EFFECT OF VINEYARD MANAGEMENT ON CABERNET SAUVIGNON (*VITIS VINIFERA* L.) GRAPE GLYCOSIDES

by

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Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

in

Food Science and Technology

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September 23, 1996

Blacksburg, Virginia

Key words: glycosides, Cabernet Sauvignon, cluster thinning, canopy management

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ABSTRACT

Quantification of grape glycosides has been suggested as an objective index of grape quality. Two studies were undertaken to observe the influence of vineyard management on grape glycosides. In the first study, three crop levels from mature Cabernet Sauvignon (*Vitis vinifera* L.) vines grown in eastern Virginia were evaluated for their influence on grape glycosides, expressed as red-free glycosyl glucose (GG). Crop levels averaged 3.2, 5.1, and 6.4 kg/vine resulting in leaf area to fruit weight ratios (cm^2/g) of 34.4, 27.0 and 19.2, respectively. Red-free GG was greatest in juice from fruit of the low treatment at four of seven sampling dates, including harvest. Phenolic glycosides were found to comprise as much as half of the total GG value.

In a second study, shoot thinning, mechanical and hand fruit zone leaf removal of mature Cabernet Sauvignon grapevines were evaluated for their influence on red-free GG. Two shoot densities were examined, each with no leaf removal (No LR), mechanical leaf removal (M LR) or mechanical plus hand leaf removal (M+H LR) imposed five weeks post-bloom. Red-free GG was increased by leaf removal of shoot thinned vines and was greatest with mechanical plus hand leaf removal.

ACKNOWLEDGMENTS

In addition to my committee members, I would like to thank The Williamsburg Winery, Ltd. for support of my research work. Special thanks to Eric Capps, Richard Carmichael and Steve Warner for their assistance and support.

I would like to extend further appreciation to Scott Drake and Stephen Rigby for their help with vineyard measurements and grape harvesting and Yauching Jasinski for her assistance with laboratory procedures.

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INTRODUCTION

Potential wine quality is dependent on grape quality. The role of glycosylated secondary metabolites in grape and wine flavor and aroma has been established. The analysis of grape glycosides has been suggested as an objective index of grape quality. The quantification of these glycosides, measured as glycosyl glucose, or GG, has been positively correlated to wine quality.

Conjugated or bound flavor/aroma components consist of volatile flavor and/or aroma precursors bound to a mono- and disaccharide conjugate through a glucose molecule, rendering them nonvolatile and undetectable sensorially. Freeing these bound compounds through acid or enzymatic hydrolysis of the glycosidic bond releases the aglycones, some of which have significant beneficial sensory implications. Thus, increasing the pool of total GG components in the grape may increase potential wine quality.

Vineyard management practices, such as shoot thinning, selective leaf removal, and cluster thinning have been shown to positively influence grape chemistry, resulting in greater wine quality. However, little work has been done on the effects of these practices on the concentration of bound flavor/aroma components in grapes. The purpose of this research was to determine the influence of these specific vineyard management techniques on grape glycoside concentration, measured as `red-free' GG.

SECTION I: LITERATURE REVIEW

A. Grapevine Canopy Microclimate

The growth of shoots and leaves mainly creates the grapevine canopy. The canopy microclimate is defined by the amount and distribution of leaf area and its interaction with above-ground climate: light radiation, temperature, wind speed, humidity and evaporation (Smart, 1985). The growth and composition of grapes, with all other factors equal, are mainly due to effects of these parameters on photosynthetic and source-sink relationships. With the increasing worldwide use of virus-free rootstock, liberal use of fertilizers and pesticides, and improvements in general viticultural practices (*i.e.* soil management and irrigation), excessive vegetative growth, or growth not related to fruit maturation, has contributed to increased canopy densities (Hunter et al., 1995). An excess of interior or shaded leaves in the canopy also affects source-sink relationships within the vine (Percival et al., 1993). Vineyard management techniques for canopy manipulation such as selective leaf removal, shoot thinning and positioning, and trellising have all been shown to be effective at improving grape quality and subsequent wine quality (Bledsoe, et al., 1988, Kliewer, et al., 1988, Reynolds and Wardle, 1989, Hunter et al., 1991). Thus, canopy microclimate greatly influences vine physiology, affecting both fruit composition and wine quality.

Canopy management is defined by Smart et al. (1990) as a deliberate manipulation of the canopy to produce some desired effect. They outlined three principles of canopy

management to specifically improve grape quality. These include: 1) maintenance of healthy, well-exposed leaf area, 2) moderately to well-exposed fruit, and 3) fruit should be the major physiological sink during ripening (Smart et al., 1990).

The photosynthetic efficiency of a leaf is primarily determined by its location within the canopy (Boardman, 1977). Leaves are of different ages and are subject to varying light intensities throughout the growing season influencing photosynthetic rate (Hunter and Visser, 1988b). Reductions of photosynthetic activity occur with excessive vine growth (Smart, 1985, Hunter and Visser, 1988a). Yield, grape composition and wine quality are also adversely affected (Hunter et al., 1995).

Smart (1987) discussed leaf shading and its effects on light quality reaching the canopy interior, or the ratio of red to far red radiation (R:FR). Absorption of light in the photosynthetically active range of 400 to 700 nm is approximately 90 percent, compared to 10% absorption in the near infrared (750-1100 nm) range (Smart et al., 1988). Leaves absorb about 95% of red light (660 nm) and about 21% of far red (730 nm) radiation, resulting in reduced R:FR ratio (light quality) in canopy interiors. The R:FR ratio is important to phytochrome reactions in the plant, key to regulating grape ripening (Smart, 1987). The canopy interior, when compared to the canopy exterior, also demonstrates reduced light quantity, as measured by the photosynthetic photon fluence rate (PPFR) (Smart, 1987).

The structure and density of the grapevine canopy will determine the quality and

quantity of light reaching the fruit zone. In turn, the quality and quantity of light received by the fruit zone affects vine physiology, especially during ripening (Smart, 1987), affecting overall grape quality. Smart et al. (1988) showed shading causes significant alterations in grape chemistry which reduces wine quality. In addition, Smart (1985) reviews several trellising systems and their means of improving canopy microclimate and for better yield and quality. More specifically, Smart and Smith (1988) state that shading causes inferior fruit composition, including increased pH, K, and malate concentrations, and reduced °Brix, anthocyanin, phenol, and flavor compound concentrations. Excessive fruit exposure on the Geneva Double Curtain (GDC) training system did, however, produce undesirable wine aromas (Carbonneau et al., 1978). Thus, the quality and quantity of light transmission, within limits, has been shown to positively affect photosynthetic activity, grape quality, and ultimate wine quality.

Smart (1985) reviewed the advantages of low canopy density for higher quality and quantity of grapes. Reduced cluster and leaf shading typically produces higher quality fruit with increased soluble solids (SS), total phenols and anthocyanins and decreased acidity (TA), pH, and potassium (K) (Jackson and Lombard, 1993). In addition, a lower canopy density also helps to minimize fruit rots such as *Botrytis cinerea* (Gubler et al., 1987, Zoecklein et al., 1992) due to increased windspeed and decreased relative humidity in the fruit zone (Hunter and Visser, 1990).

Selective fruit zone leaf removal has been shown to be an effective means of altering the grapevine microclimate. Zoecklein et al. (1992) found that selective leaf removal increased photosynthetic photon flux density (PPFD), causing reductions in TA due mostly to reduced malate concentration. Kliewer and Bledsoe (1986) reported that timing and severity of leaf removal produced no significant effects on yield, cluster number, cluster weight, and berry weight. Desirable alterations in grape composition, such as increased SS and decreased TA, malic, pH, and K concentrations were enhanced with more severe leaf removal. In a later study, TA, malate, pH, and K concentrations were significantly reduced with leaf removal, with increased severity resulting in further reduced pH and K concentrations (Bledsoe et al., 1988).

Canopy shading produced significant differences in wine aromas, with more varietal character being detected with lower density canopies (Morrison and Noble, 1990). Removal of basal leaves increased monoterpene concentrations in Gewurztraminer (Reynolds and Wardle, 1989) and Riesling (Reynolds et al., 1991). Hunter et al. (1991) reported no significant changes in berry composition or volume with partial canopy defoliation; however, both varietal character and overall wine quality were significantly higher.

Phenylalanine ammonia lyase (PAL) activity, influential in the formation of phenolic compounds important to flavor and color, is responsive to light exposure in Cabernet Sauvignon grapes (Morrison and Noble, 1990). Hunter et al. (1991) reported

that improved light conditions from exposed fruit may play a role in higher pigmentation in Cabernet Sauvignon, with increased anthocyanin concentrations with defoliation of the fruit zone. In a related study (Hunter and Visser, 1990), excessive vegetative shoot growth became a strong sink for photosynthetic products, with other parts (i.e. berries) receiving little photosynthesis. Partial defoliation did not change the accumulation of sugars and acids through the season, but a more favorable source/sink ratio was created, resulting in more efficient photosynthesis (Hunter et al., 1991). Grape color was negatively correlated with canopy shading as well as with yield/vine with Pinot Noir in Australia (Iland and Marquis, 1990).

Shoot thinning of grapevines provides another means for reducing canopy densities and, thus, improving canopy microclimate. Shoot thinning involves removal of shoots on the cordon to some desired shoot density, including vegetative and clustered shoots. Thus, shoot thinning can also serve as a partial alternative to cluster thinning (Reynolds, 1989), a method discussed in a later section. When distance between shoots is small, leaf area overlaps and canopy shading is high (Smart, 1990). Therefore, removal of shoots would decrease effective leaf area and favor greater fruit exposure. Alterations in source-sink relationships of the vine also occur with shoot removal, allowing vines to dedicate more products of photosynthesis to fruit rather than vegetative shoots (decreasing fruit source/sink ratio). In addition to an improved microclimate, Reynolds et al. (1994) cites higher SS, lower TA and pH, enhanced

variety character and minimization of vegetal ("green") flavors with shoot density limited from 15 to 25 shoots/m cordon. Earlier work of Reynolds et al. (1986) with the hybrid Seyval blanc showed an inverse linear relationship of °Brix:acid ratio, pH, and tartrate concentration to shoot density, while malate and TA, however, increased linearly with shoot density. Wine quality was not strongly influenced by shoot density or cluster thinning level.

The degree of canopy shading can be modified by varying the shoot number (shoot thinning), vine vigor, and trellis system employed. The concept of leaf layer number (LLN) as described by Smart (1985) is an attempt to quantify grapevine canopy microclimate. LLN is defined by the following equation:

$$LLN = \gamma \sin\theta/D,$$

where γ = the ratio of leaf area to shoot length, θ = the mean leaf angle to the horizon, and D = the distance between shoots of the canopy. Smart (1985) determined LLN = 3, or two exterior and one interior leaf layer, is sufficient for photosynthetic and PPFR considerations. The introduction of point quadrat analysis (Smart and Smith, 1988) simplifies the calculation of LLN. This involves insertion of a thin rod, simulating a beam of light, through the fruit zone and recording contacts or gaps. The LLN is then simply the total number of leaf contacts divided by the total number of insertions. The ideal LLN is 1.0 or less (Smart and Sharp, 1987). In addition, the percentage of gaps, interior fruit and interior leaves can be calculated. With these

tools, the canopy can be accurately described, facilitating the matching of ideal canopy for optimum fruit and wine quality for the respective training system and microclimate.

B. Grapevine Source/Sink Relationships

Source-sink relationships of grapevines play a major role in grape composition and quality. Ho (1988) defines sink organs in plants as net importers of assimilate. The ability of sink organs to import dry matter is sink strength. Earlier work of Warren-Wilson (1972) defined the sink strength as a product of sink size and sink strength. Ho (1988), however, related sink size as a physical constraint and sink activity as a physiological constraint upon sink organ assimilate import. Thus, with a constant sink strength, reducing the physical constraint of sink size (i.e. crop thinning) allows for an increase in sink activity (i.e. solute accumulation). In addition, the photosynthetic efficiency of leaves increases when the source size (leaf area) is reduced with respect to the sinks (roots, trunk, shoots, fruit) (Hunter and Visser, 1988).

Grape berries are strong sinks, with a four-fold increase in dry mass over six weeks with little accumulation elsewhere in the plant (Conradie, 1980). Most of this dry mass is synthesized in leaves (source), translocated to the berries (sink) and accumulated as solutes in grape juice (Coombe, 1989). In addition, reduction of source size relative to sink increases the photosynthetic efficiency of leaves (Hunter and Visser, 1988b). Berry volume per vine (sink size) limits crop production (Coombe, 1989) and can be

physically adjusted by crop thinning.

C. Grapevine Fruit Yield

The concept of crop yield is straightforward, typically measured by fruit weight per vineyard area (kg/ha). Kliewer and Weaver (1971) used the concept of crop load as leaf area to fruit weight ratio. They concluded the effect of crop levels on fruit and wine quality is not always consistent. Therefore, Bravdo et al. (1984, 1985) suggested crop load as the ratio of crop yield to pruning weights, implying that crop level alone is an insufficient measure for cropping.

Vineyards producing high quality wines tend to have low to moderate yields (Jackson and Lombard, 1993). Also, low yields tend to produce higher quality grapes (high SS and low pH) by increasing the rate of grape maturation (Winkler, 1954). Grape composition is affected by the leaf area/fruit weight ratio and by the rate of maturity (Kliewer and Weaver, 1971). Since the major source of sugars is leaf photosynthesis, the leaf area/fruit weight ratio is critical to berry sugar level (Jackson and Lombard, 1993).

Berry sugar level begins to decline when the leaf area/fruit weight ratio is in the range of 7 to 10 cm^2/g (Kliewer and Weaver, 1971, Smart, 1985, Kaps and Cahoon, 1992). Grapes require 8 to 12 cm^2 of exposed, healthy leaf area to fully ripen one gram of fruit properly (Lakso, 1994). Jackson (1986) confirmed that by increasing the

leaf area/fruit weight ratio above 10 cm^2/g, crop load had little effect on SS though TA continued to decline while pH increased.

Vine vigor is defined essentially as the rate of growth. Capacity refers to the vine's ability to totally produce with respect to growth and crop production. Crop level controlled entirely by thinning and without pruning produced twice as much fruit as normally pruned vines, exhibiting both high vigor and capacity (Winkler et al., 1974). Thinning increased the vigor of de Chaunac vines, measured by annual pruning weights, while producing grapes of superior quality (Fisher et al., 1977).

Yield may be adjusted by regulating bud numbers at pruning, however cluster thinning prior to veraison is effective as well (Jackson and Lombard, 1993). Cluster thinning is the easiest means of reducing crop load on highly productive vines, allowing the remaining fruit to develop and mature correctly (Winkler, 1974). Thinning increases the vine's capacity for growth and helps to properly balance supply and demand for growth metabolites (Winkler, 1930). Cluster thinning changes translocation of photosynthetates (decreases source/sink ratio) to the advantage of the fruit or plant, dependent on timing and amount of clusters removed (Fisher et al., 1977). Timing of cluster thinning either at fruit set or near veraison, however, produced no differences in must quality (Morando et al., 1991). On the other hand, cluster thinning for three consecutive seasons increased bud fertility and raised the yield of thinned vines to that of the unthinned level (Lavezzi et al., 1995).

Jackson and Lombard (1993) concluded that SS will increase after thinning and low fruit loads increase aromatic constituents such as potential volatile terpenes (PVT), possibly enhancing wine quality. Balasubrahmanyam et al. (1979) showed high yielding Riesling vineyards produced grapes with less aroma and flavor constituents. Cluster thinning was shown to markedly increase SS without accelerating acid degradation in a recent Italian study (Amati et al., 1994). Varietal character was enhanced by cluster thinning of Cabernet Sauvignon (Iacono and Scienza, 1995). However, with Cabernet Sauvignon in California, differences in grape compositions and wines were small with three levels of cluster thinning (Ough and Nagaoka, 1984). McCarthy et al. (1987) found that PVT were higher in crop-thinned Riesling vines. Reduction in crop load of Carignane grapes, within limits, increased wine quality (Bravdo et al., 1984). Lower yield vines have been shown to produce higher acetate esters and lower secondary alcohols, and, thus, more intense aromatics (Sinton et al., 1978).

D. Glycosylated Flavor and Aroma Compounds

Flavor is the result of complex reactions of taste and olfactory receptors (Winkler, 1974). Aroma can be defined as the olfactory perception of volatile compounds while outside of the mouth (Acree, 1993). Flavor and aroma compounds are present in trace amounts, but the hypothesis that more can be generated from flavorless, nonvolatile

precursors has existed for 40 years (Hewitt et al., 1956).

The role of glycosylated flavor and aroma compounds in grapes and their effects on overall wine quality is of current interest to the industry. Two general categories of grape flavor compounds exist: free volatiles and sugar conjugates. Free volatiles are responsible for the typical varietal flavor and aroma of the wine. Glycosidic conjugates consist of free volatiles bound to sugar molecules, typically glucose and other mono- and disaccharides (Abbott et al., 1990).

Glycosylation is believed to be the terminal step in the biosynthetic pathway, stripping the fruit of the flavor and aroma components by rendering them nonvolatile. However, conjugation provides a potential source of flavor and aroma following release through acid or enzymatic hydrolysis. Thus, conjugated glycosides represent flavor and aroma precursors (Williams et al., 1989). Some limited hydrolysis occurs during vinification and aging, due to the acid hydrolysis of grape juice and wine, by yeast producing ß-glucosidase, and by the addition of pectinolytic enzymes with high ß-glucosidase content (Williams et al., 1987).

Most of the research on grape glycosides has been with monoterpenes (Strauss et al., 1986). These compounds are associated with floral grape varieties, of which Cabernet Sauvignon and the majority of the world's winemaking grapes are not. However, a similar analytical approach can be utilized for non-floral, non-terpene varieties to determine other compounds responsible for varietal flavor and aroma

(Williams et al., 1989). Research is underway in Australia, in conjunction with Williams' group, to identify bound components in Cabernet Sauvignon juice and skins (Naiker and Cabalda-Crane, 1994). The biochemical mechanisms utilized for monoterpene production are also present in non-terpene varieties. These non-terpene varieties also derive their aromas from free volatiles while the majority of the grape potential is locked up in the form of conjugates.

Abbott et al. (1987) found that high quality juice, as defined by experienced enologists and viticulturists, had a high concentration of free volatiles. Acid hydrosylates of glycosidic precursor fractions, when added back to neutral wines demonstrated aroma sensory significance (Williams et al., 1989).

Abbott et al. (1993) used glycosyl glucose (GG) concentrations as a measure of grape quality. The same study suggested the assumption that flavor develops late in the season is justified, with the GG content increasing 50 percent in the one week when °Brix rose from 20-24°. Also, GG concentration was found to show a direct correlation with volatiles released during enzymatic treatment. Finally, the authors proposed that GG measurements offer the possibility of rating juice samples without the need for subjective sensory evaluation. Quantification of glycosides using the GG procedure was outlined by Williams et al. (1995).

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SECTION II: EFFECT OF CROP LEVEL ON CABERNET SAUVIGNON (*VITIS VINIFERA* L.) GRAPE GLYCOSIDES

ABSTRACT

Quantification of grape glycosides has been suggested as an objective index of grape quality. Three crop levels from mature Cabernet Sauvignon (*Vitis vinifera* L.) vines grown in eastern Virginia were evaluated for their influence on grape glycosides, expressed as red-free glycosyl glucose (GG). Vines were trained to a mid-wire (90 cm) bilateral cordon system and cluster thinned by hand five weeks post-bloom. Crop levels averaged 3.2, 5.1, and 6.4 kg/vine resulting in leaf area to fruit weight ratios (cm^2/g) of 34.4, 27.0 and 19.2, respectively. Cluster thinning influenced fruit zone porosity as measured by the percentage of sunlight penetration and point quadrat analysis. Berry weight was unaffected by treatment, though soluble solids differed between the highest and lowest treatments at each of seven sampling dates. Red-free GG was greatest in juice from fruit of the low treatment (3.2 kg/vine) at four of seven sampling dates, including harvest. Phenolic glycosides were found to comprise as much as half of the total GG value.

INTRODUCTION

Soluble solids, total acidity and pH, traditional indicators of grape maturity, may not be reliable indices of potential wine quality, particularly in warm climates. The analysis of grape aroma and flavor components has been suggested as a possible alternative to `index' potential grape and wine quality (Abbott et al., 1993, Williams et al., 1995). Two general categories of grape flavor and aroma compounds exist: free volatiles and bound conjugates (mainly glycosides). Free volatiles are responsible for the varietal flavor and aroma of wines, while glycosidic conjugates are nonvolatile precursors. Grape glycosides are composed of aliphatic residues, monoterpenes, sesquiterpenes, norisoprenoids and shikimic acid metabolites (Plank and Zent, 1993). These glycosides represent, in part, the potential flavor and aroma of a grape variety. The measure of glycosyl glucose (GG) provides an estimation of the total pool of these bound glycosides in juice and wine.

Vineyards producing high quality wines tend to have low to moderate yields (Jackson and Lombard, 1993). High quality grapes with low pH and high soluble solids (°Brix) are produced with low crop levels due to increased rate of fruit maturation (Winkler, 1954). Cluster thinning of grapevines helps to properly balance supply and demand for growth metabolites (Winkler, 1931). Low fruit yields have been shown to increase potential volatile terpenes (McCarthy et al., 1987, Jackson and Lombard, 1993), intensify other aromatics (Sinton et al., 1978) and enhance varietal

character (Iacono and Scienza, 1995).

Although phenolics are important components of grapes and resultant wines, the impact of phenolic glycosides on flavor and aroma is minimal (Singleton and Noble, 1976). Therefore, an effort was made to quantify the GG fraction containing flavor and aroma constituents, termed `phenolic-free' GG (P. J. Williams, personal communication, 1996).

The purpose of this research was to examine the influence of three crop levels of Cabernet Sauvignon on general fruit chemistry and grape glycosides, expressed as `red-free' GG. In addition, two methods for the quantification of `phenolic-free' GG are examined and discussed, offering further insight into the potential validity of the GG assay as an overall quality index.

MATERIALS AND METHODS

This research was conducted in 1995 using a mature Cabernet Sauvignon (*Vitis vinifera* L.) vineyard in eastern Virginia. Weather data was recorded at a station of the National Climatic Data Center (Williamsburg 2N), approximately 5 km north of the vineyard site. Precipitation during the growing season averages 10 cm per month. The mean temperature of July is 25 °C and the mean maximum temperature is 31 °C. During the 1995 sampling period (30 August to 10 October), precipitation averaged 9 cm per month, the mean temperature was 21 °C and the mean maximum temperature was 27 °C.

Treatments were implemented on vines grafted on cv. SO4 rootstock and planted in 1988. Vines were spaced 2.1 m apart in north-south rows 3.0 m wide and trained to a mid-wire (90 cm) bilateral cordon system. Three foliage catch wires were located 30, 60 and 90 cm above the cordon, with fruit borne in a discrete band just above the cordon. Shoots were hedged once in June, removing 10-12 nodes and a second time in early August, removing 6-7 nodes. Typical yields from this vineyard are about 6.0 kg fruit per vine with an average cluster weight of 0.66 kg.

Disease control programs included applications of downy mildew, powdery mildew and *Botrytis* fungicides. Insecticides were applied to the vineyard and copper sulfate, as a Bordeaux mix, was utilized throughout the growing season.

A. Treatments

Cluster thinning was performed by hand five weeks post-bloom to reduce fruit yields by 25% and 50% in a completely randomized block consisting of three-vine plots of six replications per treatment. The treatments included: 1) low (L), approximately 50% clusters removed, 2) medium (M) approximately 25% clusters removed and 3) high (H), no cluster thinning.

B. Vineyard Measurements

A Li-Cor quantum sensor (Li-Cor,Lincoln, NE 68504) was employed as described by Zoecklein et al. (1992) to measure photosynthetically active radiation (PAR) within and outside the canopy of each treatment replication to quantify fruit zone light penetration. Three canopy interior readings per vine were taken: vertically upright, 45° right of vertical and 45° left of vertical. Those three readings were averaged and divided by a single reading taken above the canopy (ambient) to determine the percentage of available PAR that penetrated the canopy. Measurements were completed between 1000 and 1500 hours on 23 August 1995.

Canopy density was assessed using point quadrat analysis as outlined by Smart and Robinson (1991). A thin probe was inserted horizontally into the fruit zone to simulate a beam of light with contacts being recorded as leaf, stem, fruit or gap/ground. Approximately 20 insertions per vine were made. Percentages of gaps, interior leaves,

and interior clusters were calculated to determine grape vine canopy microclimate differences, if any, among treatments. Point quadrat measurements were completed 24 August 1995.

Leaf area measurements were made using a Li-Cor leaf area meter (Li-Cor, Lincoln, NE 68504) on 10 October 1995. The mean leaf area was used to calculate the leaf area per vine and leaf area per fruit weight ratio.

C. Grape Sampling

Beginning 30 August 1995, 50 berries per treatment replication were collected weekly. Samples were frozen (-25°C) until analysis. At harvest on 3 October 1995, total clusters per vine, total fruit weight per vine and total weight per cluster were determined. Fruit from one vine per treatment replication remained on the vine and was collected 10 October 1995 (post-harvest).

D. Grape Analysis

Individual samples were warmed to 10 °C, weighed and macerated for 15 seconds in a laboratory blender (Waring Products Division, Model 31BL91, New Hartford, CT 06057). Juice was expressed from the skins and pulp by hand in stomacher filter bags (Seward, London, UK) and centrifuged at 27 000 g for 15 minutes. Soluble solids (°Brix) were measured using a temperature-compensating refractometer (American

Optical, Model 10430, Keene, NH), pH with a Fisher model 815 meter and titratable acidity (TA) by titration with NaOH to an endpoint of pH 8.2 (Zoecklein et al., 1995). Anthocyanins were measured spectrophotometrically as described by Iland (1988). Filtered (0.45 μm Acrodiscs, Gelman Sciences, Ann Arbor, MI 48106) samples (100 μL) were incubated from 3 to 4 hours at room temperature with 900 μL 1.0 N HCl in 10 mm disposable cuvettes. Absorbance was measured at 520 nm on a UV-VIS spectrophotometer (Bausch and Lomb, Model Spectronic 21, Rochester, NY).

Glycosyl glucose (GG) was quantified by a modified method of Williams et al. (1995). Glycosidic fractions were isolated using reverse-phase (RP) C-18 absorbent cartridges (Millipore Corp., Milford, MA 01757). Isolates were acid hydrolyzed at 100 °C, cooled to room temperature, and passed through RP C-18 a second time to remove phenolics inhibitory to the enzyme assay. An enzymatic assay of D-glucose (Boehringer Mannheim GmbH, Germany) was employed to quantify glucose released spectrophotometrically. Color GG was determined by quantification of anthocyanins as previously discussed. Since the molar relationship between anthocyanin content and glucose is 1:1, subtraction of the color GG from the total value provides an estimation of the concentration of the non-colored or `red-free' GG expressed as μmol glucose (McCarthy et al., 1996).

Two techniques were utilized to quantify the phenolic fraction of the total GG at commercial harvest (3 October 1995). Quantification of phenolic-free GG consisted of

removal of phenolic compounds from berry homogenate with 10% (w/v) polyvinylpolypyrrolidinone (PVPP). GG values were quantified as outlined above. Phenolic-free GG was determined from PVPP extracted samples and compared to non-PVPP treated controls.

A second method was used to determine the phenolic-free GG concentration. Juice samples were adjusted to pH 10.0 with 2.5 M sodium carbonate buffer as described by Williams (P. J. Williams, personal communication, 1996). Phenolic fractions are ionized at this pH and are not retained on RP C-18 absorbent. GG was quantified as discussed above, and phenolic-free GG compared to total GG controls.

E. Statistical Analysis

Individual vine data were averaged by treatment replicate and treatment means were compared using the least significant difference (LSD) procedures of SAS (SAS Institute, Cary, NC 27511). Significance was tested at the 5 percent level for all data.

RESULTS AND DISCUSSION

A. Canopy Density and Insolation

Canopy point quadrat analysis data is shown in Table 1. No differences were found in canopy gaps, leaf layer number (LLN) and the percentage of interior clusters among treatments. Cluster thinning reduced the percentage of exposed leaves of the low (L) and medium (M) treatments compared to the high (H) treatment. Leaf contacts in the fruit zone increased with removal of clusters. Medium (M) level vines received greater PAR compared to H, although no differences were measured between the L and M and L and H treatments.

Leaf area per vine was not affected by treatment (Table 1). Leaf area per fruit weight (LA/FW) ratios for H, M and L were 34.4, 27.0 and 19.2 cm^2/g, respectively. The LA/FW ratio of the L treatment was significantly greater than that of the H, while LA/FW of the M treatment was not different from that of both L and H. This ratio is critical to berry sugar development (Jackson and Lombard, 1993), yet the effect of yield on fruit and wine quality is unclear (Kliewer and Weaver, 1971). The amount of leaf area required to properly ripen grapes varies according to climate and cultivar (Kliewer and Weaver, 1971). A range of adequate leaf area to fruit weight from 7 cm^2/g for Thompson Seedless (May et al., 1969) to 17 cm^2/g for potted Muscat of Alexandria vines (Winkler, 1930) has been reported. The range in this study, therefore, was deemed ample for proper fruit development. Measured canopy

differences were minimal between treatments.

B. Yield Components

The number of shoots per vine were similar among treatments as a result of shoot thinning to the same level (Table 2). Clusters per vine were reduced in the L treatment, although no difference occurred between M and H. The yields per vine for H, M and L were 6.4, 5.1 and 3.2 kg/vine, respectively. Differences in yield between H and L reflected differences in the number of clusters per vine, as average cluster weight among treatments were not different. Yield difference between the M and H treatments was 20%, while a reduction of 50% was achieved between the H and L treatments.

C. Grape Composition

Berry weight was unaffected by treatment (Figure 1). Berry weight for the L and M treatments peaked on 27 September, while the H treatment crested 3 October, indicating the delayed maturity of the higher crop level. Berry weight declined for L and M beginning October 3 due to increasing maturity and dehydration (berry shriveling). Weight reduction at the end of the sampling period for all treatments was a result of berry shriveling due to dehydration.

Soluble solids (°Brix) content at seven sampling dates is shown in Figure 2.

Degrees Brix of H was less than L at each sampling presumably due to delayed fruit maturity at higher cropping levels, similar to the findings of Winkler (1954) and Bravdo et al. (1984). Additionally, treatment response may be due to low crop levels altering vine photosynthesis, with photoassimalates shifted to fruit sinks to meet carbon demand (Edson et al., 1993, 1995a, 1995b). Increases in secondary metabolites, including those responsible for potential flavor and aroma, may be due to greater fruit maturity and changes in leaf and vine photosynthesis. Reductions in °Brix on 27 September and 10 October were due to dilution resulting from accumulations of 3.8 cm and 7.3 cm of rainfall, respectively, preceding each sampling date. Slight reductions (1%) in °Brix for both the M and H treatments while L increased 2% on 13 September were recorded but were not important. Reductions in sugar per berry (Figure 3) along with weight and °Brix on 10 October (post-harvest) indicate shriveling and sugar export.

The pH of the L and H treatments were different at three of seven sampling dates (Figure 4). Increased crop level depressed pH elevation, attributable to delayed fruit maturity. Titratable acidity (TA) was not affected by treatment at six of seven sampling dates, the exception being 20 September (Figure 5). Studies with Carignane and Cabernet Sauvignon (Bravdo et al., 1984, 1985) showed reduction in TA with increased crop. However, Kliewer and Weaver (1971) reported reduction in TA with reduced crop in Tokay vines with leaf area to fruit weight (LA/FW) ratios between 11

and 12 cm^2/g. Lack of treatment influence on TA in the current study may be due to more than sufficient LA/FW ratios for each treatment, allowing for similar acid levels in each treatment. TA increased 3 October for all treatments and again 10 October for L and M possibly due to berry shriveling which may have contributed to concentration of organic acids and, hence, increased TA.

The analysis of red-free GG removes the impact of the non-aroma/flavor anthocyanidic GG fraction from the total GG, allowing for a more accurate estimation of bound flavor and aroma. Red-free GG was greater in the L than H treatment at five of seven sampling dates (Figure 6). Iland et al. (1996) suggested it may be necessary to reduce crop level by half to produce a significant increase in red-free GG. The GG concentration in the L treatment was greater than in the M crop level at two of seven sampling dates. The higher concentration of bound glycosides in the L treatment may support the concept that low yields produce fruit with more flavor and/or aroma constituents (Sinton et al., 1978, McCarthy et al., 1987). Reductions in crop level have been reported to increase potential volatile terpenes (PVT) (McCarthy, 1986) and may increase other glycosidic flavor and aroma precursors. The sharp rise in red-free GG late in the ripening period supports other evidence that flavor development in grapes occurs late in the growing season (Abbott et al., 1993). Between 27 September and 3 October (commercial harvest) the average increase in °Brix for all treatments was 1.5° (8%) while red-free GG increased by an average 26 percent. The greatest

increase (48%) was noted in the most mature, L fruit.

Red-free GG per gram of berry weight was different between the L and H treatments at four of seven sampling dates (Figure 7). Red-free GG per gram differed between the L and M treatments in one of seven sampling dates. Decreases between 6 September and 20 September for the M and H treatments paralleled reductions in °Brix. The reduction in red-free GG per gram in the H treatment on 20 September may be due to dilution resulting from 3.5 cm rainfall immediately preceding sampling. A summary of the total and red-free GG per gram for each treatment at seven sampling dates is provided in Table 3. Over the sampling period, red-free GG per gram averaged 91%, 91% and 89% of the total GG per gram for the L, M and H treatments, respectively. Therefore, color GG averaged 9%, 9% and 11% of the total GG for L, M and H, respectively, on a per gram of berry weight basis.

Treatments harvested at different dates were compared to reduce the effects of maturity on GG concentration. Grape composition was observed at similar fruit maturity levels (°Brix) (Figure 8). Comparable red-free GG concentrations were measured for the L and H treatments. The delay in maturity which occurred with increased crop supports work of Winkler (1954), Winkler et al. (1974) and Bravdo et al. (1984). Each confirmed that low cropped vines mature fruit earlier as measured by increased °Brix and pH. In the current study, longer maturation was required for the high crop level vines to obtain the same red-free GG as the low cropped vines. Timing

of harvest, therefore, is of critical importance particularly in the warm, humid climate of Virginia, where the incidence of fruit rots may be important (Zoecklein et al., 1992). Crop reduction, therefore, may allow producers to harvest fruit with higher GG before loss of crop due to rot or severe weather.

While phenolic compounds are important color and structural components of wines, their impact on flavor and aroma is minimal (Singleton and Noble, 1976). The phenolic-free GG assay allowed separation of the total GG value into components involved mainly in flavor and aroma. Total and phenolic-free GG for fruit from 3 October are shown in Table 4. Phenolic-free GG comprised 45% of the total GG using the PVPP method. This implies that 55% of grape glycosides do not contribute to potential flavor and aroma. Using the carbonate buffer method, phenolic-free GG constituted 56% of the total GG. Therefore, 44% of the total may be related to phenolic glycosides. Differences in the two assays may be due to the efficiency of the phenolic separation. Similar results were obtained recently with Australian Shiraz, where phenolic glycosides were found to comprise about half of the total (P. J. Williams, personal communication, 1996). Climate, varietal and canopy microclimate differences, however, may play a role in the amount of phenolic and non-phenolic glycosides. Nonetheless, of the total grape glycoside concentration, half may not be related to potential flavor and aroma.

CONCLUSIONS

Crop level was examined for its influence on glycoside concentration and general fruit chemistry of Cabernet Sauvignon grapes from an eastern Virginia vineyard. Yield components were affected mainly by differences in the number of clusters. Glycosides, expressed as red-free GG and red-free GG per gram of berry weight were frequently greatest in fruit from the lowest crop level. Reduction in crop level enhanced the rate of fruit maturity resulting in increases in bound aroma and flavor components. Two methods for quantification of `phenolic-free' GG were evaluated. Phenolic glycosides may comprise as much as half of the total, offering a further refinement of the GG procedure and enhancement of its potential use as an overall fruit quality index.

Glycoconjugate increases due to cluster thinning represent an increase in the total pool of potential aroma components. These products themselves have no immediate flavor or aroma value but, following hydrolysis, can increase aromatic constituents and, hence, overall wine quality. The validity of the GG assay as a quality index and the sensory significance of changes in GG, however, remain unclear. Nonetheless, the GG assay seems to be a valuable contribution which will provide insight into the relationships between vineyard management and wine quality.

Table 1. Canopy descriptor components of Cabernet Sauvignon grapevines cluster thinned to a Low (3.2 kg/vine), Medium (5.1 kg/vine) and High (6.4 kg/vine) crop level in 1995[1].

Canopy descriptors	Relative Crop Level		
	Low	Medium	High
Canopy gaps (%)	1.1a	0.8a	0.3a
Leaf layers	2.7a	2.5a	2.9a
Exposed leaves (%)	61.5a	58.5a	53.2b
Exposed fruit (%)	24.7a	36.2a	29.7a
PAR in fruit zone[2] (%ambient)	7.8ab	9.4a	7.2b
Leaf area per vine (1000 cm^2)	99.0a	104.4a	106.8a
Leaf area per fruit weight (cm^2/g)	34.4a	27.0ab	19.2b

[1]means with the same letter are not significantly different at $P \leq .05$, N=18
[2]measurement, sky conditions, and time of measure:
23 August, sunny and clear, 1000 h to 1500 h EDT.

Table 2. Yield components of Cabernet Sauvignon grapevines cluster thinned to a Low (3.2 kg/vine), Medium (5.1 kg/vine) and High (6.4 kg/vine) crop level in 1995[1].

Parameter	Relative Crop Level		
	Low	Medium	High
Shoots per vine	51.6a	54.4a	55.7a
Clusters per vine	52.5b	68.7a	77.7a
Shoots per meter	27.5ab	26.2b	28.7a
Clusters per shoot	1.0b	1.3a	1.4a
Yield (kg/vine)	3.2b	5.1a	6.4a
Cluster weight (g)	68.7a	69.9a	81.9a
Berry weight (g)	1.6a	1.5a	1.6a

[1]means with the same letter are not significantly different at $P \leq .05$, $N=18$

Table 3. Total and red-free GG per gram of berry weight (μmol glucose/g) of Cabernet Sauvignon grapevines cluster thinned to a Low (L, 3.2 kg/vine), Medium (M, 5.1 kg/vine) and High (H, 6.4 kg/vine) crop level at seven sampling dates in 1995[1].

Sampling Date	Total GG/gram			Red-Free GG/gram		
	L	M	H	L	M	H
8/30/95	6.3a	5.3a	5.7a	5.8a	4.8a	5.1a
9/6/95	6.5a	6.5a	6.4a	6.0a	5.7a	5.6a
9/13/95	6.8a	5.9ab	5.1b	5.9a	5.1ab	4.3b
9/20/95	7.4a	6.4b	4.9c	6.5a	5.6b	4.1c
9/27/95	7.8a	7.8a	7.0a	7.2a	7.2a	6.4a
10/3/95	11.8a	10.1a	7.4b	11.1a	9.6a	6.7a
10/10/95	13.4a	14.2a	10.7b	12.0a	12.9a	9.9b

[1]means with the same letter are not significantly different at $P \leq .05$, N=18

Table 4. Total and phenolic-free GG concentration (μmol glucose) in Cabernet Sauvignon juice from 3 October (harvest) 1995. Grapevines were cluster thinned to a Low (3.2 kg/vine), Medium (5.1 kg/vine) and High (6.4 kg/vine) crop level. Two methods of phenolic glycoside extraction (PVPP and carbonate buffer) are compared.

Relative Crop Level	Total GG		Phenolic-Free GG		% of Total GG	
	PVPP	Carbonate Buffer	PVPP	Carbonate Buffer	PVPP	Carbonate Buffer
Low	2026	1844	885	900	44	49
Medium	1668	1605	858	1060	53	66
High	1812	1730	702	908	39	52

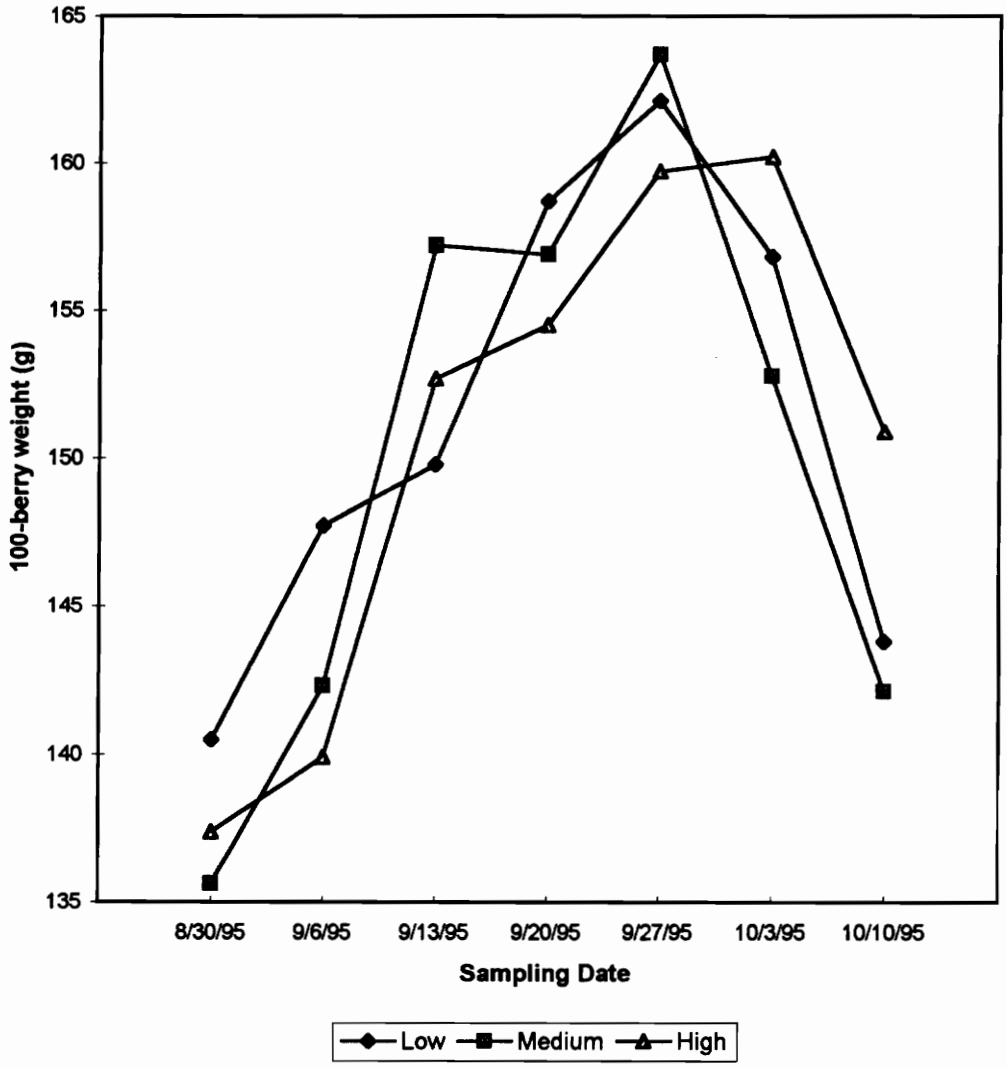

Figure 1. 100-berry weight (g) of Cabernet Sauvignon grapevines cluster thinned to a Low (3.2 kg/vine), Medium (5.1 kg/vine) and High (6.4 kg/vine) crop level at seven sampling dates in 1995. No significant differences ($P \leq .05$) were measured between treatment means at each sampling date.

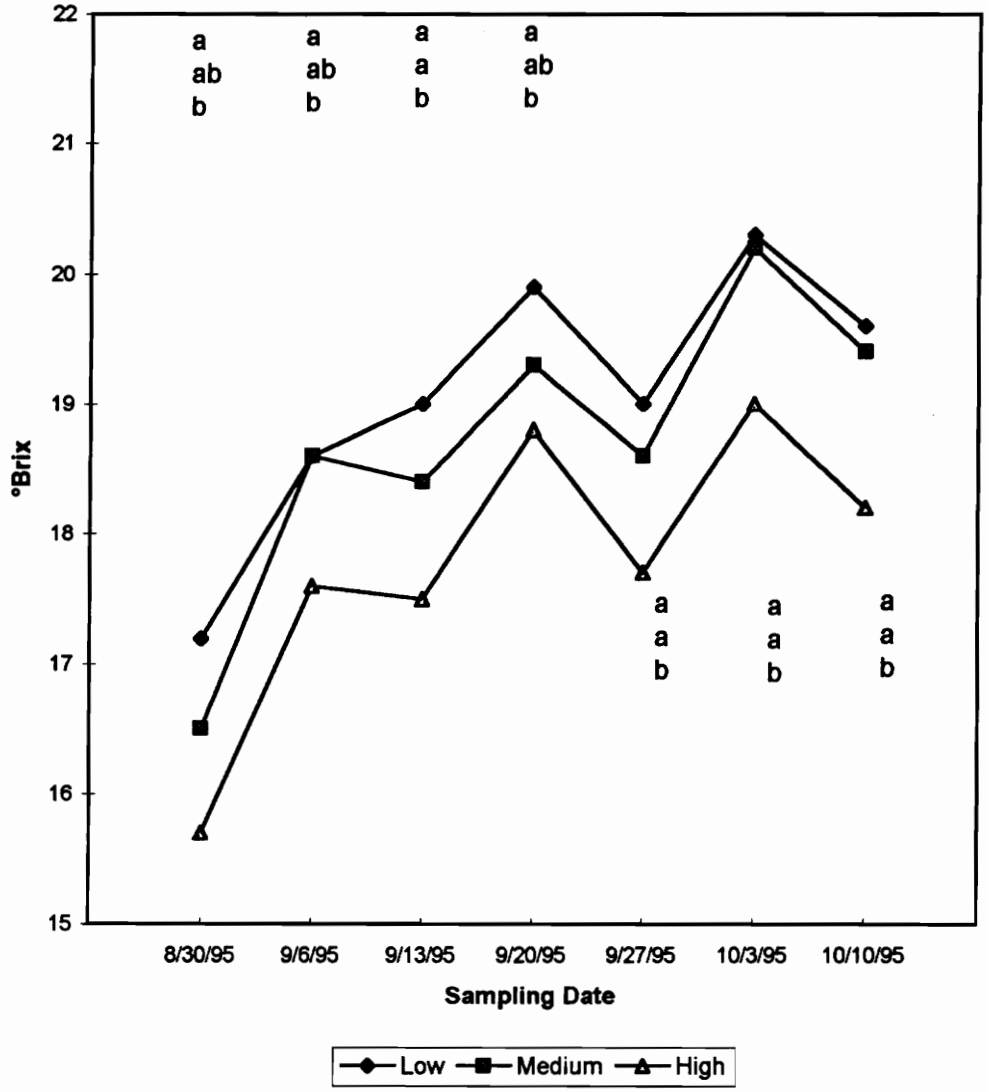

Figure 2. Soluble solids (°Brix) of Cabernet Sauvignon grapevines cluster thinned to a Low (3.2 kg/vine), Medium (5.1 kg/vine) and High (6.4 kg/vine) crop level at seven sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

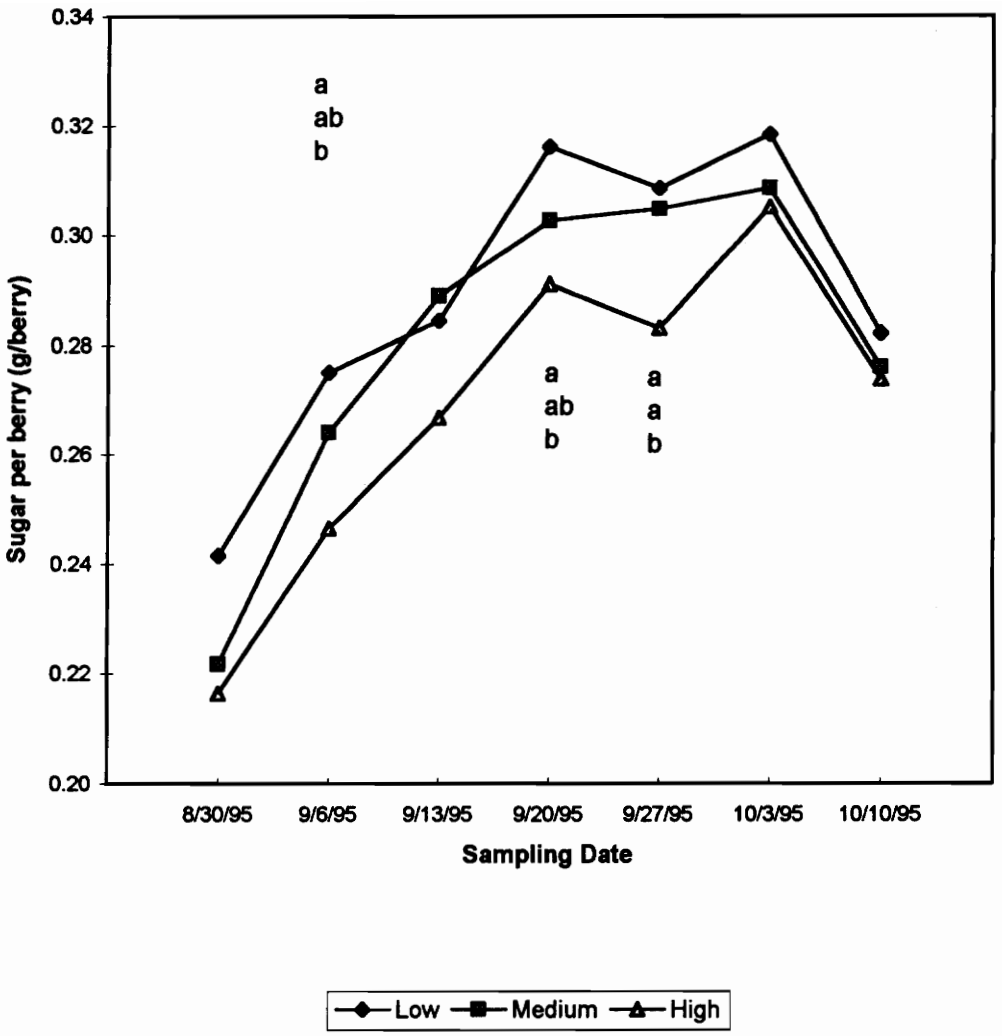

Figure 3. Sugar per berry (g/berry) of Cabernet Sauvignon grapevines cluster thinned to a Low (3.2 kg/vine), Medium (5.1 kg/vine) and High (6.4 kg/vine) crop level at seven sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

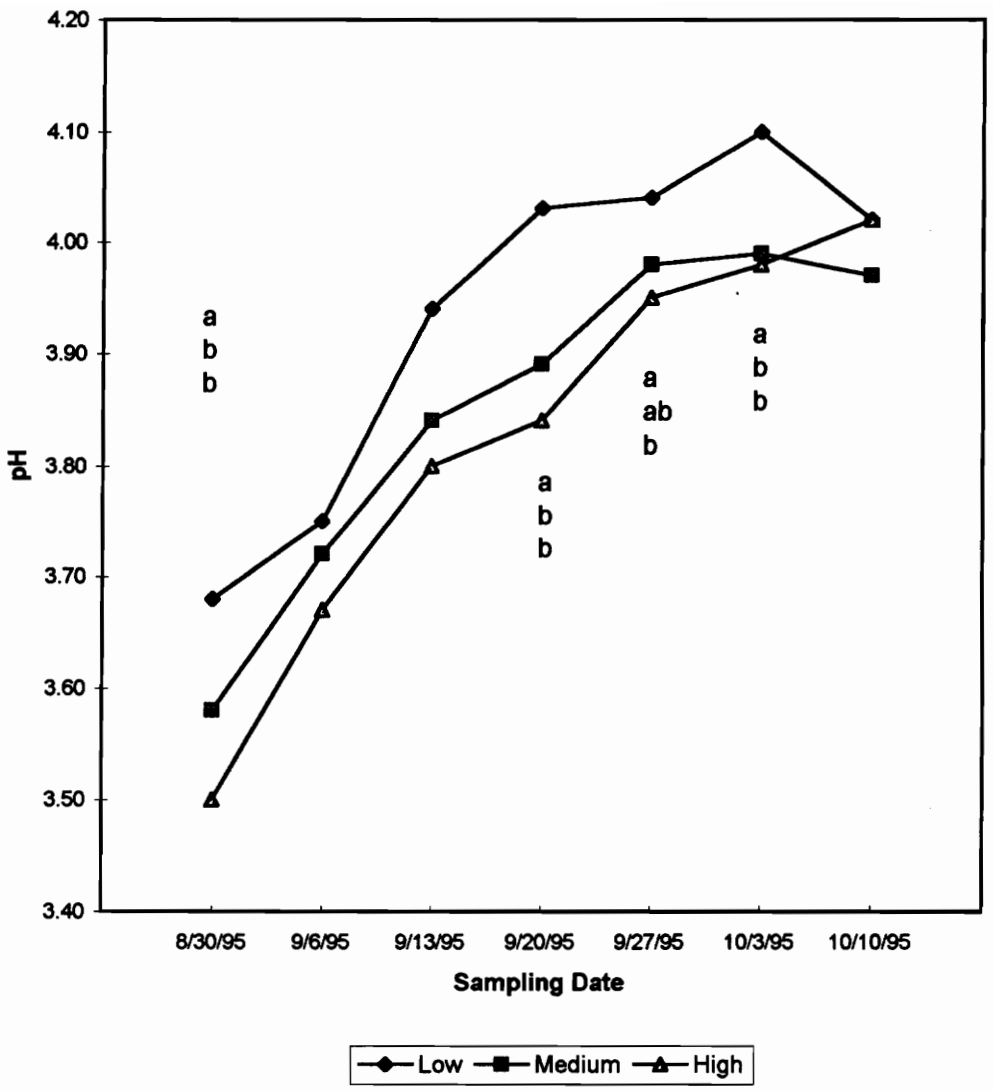

Figure 4. pH of Cabernet Sauvignon grapevines cluster thinned to a Low (3.2 kg/vine), Medium (5.1 kg/vine) and High (6.4 kg/vine) crop level at seven sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, N=18. Unlabelled points have no significant differences between treatment means.

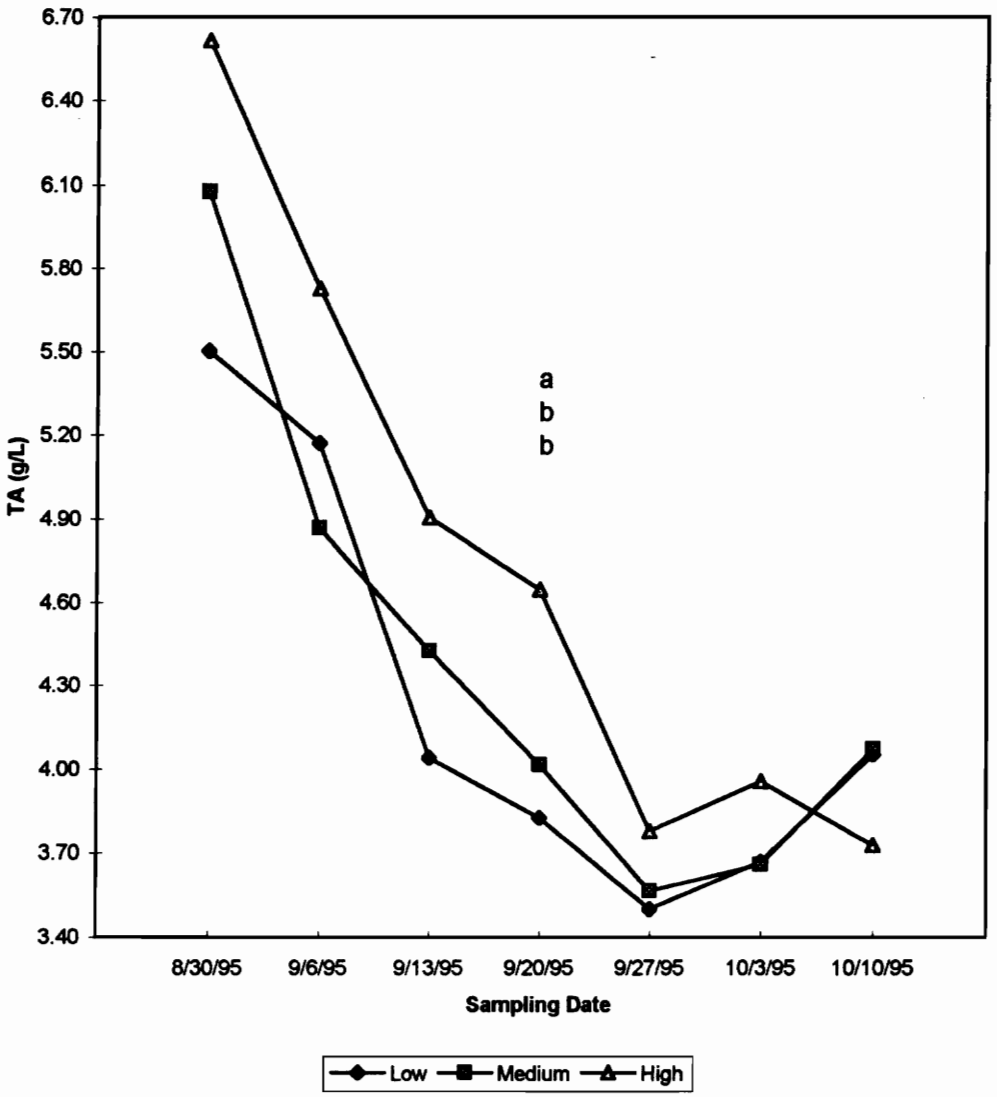

Figure 5. Titratable acidity (TA) of Cabernet Sauvignon grapevines cluster thinned to a Low (3.2 kg/vine), Medium (5.1 kg/vine) and High (6.4 kg/vine) crop level at seven sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

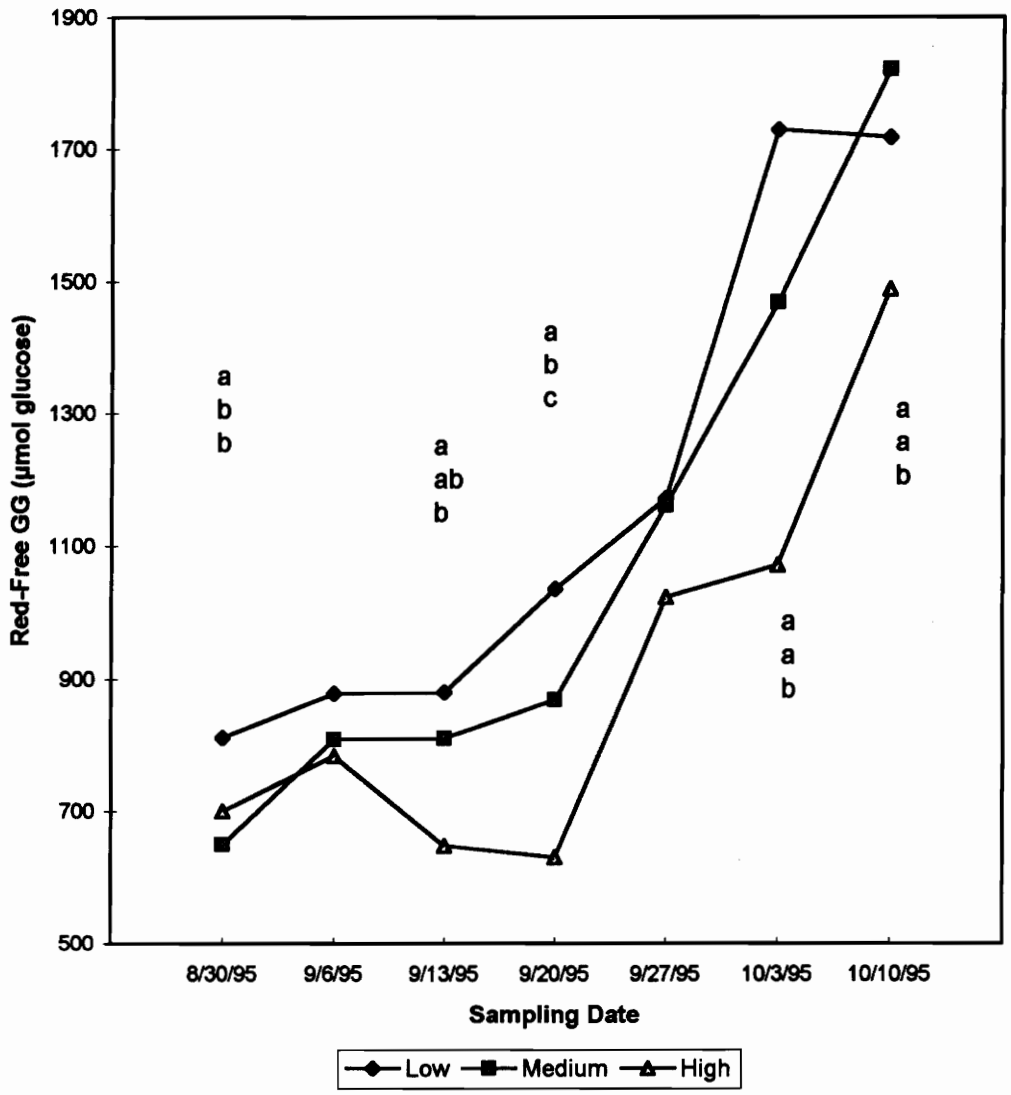

Figure 6. Red-free glycosyl glucose (GG) concentration (μmol glucose) of Cabernet Sauvignon grapevines cluster thinned to a Low (3.2 kg/vine), Medium (5.1 kg/vine) and High (6.4 kg/vine) crop level at seven sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

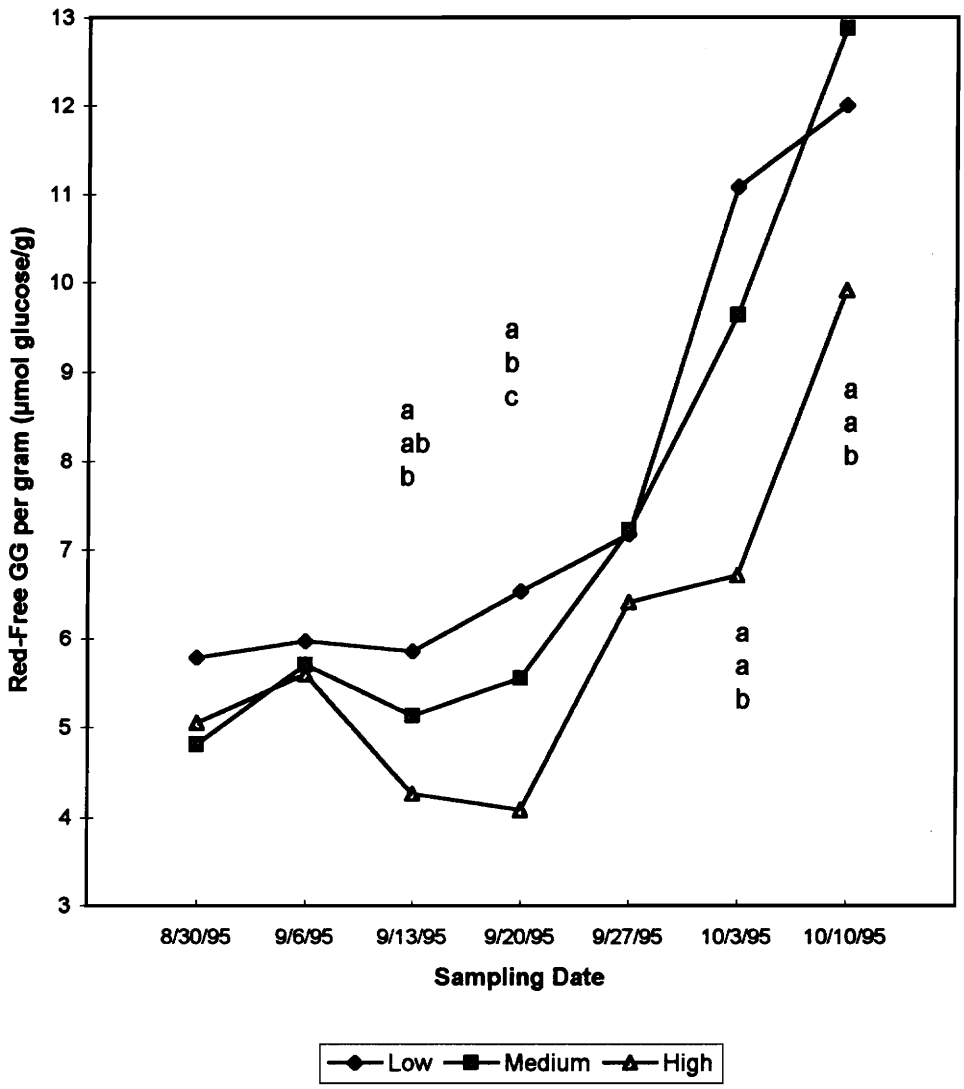

Figure 7. Red-free glycosyl glucose (GG) per gram berry weight (μmol glucose/g) of Cabernet Sauvignon grapevines cluster thinned to a Low (3.2 kg/vine), Medium (5.1 kg/vine) and High (6.4 kg/vine) crop level at seven sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, N=18. Unlabelled points have no significant differences between treatment means.

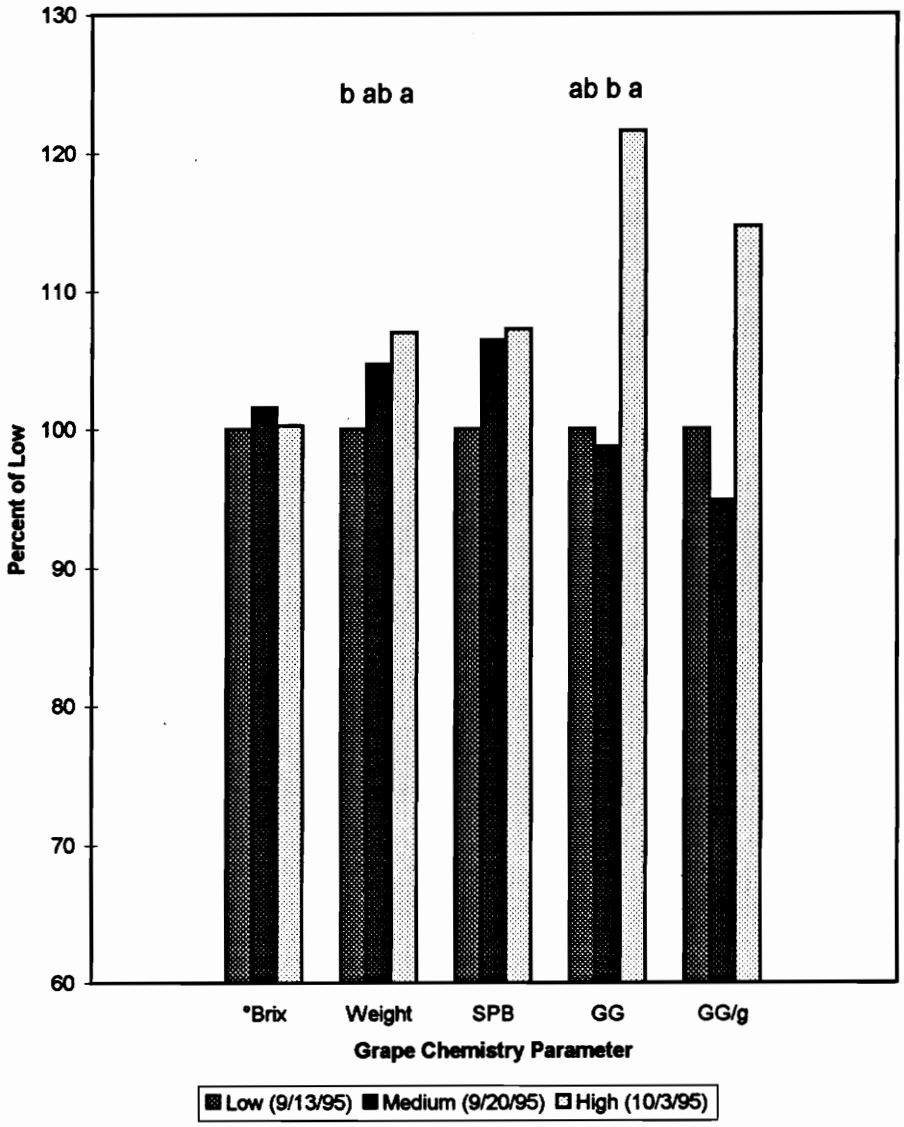

Figure 8. Comparison of grape composition parameters of Cabernet Sauvignon fruit harvested at similar maturity (equal °Brix). Grapevines were cluster thinned to a Low (3.2 kg/vine), Medium (5.1 kg/vine) and High (6.4 kg/vine) crop level and compared at three different sampling dates in 1995. Data is presented on a relative scale, with the Low treatment values assigned 100%. Letters indicate significant difference at $P \leq .05$, $N=18$.

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SECTION III: EFFECT OF FRUIT ZONE LEAF REMOVAL ON SHOOT THINNED AND NON-SHOOT THINNED CABERNET SAUVIGNON (*VITIS VINIFERA* L.) GRAPE GLYCOSIDES

ABSTRACT

The analysis of glycosides has been suggested as an objective index of grape quality. Mechanical and hand fruit zone leaf removal of mature Cabernet Sauvignon grapevines were evaluated for their influence on grape glycosides expressed as 'red-free' glycosyl glucose (GG). Two shoot densities were examined, each with no leaf removal (No LR), mechanical leaf removal (M LR) or mechanical plus hand leaf removal (M+H LR) imposed five weeks post-bloom. Shoot thinning reduced leaf area per vine by 50% and fruit yields by 25% compared to non-shoot thinned vines while canopy insolation was increased almost ten-fold. The M LR and M+H LR treatments increased fruit zone porosity among non-shoot thinned vines measured by a decrease in leaf layer number (LLN) and an increase in exposed fruit. The percentage of sunlight penetration was unaffected by leaf removal. Red-free GG was increased by leaf removal among shoot thinned vines and was greatest in the most exposed treatment, mechanical plus hand leaf removal.

INTRODUCTION

The use of virus-free rootstock, fertilizers, pesticides and improved general viticultural practices (i.e. soil management and irrigation), has contributed to excessive vegetative growth resulting in increased canopy densities (Hunter et al., 1995). The grapevine canopy microclimate is defined by the amount and distribution of leaf area and its interaction with above-ground climate: light radiation, temperature, wind speed, humidity and evaporation (Smart, 1985). The composition of grapes, with all other factors equal, is mainly due to the effects of these parameters on photosynthetic and source-sink relationships. Sink organs in plants are net importers of assimilates (Ho, 1988). The interactions that describe changes in accumulation of dry matter from the source (leaves) to the sinks (roots, trunk, shoots, and fruit) are termed "source-sink" relationships. Canopy microclimate and source-sink relationships are adversely affected by excessive vegetative growth and dense canopies (Smart, 1985, Hunter and Visser, 1988a, Percival et al., 1993). Reductions of photosynthetic activity occur with excessive vine growth (Smart, 1985, Hunter and Visser, 1988a, Hunter and Visser, 1988b), diminishing grape quality and wine quality (Smart et al., 1990, Hunter et al., 1991a, Hunter et al., 1991b). Minimizing vegetative growth can help both sources and sinks function to full capacity (Hunter et al., 1995). Therefore, producers are concerned with minimizing excessive vine growth to produce quality grapes.

Vineyard management techniques that reduce excessive canopy have been

reported to improve grape quality and potential wine quality, but not consistently. Selective fruit zone leaf removal has been shown to enhance grape and wine quality (Smart, 1985, Kliewer and Bledsoe, 1986, Hunter and Visser, 1988b, Hunter and Visser, 1989, Hunter and Visser, 1990, Smart et al., 1990, Hunter, et al., 1991a, Hunter et al., 1991b, Zoecklein et al., 1992). However, despite the widespread use of selective leaf removal, not all studies have reported improvements in grape composition or wine quality.

Shoot thinning is an effective means of reducing grapevine canopy density (Smart, 1985) while favorably altering source-sink ratios (Reynolds, 1989). Reynolds et al. (1986) demonstrated that grape quality, as measured by °Brix to acid ratio, pH, and tartrate concentration, correlated inversely with shoot density. Wine quality, however, was not strongly influenced by shoot density, although enhanced varietal character and minimization of vegetal (green) flavors was noted with shoot density limited to 15-25 shoots per meter (Reynolds et al., 1994).

Traditional measures of grape maturity such as °Brix, acidity and pH may not be reliable indicators of potential wine quality, particularly in warm climates. Quantification of grape glycosides has been suggested as a possible alternative to 'index' grape and potential wine quality (Abbott et al., 1993, Williams et al., 1995). Grape aroma compounds are important contributors to wine quality. They are present as free volatiles, which may contribute directly to odor or as bound conjugates (mainly glycosides), which are nonvolatile precursors. Grape glycosides are

composed of aliphatic residues, monoterpenes, sesquiterpenes, norisoprenoids and shikimic acid metabolites (Plank and Zent, 1993). These represent, in part, the potential flavor and aroma of a grape variety.

The measure of glycosyl glucose (GG) provides an estimation of the total pool of bound glycosides in a juice or wine (Williams et al., 1995). Anthocyanins, responsible for red color, are glycoconjugates included in the analysis of total GG. Because the molar relationship between anthocyanin and glucose content is 1:1, subtraction of the color GG from the total GG provides an estimation of non-colored or 'red-free' GG (McCarthy et al., 1996). Phenolic glycosides do not contribute directly to flavor and aroma, therefore, red-free GG is a better measure of potential flavor and aroma compounds.

The purpose of this research was to examine the influence of selective leaf removal and shoot thinning on general fruit chemistry and the total pool of bound Cabernet Sauvignon (*Vitis vinifera* L.) glycosides, expressed as 'red-free' GG.

MATERIALS AND METHODS

Research was conducted on mature (planted in 1987) Cabernet Sauvignon (*Vitis vinifera* L.) vines grown in eastern Virginia grafted on cv. SO4 rootstock. Vines were spaced 2.4 m apart in north-south rows 3.0 m wide and trained to a bilateral cordon system with the cordon wire 90 cm above ground. Three foliage catch wires were located 30, 60 and 90 cm above the cordon wire. Fruit was borne in a discrete band just above the cordon. Shoots were hedged once in June, removing 10-12 nodes and a second time in early August, removing 6-7 nodes.

Precipitation in eastern Virginia averages about 10 cm per month, the mean temperature for July is 25 °C with a mean maximum temperature of 31 °C. During the 1995 sampling period (4 September to 2 October), precipitation averaged about 9 cm per month. The mean temperature was 21 °C and the mean maximum temperature was 27 °C.

Disease control programs included applications of fungicides to control powdery and downy mildew and *Botrytis*. Insecticides were applied while copper sulfate, as a Bordeaux mix, was also utilized.

A. Treatments

Treatments were arranged in a randomized design consisting of 6 replications of three-vine plots. Shoot thinning consisted of the following: 1) non-shoot thinned which resulted in a mean value of 25.4 shoots per cordon meter (SPM) and 2) shoot-

thinned (ST) to a mean of 14.0 SPM. Three leaf pulling treatments were arranged in a randomized design within the non-shoot thinned and shoot thinned vines. Leaf removal treatments included: 1) no leaf removal (No LR), 2) mechanical leaf removal (M LR), and 3) mechanical and hand leaf removal (M+H LR). Shoot thinning, mechanical leaf removal and mechanical plus hand leaf removal were completed five weeks post-bloom. Mechanical leaf removal was conducted using a tractor-mounted mechanical leaf removal unit (Gallagher Engineering, Ltd., Model 90LR400A, Hamilton, NZ).

B. Canopy Density and Insolation

Point quadrat analysis was performed to characterize canopy density as outlined by Smart and Robinson (1991). This entailed insertion of a thin probe horizontally into the fruit zone to simulate a beam of light with contacts recorded as leaf, stem, fruit or gap/ground. Approximately 20 probes per vine were made. Percentages of canopy gaps, leaf layer number (LLN), interior leaves and exposed clusters were calculated to determine grape vine canopy microclimate differences among treatments. Point quadrat measurements were conducted 23 August 1995.

Photosynthetically active radiation (PAR) was measured within and outside the canopy of each treatment replication using a Li-Cor quantum sensor (Li-Cor, Lincoln, NE 68504) as described by Zoecklein et al. (1992) to provide a measure of fruit zone light penetration. Three canopy interior readings per vine (vertical, 45° right of

vertical, and 45° left of vertical) were averaged and divided by a single reading taken above the canopy (ambient) to determine the percentage of available PAR that penetrated the canopy. Measurements were completed between 1000 and 1500 hours on 23 August 1995.

Leaf area measurements were made using a Li-Cor leaf area meter (Li-Cor, Lincoln, NE 68504) on 11 October 1995. The mean leaf area per shoot was used to calculate the leaf area per vine and leaf area per fruit weight ratio.

C. Grape Sampling

Beginning 4 September 1995, 50 berries per treatment replication were collected weekly. Samples were maintained at -25 °C until analysis. At harvest (2 October 1995) total clusters per vine, total fruit weight per vine, and total weight per cluster were determined.

D. Grape Composition

Frozen samples were warmed to 10 °C, weighed and macerated for 15 seconds in a laboratory blender (Waring Products Division, Model 31BL91, New Hartford, CT 06057). Juice was expressed from the skins and pulp by hand in stomacher filter bags (Seward, London, UK) and centrifuged at 15 000 RPM for 15 minutes. Soluble solids (°Brix) were measured using an American Optical model 10419 temperature-compensating refractometer, pH with a Fisher model 815 meter, and titratable acidity

(TA) by titration with NaOH to an endpoint of pH 8.2 as described by Zoecklein et al. (1995). Anthocyanins were measured spectrophotometrically as described by Iland (1988) and involved incubating clear samples (100 μL) 3 to 4 hours at room temperature with 900 μL 1.0 N HCl in 10 mm disposable cuvettes. Absorbance was measured at 520 nm using a Bausch & Lomb model Spectronic 21.

Glycosyl glucose (GG) was determined by a modified method of Williams et al. (1995). Glycosidic fractions were isolated using reverse-phase (RP) C-18 extraction cartridges (Millipore Corp., Milford, MA 01757). Isolates were acid hydrolyzed at 100 °C, cooled to room temperature, and passed through RP C-18 a second time to remove phenolics. An enzymatic assay of D-glucose (Boehringer Mannheim GmbH, Germany) was employed to quantify glucose released spectrophotometrically. Since the molar relationship between anthocyanin content and glucose is 1:1, subtraction of the color GG from the total value provided an estimation of the concentration of the non-colored or 'red-free' GG expressed as μmol glucose (McCarthy et al., 1996).

E. Statistical Analysis

Individual vine data were averaged by treatment replicate and treatment means were compared using the least significant difference procedures of SAS (SAS Institute, Cary, NC 27511).

RESULTS AND DISCUSSION

A. Canopy Density and Insolation

1. Non-Shoot Thinned Vines

Canopy point quadrat analysis of non-shoot thinned vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) are shown in Table 1. Three of four canopy density measures were influenced by treatment. The M+H LR treatment increased the percentage of canopy gaps, although an optimum minimum level of 40% gaps was not achieved. Treatment reduced leaf layer number (LLN) but had no effect on the percentage of exposed leaves. Leaf removal increased the percentage of exposed fruit. Smart (1985) suggested optimal fruit zone canopy gaps of about 40%, leaf layers 1.0 or less, the percentage of exposed leaves 80% or greater and the percentage of exposed fruit 60% or greater. The measurements of LLN, exposed leaves and exposed fruit for M+H LR were in accordance with the parameters recommended for production of high quality grapes (Smart, 1985, Smart et al., 1990).

The percentage of photosynthetically active radiation (PAR) reaching the fruit zone was not affected by treatment (Table 1). The PAR levels indicate shading was prevalent in the fruit zone. Low PAR may reduce photosynthetic activity of middle and basal leaves (Hunter et al., 1995). The PAR levels were less than 5% of ambient for each treatment and could indicate cluster shading due to shoot density (Zoecklein et al., 1992).

2. Shoot Thinned Vines

Leaf removal influenced three of four canopy density parameters among shoot thinned vines (Table 2). Canopy gaps were greatest in the M+H LR treatment. The LLN was reduced by M+H LR compared No LR and M LR. The percentage of exposed leaves was unaffected by treatment. Among shoot thinned vines, M+H LR increased the percentage of exposed fruit compared to both M LR and No LR. Shoot thinning and leaf removal may have benefits including improved canopy microclimate (Smart, 1985) and reduction of vegetative sinks (Reynolds, 1989). Removal of parasitic, vegetative shoots or leaves alters source-sink relationships by enabling the vine to dedicate more photosynthetates to fruit, possibly increasing secondary metabolites (Reynolds et al., 1994).

The PAR in the fruit zone of shoot thinned vines was greatest in the M+H LR treatment. The M LR treatment had a lower PAR compared to both No LR and M+H LR. Increased light inception in the fruit zone has been reported to increase anthocyanins (Hunter et al., 1991b) and aroma compounds (Morrison and Noble, 1990). Overexposure of fruit, however, has been shown to produce undesirable wine aromas (Carbonneau et al., 1978).

B. Leaf Area and Fruit Yield

1. Non-Shoot Thinned Vines

Leaf area for non-shoot thinned vines was reduced by leaf removal although

treatment did not affect fruit yield per vine (Table 3). The amount of leaf area required to properly ripen grapes varies according to climate and cultivar (Kliewer and Weaver, 1971). A range of adequate leaf area to fruit weight from 7 cm^2/g for Thompson Seedless (May et al., 1969) to 17 cm^2/g for potted Muscat of Alexandria vines (Winkler, 1930) has been reported. Photoassmimilate accumulation in grapes has been reported to begin declining when this ratio is between 7 cm^2/g and 10 cm^2/g (Kliewer and Weaver, 1971, Smart, 1985). The range in this study, therefore, was considered adequate for proper fruit development. Leaf removal did not affect shoots per vine, clusters per vine, clusters per shoot, and cluster weight (data not shown).

2. Shoot Thinned Vines

Leaf area differences were not observed among shoot thinned vines (Table 4). Yield was unaffected by leaf removal treatments and leaf area to fruit weight ratios (LA/FW) were considered sufficient for proper ripening.

Shoots per vine, clusters per vine, clusters per shoot and yield of shoot thinned vines were also unaffected by leaf removal (data not shown). Cluster weights of the M+H LR treatment were reduced by 15% compared to the M LR treatment (data not shown). This may be attributed to enhanced dehydration of the fruit, resulting from the relatively high percentage of light (33% of ambient) in the fruit zone.

C. Fruit Composition

1. Non-Shoot Thinned Vines

Berry weights from No LR were less than M LR and M+H LR vines at four of five sampling dates (Figure 1). The No LR treatment had the lowest relative percentage of exposed fruit among treatments. Hunter and Visser (1988a) observed reduced berry weight as a result of reductions in photosynthetic activity due to excessive canopy density and growth. Reynolds et al. (1986) reported highly shaded and highly exposed fruit tended to have smaller berries than partially shaded fruit, which may account for reduced berry weight of the No LR treatment. Berry weight reductions from 24 September to 2 October for M LR and M+H LR treatments may be the result of canopy differences. Fruit from these treatments had greater solar exposure than control vines.

Fruit soluble solids (°Brix) were not affected by leaf removal throughout the sampling period (Figure 2). A reduction in °Brix occurred between 24 September and 2 October for No LR while M LR and M+H LR °Brix continued to rise. During that time period, approximately 3 cm of rainfall was recorded, possibly resulting in dilution of berries and reduction in °Brix for No LR. The M LR and M+H LR treatments had greater fruit exposure which may offset the effects of dilution, resulting in continued increase in soluble solids.

Sugar per berry (SPB) expresses solute accumulation as a function of both soluble solids and weight, and is, therefore, a better indicator of solute accumulation than °Brix (Zoecklein et al., 1995). The SPB was lowest in the No LR treatment

throughout the sampling season (Figure 3). The reduction in SPB from 24 September to 2 October in M LR was due to weight reduction from dehydration. Berry weight of No LR increased with a corresponding drop in °Brix, indicating both dilution and sugar export as demonstrated by reduced SPB for the No LR treatment on 2 October. Excessive fruit zone shading can result in leaves (below the light compensation point) consuming as many carbohydrates as they produce (Smart, 1973). The SPB at each sampling date was greatest in M LR and M+H LR and corresponded to the treatments with the greatest fruit exposure.

The pH of the No LR treatment was lower than M LR at three of five sampling dates and lower than the M+H LR treatment at each sampling date (Figure 4). This could indicate an increased rate of maturity for the M LR and M+H LR treatments even though differences in °Brix were not observed. The increased pH with increased exposure due to leaf removal contrasts with other studies (Smart, 1985, Smart et al., 1988) that reported increased pH with shading.

Titratable acidity (TA) of non-shoot thinned vines showed limited treatment differences. Fruit from the M LR treatment had a greater TA than No LR and M+H LR on one of five sampling dates (Figure 5). The increase in TA for M LR on 24 September corresponded to a decline in pH. Clusters exposed to more sunlight typically have a lower malate concentration than shaded clusters (Smart, 1985, Reynolds et al., 1986), and hence a lower TA. Increased PAR has been shown to reduce TA due mostly to decreased malate concentration (Zoecklein et al., 1992).

This may explain the reduction in TA for M+H LR.

Red-free glycosyl glucose (GG) generally increased throughout the sampling period and was influenced by treatment at three of five sample dates (Figure 6). At harvest, the No LR treatment had the greatest red-free GG, despite differences in canopy microclimate among treatments. Between 18 September and 24 September a 38% increase in M+H LR red-free GG was observed compared to an 18% and 19% increase in the No LR and M LR treatments, respectively. Fruit zone shading was evident in each treatment as indicated by low PAR levels, yet the M+H LR treatment had the greatest percentage of fruit exposure. Degrees Brix and sugar per berry (SPB), however, did not increase at the greatest rate for M+H LR during the same period, indicating accumulation of red-free GG did not directly parallel increases in total soluble solids. Between 24 September and 2 October, red-free GG remained the same for M+H LR and decreased by 2% for M LR, corresponding to a leveling-off and reduction in SPB for the M+H LR and M LR treatments, respectively.

Red-free GG per gram of berry weight was influenced by treatment at four of five sampling dates (Figure 7). Red-free GG per gram was greatest in the No LR treatment at two of four dates, including harvest. The greatest change (36%) in red-free GG per gram was noted in the M+H LR treatment between 18 and 24 September. Meanwhile, red-free GG per gram of the No LR and M LR treatments increased 21% and 15%, respectively. Despite similar or decreasing red-free GG for M+H LR and M LR on 2 October, respectively, red-free GG per gram continued to

increase due to reductions in berry weight. The increases in red-free GG per gram of berry weight for all treatments at the end of the sampling period did not correspond to reductions or leveling-off of SPB, showing red-free GG per gram accumulation was not analogous to total solids accumulation.

Leaf removal has been shown to increase GG concentration and GG per gram in other studies (B. W. Zoecklein, unpublished data, 1996). The low PAR levels of each treatment of have been reported to reduce fruit temperatures (Zoecklein, et al., 1992). Temperature reductions in the fruit zone due to reduced light penetration have been reported to lower metabolic activity in fruit and leaves (Jackson and Lombard, 1993). Basal leaf removal has been shown to increase glycosidically bound monoterpene aroma concentrations in Gewurztraminer (Reynolds and Wardle, 1989) and Riesling (Reynolds et al., 1991), but did not affect red-free GG concentrations in fruit from non-shoot thinned vines in the current study presumably as result of low fruit zone PAR.

2. Shoot Thinned Vines

Berry weights from shoot thinned vines did not differ at four of five sampling dates (Figure 8). The reduction in berry weight for No LR and M+H LR on 2 October corresponded to increased fruit zone PAR for each treatment compared to M LR (Table 2). Reductions in weight may have been due to greater light and heat exposure of fruit and the resultant berry shriveling.

Soluble solids of fruit from shoot thinned vines was lowest in the M LR treatment at four of five sampling dates (Figure 9). The lower °Brix was not due to insufficient retained leaf area, as the suggested minimum 10 cm^2/g fruit required (Lakso, 1994) was achieved (Table 2). Sunlight levels (PAR) were greatest in No LR and M+H LR, which, in turn, can increase soluble solids (Reynolds et al., 1986, Bledsoe et al., 1988). Sugar per berry was not different among treatments at each sampling date for shoot thinned vines (Figure 10). Reductions in SPB on 2 October for the No LR and M+H LR treatments corresponded to reductions in berry weights likely due to increased light and heat exposure resulting in berry shriveling. In addition, a 5% increase in °Brix for the M LR treatment was observed between 24 September and 2 October, compared to a 2% and 1% increase for No LR and M+H LR, respectively. The SPB decreased for No LR and M+H LR, while SPB increased for the M LR treatment during the same period.

The pH was greatest in No LR at two of five sampling dates among shoot thinned vines (Figure 11). Titratable acidity (TA) was greatest for the M LR treatment at three of five sampling dates for shoot thinned vines (Figure 12). Both No LR and M+H LR had similar TA's during the entire sampling period. Increased fruit exposure and canopy PAR (Table 2) likely decreased TA for these two treatments compared to M LR, consistent with other reports (Smart, 1985, Reynolds et al., 1986).

Red-free GG was increased at each sample date except for a slight reduction in

the M LR treatment at harvest (Figure 12). The greatest red-free GG was measured in M+H LR, the most exposed canopy, at each sampling. Red-free GG increased with each treatment an average of 224% from 4 September to 24 September. From 24 September to 2 October, red-free GG increased 15% and 9% for M+H LR and No LR, respectively, while declining 1% in the M LR treatment. Increases in red-free GG for M+H LR and No LR corresponded to increased PAR in the fruit zones of those treatments versus M LR (Table 2). Smaller increases in °Brix and reductions in sugar per berry (SPB) for both the M+H LR and No LR treatments compared to M LR between 24 September and 2 October suggest that red-free GG accumulation did not coincide with total solute accumulation. The reduced canopies due to shoot thinning and leaf removal can alter photosynthetic activity (Smart, 1985, Hunter and Visser, 1988a, 1988b) and source-sink relationships (Reynolds, 1989) in the vine and may influence glycoside concentration as a result.

Red-free GG per gram of berry weight generally increased and was greatest in the M+H LR treatment at each sampling date (Figure 13). A 27% increase was observed in the M+H LR treatment from 24 September to 2 October while a 17% increase and 1% decline in red-free GG per gram were noted for No LR and M LR, respectively. Reduction in red-free GG per gram of the M LR treatment on 2 October corresponded to a decrease in red-free GG and an increase in weight possibly due to reduced fruit zone PAR as previously explained.

A portion of red-free GG contains phenolic glycosides, and increases in red-free

GG may partially be a result of this. Leaf and cluster exposure has been reported to increase phenolics, including anthocyanins (Smart and Smith, 1988, Hunter et al., 1991), with fruit exposure considered most important for increased phenolics (Crippen and Morrison, 1986). The activity of phenylalanine ammonia lyase (PAL), important for development of phenolic compounds, is influenced by light exposure of Cabernet Sauvignon (Morrison and Noble, 1990). Increases in glycosides noted in the current study parallel others which have shown less dense canopies resulting from leaf removal (Smart and Smith, 1988, Reynolds and Wardle, 1989, Reynolds et al., 1991) and shoot thinning (Reynolds et al., 1994) may influence the development of both free and glycosidically bound grape flavor and aroma compounds. These changes are reported to be the result of increasing photosynthetic efficiency, increasing solar exposure and possibly influencing source-sink relationships. The most severe level of leaf removal increased red-free GG and red-free GG per gram in shoot thinned vines likely due to greater solar exposure of the fruit and favorable changes in grapevine source-sink.

Shoot thinning may have a two-fold influence on berry development and composition. When shoots are close together, shoot density is high, and canopy density is also increased (Smart, 1990). Removal of shoots can also serve as a form of partial crop reduction altering vine source-sink relationships (Reynolds, 1989). Removal of clusters shifts translocation of photoassimilates to fruit (Fisher et al., 1977). This enables the vine to dedicate more photosynthetates to less fruit and less

vegetative growth and increasing solute and possibly glycoside accumulation. Crop reduction may also influence bound aroma constituents (McCarthy et al., 1987). In the current study, red-free GG was greatest in fruit from the most exposed treatment with shoot thinned and mechanical plus hand leaf removal (M+H LR). Combinations of shoot thinning and leaf removal may have altered vine photosynthesis and source-sink favorably for increased red-free GG and red-free GG per gram of berry weight, a phenomenon that did not occur with leaf removal without shoot thinning. For this vineyard and variety, the greatest level of leaf removal in conjunction with shoot thinning produced the greatest concentrations of GG.

CONCLUSIONS

Fruit zone leaf removal was evaluated in non-shoot thinned and shoot thinned vines for the influence on grape glycosides expressed as glycosyl glucose (GG) in Cabernet Sauvignon from an eastern Virginia vineyard. Grapevine canopy density and light insolation were altered with leaf removal of both non-shoot thinned and shoot thinned vines. Fruit yield components were generally unaffected by leaf removal. Red-free GG concentration was not affected by leaf removal of non-shoot thinned vines but was increased and had the highest concentration in fruit from vines with both shoot thinning and mechanical plus hand leaf removal (M+H LR).

Increases in grape glycosides, measured as red-free GG, due to shoot thinning and leaf removal represent, in part, an increase in the total pool of potential grape aroma and flavor constituents. Release of bound aglycones during vinification and wine aging may enhance overall wine quality. The effectiveness of the GG assay as a quality index and the sensory significance of changes in GG remain to be determined. This analytical tool, nonetheless, appears to be a valuable contribution which will help unravel the often tangled relationships between vineyard management, grape and potential wine quality.

Table 1. Canopy density and insolation parameters of non-shoot thinned Cabernet Sauvignon grapevines as affected by no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) in 1995[1].

Parameter	Treatment		
	No LR	M LR	M+H LR
Canopy gaps (%)	0.3b	2.3b	8.5a
Leaf layer number	2.5a	1.9b	0.7c
Exposed leaves (%)	67.2a	67.5a	72.1a
Exposed fruit (%)	24.8a	46.6b	83.0c
PAR in fruit zone[2] (%ambient)	1.8a	1.6a	3.1a

[1]means with the same letter are not significantly different at $P \leq .05$, N=18
[2]measurement, sky conditions, and time of measure:
23 August, sunny and clear, 1000 h to 1500 h EDT.

Table 2. Canopy density and insolation parameters of Cabernet Sauvignon grapevines shoot thinned to 14.0 shoots per meter as affected no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) in 1995[1].

Parameter	Treatment		
	No LR	M LR	M+H LR
Canopy gaps (%)	7.7b	6.3b	18.2a
Leaf layer number	1.6a	1.3a	0.6b
Exposed leaves (%)	77.1a	80.9a	83.5a
Exposed fruit (%)	59.6b	68.6b	89.4a
PAR in fruit zone[2] (% ambient)	17.4b	11.1c	33.1a

[1]means with the same letter are not significantly different at $P \leq .05$, N=18
[2]measurement, sky conditions, and time of measure:
23 August, sunny and clear, 1000 h to 1500 h EDT.

Table 3. Leaf area per vine, yield and leaf area per fruit weight of non-shoot thinned Cabernet Sauvignon grapevines as affected by no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) in 1995[1].

Parameter	Treatment		
	No LR	M LR	M+H LR
Leaf area per vine (1000 cm^2)	115.3a	97.8b	91.1b
Yield (kg/vine)	5.8a	6.7a	5.7a
Leaf area per fruit weight (cm^2/g)	19.5a	15.6a	16.3a

[1]means with the same letter are not significantly different at $P \leq .05$, N=18

Table 4. Leaf area per vine, yield and leaf area per fruit weight of Cabernet Sauvignon grapevines shoot thinned to 14.0 shoots per meter as affected no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) in 1995[1].

Parameter	Treatment		
	No LR	M LR	M+H LR
Leaf area per vine (1000 cm^2)	55.2a	49.5a	51.6a
Yield (kg/vine)	4.8a	4.7a	4.5a
Leaf area per fruit weight (cm^2/g)	11.6a	11.4a	12.6a

[1]means with the same letter are not significantly different at $P \leq .05$, N=18

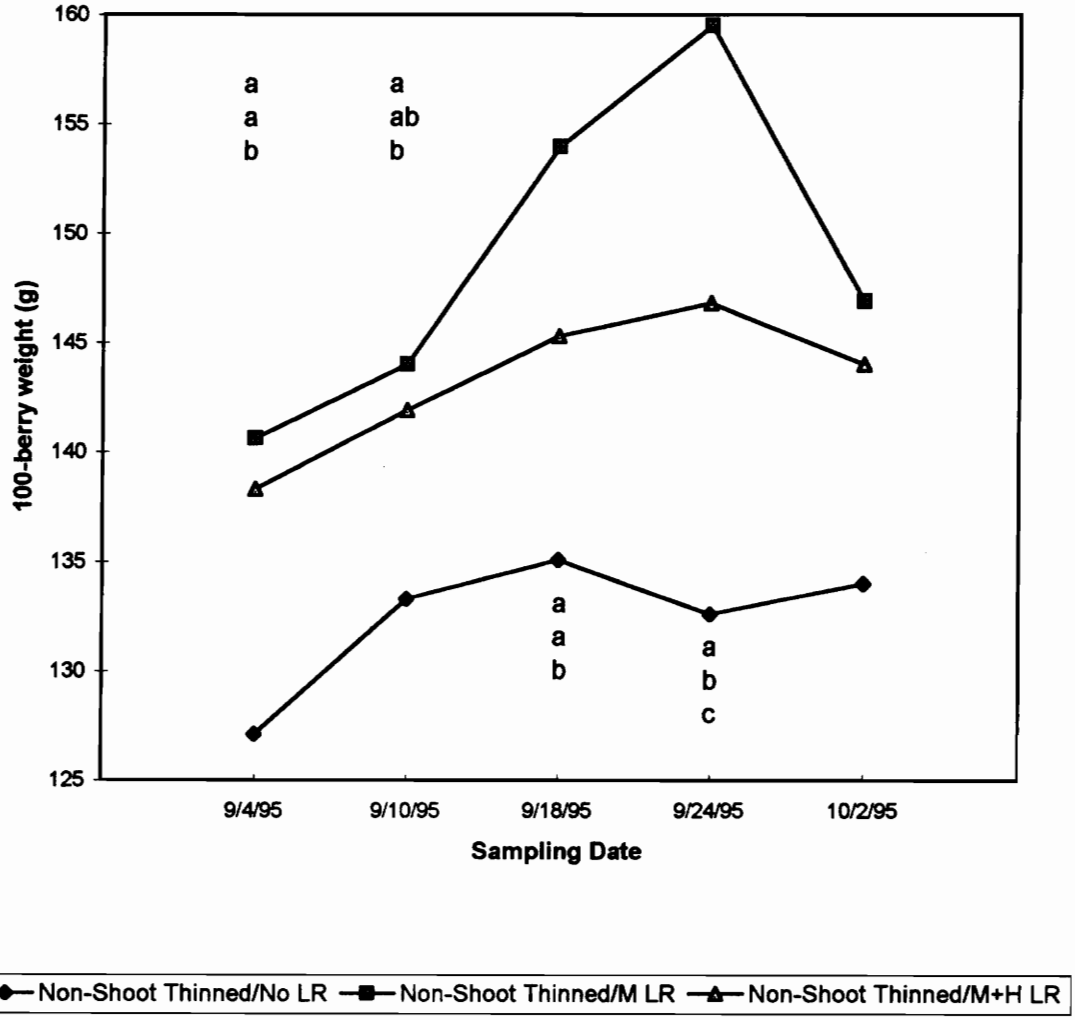

Figure 1. 100-berry weight (g) of non-shoot thinned Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

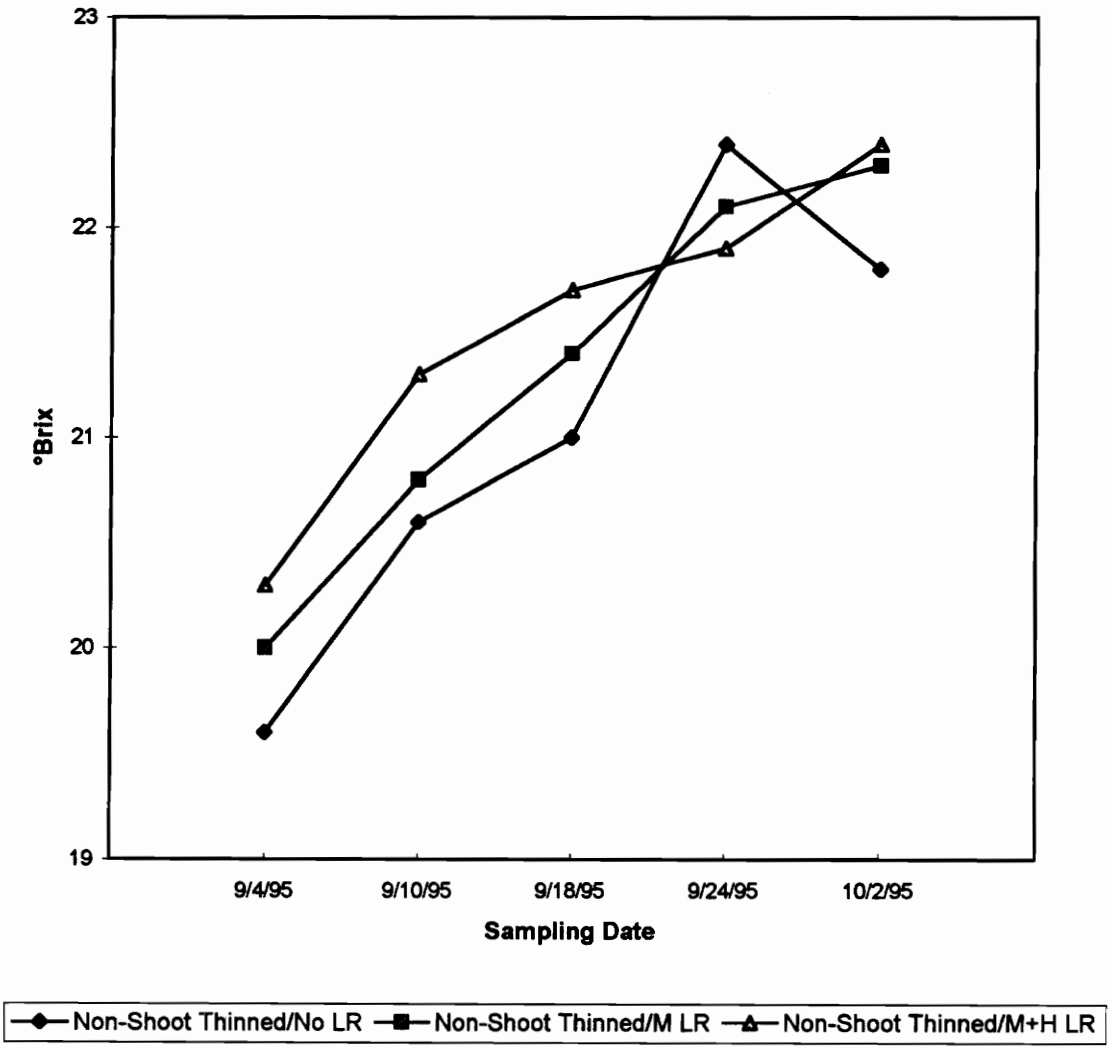

Figure 2. Soluble solids (°Brix) of non-shoot thinned Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. No significant differences ($P \leq .05$) were measured between treatment means at each sampling date.

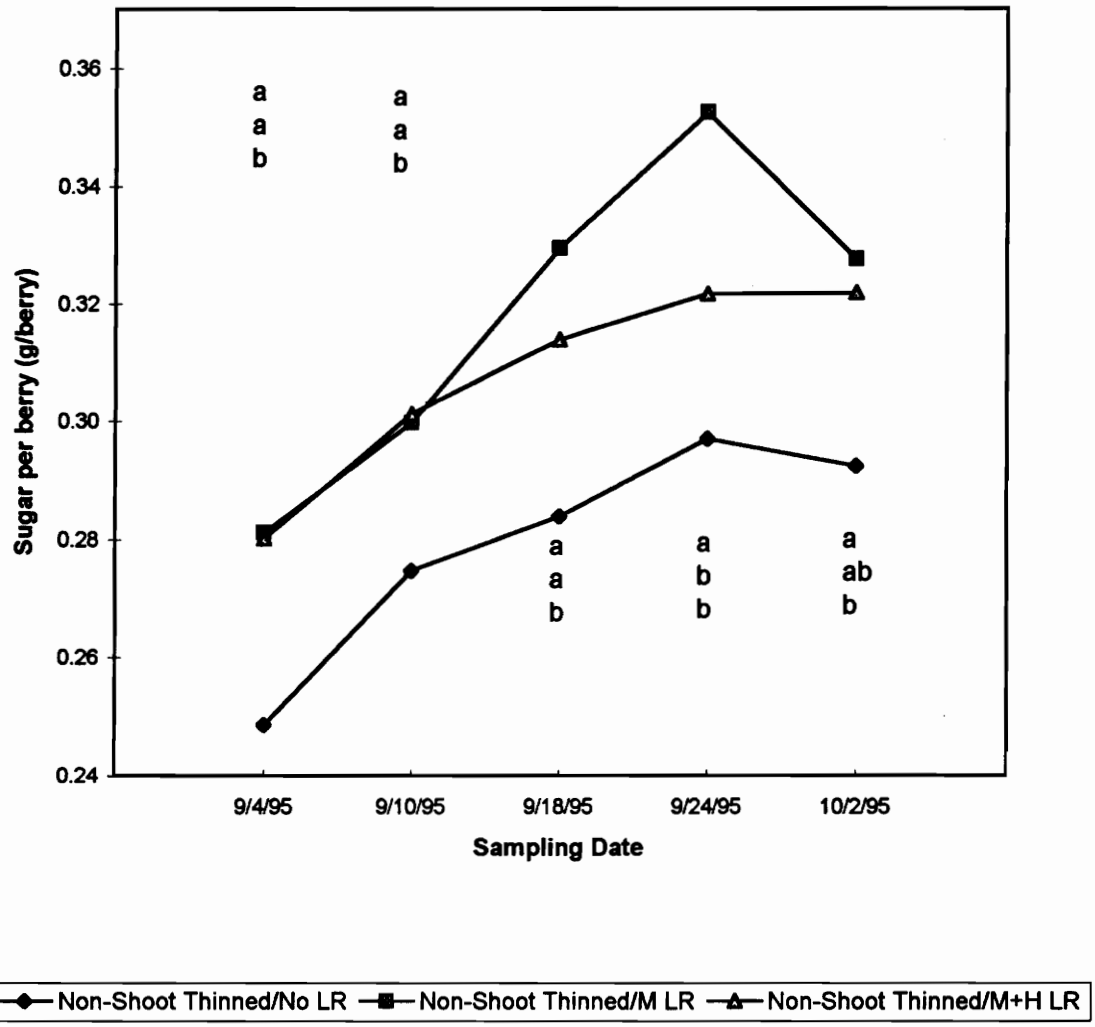

Figure 3. Sugar per berry (g/berry) of non-shoot thinned Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

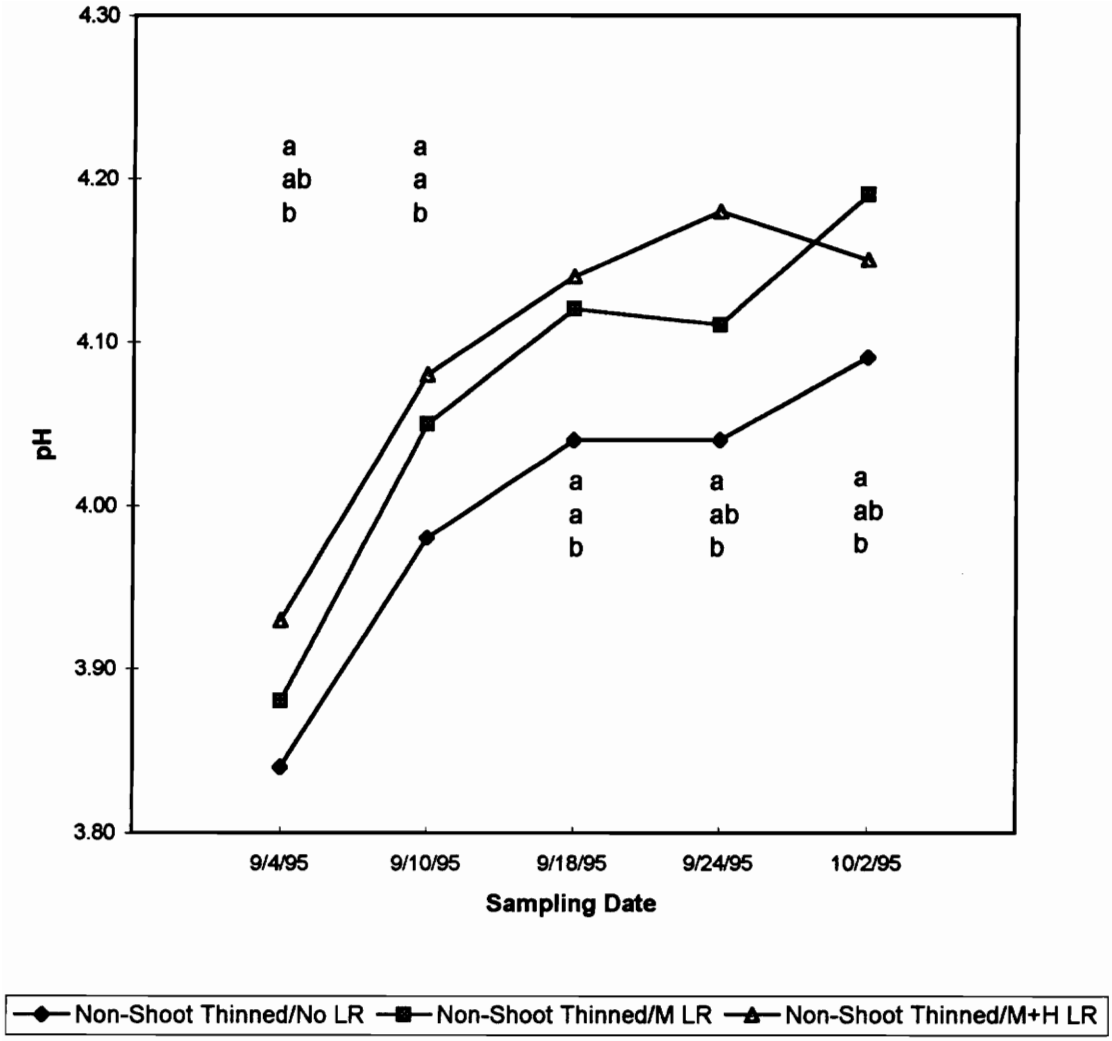

Figure 4. pH of non-shoot thinned Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

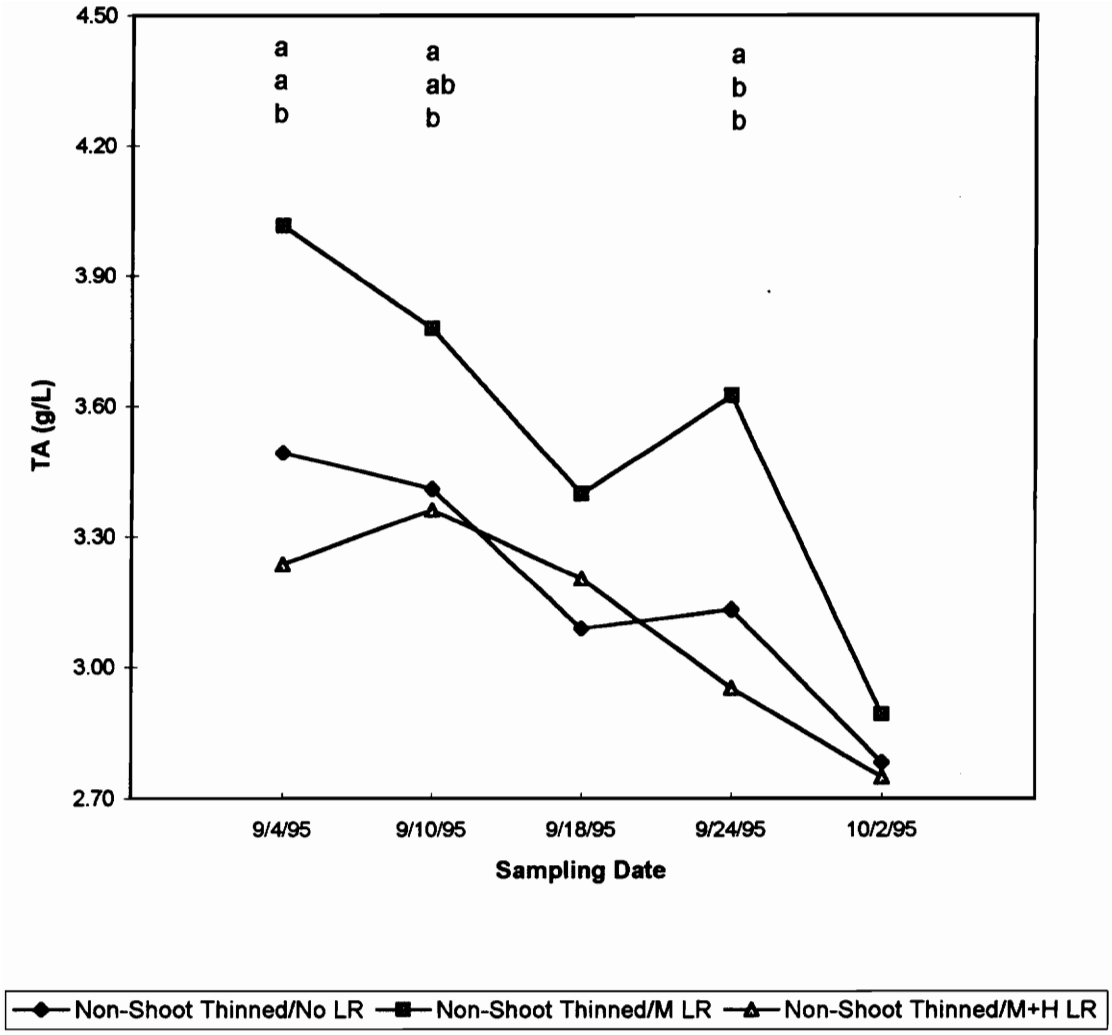

Figure 5. Titratable acidity (TA) of non-shoot thinned Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

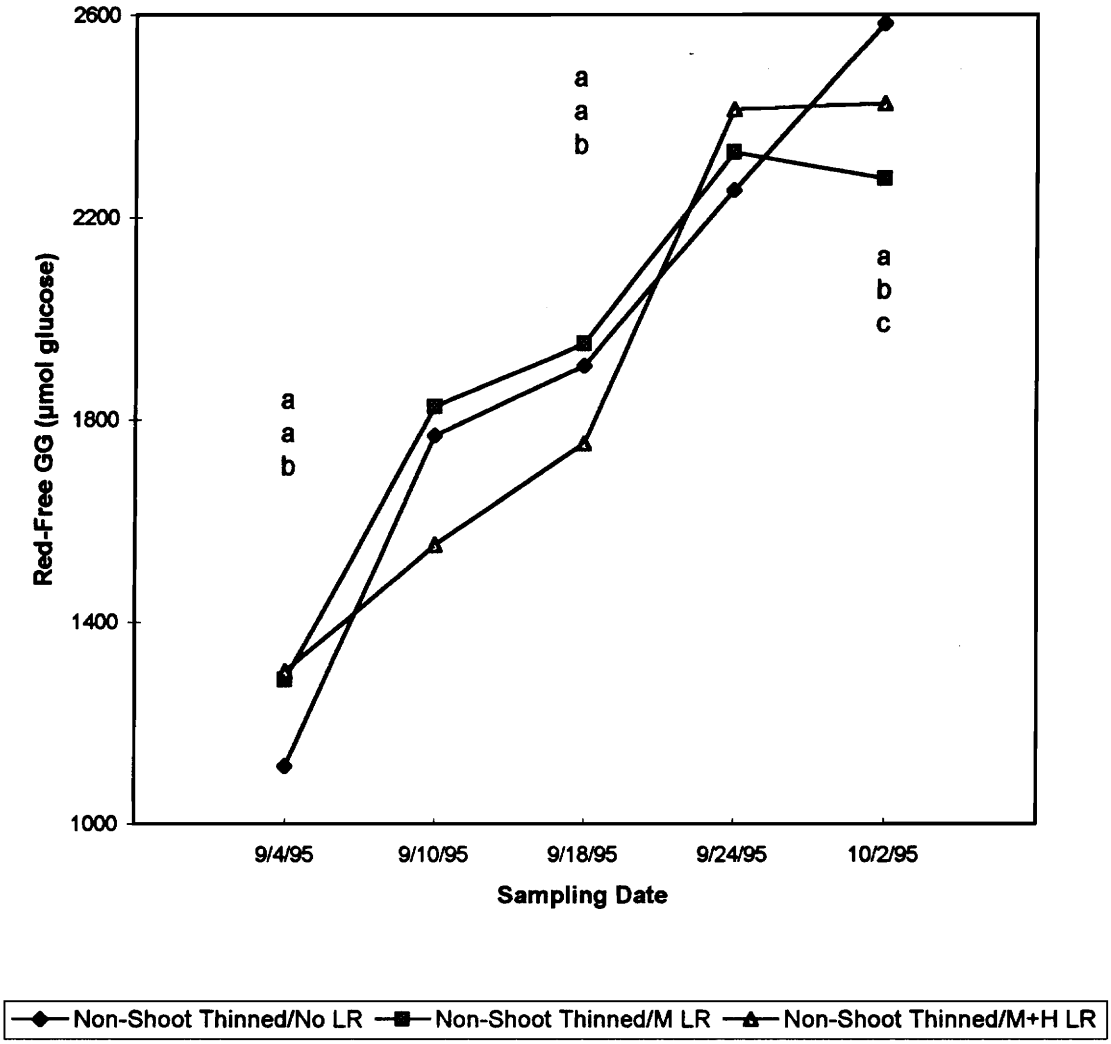

Figure 6. Red-free glycosyl glucose (GG) concentration (μmol glucose) of non-shoot thinned Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

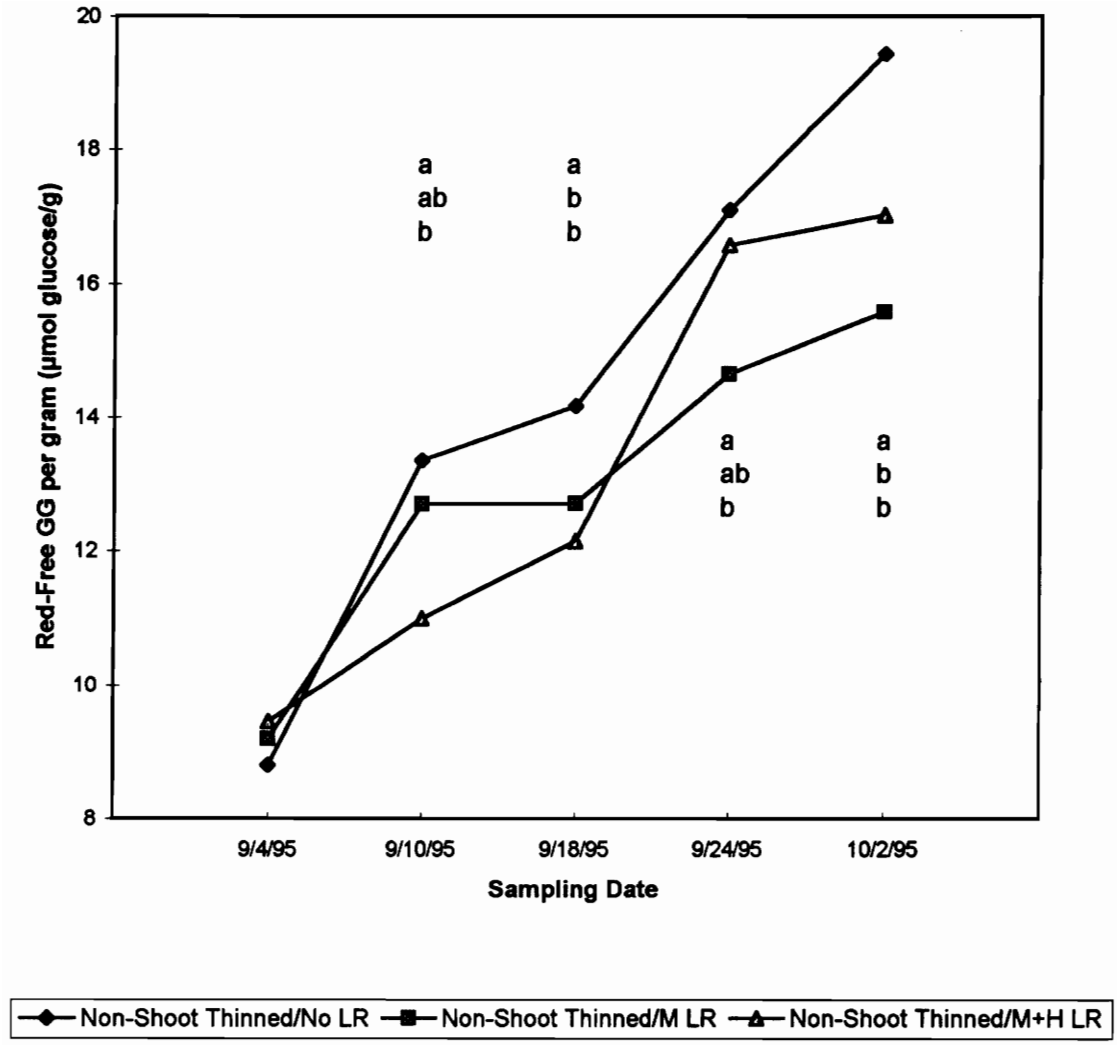

Figure 7. Red-free glycosyl glucose (GG) per gram berry weight (μmol glucose/g) of non-shoot thinned Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

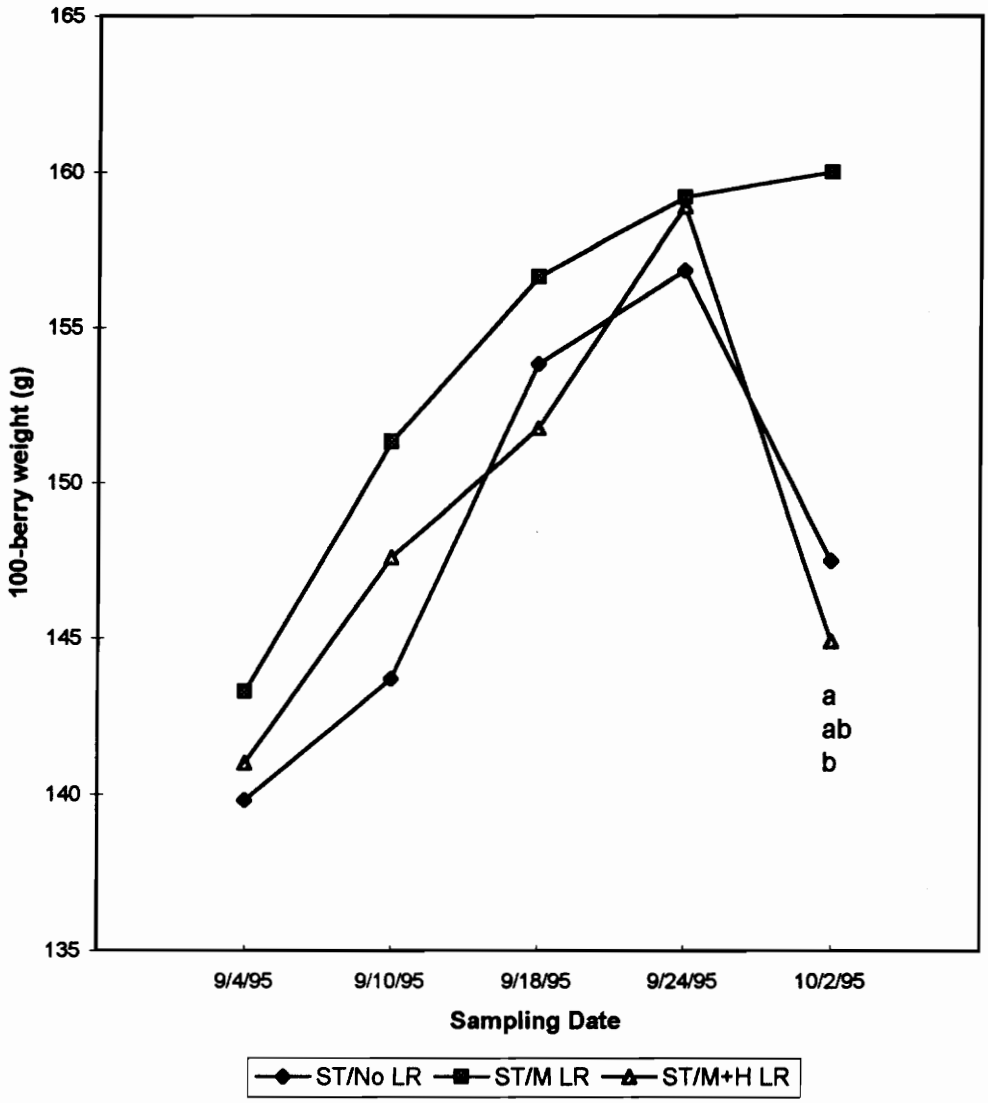

Figure 8. 100-berry weight (g) of shoot thinned (ST) Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

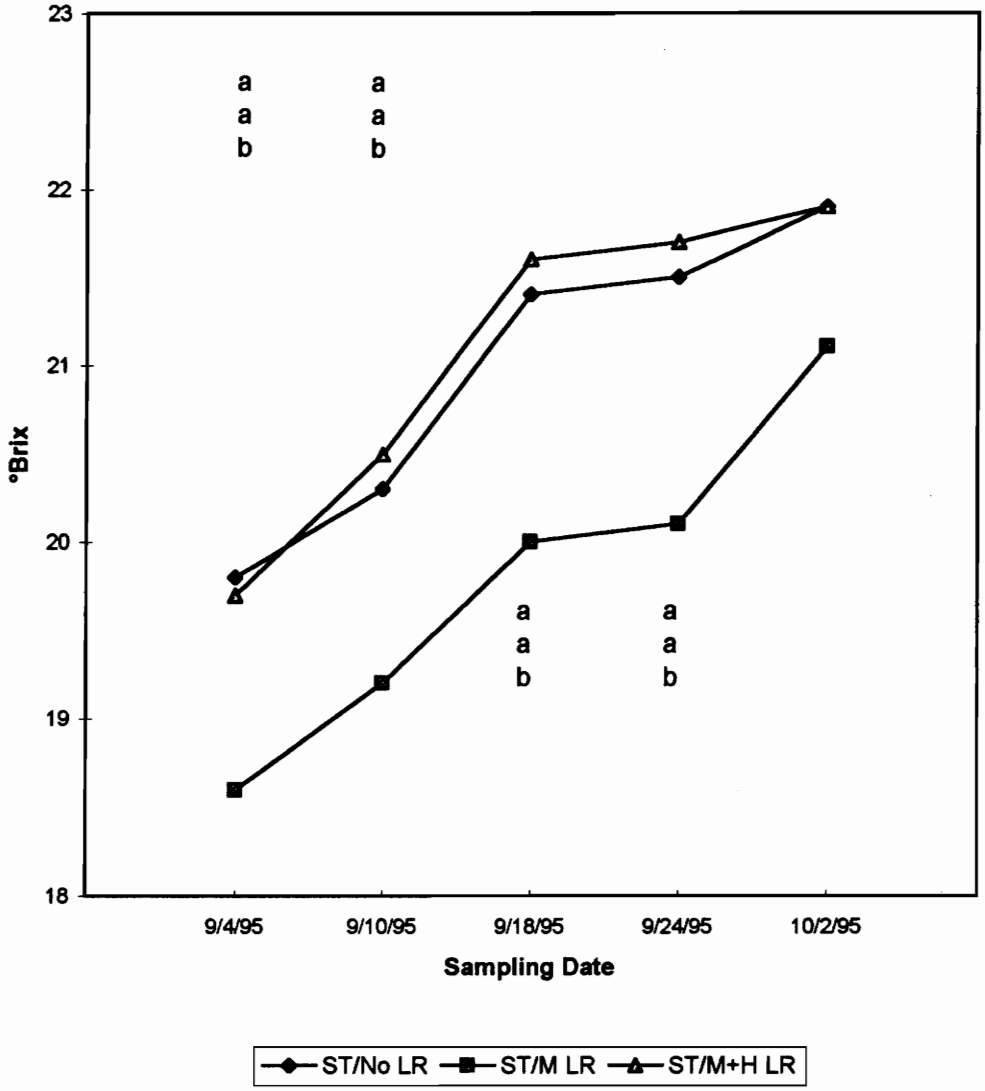

Figure 9. Soluble solids (°Brix) of shoot thinned (ST) Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

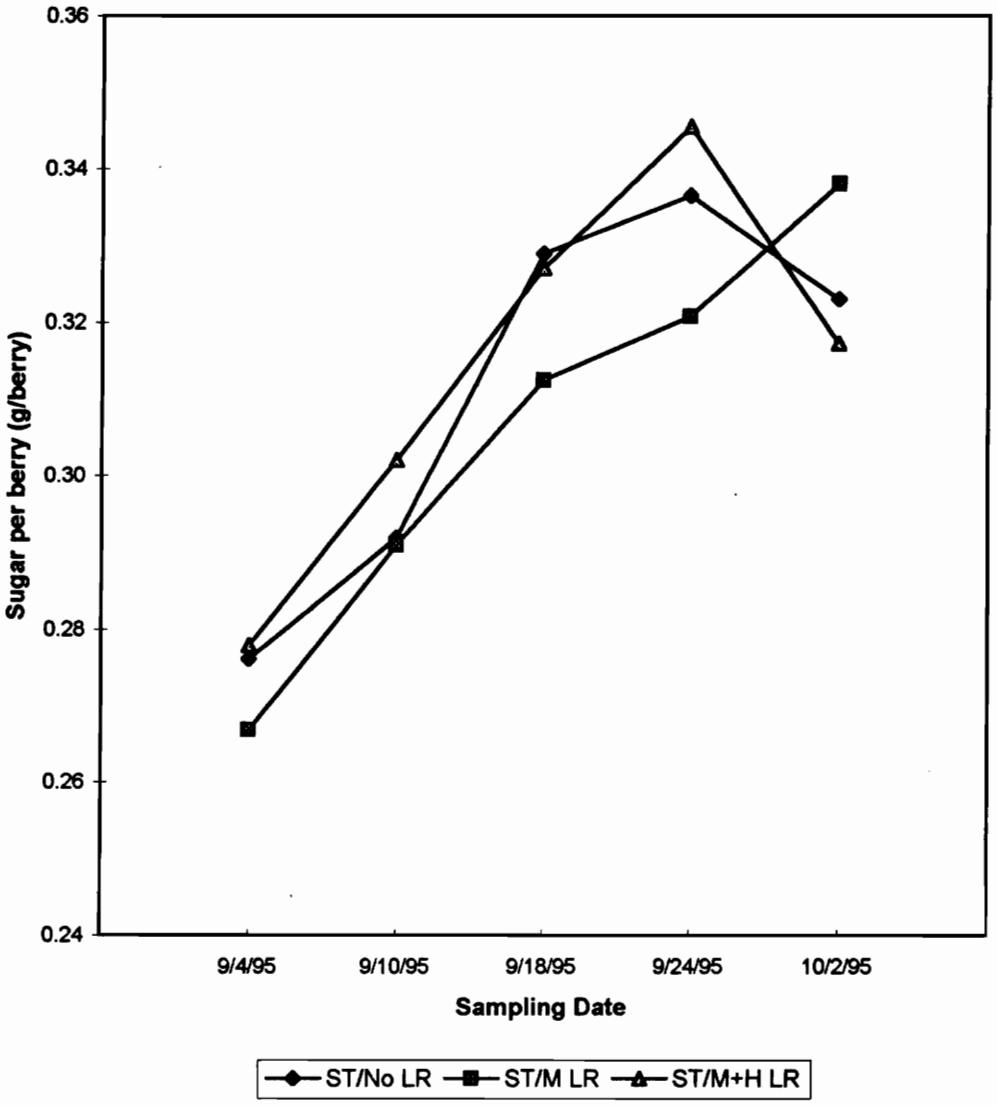

Figure 10. Sugar per berry (g/berry) of shoot thinned (ST) Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

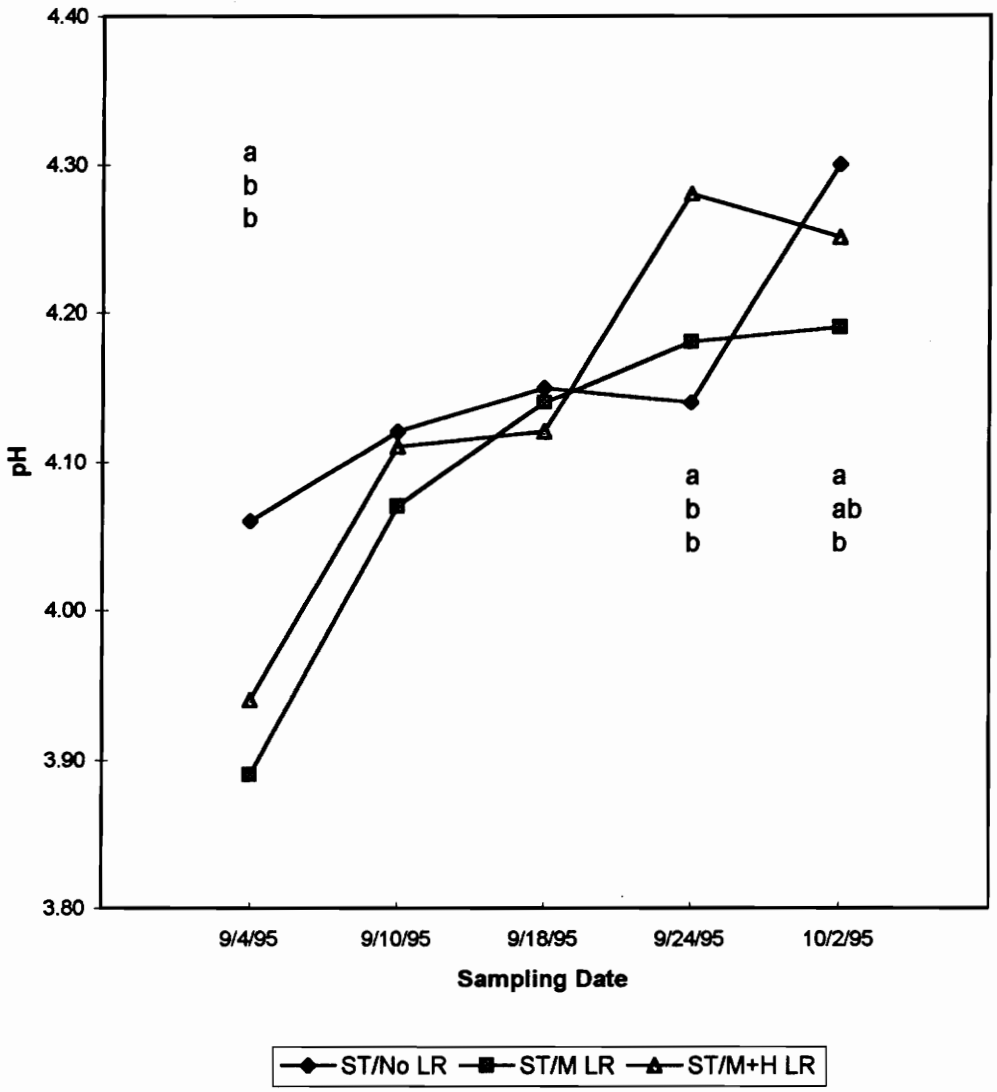

Figure 11. pH of shoot thinned (ST) Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

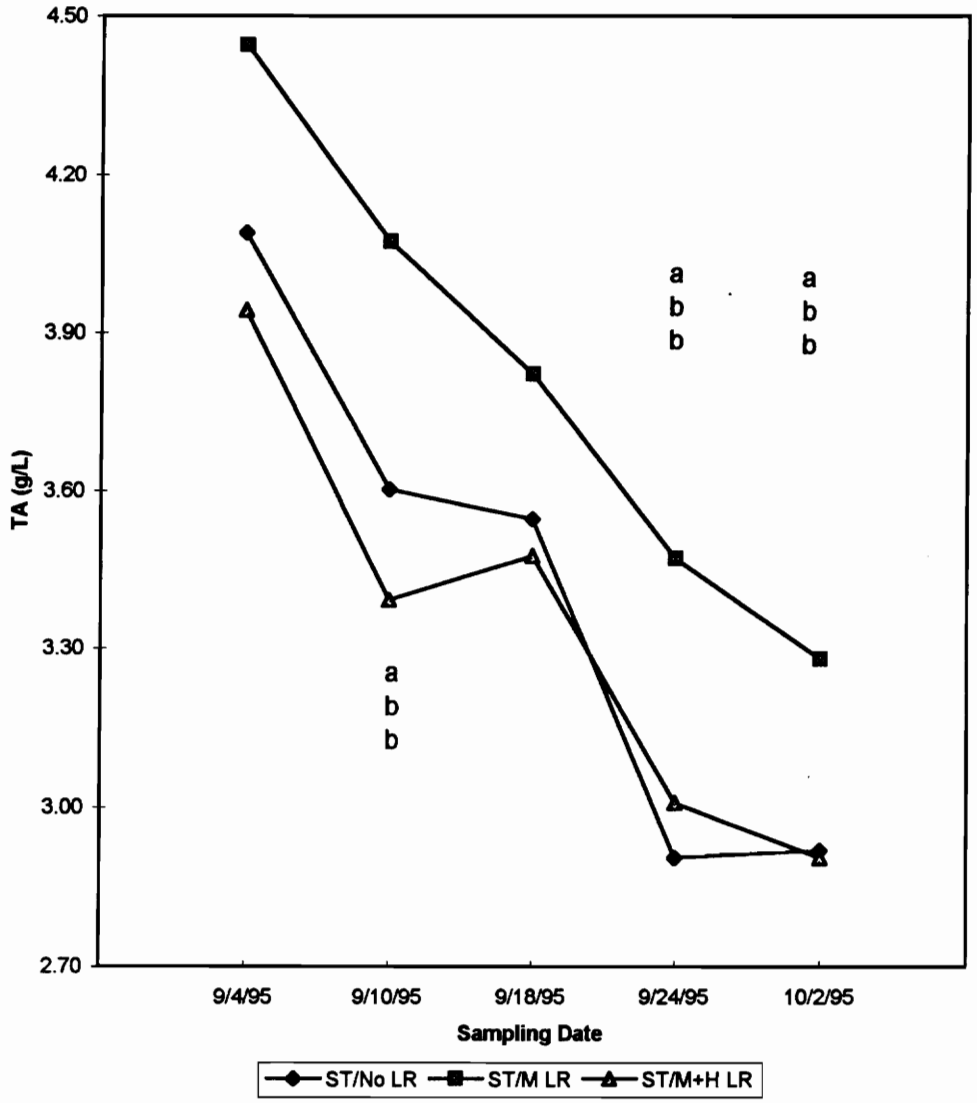

Figure 12. Titratable acidity (TA) of shoot thinned (ST) Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

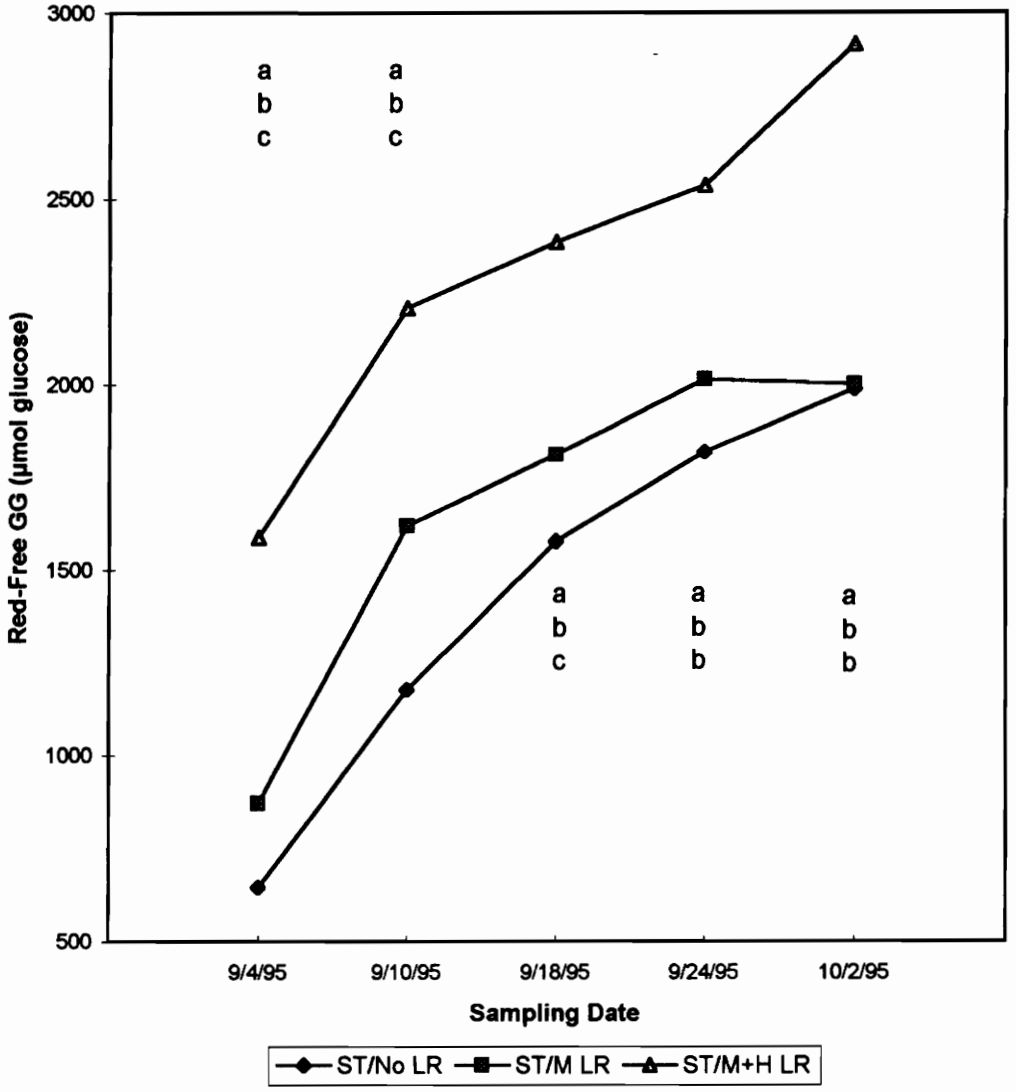

Figure 13. Red-free glycosyl glucose (GG) concentration (μmol glucose) of shoot thinned (ST) Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

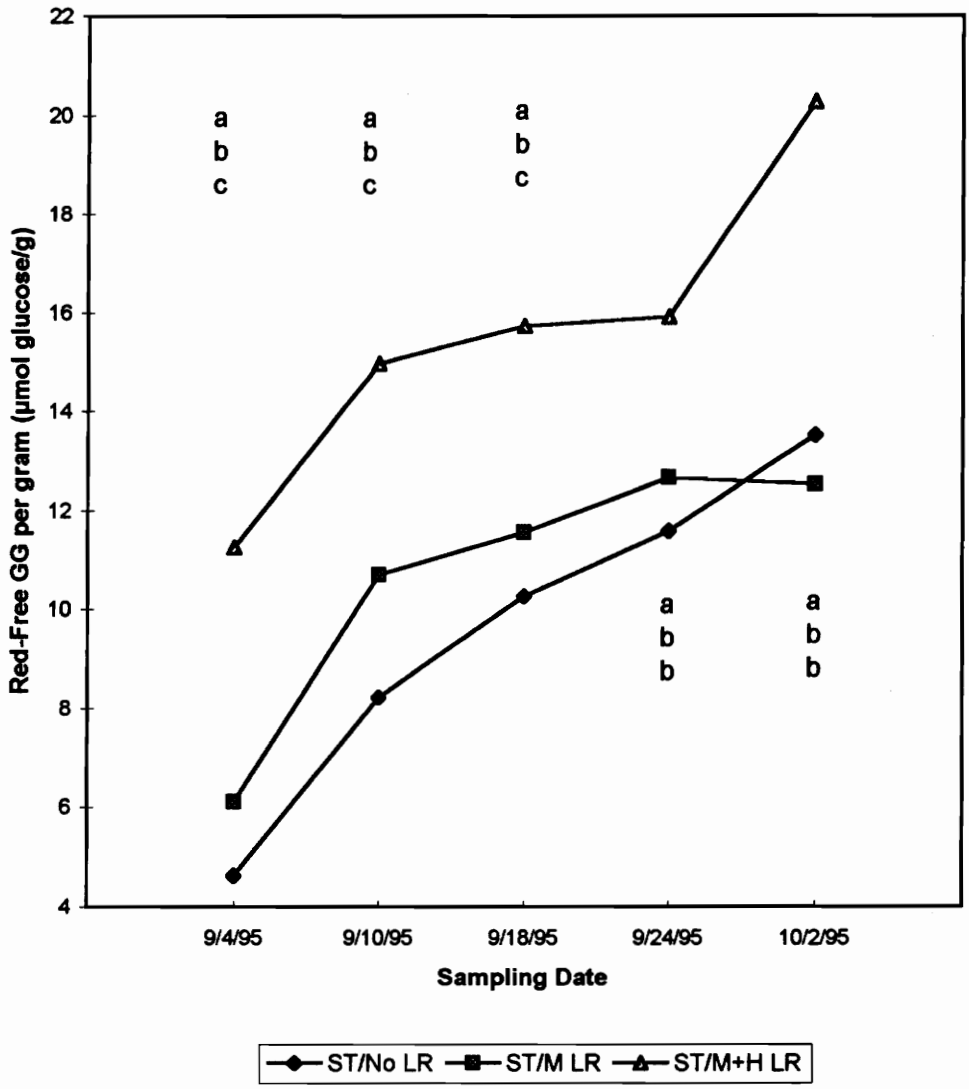

Figure 14. Red-free glycosyl glucose (GG) per gram berry weight (μmol glucose/g) of shoot thinned (ST) Cabernet Sauvignon vines with no leaf removal (No LR), mechanical leaf removal (M LR) and mechanical plus hand leaf removal (M+H LR) at five sampling dates in 1995. Treatment means with the same letter are not significantly different at $P \leq .05$, $N=18$. Unlabelled points have no significant differences between treatment means.

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SUMMARY

Two separate studies were undertaken to determine the influence of specific vineyard management techniques on grape glycoside concentration, measured as red-free glycosyl glucose (GG). In the first study, crop level was examined for its influence on glycoside concentration and general fruit chemistry of Cabernet Sauvignon grapes from an eastern Virginia vineyard. Red-free glycosyl glucose (GG) and red-free GG per gram of berry weight were frequently greatest in fruit from the lowest crop level. Reduction in crop level enhanced the rate of fruit maturity resulting in increases in bound aroma and flavor components. Two methods for quantification of 'phenolic-free' GG were evaluated and it was determined that phenolic glycosides may comprise as much as half of the total GG value. This offered a further refinement of the GG procedure and enhancement of its potential use as an overall fruit quality index.

In a second study, fruit zone leaf removal was evaluated in non-shoot thinned and shoot thinned vines for the influence on grape glycosides in Cabernet Sauvignon. Grapevine canopy density and light insolation were altered with leaf removal of both non-shoot thinned and shoot thinned vines. Red-free GG concentration was not affected by leaf removal of non-shoot thinned vines but was increased and had the highest concentration in fruit from vines with both shoot thinning and mechanical plus hand leaf removal (M+H LR), presumably due to increased solar exposure, enhanced vine photosynthesis and favorable alterations in vine source-sink relationships.

Quantification of grape glycosides by analysis of red-free GG has been proposed as an index of fruit quality as related to potential wine quality. Compared to gas chromatography/mass spectrometry (GC/MS), the GG method is simple and rapidly quantifies all secondary metabolites in a sample, giving an estimation of bound flavor and aroma. The future of its use a quality index is dependent on further research to determine how processing and aging of wines influences GG and the sensory significance of differences in GG.

VITA

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