rot severity (A) and incidence (C) and sour rot severity (B) and incidence (D) at harvest 2017 and 2018.

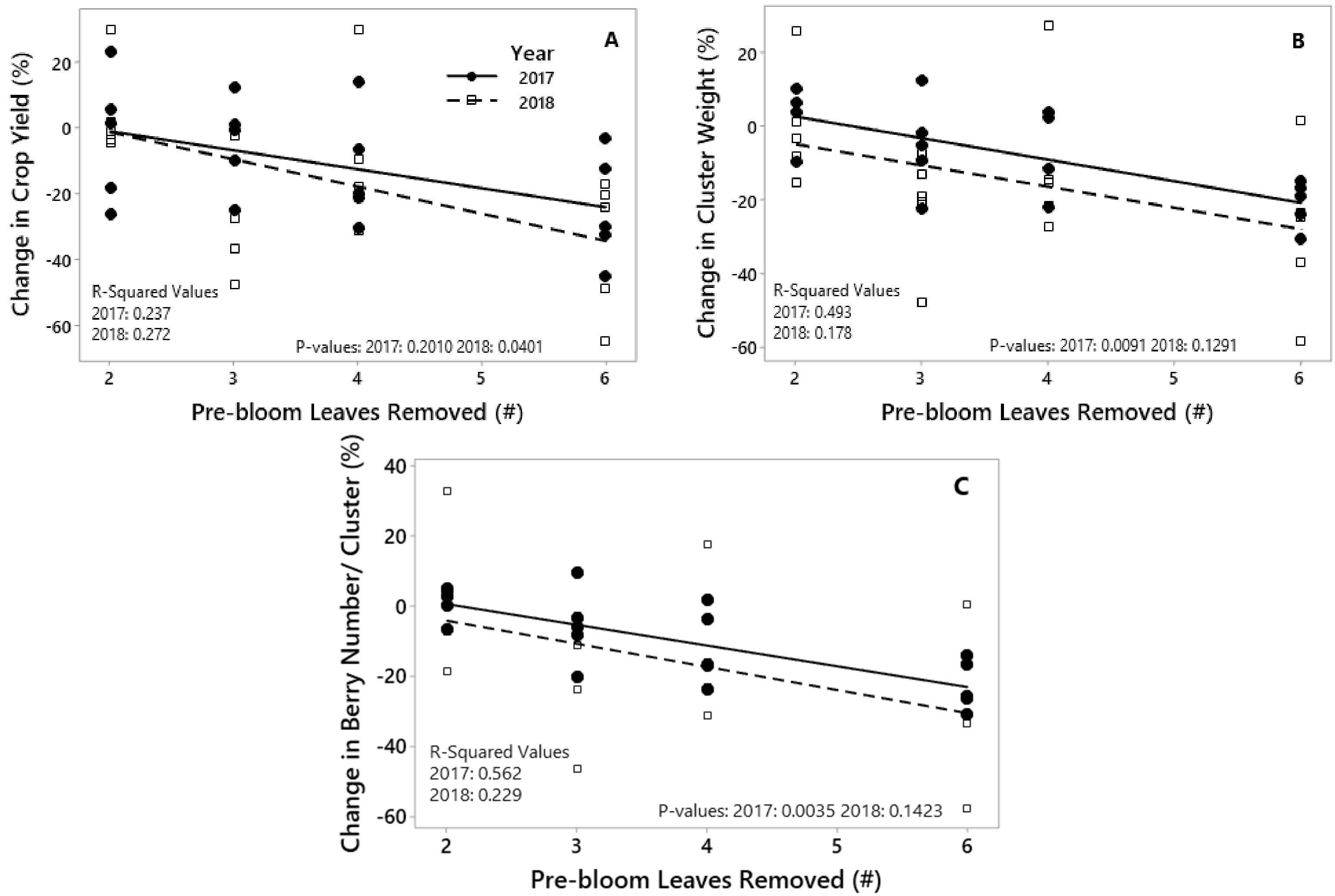


Figure 2.5 Pre-bloom leaf removal effect on the percent reduction in crop yield (A), cluster weight (B), and berry number per cluster (C) at harvest in 2017 and 2018. Percent reductions were based off of the average yield on all non-pre-bloom leaf removal treatments in our study: NO, PFS-4, and PFS-6.

CHAPTER 3

PRE-BLOOM LEAF REMOVAL IMPACTS CANOPY SIDE-SPECIFIC CROP YIELD AND FRUIT COMPOSITION IN CABERNET FRANC GROWN ON A LYRE TRELLISING SYSTEM[2]

[2] Vogel, A., R. White, S. Breeden, C. Hickey. To be submitted to *Hortscience*

Abstract. Fruit zone leaf removal is used to decrease rot and enhance varietal character. If implemented to extremes, leaf removal before bloom can create a source-sink imbalance that limits fruit set, berry number per cluster and thus crop yield. A divided canopy trellising system offers a unique platform to question if leaf removal implementation in the treated fruit zone produces similar or variable responses in the opposite, non-treated fruit zone. The aim of this work was to evaluate if pre-bloom removal of seven fruit zone leaves from only the east side, only the west side, both canopy sides, and neither canopy side of a lyre system would differentially impact crop yield and fruit composition of Cabernet franc grapes harvested from either canopy side. Pre-bloom leaf removal from both canopy sides reduced crop yield on both canopy sides relative to commercial leaf removal (CLR). Pre-bloom leaf removal from the east or west canopy sides tended to depress crop yields on the pre-bloom-treated canopy side compared to the CLR-treated canopy side. Pre-bloom leaf removal tended to depress titratable acidity relative to CLR but effects were inconsistent across years. Pre-bloom leaf removal from the east or west canopy sides tended to increase total grape anthocyanins and phenolics on the pre-bloom-treated canopy side compared to the CLR-treated canopy side. Trends show that pre-bloom leaf removal affected source-sink dynamics and secondary metabolites on the canopy side where it is implemented but not on the opposite side of a divided canopy system. Thus, source limitations imposed on spatially separated vegetative tissues may not affect whole vine source-sink dynamics.

Introduction

Canopy management is essential to produce grapes of high quality potential in challenging environments such as those found in the southeast US. The humid environment of the southeast US combined with high vine vigor can intensify disease severity and incidence (Giese et al., 2014; Hatch et al., 2011; Hickey et al., 2016). Cluster exposure to air movement and radiation creates a microclimate that is less favorable to fungal disease and improves pesticide coverage, which together assist in disease management (English et al., 1989; Hed et al., 2009; Hickey et al., 2018; Wolf et al., 1986). Fruit zone leaf removal is a management strategy that can be used to manage grape disease and composition. Standard fruit zone management practice is to remove leaves after fruit set and before bunch closure. The retention of one to two fruit zone leaf layers is recommended for wine grape production in the eastern US (Reynolds and Wolf, 2008). Recent studies in the eastern US have shown that a fruit zone devoid of leaves could offer better disease management relative to a shaded fruit zone (Hickey et al., 2018; Hickey and Wolf, 2018; Wolf et al., 1986).

Total soluble solids (TSS), titratable acidity (TA), and phenolics are affected by fruit zone leaf removal. For example, it was found that pre-bloom leaf removal increased the concentration of TSS and phenolics in must when compared to no leaf removal (Palliotti et al., 2012). Radiant heating of berries can reduce malic acid through increased respiration (Lakso and Kliewer, 1975) which can ultimately decrease TA and may increase the TSS: TA ratio. Leaf removal does not consistently impact TSS (Hickey and Wolf, 2018; Yue et al., 2019), however, the decrease in acidity in exposed fruit could hasten the achievement of a balanced sugar-acid ratio and enable an earlier harvest. Grape anthocyanins can be limited under high radiant heat loads (Bergqvist et al., 2001; Spayd et al., 2002; Tarara et al., 2008). Still, phenolics and anthocyanins are maintained or increased in exposed relative to shaded grapes grown under the variably cloudy sky conditions during growing seasons in humid climates (Hickey et al., 2018; Hickey and Wolf, 2019) as well as some arid climates (Iacono et al., 1995; Chorti et al., 2010).

The goal of canopy management is often to maintain crop yield and improve fruit quality, but specific methods should be refined for climate, cultivar, and trellising system. Fruit zone leaf removal effects on crop yield and fruit composition are not only affected by sun exposure but also the source-sink balance within a vine (Frioni et al., 2019). Leaf removal methods have been studied in a number of different settings and continue to be refined for specific climates and cultivars. However, to our knowledge, no research has evaluated leaf removal effects on crop yield and fruit composition on spatially separated canopies within a divided trellis system.

Trellising system choice impacts vine management, productivity, and source-sink balance (Reynolds and Vanden Heuvel, 2009) and can thus affect sugar accumulation (Dai et al., 2009). Trellis systems are classified as either single canopy or divided canopy. A common benefit of a divided canopy system is increased crop yield per vine relative to a single canopy system (Carbonneau and Casteran, 1987; Reynolds and Vanden Heuvel, 2009). When the canopy is divided, greater leaf area is exposed to sunlight relative to single canopy systems, often resulting in greater carbon assimilation to produce and ripen a crop (Gladstone and Dokoozlian, 2003; Kliewer and Dokoozlian, 2005). The Lyre trellis is a divided canopy system in which bilateral cordons are trained to form two horizontally-divided fruit zones; shoots are trained vertically from each fruit zone to essentially create two VSP canopies from one trunk and system (Dokoozlian, 2003). The horizontally-divided fruit zones are usually spaced between 0.91 and 1.22 m apart which necessitates wider row spacing compared to systems that take up less horizontal space (Dokoozlian, 2003). Divided trellis systems that have two separate fruit zones are often harvested in composite. Similarly, management practices, such as fruit zone leaf removal, are often implemented on both canopy sides of divided trellis systems.

The spatially separated canopies in a Lyre system provides an opportunity to explore source-sink relationships of the whole vine as well as specific vine tissues (e.g. shoots; clusters). For example, to the authors' knowledge, it is unknown if limiting source tissues in one canopy would impact crop yield or fruit composition on the "treated" canopy, the opposite canopy, both canopies, or neither canopy. Since both canopies are connected by the same roots and trunk, it is possible that the effects of a reduction in carbon source tissues on one canopy side could be "offset" by a translocation in resources from the opposite canopy. Evaluation of carbon translocation evaluation was beyond the scope of our study. However, to gain insight into source-sink dynamics, we evaluated the effects of pre-bloom removal of seven fruit zone leaves from the east, west, both, and neither canopy sides on the following responses in both fruit zones within a Lyre trellis system: Leaf layer number, crop yield, cluster weight, berry number per cluster, soluble solids, titratable acidity, anthocyanin concentration, and total phenolic content. We hypothesized that pre-bloom source tissue removal would increase phenolics and decrease titratable acidity and crop yield components where implemented when compared to commercial leaf removal implemented after fruit set.

Materials and Methods

Experimental vineyard and treatments. The experiment was implemented in a commercial Cabernet franc vineyard in Dahlonega, GA. Most vines were planted in 1998 with 1.83 m (vine) x 3.51 m (row) spacing between posts in generally north-south oriented rows. While not every vine was the same age, the experimental site was chosen for its relative uniformity in growth and management. Vines were trained to bilateral cordons on each side of the Lyre system; the total distance between the two fruit zones was 1.22 m. In the dormant season, vines were spur pruned followed by shoot thinning to 28 shoots per canopy side in the spring, at modified EL stage 12 (Dry and Coombe, 2004). Pest management was uniform across the experiment. Shoots were mechanically hedged throughout the season.

A randomized complete block design was replicated in five blocks. Experimental units were three-vine panels. Treatments were implemented in the same experimental units in the 2018 and 2019 growing seasons. Treatments were as follows:

> *Commercial leaf removal*: CLR [Post-fruit set commercial fruit zone leaf removal to quantified leaf layer number, implemented by vineyard crew at modified EL stage 31 when berries are pea sized (Dry and Coombe, 2004)].
>
> *Pre-bloom leaf removal*: EAST [seven leaves and laterals removed on east canopy side from primary shoot nodes 1-7 at modified EL stage 17 when single flowers are well separated and commercial leaf removal on the west canopy side]; WEST [seven leaves and laterals removed on west canopy side from primary shoot nodes 1-7 at modified EL stage 17 and commercial leaf removal on the east canopy side]; BOTH [seven leaves and laterals removed on both canopy sides from primary nodes 1-7 at modified EL stage 17].

Meteorology. Temperature and rainfall data were recorded from 1 April to 31 October 2018 and 2019 using a weather station located approximately 1 km from the experimental site. The weather station was comprised of a HMP35 temperature and humidity probe (Vaisala, Helsinki, Finland) and a TB4 rain gauge (Hydrological Services America, Lake Worth, FL), which were logged with a CR1000 data logger (Campbell Scientific, Logan, UT).

Fruit zone architecture. Point quadrant analysis (PQA) data was collected at veraison, modified EL stage 35 (Dry and Coombe 2004), using previously described methods (Smart and Robinson

1991). Briefly, a thin metal rod was inserted through each fruit zone and perpendicular to the cordon at a frequency of three times every meter in each experimental unit, equating to approximately 20 to 24 total probe insertions per canopy side. These methods were performed in order to quantify leaf layer number (LLN), which was calculated using enhanced point quadrant analysis software (EPQA version 1.6.2) (Meyers and Vanden Heuvel 2008).

Components of crop yield. At commercial harvest (24 Sept 2018 and 27 Sept 2019), crop yield was measured from each canopy side on a per-panel basis using a field scale. Cluster number was recorded from each canopy side on a per panel basis. Average cluster weight was determined as the quotient of crop weight and cluster number per canopy side. Prior to harvest, a random composite sample of 100 berries collected equally from both canopy sides was used to calculate average berry weight. Berry number per cluster was determined as the quotient of average cluster weight and average individual berry weight. Crop yield per canopy side, and components thereof, were averaged within each experimental unit to maintain statistical integrity.

Primary Juice Composition at Harvest. The above-mentioned 100-berry composite sample that was randomly sampled from both canopy sides was used to measure juice TSS, TA, and pH at harvest. Juice was expressed from fresh berries that were hand-pressed in bags and then poured into test tubes before centrifuging for 5 min at 5000 RPM. One mL of juice was used to measure TSS with a digital refractometer (Pocket PAL-1, ATAGO USA, Inc., Bellevue, WA). A Titrino 848 Plus auto titrator (Metrohm, USA, Riverview, FL) was used to measure pH on undiluted juice, and total titratable acidity (TA) on 5 mL of juice diluted with 40 mL of deionized water. TA was measured by titration to a pH endpoint of 8.2 using 0.1 M sodium hydroxide (NaOH) base.

Anthocyanin and total phenolic concentrations. The 100-berry composite samples that were randomly collected at harvest were stored at -80 °C until ready to be processed. Berries were collected by canopy side on a per panel basis and kept separate so that anthocyanin content could be evaluated by canopy side. Anthocyanin content was measured on homogenate made from the thawed berry samples. Using a modified method (Lee et al., 2005), anthocyanin content was measured at 700 nm, 520 nm, and total phenolic content was measured at 280 nm using a Genesys 10S Spectrophotometer (ThermoElectron North America, Madison, WI). Homogenate was made by blending thawed grapes for 1 minute ensuring consistent texture. For each treatment, 1 g of homogenate was added into two tubes, and 0.5 g was added to a third tube. Two buffers were prepared using 37% hydrochloric acid, a potassium chloride (KCl) buffer adjusted to a pH of 1 and sodium acetate (CH_3CO_2Na) buffer adjusted to a pH of 4.5. 30 mL of 0.025 M KCl buffer was added to one of the tubes with 1 g homogenate and the only tube with 0.5 g homogenate, and 30 mL of 0.4 M sodium acetate buffer was added to the remaining tube with 1 g homogenate to separate anthocyanins and phenolics at different wavelengths. Once buffers were added, the reagents were mixed by placing tubes in a Burrell 075-775-12-39 wrist-action shaker (Burrell Scientific, Pittsburgh, PA) for 20 minutes and then centrifuged for 5 minutes at 4000 rpm. To quantify anthocyanins, 500 µl of supernatant was pipetted into two 10 mm path length methacrylate cuvettes (Thermo Fisher Scientific Inc.), and its absorbance at 520 and 700 nm was measured with a Genesys 10S UV-Vis spectrophotometer. To measure phenolics, the sample with two-part sample: one-part buffer ratio was used, and its absorbance at 280 nm was measured in duplicate. Phenolic content was expressed on an absorbance unit/g berry-basis by multiplying the absorbance by the 2:1 dilution factor. Anthocyanins were expressed on a mg/g berry basis by multiplying the absorbance by the dilution factor and the molar mass of malvidin-3-glucose all divided by 28000 (using the molar mass and coefficient of malvidin-3-glucose).

Statistical analysis. Statistical computation was performed using JMP Pro v. 13. A mixed model was used to evaluate the random block effect and fixed treatment effect using 2-way ANOVA for EPQA, components of crop yield, primary chemistry, and anthocyanin and phenolic concentrations. Each variable was evaluated separately by canopy side. Significance ($\alpha \leq 0.05$) was determined with Tukey's HSD for all treatment effects.

Results and Discussion

Meteorology. In 2018, 2407 GDD accumulated and 1081 mm of rain fell from 1 April to 31 October (Figure 3.1). In 2019, 2451 GDD accumulated and1086 mm of rain fell from 1 Apr to 31 Oct. The greatest monthly rainfall was in May 2018 and Apr 2019; however, total rainfall was similar between years. Considering harvest dates of 24 Sept 2018 and 27 Sept 2019, more rain fell before harvest in 2018 compared to 2019, and more GDD were accumulated before harvest in 2019 than in 2018. At harvest in 2018, 917.4 mm of rain had accumulated along with 2120 GDD, while 805.7 mm rain fell and 2189 GDD were accumulated by harvest in 2019.

Fruit zone leaf layer number. Leaf removal treatments reduced fruit zone leaf layer number (LLN) on pre-bloom-treated sides by at least 99% compared to CLR-treated sides in both 2018 and 2019 (Table 3.1). While pre-bloom leaf removal of seven leaves effectively lowered LLN compared to commercial leaf removal (CLR), all fruit zones had lower LLN than a previous recommendation of retaining one to two leaf layers (Reynolds and Wolf, 2008).

Components of crop yield. Pre-bloom leaf removal consistently reduced crop yield compared to CLR (Table 3.2). On canopy sides where leaves were removed before bloom, crop weight, cluster weight and berry number per cluster were reduced relative to the canopy side with CLR.

Crop weight was reduced by an average 42% and 45% by BOTH compared to CLR in 2018 and 2019, respectively. In 2018, WEST reduced crop weight by 38% on the west canopy side compared to CLR. In 2019, EAST reduced crop yield by 33% and WEST reduced crop by 46% when compared to CLR on east and west canopy sides, respectively. BOTH reduced cluster weight by an average of 38% when compared to CLR in both years. EAST reduced cluster weight by 34% and 35% relative to CLR on the east canopy side in 2018 and 2019, respectively; WEST reduced cluster weight by 30% and 39% when compared to CLR in 2018 and 2019, respectively. Berry number per cluster was reduced by pre-bloom leaf removal in a similar fashion to the reduction in cluster weight and crop yield. BOTH reduced berry number per cluster by an average of 32% in 2018 and 24% in 2019 when compared to CLR. Pre-bloom leaf removal on the corresponding canopy side reduced berry number per cluster by 27% on the east and 22% on the west when compared to CLR in 2018. In 2019, EAST reduced berry number per cluster by 25% on the east canopy side compared to CLR.

Our trends suggest that crop yield components were depressed only on the canopy side in which pre-bloom treatments were implemented and confirm that pre-bloom leaf removal reduces crop yield components (Diago et al., 2012). When leaves are removed before bloom, the reduction in source tissues can decrease crop yield as a function of decreased fruit set, berry number per cluster, and berry size (Diago et al., 2012; Frioni et al., 2019; Hickey and Wolf, 2018; Matthews and Shackel, 2005; Poni et al., 2006, 2009). Post-fruit set leaf removal treatments (like CLR), however, do not affect crop yield (Hickey and Wolf, 2018; VanderWeide et al., 2018). A crop yield reduction could be beneficial in particularly high cropping cultivars (Diago et al., 2012) or may be offset by a positive response if decreased cluster compactness improves rot management (Hed and Centinari, 2018; Sabbatini and Howell, 2010). The effects of climate, vine age, and vigor are notable in the experimental setting since the storage of excess carbohydrates in an older vine grown on a vigorous site could curtail the impact of limiting source tissues on one canopy side. A decrease in crop yield on both the pre-bloom-treated and CLR-treated sides may have been experienced if similar magnitudes of pre-bloom leaf removal were implemented on young vines or vines with low vine capacity and less carbohydrate availability.

Primary Juice Composition at Harvest. Though leaf removal can have a large impact on primary composition, effects are often inconsistent between sites, cultivars, and years (Hickey and Wolf, 2018). EAST increased juice TSS from berries sampled from the east canopy side by a range of 2% to 3% when compared to CLR in 2018 (Table 3.3). There was no effect on pH (data not shown), however pH ranged from 3.81-3.97 in 2018 and 3.84-3.99 in 2019. In 2018, TA was decreased by 12% by EAST on the east canopy side, 10% by WEST on the west canopy side, and 14% by BOTH on the west canopy side, relative to CLR. Primary metabolites were unaffected by treatment in 2019, possibly because of lower crop yield in 2019 compared to 2018. The lack of consistency in treatment effect on fruit composition was potentially because the CLR treatment resulted in enough fruit exposure to produce similar composition to grapes sampled from pre-bloom-treated canopy sides. Fruit zone leaf removal can decrease TA and maintain or increase TSS relative to shaded fruit zones, regardless of leaf removal timing (Palliotti et al., 2012). The more open fruit zones characteristic of pre-bloom treatments tended to reduce juice TA when compared to CLR, and this was putatively a function of a reduction in malic acid due to heat-induced respiration (Lakso and Kliewer, 1975).

Anthocyanin and total phenolic concentrations. In 2018, EAST increased anthocyanin concentration on the east canopy side, and BOTH increased anthocyanin concentration on both canopy sides, when compared to CLR (Table 3.4). Similarly, EAST and WEST increased total phenolics when compared to CLR on corresponding canopy sides and BOTH increased total phenolics on both canopy sides relative to CLR. In 2019, treatment had fewer effects on anthocyanins and total phenolics. WEST increased anthocyanin concentration on the west canopy side and EAST increased total phenolics on the east canopy side, when compared to CLR. In 2019, weather was warmer and sunnier and treatments had similar or higher anthocyanin concentrations and total phenolics when compared to 2018. Like previous work (Frioni et al., 2017), our study suggests that the fruit zone leaf removal in a cloudier year, like 2018, may register an increase in phenolic compounds relative to shaded clusters. Leaf removal may have a lesser impact on fruit composition in sunnier and drier years, like 2019, when even shaded fruit zones may receive the critical thresholds of light and temperature that are required to maintain grape phenolic production. Climate should dictate fruit zone leaf removal methods to optimize grape anthocyanin and phenolic content. The cited critical berry temperature thresholds for grape anthocyanin accumulation are 30 and 35°C (Spayd et al., 2002; Tarara et al., 2008). In growing regions with high radiant heating capacities, the temperatures experienced by exposed clusters may limit their anthocyanin content. In the southeast US, frequent cloudiness lessens radiant heating magnitude; the result is that anthocyanin and phenolic content is often maintained or increased in exposed relative to shaded grape clusters (Hickey and Wolf, 2019). Here, the more extreme levels of pre-bloom leaf removal tended to improve fruit composition when compared to a more conservative approach in the post-fruit set period (CLR). Previous studies reported that pre-bloom leaf removal increased anthocyanins and phenolics via increases in grape skin thickness (Poni et al., 2009). While beyond the scope of our study, it was possible that extended exposure to sunlight and radiant hating resulted in thicker grape skins in the pre-bloom leaf removal plots when compared to the CLR treatment. Because variable cloudiness is characteristics of the humid climate of the southeast US, fruit zone leaf removal to less than one leaf layer may impose less risk for fruit quality reduction than in more arid climates and may further produce fruit with high wine quality potential and with less rot (Hed and Centinari, 2018; Sabbatini and Howell, 2010; Smith and Centinari, 2019).

Conclusion

Leaf removal is a tool to manage crop yield and wine quality potential. Some effects of leaf removal are desirable such as balanced fruit composition and disease management. However, extreme levels of pre-bloom leaf removal can considerably reduce crop yield. The effect of pre-bloom leaf removal on each side of a divided canopy system is reliant on environmental and cultural factors and thus should be curated by region. Our results illustrated that pre-bloom removal of source tissues impacts the source-sink balance on a shoot-, or canopy-side-specific, basis. Our study was conducted in a 20-year-old vineyard in a growing region with ample resources to produce vigorous vegetation; results may differ in other scenarios with less vigorous vines, as perhaps due to limited water or mineral nutrient resource availability. In the interest of crop yield maintenance, future research should evaluate if pre-bloom leaf removal to lesser extents could produce similar fruit composition benefits with being deleterious to crop yield.

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Table 3.1. Leaf removal effects on leaf layer number (LLN) on east and west canopy sides of Cabernet franc trained to a Lyre trellis.

	2018	
	EAST	**WEST**
Treatment[a]	**LLN**	
CLR	0.7 a	0.8 a
EAST	0.0 b	0.7 a
WEST	0.6 a	0.0 b
BOTH	0.0 b	0.0 b
Significance[b]	<0.0001	<0.0001
	2019	
	EAST	**WEST**
Treatment	**LLN**	
CLR	1.0 a	0.9 a
EAST	0.0 b	1.0 a
WEST	0.8 a	0.0 b
BOTH	0.0 b	0.0 b
Significance	<0.0001	<0.0001

[a]CLR=commercial leaf removal; EAST= pre-bloom leaf removal of seven leaves on east canopy, WEST= pre-bloom leaf removal of seven leaves on west canopy, BOTH= pre-bloom leaf removal of seven leaves on both canopy sides
[b]Significance of treatment effects ($p > F$; ns = not significant at 0.05 level). Means in the same treatment group (columns) not sharing a letter are significantly different, and means in the same column without letters are not significantly different, at 0.05 level based on adjusted p-values using Tukey HSD.

Table 3.2. Pre-bloom leaf removal effects on components of crop yield on east and west canopy sides in Cabernet franc trained to a Lyre trellis.

	2018					
	EAST	**WEST**	**EAST**	**WEST**	**EAST**	**WEST**
Treatment[a]	**Crop weight (kg/panel)**		**Cluster weight (g)**		**Berry #/cluster**	
CLR	15.7 a	15.3 a	132.6 a	124.2 a	74 a	74 a
EAST	11.1 ab	14.2 a	87.5 b	113.2 ab	54 bc	70 ab
WEST	12.5 ab	9.6 b	107.6 ab	86.6 bc	64 ab	58 bc
BOTH	9.3 b	8.9 b	79.8 b	80.5 c	48 c	53 c
Significance[b]	0.0151	0.0024	0.0007	0.0023	0.0014	0.0048
	2019					
	EAST	**WEST**	**EAST**	**WEST**	**EAST**	**WEST**
Treatment	**Crop weight (kg/panel)**		**Cluster weight (g)**		**Berry #/ cluster**	
CLR	11.7 a	13.5 a	112.1 a	121.4 a	64 a	66
EAST	7.9 b	11.6 a	72.4 b	88.4 b	48 b	51
WEST	9.7 ab	7.3 b	96.4 ab	74.8 b	59 ab	51
BOTH	6.9 b	6.8 b	71.5 b	74.4 b	48 b	51
Significance	0.0051	0.0007	0.0018	0.0025	0.0090	NS

[a]CLR=commercial leaf removal; EAST= pre-bloom leaf removal of seven leaves on east canopy, WEST= pre-bloom leaf removal of seven leaves on west canopy, BOTH= pre-bloom leaf removal of seven leaves on both canopy sides
[b]Significance of treatment effects (p > F; ns = not significant at 0.05 level). Means in the same treatment group (columns) not sharing a letter are significantly different, and means in the same column without letters are not significantly different, at 0.05 level based on adjusted p-values using Tukey HSD.

Table 3.3. Pre-bloom leaf removal effects on total soluble solids (TSS) and titratable acidity (TA) on Cabernet franc harvested from east and west canopy sides of a Lyre trellis.

	2018			
	EAST	**WEST**	**EAST**	**WEST**
Treatment[a]	**TSS (°Brix)**		**TA (g/L)**	
CLR	22.7 b	22.6	3.10 a	3.07 a
EAST	23.4 a	22.8	2.73 b	3.02 a
WEST	22.9 b	22.8	3.10 a	2.75 b
BOTH	22.7 b	22.8	2.84 ab	2.65 b
Significance	0.0052	NS	0.0462	0.0003

	2019			
	EAST	**WEST**	**EAST**	**WEST**
Treatment[a]	**TSS (°Brix)**		**TA (g/L)**	
CLR	23.4	23.1	4.22	4.09
EAST	23.4	23.5	4.00	4.04
WEST	23.2	22.6	4.27	4.17
BOTH	23.1	23.2	3.99	3.91
Significance	NS	NS	NS	NS

[a]CLR=commercial leaf removal; EAST= pre-bloom leaf removal of seven leaves on east canopy, WEST= pre-bloom leaf removal of seven leaves on west canopy, BOTH= pre-bloom leaf removal of seven leaves on both canopy sides
[b]Significance of treatment effects (p > F; ns = not significant at 0.05 level). Means in the same treatment group (columns) not sharing a letter are significantly different, and means in the same column without letters are not significantly different, at 0.05 level based on adjusted p-values using Tukey HSD.

Table 3.4. Pre-bloom leaf removal effects on grape anthocyanin concentration and total phenolics of Cabernet franc harvested from east and west canopy sides of a Lyre trellis.

		2018		
	EAST	**WEST**	**EAST**	**WEST**
Treatment[a]	**Total anthocyanins (mg/g berry)**		**Total phenolics (au/g berry)**	
CLR	0.34 c	0.32 b	81.1 b	74.6 c
EAST	0.44 a	0.34 ab	100.1 a	76.6 bc
WEST	0.36 bc	0.38 ab	78.9 b	90.0 ab
BOTH	0.43 ab	0.41 a	101.4 a	98.5 a
Significance[b]	0.0032	0.0355	<0.0001	0.0006
		2019		
	EAST	**WEST**	**EAST**	**WEST**
Treatment	**Total anthocyanins (mg/g berry)**		**Total phenolics (au/g berry)**	
CLR	0.41	0.30 b	95.2 b	85.8
EAST	0.47	0.31 b	119.6 a	97.8
WEST	0.34	0.44 a	96.1 b	104.0
BOTH	0.44	0.33 ab	111.4 ab	91.4
Significance	NS	0.0148	0.0061	NS

[a]CLR=commercial leaf removal; EAST= pre-bloom leaf removal of seven leaves on east canopy, WEST= pre-bloom leaf removal of seven leaves on west canopy, BOTH= pre-bloom leaf removal of seven leaves on both canopy sides
[b]Significance of treatment effects (p > F; ns = not significant at 0.05 level). Means in the same treatment group (columns) not sharing a letter are significantly different, and means in the same column without letters are not significantly different, at 0.05 level based on adjusted p-values using Tukey HSD.

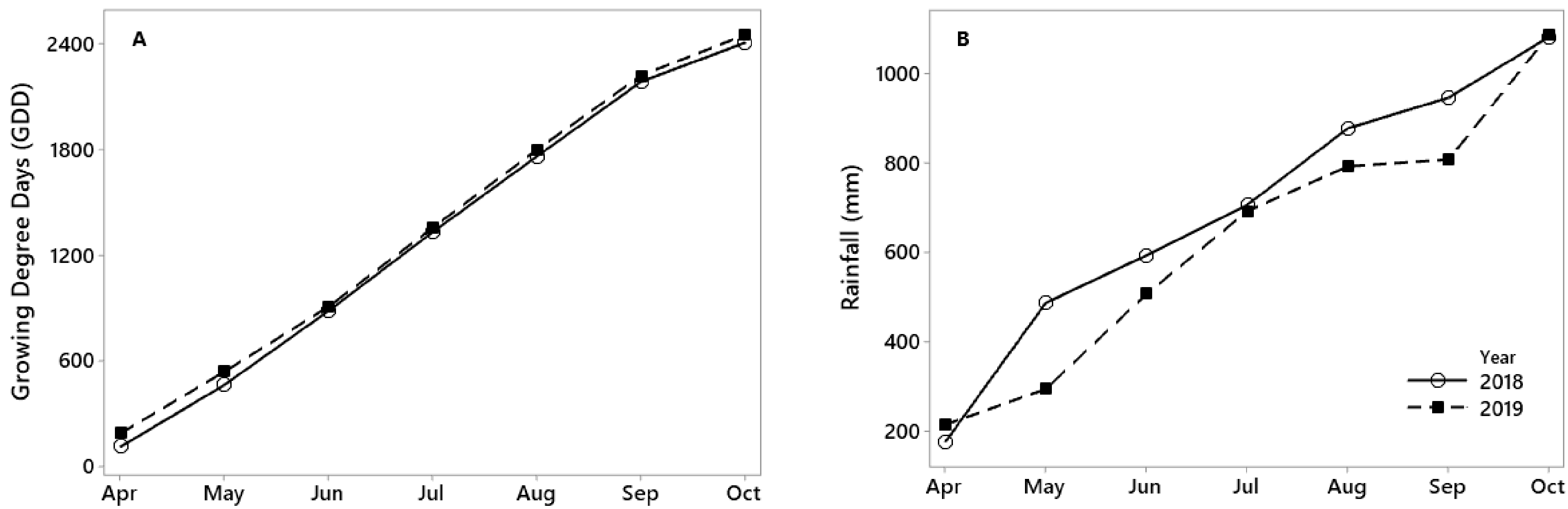

Figure 3.1. Growing degree day (A) and rainfall (B) accumulation for 2018 and 2019 at the experimental vineyard in Dahlonega, GA. Growing degree days were calculated using a base of 10˚C.

CHAPTER 4

EVALUATION OF THE PREDICTABILITY OF GRAPE TEMPERATURE USING WIRELESS DATALOGGERS CONTAINED WITHIN A BERRY MIMIC[3]

[3] Vogel, A., S. Breeden, M. Van Iersel, C. Hickey. To be submitted to *HortTechnology*

Abstract. Fruit zone leaf removal effects on vine productivity and fruit quality have been widely researched. Many fruit zone leaf removal studies cite that grape temperature influences grape composition. However, few of studies have quantified grape berry temperature over time. It is suspected that grape temperature is infrequently quantified due to limitations such as cost and intensive programming requirements. An efficient and economical way to estimate grape berry temperature would be desirable for researchers and industry members. Consistent quantification of grape temperature would allow researchers to compare the effects of leaf removal and incident radiation on grape composition across varying climates and regions. A cost-effective means to quantify berry temperature would also provide industry members site-specific feedback on berry temperature patterns and guide leaf removal practice. We evaluated the ability of wireless temperature sensors, submerged in various volumes of water within black and white balloons, to predict berry temperature. Treatments included 0, 10, 30, 50, and 70 mL volumes of DI water in black and white balloons and a control of 0 mL water in a clear plastic bag. Regression analysis was used to determine the relationship between sensor-logged temperatures and Carminare noir berry temperatures. Nighttime berry temperatures were accurately predicted by all treatments including the control, suggesting that solar radiation is an explanatory variable. Using a seasonal model, one that behaves differently in two or more points of time, to compensate for the variation in day and night temperatures, the 30 ml-white- and 30 ml-black- balloon treatments similarly predicted berry temperature with the greatest accuracy (R^2 values of 0.96). Housing temperature sensors in balloons proved to be an accurate, practical, and cost-effective solution to estimate berry temperature. Further refinement of methods in different regions, row orientations, training systems, and cultivars is necessary to increase adoption of methods presented herein.

Fruit zone leaf removal is a vineyard management practice that can reduce fungal rots (English et al., 1989; Hed et al., 2009; Hickey et al., 2018b; Wolf et al., 1986) and manipulate primary and secondary berry metabolite concentrations (Crupi et al., 2010; Hunter et al., 1991; Jackson and Lombard, 1993; Lee et al., 2005; Ryona et al., 2008). Leaf removal changes the fruit zone microclimate such that radiation and temperature patterns are higher than those of shaded fruit zones (Bledsoe et al., 1988; Guidoni et al., 2008; Ristic et al., 2007; VanderWeide et al., 2018; Zoecklein et al., 1992). High temperatures over an extended period of time can reduce grape anthocyanin content and limit color development (Bergqvist et al., 2001; Spayd et al., 2002; Tarara et al., 2008). However, variable cloudiness is anticipated to moderate radiant grape heating relative to conditions experienced in arid, less cloudy growing regions (Faust and Logan, 2018), thus maintaining or increasing grape anthocyanins (Hickey and Wolf, 2019).

Leaf removal alters grape metabolites via changes in berry temperature (Bergqvist et al., 2001; Hickey and Wolf, 2018; Spayd et al., 2002; Tarara et al., 2008), but treatment effects can only be attributed to berry temperature if it is quantified. Grape temperature models have been published (Cola et al., 2009), but such methods are likely more precise in regions characterized by predictable sky conditions. Meteorological modeling may not be reliable across all grape growing regions (Faust and Logan, 2018). For example, cloud coverage is highly variable in humid climates making it difficult to predict the temperatures of objects in such conditions. Therefore, quantifying berry temperature would allow comparisons between studies conducted in divergent climates and advance our understanding of how leaf removal affects berry temperature and metabolite profiles. To the authors knowledge, only four studies have recorded berry temperature over time during the course of fruit development from veraison to harvest (Bergqvist et al., 2001; Hickey and Wolf, 2018; Spayd et al., 2002; Tarara et al., 2008). The studies employed handheld thermometers or data loggers to record temperatures with hypodermic thermocouples inserted beneath the skin of grape berries. Where the handheld thermometers were used, berry temperatures had to be manually recorded throughout the day on multiple dates. While the use of data loggers reduces time spent manually recording data, the set-up of semi-permanent thermocouples inserted in berries requires continuous monitoring. These current methods to measure berry temperature over time are costly and require advanced skills to program data loggers. Easy and cost-effective means of estimating berry temperature would likely increase the frequency of berry temperature quantification in future canopy management studies. Reliable methods are needed to measure berry temperature precisely across climates. Additionally, industry practitioners may benefit from evaluating berry temperature patterns in commercial vineyards to determine optimal leaf removal practices on a site-specific basis.

The volume and color of an object have bearing on its temperature. Because these parameters are important to determine grape temperature we sought to account for them in our study. We evaluated if small temperature loggers submerged in water in various volumes of black and white balloons could accurately predict grape berry temperature.

Materials and Methods.

Experimental vineyard. The experiment was conducted at University of Georgia's Durham Horticulture Farm in Watkinsville, Georgia. Carminare noir was planted in 2018 with 6 ft vine x 12 ft row spacing in north-south oriented rows. Vines were intended to be trained to a single canopy, vertical-shoot-positioned (VSP) system with low, bilateral cordons but the age of the vineyard limited full realization of canopy training. Shade cloth (Griffin Greenhouse Supplies Inc, Green-Tek 80% Black 26' Wide Shade Cloth) was positioned around the middle and top catch wire to uniformly represent an above-head canopy in the canopy-sparse, two-year old vines. The fruit zone was cleared of leaves on 9 Aug 2019. The first experimental period was 10 Aug at 0800 $_{HR}$ to 23 Aug 2019 0730 $_{HR}$ and the second was 24 Aug 1000 $_{HR}$ to 6 Sept 2019 0730 $_{HR}$. Data from both periods was combined for analysis.

Two vines were used in the experiment. The project was set up as a randomized complete block design replicated in four blocks, each of which consisted of one arm (cordon) of a vine. Due to block proximity and therefore similar environmental conditions, blocks were treated as replicates because there was little variability across blocks (Figure 4.1). Four clusters, one from each cordon, were chosen for berry temperature recording based on location and exposure on both canopy sides.

"Logged" berry temperature, ambient temperature, and ambient radiation. During the first experimental period, from 10 Aug 2019 to 23 Aug. 2019, each cluster had four thermocouples inserted into four total berries (two on the east and two on the west sides of clusters) per cluster, totaling sixteen thermocouples across the four blocks. In the second experimental period, from 24 Aug. 2019 to 6 Sept. 2019, two more thermocouples were added to each cluster, resulting in three thermocouples inserted into six total berries (three on the east and three on the west sides of clusters) per cluster, totaling twenty-four thermocouples across the four blocks. The split wire ends of hypodermic thermocouples (OMEGA, HYP1-30-1/2-T-G-60-SMP-M) were inserted into an AM25T multiplexer (Campbell Scientific); the needle probe ends of those thermocouples were inserted approx. 0.125 inches into grape berries at modified EL stage 35 (veraison; berry softening and sugar accumulation) (Dry and Coombe 2004). The AM25T multiplexer was logged with a CR1000 data logger (Campbell Scientific). Ambient total solar radiation was measured with a pyranometer (Apogee Instruments, SP-110-SS) and ambient air temperature was measured with a thermistor (Apogee Instruments, ST-110-SS) housed in an aspirated

radiation shield (Apogee Instruments, TS-110-SS). Both the pyranometer and thermistor were logged with the above-mentioned CR1000 datalogger. Berry temperatures and ambient conditions were logged every 10 minutes as a sample to mirror the ibutton logging method (below).

Berry mimic temperature measured by ibutton. White and black 30.4 cm balloons were filled with five different volumes of deionized water using a 1-10ml pipette (Eppendorf). Ibuttons (ibuttonlink, DS1921G#F) were programmed to log temperatures as a sample every 10 minutes during the same two experimental periods mentioned above. The program software (ExpressThermo, Eclo) was downloaded to a computer with a USB to 1-Wire adapter (DS9490R) and probe (DS1402-DR8+) used to connect the ibuttons to the system.

After programing, the ibuttons were placed into miniature zipper sealable plastic bags (1.5” x 1.5” 2 MIL) and randomly assigned to treatments. The treatments were as follows:

Control: C [One ibutton in a plastic bag]

Volume and Color: B0, B10, B30, B50, B70 [One ibutton in a plastic bag, each placed into one black balloon and filled with 0, 10, 30, 50, and 70 mL of water, respectively]; W0, W10, W30, W50, W70 [One ibutton in a plastic bag, each placed into one white balloon and filled with 0, 10, 30, 50, and 70 mL of water, respectively].

Each block consisted of eleven ibuttons, ten of which were submerged in five different volumes of deionized water and contained in either white or black balloons; the other ibutton was a control in a clear plastic bag. Berry mimics were randomly placed along the fruit zone within each block and attached to the catch wire using zip ties so that they hung in the open fruit zone, directly above the grape clusters from which berry temperature was being measured, and directly under the above-head shade cloth.

Data collection. After each two-week experimental period, the ibuttons were taken down, the balloons were cut open and the data was downloaded onto a computer using the ExpressThermo program. The ibuttons then were re-programmed for the second experimental period. The ibuttons were placed in new plastic bags and randomly assigned treatments with new sets of balloons and deionized water before being randomly placed within each block. During the course of the experiment, berry temperature, solar radiation, and ambient temperature data was downloaded weekly from the CR1000 data logger.

Berry diameter and weight. A random sample of 100 total Carminare noir berries was taken across the entire experiment on 6 Sept 2019; individual berry weight was measured using a mass scale and individual berry diameter was measured using digital calipers. Only 83 of the sampled berries were measured; 17 berries incurred damage during sample transportation to the lab. A regression equation was created to estimate the weight of berries from diameter measurements. Diameters were measured on the berries with thermocouples inserted ("logged berries"); the regression equation developed from the relationship between berry weight and diameter of the sampled berries was then used to estimate the weight of the "logged berries". Temperature was recorded on only turgid berries throughout the experiment.

Statistical analysis and model development. Exploratory data analysis was conducted to determine the best method to model grape temperature. Regressions were run to evaluate the relationship of berry temperature and treatment temperatures at times of day when there was no radiation and at times of day when radiation was greater than zero. Between-block variation was analyzed to ensure homogeneity (Figure 4.1). A seasonal linear model created with a piecewise function was used to separate the day/night cycle because the variance in nighttime temperatures was lesser than that of daytime temperatures. Day/night cycle was based on solar radiation levels. The breakpoint between day and nighttime data was found to be 1000 $_{HR}$ based on bootstrapping, a statistical method that randomly samples from the data and makes inferences about a population. Separate models were analyzed for each treatment, and best fit was determined through model selection process of corrected Akaike Information Criterion (AICc) and Bayesian Information Criterion (BIC) for daytime temperatures. The lowest AICc value indicates the least amount of error in the model. Only daytime temperatures were used to create the model because the absence of radiation at night resulted in similarity between all treatments and thus adaptability to any model. BIC values were also recorded and agreed with AICc on best fit model. Data from block three was used as the test data, and data from blocks one, two, and four were used as a training set to create the final prediction models.

Results and Discussion

Ambient temperature and radiation. Ambient temperature and solar radiation varied diurnally (Figure 4.2). During the night, when solar radiation was lowest, ambient temperature fell between 15.4 and 26.5 °C. Ambient temperature was highest from 1200 to 1900 $_{HR}$ when it ranged from 21.8 to 35.6 °C. Diurnal solar radiation trends were bell shaped. The period of greatest ambient solar radiation was recorded between 1000 and 1600 $_{HR}$ and ranged from 79.6 to 1039.0 W/m^2 during that timeframe. Similar ambient temperature and solar radiation patterns were reported at Virginia Tech's Agricultural Research and Extension Center near Winchester, VA (Hickey and Wolf, 2019). Ambient temperature and solar radiation can affect berry temperature. Exposed grape clusters will be subjected to higher ambient temperature and solar radiation compared with shaded clusters, and therefore internal temperature will increase throughout the day (Hickey and Wolf, 2019). Exposure to ambient temperature and radiation especially between 1000 $_{HR}$ and 1900 $_{HR}$ hours may increase critical berry temperature thresholds for grape anthocyanin accumulation, which thought to occur between 30 and 35°C (Spayd et al., 2002; Tarara et al., 2008). Based on the diurnal ambient temperature and radiation patterns in Figure 4.2, measuring grape berry temperature between 1200 and 1600 $_{HR}$ would provide the best opportunity to determine if critical berry temperature thresholds are being reached or exceeded in our experimental conditions. Measuring point-in-time berry temperature between 1200 and 1600 $_{HR}$ may help industry members determine best leaf removal practices at their site. However, training system and row orientation will change the time of day when the greatest radiation penetration to the fruit zone is experienced.

Berry diameter and weight. Berry size is an important metric when considering how methods evaluated herein could be used to predict temperature in other grape cultivars. An equation representing the relationship between weight and diameter was developed using a linear regression (Figure 4.3). These results indicated a positive relationship between berry weight and berry diameter. Berry weights ranged 1.25 to 4.29 g and berry diameter ranged 12.19 to 19.99 mm. The equation allowed for the estimation of the weights of logged berries using the measured diameter at the end of data collection (Table 4.1). The average diameter and weight of Carminare noir grapes were 16.82 mm and 2.86 g, respectively, at our experimental site.

Regression analysis between treatments and berry temperature. Berry temperature was closely related to ambient temperature throughout the experimental period (Figure 4.4). Regression analysis resulted in equations for the estimation of east and west side berry temperatures with R^2 values of 0.891 and 0.886, respectively. Thus, while ambient temperature could be used to predict berry temperature, solar radiation confounds this relationship and thus needs accounted for to refine the predictability of berry temperature. There were high R2 values when regressions were analyzed between all recorded berry temperatures and treatments throughout the experimental period (Table 4.2). For example, R^2 values were at least 0.820 across all treatments regardless of time, and the lowest R^2 values for 0 White, 10 White, and 30 White were 0.925, 0.920, and 0.913, respectively. The treatments with black balloons were not as closely correlated to berry temperature as those with white balloons, but 30 Black had an R2 value of 0.961. The variation of R^2 values between treatments was lower at night than during the day. All treatments effectively estimated berry temperature in the absence of solar radiation. For example, the model for the control treatment accounted for 97.8% of the variance at night when the models for 30 White and 30 Black accounted for 96.8% and 96.5%, respectively. In the presence of radiation, however, the regression for the control treatment only accounted for 87.9% of the variance while 30 White and 30 Black accounted for 91.3% and 91.2% of the variance, respectively. A seasonal model was used to optimize berry temperature prediction by accounting for radiation levels in conjunction with time of day; the linear regressions used to develop the data in Table 4.2 did not simultaneously account for radiation and time.

Statistical insights and model development. During exploratory analysis, the AICc model selection indicated that treatments 30 White and 30 Black had the lowest error and, therefore, the best fit for berry temperature prediction. Prediction models were created for these treatments and tested for model quality. For the models to function properly, day and night hours were adjusted and shifted to have only one breakpoint instead of two. Taking the last four hours of a day (2000-2300 $_{HR}$) and shifting it to the beginning of the next day allowed for all night hours to be together on one side of the breakpoint instead of broken up on either side of daytime hours. The hour adjustment to create one breakpoint also resulted in two equations for estimated berry temperature, one for day and one for night. When choosing an equation, actual hour must be converted to an adjusted hour to account for the new breakpoint. Using a 24-hour scale, when actual hour is less than 20, the adjusted hour equals actual hour plus 4. When actual hour is 20 or more, the adjusted hour equals actual hour minus 20. For example, when the actual hour is 1000 $_{HR}$ the adjusted hour would be 1400 $_{HR}$, or if the actual hour is 2100 $_{HR}$ the adjusted hour would equal 0100 $_{HR}$. The adjusted hour will confirm which equation should be used for the model. The formula for the model is explained in Figure 4.5a. The equation coefficients for 30 White and 30 Black were based on the training data which produced the prediction models; the coefficients differ because the treatments had different effects on estimated grape berry temperature (Table 4.3). Because 1000 $_{HR}$ is the breakpoint and 10 + 4 = 14, c = 14. With the parameter estimates from Table 4.3, the model for 30 White, in piecewise function notation, is equivalent to the model in Figure 4.5b, and the model for 30 Black, in piecewise function notation, is equivalent to the model in Figure 4.5c. When the predicted temperature is found, the adjusted hour can be converted back to get time of the predicted temperature.

Once the models were created with blocks one, two, and four as a training set, they were then tested with block three to evaluate accuracy when compared to the berry temperature measurements from thermocouples. Mean Absolute Percentage Error (MAPE) was used to evaluate the quality of the model. MAPE is used to estimate how close average predicted values are to actual values and the quality of the regression (R^2). 30 White had a MAPE of 0.0275 which means there is 2.75% error in the model; 30 Black had a MAPE of 0.0345, or a 3.45% error in the model. The R^2 values for the 30 White and 30 Black regressions were 0.9663 and 0.9646, respectively. Based on these findings, the treatment with the best fit model was 30 White.

The models developed herein may be suitable for grapes grown in Watkinsville, Georgia and proximate locations as diurnal solar radiation will vary on a latitudinal and longitudinal basis.

Carminare noir vines were two-years old at the time of the experiment and were loosely trained to a VSP trellis system which was oriented in rows that were planted north-south. The experiment was only run during one growing season. Models will vary across cultivars, training systems, row orientations, and locations. However, models could be developed on a case-specific basis using the statistical methods presented.

Conclusion

New methods for estimating grape berry temperatures in field research are essential for quantifying how temperature affects response variables., such as grape metabolites. While a solution was explored and evaluated through this study, experimental limitations remained. Current methods for measuring berry temperature are time-consuming, costly, and difficult to control in a field setting due to the impact that environment and pests have on experimental set-up. All treatments, including the control, could estimate berry temperature with a high degree of accuracy in the absence of solar radiation. During the day, the increased variation in models and lowest percent error in estimated temperature narrowed the best treatment to 30 White. Using the developed model, an ibutton could be inserted in a white balloon with 30 mL of water and hung in a fruit zone to predict grape temperature with 97.3% accuracy. The ability to estimate berry temperature will give researchers the opportunity to cost-effectively report berry temperature and its relationship with berry physiology. Berry temperature quantification could aid in the development of regionally specific management canopy management recommendations. Methods would benefit from further refinement if evaluated across several growing seasons and in different cultivars, training systems, row orientations and growing regions. Future work should evaluate the ability of methods described herein to predict berry temperature in a variety of unique growing situations.

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Table 4.1. Average estimated berry weight from diameter measurements		
Replicate[z]	**Diameter (mm)**	**Weight[y] (g)**
1	16.49	2.71
2	16.81	2.85
3	17.02	2.95
4	16.95	2.91

[z]Replicates consist of six berries three on east canopy side and east on west canopy side which were averaged to compare values.

[y]Weights were estimations determined using the equation from the linear regression (Figure 4.2); Diameter = 10.602 + 2.175 x Weight

Table 4.2. R^2 values of the relationship between treatment and berry temperature during daytime hours, nighttime hours, and the combination of both.

Treatment[z]	$0700_{HR} – 2100_{HR}$[y]	$2100_{HR} – 0700_{HR}$[x]	$0000_{HR} – 2400_{HR}$
0 White	0.925	0.981	0.963
10 White	0.920	0.980	0.965
30 White	0.913	0.968	0.958
50 White	0.892	0.961	0.945
70 White	0.820	0.958	0.946
0 Black	0.875	0.976	0.942
10 Black	0.833	0.902	0.879
30 Black	0.912	0.965	0.961
50 Black	0.899	0.954	0.946
70 Black	0.878	0.945	0.942
Control	0.879	0.978	0.939

[z]Treatment titles consist of volume of water (mL) followed by color of balloon. Control was a clear plastic bag with no water added.

[y]Daytime is considered the hours between 0700_{HR} and 2100_{HR}.

[x]Nighttime is considered hours between 2100_{HR} and 0700_{HR}.

Table 4.3. Equation terms, meanings, and values used in the grape berry temperature prediction models developed for 30 White and 30 Black treatments.

Term	Meaning	Value[z]
	30 White	
β_0	Intercept	-3.9988
β_1	Coefficient for 30 W	1.1124
β_2	Coefficient for hour ≤ 14	0.1790
β_3	Coefficient for hour >14	-0.2812
	30 Black	
β_0	Intercept	1.26564
β_1	Coefficient for 30 B	0.89509
β_2	Coefficient for hour ≤ 14	0.04772
β_3	Coefficient for hour >14	-0.23817
c	Constant	14
y	Predicted grape temperature	
x_1	Temperature from ibutton	
x_2	Adjusted hour	

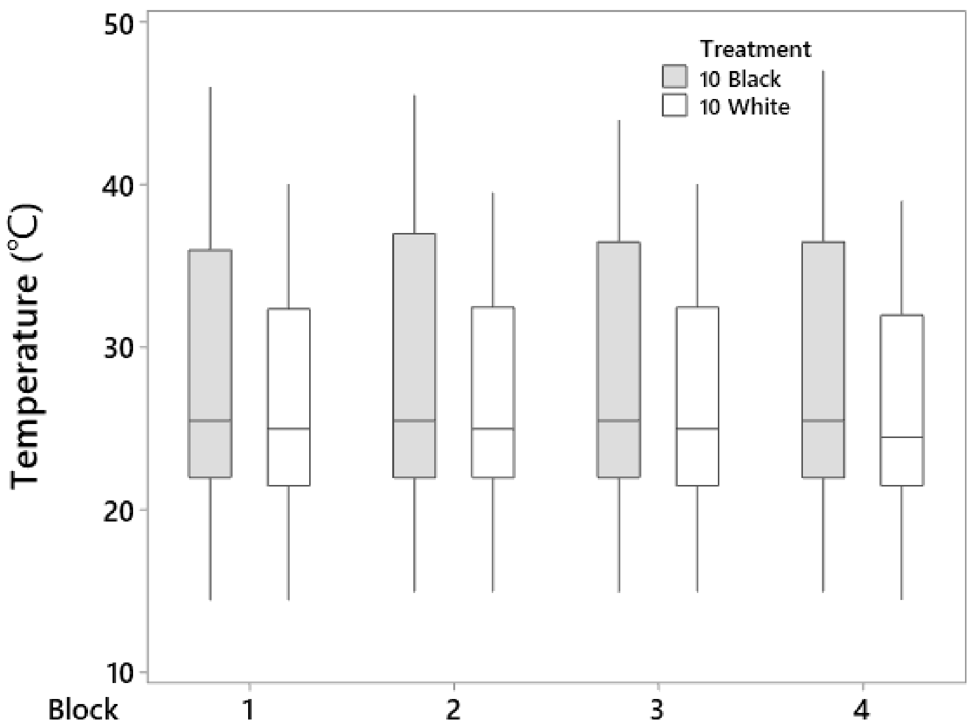

Figure 4.1. Between-block variation in sensor temperature from 10 B and 10 W; n = 1.

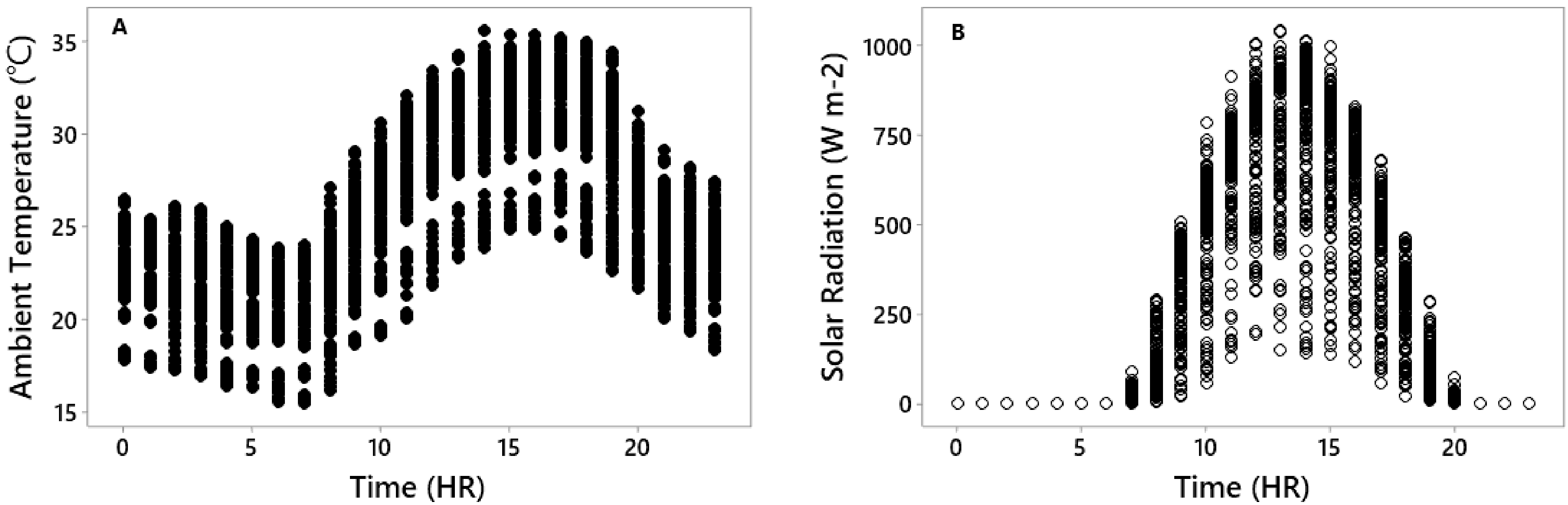

Figure 4.2. Diurnal ambient temperature (A) and solar radiation (B) patterns at experimental site in Watkinsville, GA.; Data recorded over entire experimental period. °C

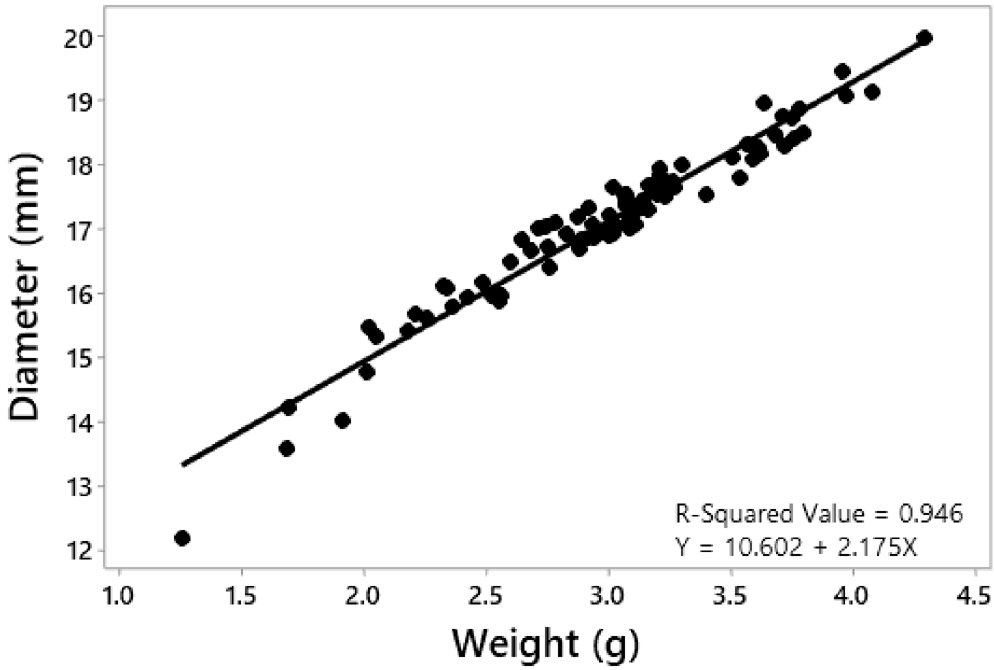

Figure 4.3. The relationship between berry weight and berry diameter of Carminare noir grapes on 6 August 2019; n = 83.

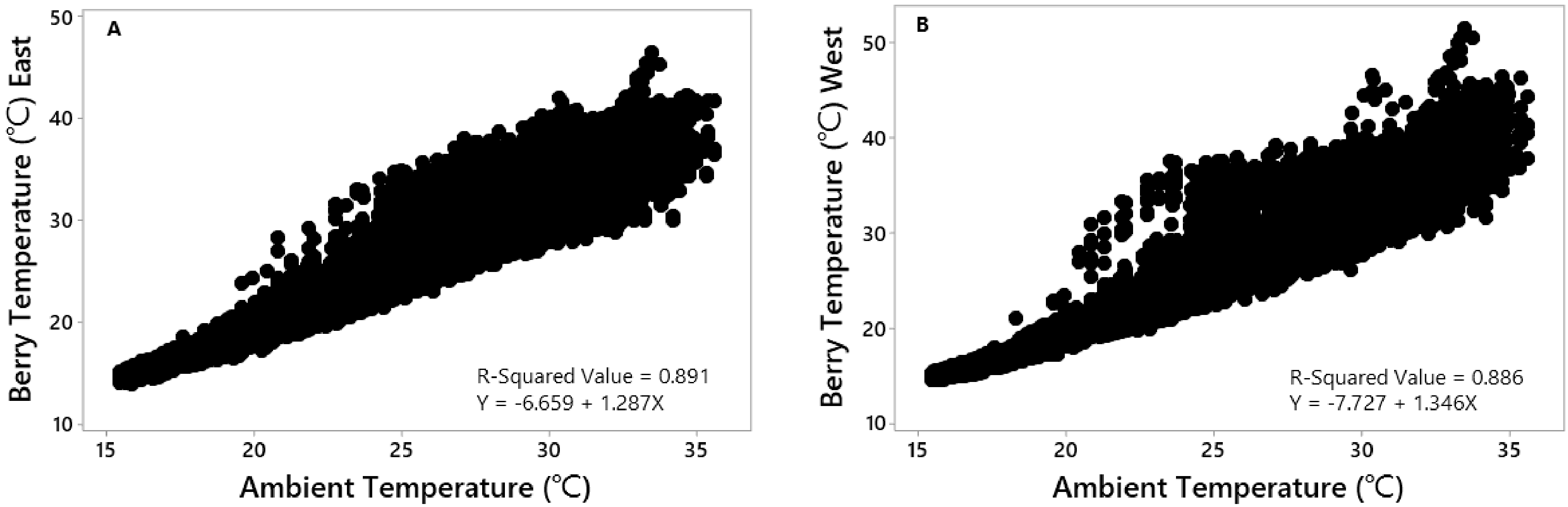

Figure 4.4. The relationship between ambient temperature and "logged" berry temperature on the east canopy side (A) and west canopy side (B).; Data recorded in berries from one block over the entire experimental period; n = 3

a. $y = \begin{cases} \hat{\beta}_0 + \hat{\beta}_1 x_1 + \widehat{beta}\hat{\beta}_2 x_2, \ x_2 \leq c \\ \hat{\beta}_0 + \hat{\beta}_1 x_1 - (\hat{\beta}_3 - \hat{\beta}_2)c + \hat{\beta}_3 x_2, \ x_2 > c \end{cases}$

b. $y = \begin{cases} -3.9988 + 1.1124x_1 + 0.1790x_2, \ x_2 \leq 14 \\ 2.4446 + 1.1124x_1 - (-0.2812 - 0.1790)14 + -0.2812x_2, \ x_2 > 14 \end{cases}$

c. $y = \begin{cases} 1.26564 + 0.89509x_1 + 0.04772x_2, \ x_2 \leq 14 \\ 5.2681 + 0.89509x_1 - (-0.23817 - 0.04772)14 + -0.23817x_2, \ x_2 > 14 \end{cases}$

Figure 4.5. Basic model formula (a), model for 30 White (b), and model for 30 Black (c) were all developed using training data from logged berry temperature. Parameter estimates from Table 4.3 were used to express models b and c.

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APPENDIX A

GRAPE SOUR ROT: EXTENSION PUBLICATION

Introduction/background

Though grape sour rot can occur in drier climates, it is a disease complex that can be especially problematic during the ripening of wine grapes in wet, humid regions. The mechanism of infection and role that multiple causal organisms play in sour rot etiology are not fully understood. However, grape sour rot has been described by Hall et al. (2018a) as a disease that only exists in the presence of damaged fruit, ethanol producing yeast (*Metschnikowia* spp., *Pichia* spp., and *Saccharomyces* sp.), acetic acid bacteria (*Acetobacter* sp. and *Gluconobacter* spp.) (AAB), and *Drosophila* spp. (fruit flies). Sour rot infections appear to be a function of AAB, yeast, and fruit flies on damaged grape berries that encourages disease progression throughout the entire cluster as ripening progresses. Secondary or simultaneous invasion, from fungal pathogens such as from *Botrytis cinerea*, can be observed in sour-rotted clusters (Figure 1). Browning and disintegrating berries and the aroma of vinegar (acetic acid) are a few symptoms that characterize grape sour rot. Sour rot ultimately results in crop yield reduction as damaged berries often "shatter," or fall off the clusters. Sorting out clusters with sour rot that are not suitable for winemaking causes a further reduction in return revenues as less wine is produced. Though it has only recently been a topic of defined research, sour rot has been a prominent concern in eastern US vineyards as: (1) it is consistently observed in vineyards, particularly in white-berried cultivars; and (2) questions remain about how to best manage it, particularly with the threat of insecticide resistance development in targeted fruit flies (Loeb and Walter-Peterson 2019).

Figure 1. *Drosophila spp*. (left) and *Botrytis* bunch rot (right) on sour rot infected clusters. Note the fungal growth which is not part of the sour rot complex but exacerbates the damage. Left photo Courtesy Wendy McFadden-Smith, OMAFRA.

Range and causal conditions

Grape sour rot is especially prevalent in wet, humid environments, like those observed in the eastern US. All four of the major disease components (damaged fruit, yeast, bacteria, and fruit flies) are often present in eastern US vineyards. The sour rot disease pyramid (Figure 2) portrays the disease-causing agents that are currently understood to cause grape sour rot. Sour rot symptoms generally begin when berries are around 15 Brix (Figure 3) and daily temperatures are at least 68 °F (Hall et al. 2018a). The disease infiltrates through damaged berry skins (Figure 4). Thin-skinned, tight-clustered cultivars (e.g. Vignoles, Sauvignon blanc, Blanc du Bois) are at greatest risk when compared to those that are thick skinned and loose clustered (e.g. Petit Manseng and Petit Verdot), although field observations of Chardonnay suggest that clones can vary in their susceptibility to sour rot, possibly related to cluster morphology. Due to the ease with which insects can penetrate thin-skinned cultivars, these cultivars experience greater insect damage, which can manifest in increased sour rot. In addition, thin-skinned cultivars have a propensity to crack with an influx of water due to late-season rains. Regardless of cultivar, rainy and cloudy conditions exacerbate sour rot symptoms. Sour rot levels can increase when harvest is delayed late into the fall in an attempt to increase Brix, when sometimes the only fruit compositional changes are increased pH and decreased acidity.

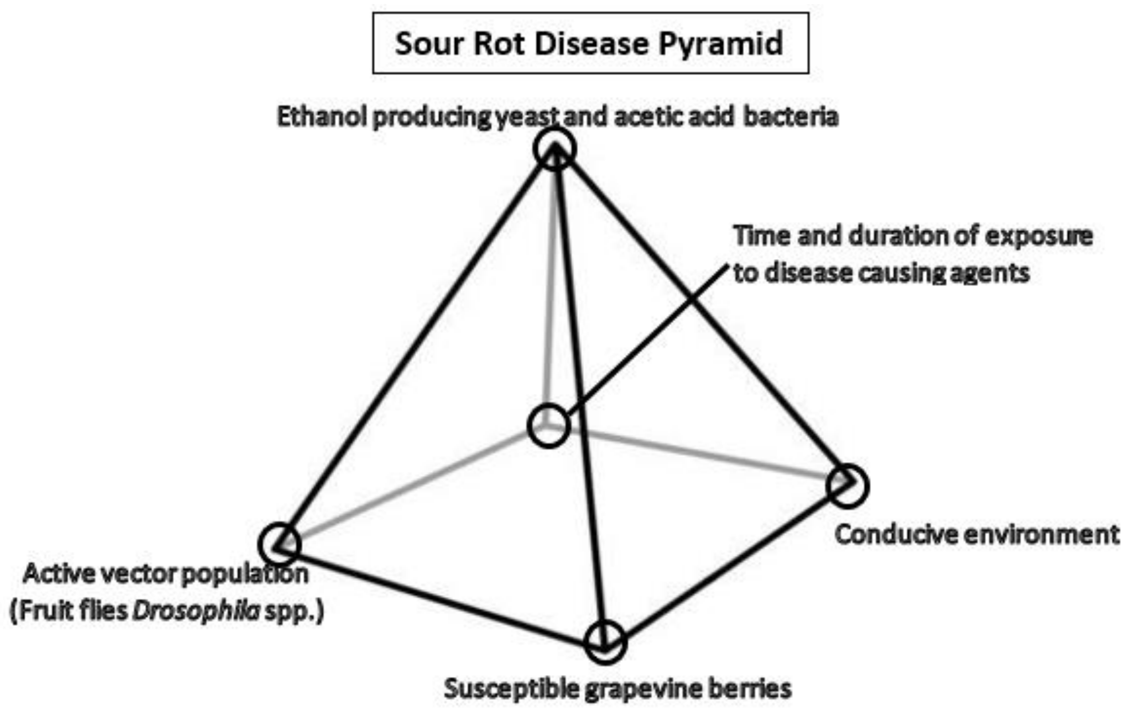

Figure 2. The sour rot disease pyramid as the complex and causal agents are currently understood. Susceptible grapevine berries, *Drosophila* spp. vector, ethanol producing yeast, AAB, a conducive environment for pathogenicity, and time and duration of exposure to the disease-causing agents

Figure 3. Sour rot infection early in ripening (~15 Brix)

Symptoms and identification

Barata et al. (2012) suggested that sour rot initiates with any damage to the berries. The causal agent of the damage is not relevant to sour rot; rather, any damage results in exploitation of the berry pulp by fruit flies AAB, and yeast. Fruit flies are attracted to the damaged berries, and thus act as vectors that transport AAB and yeast to injury sites in unaffected fruit clusters (Barata et al. 2012). Sour rot is then initiated through uncontrolled fermentation of the berry juice into ethanol. Ethanol is oxidized by AAB into acetic acid, which then turns the fruit shades of brown and causes the pulp to liquefy and emit a sour-vinegar aroma, giving the disease its name. In fact, the smell of vinegar permeates the air and is indicative of sour rot. Damaged and rotting fruit attract more fruit flies that continue the cycle by spreading the AAB and yeast to unaffected fruit. The fermented pulp can also ooze and drip onto the other berries within the cluster, spreading the infection to previously undamaged berries. Sour rot can be visually distinctive with deflated tan to brown berries and no obvious fungal structures (Figure 4 and Figure 5), though the disease can often coincide with *Botrytis* bunch rot infections (Figure 1). Sour rot can resemble sunscald, but the scent of acetic acid is a diagnostic key to identifying this disease complex.

Figure 4. Vidal blanc with damaged skins and sour rot symptoms.

Figure 5. Sour rot symptoms on Chardonnay. Sour rot can manifest as browned berries with no fungal infections (left), but the vinegar smell is diagnostic of this rot complex

Management

Sour rot has historically been difficult to manage. The disease complex, its environmental requirements, and the factors that cause sour rot are still in question by plant pathologists. This lack in knowledge has limited effective chemical management options until recently. Planting cultivars that have been observed to be less susceptible to sour rot, such as *V. vinifera* cultivars like Petit Verdot and Cabernet Sauvignon, hybrid cultivars like Chambourcin and Chardonel, and Pierce's disease-tolerant hybrid cultivars like Norton and Lomanto, use the advantage of genetics to mitigate sour rot development in the vineyard. Further, judicious harvest decisions appear to be important to limit sour rot incidence. As grapes are left on the vine, grape berries tend to become softer, acidity declines, and pH increases; sour rot has been observed to increase over time in the post-veraison period. Some cultivars, like “aromatic” whites (e.g. Blanc du Bois, Sauvignon blanc, Muscat ottonel) often have varietal character at relatively low Brix levels. It is therefore important to weigh risks – “do you want to risk crop loss and/or reduced wine quality due to sour rot? – or – do you want to harvest a full crop and use winemaking tools to modify must composition to produce a well-balanced, finished wine?” Only one choice can be made in certain cultivars in some vintages.

Sour rot can be partly managed through cultural practices that improve air movement through, and spray penetration to, the fruit zone. Management strategies should focus on creating an environment that limits one or more of the disease-causing factors, such as: controlling or mitigating fruit fly infestations, preventing berry damage, choosing a trellis style that reduces canopy density, and managing the canopy to optimize spray penetration and evaporation rates. Canopy management can decrease disease pressure; Hall et al. (2018b) documented higher disease severity in research plots with denser canopies and less managed vineyard floors. Similarly, Blaauw et al. (2019 and 2020) and Hickey et al. (2018b) documented a decrease in sour rot incidence and severity with fruit zone leaf removal in Chardonnay. Good weed management and carefully mowed row middles will also increase air flow and reduce canopy/fruit drying times. In addition to cultural practices, a chemical program utilizing antimicrobials and insecticides directed at controlling yeast, AAB, and fruit flies can further minimize risk of sour rot (Hall et al. 2018b). For example, weekly applications of insecticides and antimicrobial sprays (commencing at 15 Brix) resulted in a 64% reduction of sour rot severity when compared to untreated vines (Hall et al. 2018b). However, ongoing work suggests that less frequent sprays of insecticides + antimicrobials after 15 Brix will help regulate sour rot. As with control of many vineyard diseases, an integrated approach that combines cultural and chemical programs will optimize sour rot management. However, recent work (Loeb and Walter-Peterson 2019) suggests that it is important to use insecticides judiciously in order to reduce the incidence of resistance build up in fruit flies and other insects. Resistance management should involve effective rotations of insecticides and fungicides with different modes of action.

Summary

Grape sour rot is a disease complex characterized by the smell of acetic acid and browning of grape berries. As berries ripen, the grapes begin to ooze rotting pulp. Fruit flies, which are attracted to damaged berries, are an important vector for the AAB and yeast that incite the disease. Management options are limited, but it is possible to minimize sour rot damage. Canopy management is important to prevent excessive shading, improve air flow, and increase chemical deposition to the fruiting zone. Limiting mechanical and insect fruit damage is also key in reducing the effect of the disease components. The addition of an insecticide and antimicrobial chemical program directed towards limiting sour rot casual agents (AAB, yeast, and fruit fly) will provide significantly better control than utilizing only one form of disease management alone. Finally, scouting and harvesting before sour rot incidence and severity peaks will reduce the need to sort fruit and limit microbe introduction into winemaking facilities.

Further Reading:

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APPENDIX B

VINEYARD CANOPY MANAGEMENT SERIES: FRUIT ZONE MANAGEMENT: EXTENSION PUBLICATION

Introduction

The practices collectively known as “canopy management” aim to maximize canopy leaf exposure, maintain crop yield and quality, decrease disease, and improve vineyard health. Though labor-intensive, canopy management should not be considered optional if the goal is annual production of high quality grapes and wines.

Fruit zone leaf and lateral shoot removal (fruit zone leaf removal) is often implemented in conjunction with, or slightly after, the initial shoot positioning. Fruit zone leaf removal is primarily practiced in winegrape vineyards. Failure to remove some foliage from the fruit zone can result in excessive shading of grape clusters. When foliage surrounds the fruit zone, airflow, pesticide spray penetration, and evaporation rates are reduced. Such phenomena greatly increase disease incidence and severity on grape clusters, especially in humid climates. Varietal character, positive wine aroma compounds, and color development are all generally reduced in shaded fruit zones. Fruit zone leaf removal is a tool used to manage bunch rots and wine quality potential, especially in variably cloudy, humid climates like those of the eastern US.

The fruit zone

Fruitful shoots bear two or three grape clusters, depending on cultivar. Grape clusters are typically produced from the third through fifth nodes of primary shoots (Figure 1). Fruitful, primary shoots originate from one-year old wood whereas secondary (or "lateral") shoots grow laterally from the nodes of primary shoots. The fruit zone of a grapevine canopy is defined as the region of the canopy where the greatest density of grape clusters exists. Within a training system, fruit zones comprise a confined region of the canopy in order to facilitate cultural practices, optimize spray targeting, and improve harvest efficiency (Figure 2). The fruit zone can be positioned 30 to 36" above-the-ground and confined in a linear space in popular training systems such as the vertical-shoot-positioned (VSP) system, or exist roughly 60 to 72" above-the-ground and manifested in a two-dimensional manner in divided canopy systems such as the Watson system (Figure 2). For more information on the Watson System, please see UGA Extension Bulletin 1522 (White et al. 2020).

Figure 1. A primary count (spur-originating) shoot bearing clusters at node positions 4 and 5

Figure 2. A Chardonel fruit zone in a vertical-shoot-positioned (VSP) system (left) and a Norton fruit zone in a Watson system (right).

Motivation for fruit zone management

Fruit zone leaf removal increases airflow and reduces drying time, thereby creating a microclimate that is less hospitable to fungal diseases (English et al. 1989, Wolf et al. 1986). *Botrytis* bunch rot and other late season bunch rots (Figure 3) are often better controlled when leaf removal is implemented relative to fully foliated fruit zones (Table 1). Vineyard managers do not want to harvest or cull rotten fruit, winemakers do not want to make wine with rotten fruit, and wine consumers probably do not want to drink wine made from rotten fruit. Improved rot management alone should incentivize fruit zone management to improve air flow and light exposure to clusters — especially in humid climates where fungal diseases are extremely prevalent.

Figure 3. Chardonnay clusters with *primarily Botrytis* bunch rot (left) and *primarily* sour rot (right) harvested from shaded fruit zones.

Table 1. Fruit-zone leaf removal effect on *Botrytis* bunch rot incidence and severity in two Cabernet franc clones in North Carolina in 2017.

Treatment[a]	***Botrytis* incidence (%)**[b]	***Botrytis* severity (%)**[b]
	Clone 214	
NO	54.0	4.6
PB6	30.0	1.6
PFS6	10.0	0.1
	Clone 327	
NO	44.0	0.9
PB6	32.0	0.6
PFS6	32.0	0.3

[a]Treatment = no leaf removal (NO); removal of six leaves before bloom (PB6); removal of six leaves after fruit set (PFS6). Data adapted from Hickey et al. 2018b.
[b] Incidence = visual inspection of the infection of one berry or more per cluster; severity = visual inspection of percent damage per cluster.

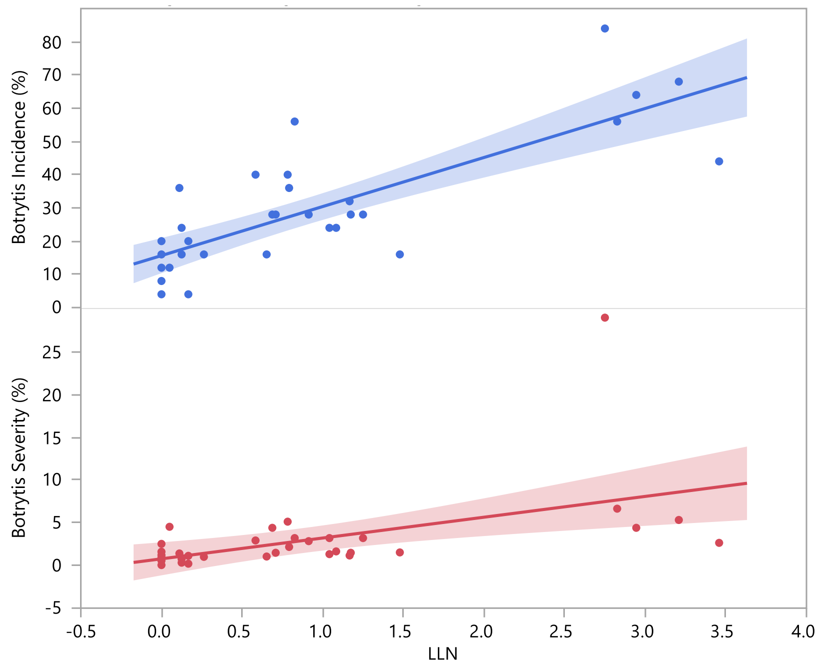

Figure 4. The relationship between fruit zone leaf layer number (LLN) and *Botrytis* bunch rot incidence and severity, summer 2017. As tissue layers surrounding clusters are reduced, *Botrytis* bunch rot severity is reduced more so than incidence. Incidence = visual inspection of the infection of one berry or more per cluster; severity = visual inspection of percent damage per cluster.

Leaf removal may indirectly increase usable crop via reducing bunch rots, and may improve fruit quality. Compounds that positively influence wine sensory perception can be increased in exposed relative to shaded grapes (Hickey et al. 2018a, Razungles et al. 1998). Compounds that produce vegetal/herbaceous aromas, such as methoxypyrazines, can be reduced by cluster exposure (Ryona et al. 2008). Exposed grape clusters tend to have lower acidity than shaded clusters due to temperature-driven respiration of malic acid (Lakso et al. 1975) (Figure 5). Therefore, a wine must (juice) comprised of exposed clusters often has a greater sugar: acid ratio. Relative to the inverse, a greater sugar: acid ratio may enable an earlier harvest, minimize wine tartness of rot-prone whites, and reduce "sharpness" or astringency in red wines.

Figure 5. The same, two Chardonnay clusters photographed from the shaded side (left) and sun-exposed side (right). The shaded grapes in the left photo are likely to be more acidic than the sun-exposed grapes in the right photo.

Due to the variance in fungal disease tolerance and differences in canopy vigor, optimal leaf removal in the southeast can depend on training system and grape cultivar, and likely even between clones (Table 1). European grapes (*Vitis vinifera*) trained on VSP trellis systems can benefit from fruit zone leaf removal when grown in humid regions, while native or hybrid cultivars, like Norton or Villard blanc, grown on high wire systems generally require less fruit zone management for successful production.

How to remove leaves from the fruit zone

Leaves surrounding clusters are commonly removed by hand in eastern US vineyards with modest acreage (< 5-10 acres). Petioles ("leaf stems") are removed from the primary shoot, thereby removing leaf blades as well. Tender and slim lateral shoots produced from nodes surrounding the fruit zone can also be removed by hand if foliage is removed between the "bloom" through "BB-size berries" growth stages. However, hand shears will be required to remove the thicker, lignified lateral shoots that are present near the "bunch closure" growth stage. Mechanical leaf removal machines are becoming popular in the eastern US (Figure 6). While the cost of such equipment can exceed $15,000, their purchase can offset labor costs spent on manual canopy management. Recent economic insight into manual vs. mechanical leaf removal showed that mechanical leaf removal can eventual result in cost savings when compared to manual leaf removal (Table 2). Cost savings with mechanical leaf removal are predicted to be realized earlier with increased vineyard acerage. For example, it is predicted that cost savings with mechanized leaf removal would be attained in year three in a 15-acre vineyard while it would take several years to realize cost savings in a 5-acre vineyard (Table 2).

Perhaps as importantly as cost savings, the strategic use of a mechanized leaf remover allows *timely* leaf removal, especially across large vineyard acreages (> 20 acres), and can aid in pesticide spray penetration during critical periods for cluster disease management (bloom through bunch closure). An experienced labor crew of two people could remove leaves and apply spray zone-targeted pesticides over approximately 6 acres of vineyard per day with the simultaneous use of two tractors (one leaf removal, one pesticide application). Thus, in situations of labor scarcity, mechanical leaf removal machines can offer an efficient solution to effectively implement fruit zone management over large acreages at a targeted growth stage. Investment in mechanical leaf removers shows industry acknowledgement that fruit zone management is an important tool to manage grape disease and quality (Table 3). However, some vineyards may not be suitable for mechanical leaf remover use. Mechanical leaf removal is most effective in training systems with defined fruit zones (e.g. VSP systems). Slope of the vineyard, ground cover, and tractor operator skills are limiting factors for the use of a mechanical leaf removal machine. For example, mechanical leaf removal will increase in difficulty in vineyards with frequent topography changes and on highly sloped sites (e.g. > 15 to 20°). Note also that some mechanical leaf removal machines use "rollers" to pull and cut foliage while others use pulses of compressed air to remove or shred foliage. To limit cluster damage, early-season leaf removal (early fruit set and before) may be best accomplished with air pulse machines; the pulses of compressed air produced by these machines may aid in the removal of floral tissue debris which could limit fungal disease prevalence, but these air pulses may also reduce fruit set during bloom if the result is reduced pollen availability. "Cutting" machines are best used after berries have enlarged (around "pea-size berries" stage) and clusters hang down in the canopy with the aid of gravity.

Table 2. Manual and mechanical fruit-zone leaf removal: Approximate costs and time savings per acre[a].

	Manual leaf removal	Mechanical leaf removal	Potential Savings
Variable cost per acre[a]	$485	$38.30	**$446.7**
Variable cost per ton[b]	$173.2	$13.7	**$159.5**
ONE-TIME COSTS	*$0*	*$20,000*	
	5-acre vineyard[c]		
Year 1	$2,425	$20,191.5	**$-17,766.5**
Year 2	$2,497.75	$197.25	**$-15,466**
Year 3	$2,572.7	$203.16	**$-13,096.4**
	15-acre vineyard[c]		
Year 1	$7,275	$20,574.5	**$-13,299.5**
Year 2	$7,493.25	$591.7	**$-6,397.5**
Year 3	$7,718	$609.5	**$711**
	30-acre vineyard[c]		
Year 1	$14,550	$21,149	**$-6,599**
Year 2	$14,986.5	$1,183.47	**$7,204.03**
Year 3	$15,436	$1,219	**$14,217**

[a]Costs are based on the 2018 costs of a 30-acre commercial vineyard in Western North Carolina. Manual labor at $12.50/hour + benefits. Mechanical costs include tractor labor ($17/hour + benefits), fuel and maintenance. It does not include depreciation of the mechanical leaf remover, but only reflects variable costs per acre.
[b]Total cost per ton of wine grapes, calculated on an average of 2.8t/acre production over a 30-acre vineyard with different cultivars.
[c]Hypothetical costs for a vineyard of different sizes. We assume that costs will increase at a 3% rate every year, based on inflation rate and salary adjustments

Table 3. Manual and mechanical fruit-zone leaf removal effect on *Botrytis* bunch rot incidence and severity in Chardonnay and total grape phenolics and anthocyanins in Merlot in North Carolina in 2018.

Chardonnay[a]	***Botrytis* incidence (%)**[b]	***Botrytis* severity (%)**[b]
NO	32.0	4.0
PFS4	15.2	0.6
PFS6	17.6	0.9
MECH	16.0	0.5
Merlot[a]	**Phenolics (au/g berry)**	**Anthocyanins (mg/g berry)**
NO	68	0.35
PFS4	79	0.38
PFS6	80	0.37
MECH	74	0.35

[a]Treatment = no leaf removal (NO); removal of four leaves after fruit set (PFS4); removal of six leaves after fruit set (PFS6); mechanical leaf removal (MECH). Chardonnay data adapted from Hickey et al. 2019.
[b]Incidence = visual inspection of the infection of one berry or more per cluster; severity = visual inspection of percent damage per cluster.

Figure 6. Tractor-mounted, mechanical leaf removal machines are becoming popular in large acreage (> 15 to 20 acres), eastern US vineyards (top photos). Such trends are indicative of the value industry places on fruit zone management as a tool to manage grape rot and wine quality potential. Mechanical leaf removal (bottom, left) relative to manual removal of six basal shoot leaves and laterals (bottom, right). Note: neither UGA nor the authors endorse the equipment in the photos.

When to remove leaves from the fruit zone

Standard protocol is to remove leaves in the post-fruit set period — a rather general timeframe. Perhaps this general recommendation is acknowledgement that leaf removal is a labor- and time-intensive practice that takes several weeks (and across several vine growth stages) to be implemented by hand over large vineyard acreages. Leaf removal to zero fruit zone leaf layers immediately after fruit-set (e.g. BB-size berries; Figure 7) has been shown to maintain crop yield, reduce bunch rot (Hickey and Wolf 2018; Hickey et al. 2018b; Hed and Centinari 2018;

Hed et al. 2015) and improve or maintain grape berry phenolics and anthocyanins (Hickey et al. 2018a; Hickey and Wolf 2018). Implementing leaf removal immediately after fruit-set will improve fungicide spray coverage on clusters throughout most of the critical period for early season cluster disease control (bloom through bunch closure). Recent research has evaluated the effect of pre-bloom leaf removal on crop quantity and quality. It is judicious to wait until roughly 10 or more leaves have unfolded before removing leaves before bloom (Figure 7); earlier implementation can damage the extremely tender shoots.

There is perceived value of mechanical over hand leaf removal regarding the precision of leaf removal timing. In its efficiency, mechanical leaf removal offers the ability to implement leaf removal within a specific growth stage, as opposed to across several growth stages. Grape sunburn was infrequently observed when leaf removal was implemented early in grape development (e.g. fruit-set through BB-sized berries) in Virginia, North Carolina, and Georgia. If fruit zone leaf removal is delayed until around pea- or marble-sized berries/or bunch closure, there may be a greater chance for sunburn to occur on the outside-facing grapes of a fully-exposed cluster. Sunburn has been observed more frequently on white-berried as opposed to red-berried cultivars. However, leaf removal several weeks after fruit set still may aid in rot control and fruit quality. Thus, it is not a “lost cause” to implement remedial fruit zone management if the busy start to the season has prevented fruit zone leaf removal from occurring between fruit-set and “BB-sized berries” stages.

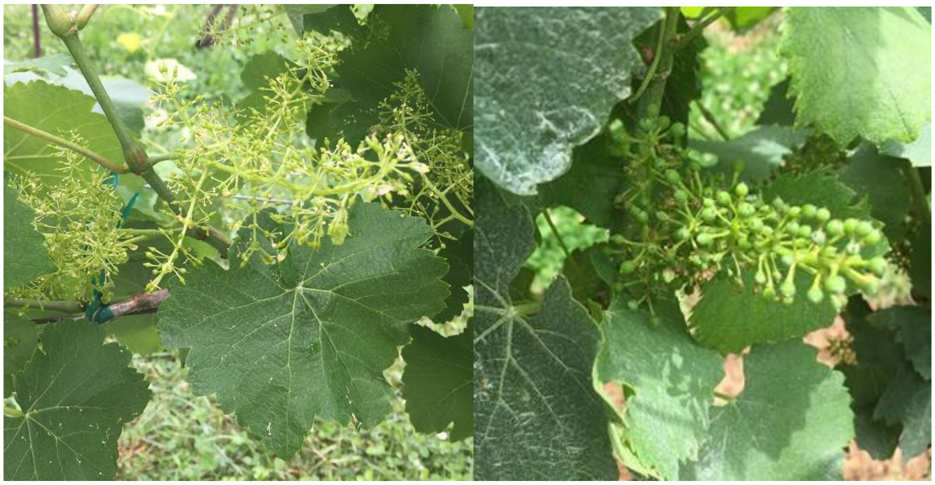

Figure 7. Grapevine growth stage when leaves would be removed before bloom (left) and after fruit-set (right). Pre-bloom leaf removal occurs at when single flowers are well separated. Post-fruit set leaf removal occurs when berries are BB-sized.

Figure 8. Pre-bloom removal of four (left), eight (center) leaves and laterals and post-fruit set removal of six leaves (right). Estimated crop yield weight per acre from those treatments are 2.12 , 1.17, and 3.52 tons, respectively.

How many leaves to remove from the fruit zone

The number of fruit zone leaves removed will depend on the amount of labor and time budgeted for fruit zone management, this is dictated by: (1) the perceived positive effects of leaf removal; (2) the acreage over which leaf removal will be implemented; and (3) the cultivars that are grown. In many regions, leaf removal efforts are primarily focused on the "morning-side" canopy (e.g. the east canopy side in north/south-oriented rows). Such practice is an attempt to avoid excessive radiant heating of grapes in the afternoon, which has been shown to reduce anthocyanins in the western US (Bergqvist et al. 2001; Spayd et al. 2002; Tarara et al. 2008). Climate greatly determines grape temperature patterns and the hours above critical berry temperature thresholds for grape anthocyanin accumulation, which is approximately 30 to 35℃ (Spayd et al. 2002; Tarara et al. 2008). In some US regions, radiation is persistent throughout the day while afternoon cloud coverage can reduce radiant heating of grapes in humid regions like Virginia (Hickey and Wolf 2018) and likely other parts of the eastern US. In regions where cloud coverage is typical, removing leaves from both sides of the canopy may increase airflow and spray penetration without reducing anthocyanin accumulation or causing sunscald (particularly when leaves are removed around bloom or BB-size berries so berries develop and acclimate to ambient radiation conditions) (Table 4). In the eastern US, extensive fruit zone leaf removal on both sides of the canopy can improve primary chemistry and increase or maintain phenolics and anthocyanins (Table 4), thus improving wine quality potential of *V. vinifera*, in comparison to no leaf removal (Frioni et al. 2017; Hickey et al. 2018a; Hickey and Wolf 2018). Removal of leaves exclusively on the vine canopy's morning side may not necessarily be the best management practice in humid regions where fungal disease control is of great importance and radiant heating is diminished compared to the western US.

Table 4. Fruit-zone leaf removal effect on Cabernet franc Brix: titratable acidity (TA) ratio and canopy-side specific total grape phenolics and anthocyanins in North Carolina, and Georgia in 2017.

		East canopy side		West canopy side	
Treatment[a]	**Brix: TA ratio**	**Phenolics (au / g berry)**	**Anthocyanins (mg / g berry)**	**Phenolics (au / g berry)**	**Anthocyanins (mg / g berry)**
			North Carolina		
NO	6.1	87	0.56	82	0.59
PB6	6.6	97	0.61	103	0.66
PFS6	6.1	98	0.59	98	0.65
			Georgia		
NO	3.7	102	0.64	101	0.67
PB6	4.2	135	0.81	131	0.75
PFS6	4.3	132	0.74	114	0.73

[a]Treatment = no leaf removal (NO); removal of six leaves before bloom (PB6); removal of six leaves after fruit set (PFS6)

Leaf removal to zero fruit zone leaf layers around clusters would require removal of approximately four to five basal leaves per shoot. Such effort is unnecessary and would not be commercially feasible. Further, intensive leaf removal before bloom can drastically reduce crop yield, while removal of a similar amount of leaves after fruit set will maintain crop yields (Figure 8). Thus, both timing and magnitude of leaf removal are important considerations for crop management (Figure 8). Leaf thinning to an average of one to two leaf layers has been widely recommended for eastern US growing regions (Reyonlds and Wolf 2008). An average of one to two leaf layers surrounding grape clusters can be achieved by removing approximately two leaves per shoot near clusters. The data in Figure 4, above, shows that *Botrytis* bunch rot is reduced in fruit zones characterized by approximately one and a half leaf layers relative to those with approximately three leaf layers. The practical goal is to find a level of leaf removal that: (1) is not limited by labor nor the number of acres that require leaf removal; (2) improves spray penetration and rot control; and (3) maintains or improves color and flavor compound development. “A little bit goes a long way” is true with leaf removal, meaning that modest fruit zone leaf removal is a good practice to aid in late season spray penetration and sensory compound development, even if implemented several weeks after fruit set.

Prioritizing leaf removal

Priorities for leaf removal are dictated by several factors (Table 5). Cultivars vary in their susceptibility to bunch rots and should therefore dictate where to prioritize fruit zone management. Chardonnay, Sauvignon blanc, Riesling, and Pinot noir are more susceptible to certain rots, and may consequently have greater need for leaf removal, relative to Petit Manseng, Petit Verdot, and Cabernet Sauvignon. Generally, hybrid cultivars have greater disease tolerance than *vinifera* cultivars; relatively modest amounts of fruit zone leaf removal can therefore improve rot management and fruit composition in hybrids. In general, leaf removal to manage rots may be more important in white-berried relative to red-berried cultivars while leaf removal to manage primary and secondary metabolites may be equally beneficial in both white- and red-berried cultivars. Budget and labor may ultimately limit the implementation of fruit zone leaf removal. Thus, if labor is limited, leaf removal priority could be based on cultivar rot susceptibility, which may be (in order of most susceptible to least susceptible): Sauvignon blanc/Riesling/Vignoles/Pinot noir > Chardonnay/Merlot > Cabernet Sauvignon/Petit Verdot > Chambourcin/Chardonel. Table 5 can be used as a general guide for determining leaf removal priority based on cultivar traits, growing and training scenarios, and region. For example - the bottom row cosuggests leaf removal is a high priority for a white-berried, *vinifera* cultivar that has compact clusters, thin skins, low rot tolerance, and is grown a in humid climate. There may be other considerations besides cultivar for prioritizing leaf removal, including training system, fruit zone architecture, climate, and targeted price premium (Table 5).

Priority	Species	Berry color	Cluster morphology	Rot tolerance	Grape skin	Training system	Fruit zone architecture	Climate	Price premium
Low	American		Loose	High	Thick	High Wire	Multi-dimensional; spacious	Dry, arid	Lower cost
Moderate	Hybrid	Red	Normal	Medium				Humid, subtropical	Higher cost
High	Vinifera	White	Compact	Low	Thin	VSP	Linear; confined	Humid, continental	Higher cost

Table 5. A generalized and relative prioritization for fruit zone leaf removal based on several factors.

Summary

Fruit zone management may have the most direct impact on fruit quality when considering all canopy management practices. The interaction of cultivar and climate will determine the need for fruit zone leaf removal. Rot-prone cultivars grown in humid environments will necessitate open fruit zones to optimize fungal disease management. Fruit zones with few leaf layers may aid wine quality potential in humid environments characterized by variable cloudiness throughout the ripening period. Extensive pre-bloom leaf removal can decrease crop yield. Post-fruit set leaf removal maintains crop yield but leaves a shorter time frame to complete canopy management in a large vineyard. Investment in a mechanized leaf removal machine could remedy the time and labor constraint that fruit zone leaf removal imposes. Careful consideration of site-specific environment and growing conditions will help to choose the level of leaf removal necessary to target fruit composition as related to winemaking goals. In the eastern US, fruit zone leaf removal benefits may outweigh the cost of time and labor, especially when leaf removal is prioritized by cultivar needs (e.g. disease susceptibility). Leaf removal methods should be chosen accordingly based on vineyard conditions, cultivars, and resources available

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