Effect of Canopy Manipulation and Fermentation on Grape Aroma Components

by

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(ABSTRACT)

Several experiments were conducted to determine optimum methods for extraction, isolation and analysis of selected aroma components and the influence of grapevine canopy manipulation and fermentation on those components.

A polymeric styrene resin (XAD-2) was evaluated for its ability to absorb and desorb five monoterpene alcohols, three monoterpene hydrocarbons, four monterpene oxides, two aromatic alcohols and a glucopyranoside from White Riesling juice at two different pH values. The percent recovery and the coefficients of variation for each compound was compared with a continuous Freon 11 extraction system. The percent recovery averaged 90% or greater for both systems with the coefficient of variation being smaller with the resin extraction.

In two separate studies canopy manipulation was evaluated for the effect on aroma components using the XAD-2 resin isolation procedure. The influence of shoot topping to 10 or 20 nodes or ethephon application on grape aroma components was measured for three seasons. Canopy modification by both topping levels and ethephon treatment increased sunlight penetration into the canopy fruiting zone. Free volatile terpenes (FVT) were increased by ethephon in two of three seasons while shoot topping increased FVT and potentially free volatile terpenes (PVT) in one of three seasons.

In the second separate three-year study, two to four leaves were removed from the fruiting zone of grapes grown on two training systems. Selective leaf removal increased sunlight penetration into the grape

canopy but generally did not influence FVT. However, PVT was frequently higher in the leaf-pulled fruit including four of six commercial harvest dates. The total quantity of the bound geraniol, nerol, linalool, and α-terpineol was higher in fruit from the leaf-pulled vines at harvest.

Four strains of *Saccharomyces cerevisiae* were evaluated for their influences on free and conjugated aroma components of White Riesling grapes, immediately following and 45 days post-fermentation with lees or *Sur lie*. Fermentation generally reduced free terpenes except for ∝-terpineol, hotrienol, citronellol, and linalool oxides. Fermentation also increased free benzyl and 2-phenylethanol. In newly fermented and aged wines the concentrations of free volatiles were always below the sensory threshold for each compound. The potentially volatile terpenes (PVT) were similar among treatments following fermentation, the exception being the Fermiblanc (FB) yeast strain. Additional hydrolysis of bound compounds occurred in each wine following lees storage, the exception being the wine fermented with the Fermiblanc (FB) strain.

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INTRODUCTION

Flavor is the combination of odor, taste and texture and is possibly the most important sensory parameter used in juice and wine evaluation. Since most or our sense of taste is attributed to smell, aroma is a particularly significant aspect of flavor. Premium wines are characterized by the intensity and complexity of their aromas.

In many commodities, the natural flavor or aroma is dominated by a single substance or a group of 'character impact' compounds. The floral aromas of Muscat, Gewurtztraminer and Riesling grapes and wines are attributed to monoterpenes, considered character impact compounds for those varieties. Monoterpenes are secondary plant metabolites, probably produced during a plant's life cycle in a balance between biosynthesis and breakdown. These opposing activities are directly controlled by several intrinsic factors such as genotype and ontogeny and extrinsic environmental properties such as temperature, light and wind. The intrinsic factors are, of course, fixed, while the extrinsic factors can presumably be manipulated. The parameters controlling monoterpene production in grapes are not well understood. However, changes in vineyard management practices which increase the monoterpene content of the fruit could significantly increase aroma and subsequent wine quality.

Excessive vegetative vine growth in Virginia vineyards is common owing to the lack of control of soil moisture and the nature of the soils. Increases in shoot growth and leaf area often create conditions of excessive density and shading in the canopy interior. Grapes maturing in densely shaded canopy interiors compared to open, exposed canopies are generally associated with the following: 1) low soluble solids, 2) high titratable acidity, 3) elevated pH and 4) increased incidence of fruit rot. Foliage management is, therefore, a major priority for the viticulturist for improvement in light conditions for photosynthesis,

fruit development and rot control.

Although selective leaf removal, together with management practices such as shoot topping are utilized by commercial grape growers, uncertainty exists regarding their influence on fruit composition. Since the growing regions, methods, levels and timing of defoliation differ greatly among published studies, divergent results have been frequently reported. Many have reported the influence of canopy management on primary grape metabolites, however, few reports have examined the effect on grape aroma components. Under conditions of excessive vegetative growth, shoots become strong sinks for photosynthesis products. Since the distribution of photosynthates is regulated by the source-sink relationship, changes in the leaf area may cause a change in the available products of photosynthesis for the different sinks. Vineyard management practices that increase grape aroma, qualitatively or quantitatively, may have an influence on enhancing wine quality.

The flavor of grapes is dependent upon a small group of free secondary metabolites that have escaped oxidation to polyols or conjugation. Glycosylation and hydroxylation are natural processes that remove components as direct contributors to flavor. There is, however, some reclamation of flavor available through acid catalyzed hydrolysis of polyols and/or enzymatic hydrolysis of glycosides. Hydrolysis can produce a wide range of volatiles that may broaden and enhance the complexity of juices and wines. The use of yeast species and strains with improved hydrolytic activity could help to 'unlock' potential flavor components produced by canopy modification.

The biochemical mechanisms utilized for monoterpene production in White Riesling are also present in non-terpene varieties. Non-terpene varieties also derive their flavor from free volatiles while the majority of the potential flavor is locked up in the form of conjugation products.

Thus, the study of changes in grape monoterpenes resulting from vineyard management and research on the influence of fermentation on White Riesling aroma compounds may have direct relevance to other grape varieties.

The objective of these studies, therefore, was to develop optimum liquid and gas chromatographic techniques for the evaluation of aroma volatiles and to evaluate the effects of grapevine canopy density and yeast fermentation on selected grape aroma components.

LITERATURE REVIEW

1. Monoterpenes

Terpene compounds are derived from a basic branched 5 carbon unit and are classified according to the number of such units present in the molecule. Monoterpenes are 10-carbon compounds derived from acyclic precursors and divided into four general classifications: acyclic, cyclopentanoid, cyclohexanoid and irregular (Croteau, 1984). Several hundred naturally occurring monoterpenes are presently known although the specific function of these compounds are not well understood. It is believed, however, that they play a role in insect pollination, seed dispersal by animals, repelling of animals, microbial resistance, microbial inhibition and germination of other plants (Croteau, 1984). Additionally, monoterpenes have been shown to convert to sugars and amino acids in the leaf. This supports the belief that they may also provide a carbon source in times of photosynthate deficiency (Croteau, 1984).

Monoterpenes are produced primarily by higher plants, and constitute the characteristic components of essential oils. They were traditionally regarded as products of a relatively limited group of plants, however, monoterpenes are ubiquitous in higher plants (Hardy, 1970). The classical essential oil bearing plants are unique only in that they possess specialized secretory structures adapted to accumulating large quantities of these compounds. According to Croteau (1984), it is generally assumed that secretory structures such as glandular hairs and resin ducts constitute the primary site of synthesis. Glandular hairs and resin ducts are usually extracellular; they occur in many types of plant tissues from flowers to roots and consist of highly specialized cells and an isolated cavity in which terpene compounds are sequestered (Fluck, 1963).

Nearly 50 terpenes have been identified in grapes and wines (Marais, 1987; Park and Noble, 1993). They include alcohols, ethers, aldehydes,

hydrocarbons and polyfunctional derivatives. Monoterpenes are related to the flavor characteristics of certain grape varieties. Typical aroma descriptors of some important monoterpenes include: floral, rose-like (geraniol, nerol, rose oxides), coriander (linalool), camphoraceous (linalool oxides), green and herbaceous (nerol oxide) (Meilgaard, 1975; Simpson, 1979). Ribereau-Gayon et al. (1975) determined the aroma threshold of eight monoterpenes and found that the two major monoterpenes of Muscat grapes, linalool and geraniol, were present in concentrations higher than their sensory threshold values. However, none of the individual terpenes had sensory properties identical to the muscat character. They concluded that a combination of the eight monoterpenes was essential to the sensory characteristics of the Muscat grape. Further, they reported that monoterpenes interact to such a great extent that one compound can decrease the aroma threshold of another, and a mixture can be more aromatic than the individual components.

The monoterpene content of non-Muscat, and non-aromatic grapes has also been studied. The flavor of varieties such as White Riesling, Sylvaner, Gewurztraminer and Muller-Thurgau is, in part, due to monoterpenes (Terrier et al., 1972; Park and Noble 1993). Schreier et al. (1976, 1977) used stepwise discriminant analysis of wine to distinguish six wine grape cultivars. Although yeast produce an abundance of volatile components during fermentation, monoterpene varieties were easily distinguished and their distinguishing properties easily noted. Thus, the importance of these grape constituents to the sensory characteristics of wine has been established.

2. Monoterpene Biosynthesis

Terpenes arise from either carbohydrate or lipid metabolism, although the metabolic pathways are not well understood. Monoterpenes are produced from mevalonic acid which in the presence of ATP produces

mevalonate-5-phosphate and mevalonate-5-pyrophosphate. This activation step precedes decarboxylation and dehydration of mevalonate to form isopentyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). These two intermediates are joined in a head to tail condensation to form linaloyl pyrophosphate, neryl pyrophosphate and likely other acyclic and cyclic monoterpenes (Croteau et al., 1972).

Monoterpenes with few exceptions are derived biogenetically from an acyclic precursor containing two isoprenoid units linked in a head-to-tail fashion. The fundamental role of prenyl pyrophosphates in the metabolism of all isoprenoid compounds is known. A proposal for monoterpene cyclization ascribes a key role to the acyclic precursor - neryl pyrophosphate or to linalool pyrophosphate (Attaway et al., 1967).

3. Forms of Monoterpenes

Williams et al. (1982c) listed two general classes of monoterpenes in grapes: 1) those bound to sugars and therefore nonvolatile (PVT or potentially volatile terpenes), and 2) free or unbound compounds (FVT or free volatile terpenes). The FVT group contains both free, odorous compounds and a group of non-odorous polyhydroxylated derivatives, e.g., polyols. The glycosides (PVT) can undergo acid or enzyme hydrolysis to yield free volatile terpenes (FVT) or alternatively, can yield odorless polyols which themselves can undergo acid hydrolysis to form aroma components.

In grapes such as White Riesling, about 85% of the total monoterpene content is tied up as glycosides and polyols - the non-aroma components or PVT. This leaves only about 15% as aroma constituents. Gunata et al. (1985b) reported that, for Muscat and Riesling varieties, bound terpenes are in concentrations 3-5 (or more) times higher than the free forms. There has not been a satisfactory justification to explain why certain varieties consistently produce more monoterpenes than others, although a

basis for varietal differences in the terpene concentration may be the oxidative complement occurring in the fruit. The less effective this metabolism is, the more aromatic the fruit. Strauss et al. (1987), reviewed pathways available for linalool, a principle monoterpene in Muscat grapes. These pathways include:

1. Glycosylation. It is believed that monoterpenes are glycosylated after synthesis and that the process may aid in transport of these compounds. Glycosylation takes place after extra hydroxyl groups are introduced into the monoterpene skeleton (Strauss et al., 1988). This is consistent with the view that Glycosylation is usually the terminal step in any biosynthetic pathway (Hosel, 1981).
2. Epoxidation. Enzymatic epoxidation of the 6, 7 double bond can occur to produce 6, 7 epoxydihydrolinalool. This epoxy can open to produce a series of polyols which can then become glycosylated or alternately epoxides can cyclize under acid conditions to produce linalool oxides.
3. Enzymatic hydroxylation. Enzymatic allylic hydroxylation can form two isomers of 8 hydroxylinalool, present mainly as glycosides.
4. Addition of water. The addition of water across the 6, 7 double bond of linalool produces an enediol. This reaction is believed to be nonoxidative and may or may not involve an enzymatic transformation.

Strauss et al. (1987) suggested that the reactions of linalool may be cultivar dependent. For example, in Muscats, glycoslation and enzymatic Epoxidation may be the most important reactions affecting linalool. In White Rieslings, metabolism of linalool mainly involves glycosylation and epoxide formation to pyran and furan oxides and the production of enzymatically catalized allylic hydroxylation to form eight hydroxylinalool derivatives. Biochemical transformations similar to those

outlined are believed to occur with citronellol, nerol, geraniol and α-terpinol (Park and Noble, 1993).

Research has revealed that flavor compounds are not end products of monoterpene biosynthesis in the grape. Oxidative pathways leading to flavorless polyhydroxylated forms of monoterpenes are active in *Vitis vinifera*. In grapes, polyols are important because they greatly decrease the sensory threshold of the particular monoterpene. According to Kjosen and Liaaen-Jensen (1973), monoterpene diols (polyols) can form by photohydroperoxide synthesis, and by photosensitized oxygen transfer onto the double bond in the carbon 6 position of acyclic monoterpenes with the subsequent reduction of the corresponding hydroperoxides to diols. Additional formation involving acid catalyzed H_2O addition to the C-7 double bond of alcohols yields hydroxyl alcohols. The oxidative products of monoterpenes are either nonodorous or have threshold levels greater than 3000 μg/L. Therefore, cultivars which do not have the biochemical mechanisms to degrade monoterpenes oxidatively would produce fruit with greater aroma intensity.

4. Significance of Polyols and Glycosides

Williams et al. (1980), and Rapp et al. (1980) and Abbott et al. (1993) have reported that polyols, though odorless, are acid labile and can form volatile flavorants. This transformation can occur at both ambient temperatures and within the pH of grape juice (3.0 to 3.8). Park and Noble (1993) and Gunata et al. (1994) reported that the enhancement of monoterpene volatiles as a result of heating grape juice (with the exception of nerol, geraniol and pyran linalool oxides) can be attributed to the rearrangement products of polyols.

Glycoside hydrolysis is a major pathway for the liberation of free aroma compounds (Williams et al., 1982c). This hydrolysis explains the observations of enrichment of linalool, geraniol, nerol and α-terpineol in

heated juice. Strauss et al. (1986) suggest that water solubility of glycosides and the presence of an allylic glycosidic linkage may determine the reactivity of these substrates. Enzymatic hydrolysis has also been utilized for the study of naturally occurring glycosides and aglycones. Williams et al. (1983) and Gunata et al. (1994) examined monoterpenes liberated by enzymatic hydrolysis which produced monoterpene alcohols with primary, secondary and tertiary hydroxyl functions. Additionally, two aromatic alcohols were found to be liberated from the disaccharides. These alcohols, benzyl and 2-phenylethanol, are not readily liberated by mild acid hydrolysis. Although these compounds may play a role in the sensory characteristics of resultant wines, grape cultivars cannot be separated based upon their aromatic alcohol content (Gunata et al., 1985b).

5. Hydrolytic Enzymes of Grapes and Glycosides

Aryan et al. (1987) isolated two β-glucosidases, a galactosidase and two weak α-glucosidases from Muscat grapes. In all cultivars studied, the hydrolysis function of β-glycosidase was active with the β-galactosidase activity dominant. The β-glucosidase was located principally in the juice with activity of this and the other enzymes increasing with maturity. β-glucosidase activity increased eight fold between 13.8°Brix and 23.6°Brix. A pH optimum for β-glucosidases was reported as 5.0.

Aryan et al. (1987) suggested that a natural inhibitor of β-glucosides must exist in grapes. Such inhibition would explain the relatively high levels of glycosides versus free monoterpenes. They also note that the main determinate of the hydrolysis rate is the structure of the aglycone. Native enzymes are unable to hydrolyze sugar conjugates of tertiary alcohols, such as linalool and dienediol 1 (3,7-dimethyloca-1,5-diene 3, 7-diol).

The glycoside mixture from Muscat grapes consisted of β-rutinosides

(6-0-α L rhamnopyranosyl-β-D gluco-pyranosides) and 6-0-β L arabinofuranosyl β-D glucopyranosides (Williams et al., 1982a,b). The hydrolysis products of these sugars were mainly linalool, nerol, and geraniol, with traces of α-terpineol. Strauss et al. (1986) suggested that linalool oxides were also present in disaccharide glycosides and Wilson et al. (1984, 1986) and Gunata et al. (1994) demonstrated that several polyhydroxylated compounds are also bound to sugars though glycosidic linkages.

6. Monoterpene Transport and Turnover

Monoterpenes are in a rather high state of flux. For example, Croteau (1984) reported a 50% transformation in several weeks, including both losses and glycosylation. The formation of glycosides has been postulated as a possible transport mechanism. In one study with peppermint, half of the terpene glycosides had been transported from the leaf to the rhizome within eight hours while thirty-six hours later, half of the terpene content of the rhizome had broken down to other metabolites (Croteau, 1984). This suggests that catabolic transformation may occur at sites other than those of synthesis. Williams et al. (1981) suggested that the grape hypoderma cells are the sites of both biosynthesis and storage of geraniol, with the precursor materials of this component produced in the leaves. Support for this belief has come from grafting trials, in which clusters of one variety were grafted to shoots of another. Winkler et al. (1974) reported Muscat Albardiens grapes grafted to Olivette Blanche vines had a characteristic, Muscat aroma and flavor. The fruit of the aroma neutral Olivette grafted to the Muscat vines were typically neutral with the characteristic Olivette aromas and flavors.

Croteau (1984) stated that there are two types of monoterpene turnover mechanisms. Short-term turnover involves the balance between photosynthesis and utilization of photosynthates. When net photosynthesis

exceeds utilization, an active intracellular pool is created. The second turnover mechanism occurs during late development and results in the net reduction of terpenes. This mechanism involves the breakdown of stored terpenes located in extra-cellular compartments. It has been suggested that conjugation and transport of monoterpenes could be a response to a collapse of oil glands late in leaf development.

7. Monoterpene Levels in Grapes

Several surveys have been conducted to determine the monoterpene content of various grape cultivars. Some studies have reported free monoterpenes, others bound. Because quantitative data were obtained by different techniques resulting in varying degrees of sugar and polyol hydrolysis, direct comparisons are difficult. Strauss et al. (1986) summarized these studies and grouped grape cultivars into three classifications: 1) intensely flavored Muscat varieties which contain monoterpenes in concentrations of up to 6 mg/L; 2) aromatic non-Muscat varieties, such as Rieslings, Gewurztraminer, Scheurebe, Muller-Thurgau, which have a monoterpene concentration of 104 mg/L.; and 3) neutral varieties such as Cabernet Sauvignon, Chardonnay, Merlot and Thompson Seedless, etc., which are not dependent upon monoterpenes for their flavor.

8. Changes in Monoterpene Concentration with Changes in Maturity

Several researchers have charted the changes in free monoterpene composition with grape development (Hardy, 1970; Bayonove and Cordonnier, 1971; Terrier et al., 1972). Linalool is absent in the unripe Muscat grapes but appears at the beginning of ripening along with the Muscat aroma. Concentrations of linalool continue to increase until fruit maturity is reached, then decrease. Little work has been reported on the changes in either glycosides or polyhydroxylated monoterpenes with increased fruit maturity. Wilson et al. (1984) noted five phases of

monoterpene development during berry ontogeny. At set, high levels of both free and bound geraniol were noted in Muscat of Alexandria. Levels of all monoterpenes studied decreased near veraison (color change). During the rapid sugar accumulation which occurs after veraison, monoterpene levels increased. Several monoterpenes reached maximum levels in overripe fruit. Of interest is the fact that, after veraison, linalool, geraniol, nerol, α-terpineol and the furan linalool oxides were present mainly as glycosides. While the total concentration of glycosides in Muscat grapes increased during ripening, only free linalool showed development that paralleled the glycoside increase. They reported that the concentration of free dienediol 1 was greater than the total concentration of all other monoterpenes in Muscat grapes. Therefore, concentrations of glycosides and polyols exceeded the free flavor compounds. Versini et al. (1981, 1987) determined the monoterpene concentration of Weisser Riesling grape during final ripening and noted both increases and decreases of different compounds. Their study showed that maximum aroma can be obtained before maximum sugar accumulation. According to Bayonove and Cordonnier (1970 ,1972), ripe Muscat grapes (approximately 22°Brix) contain about six times more linalool, five times more α-terpineol, four times more nerol and 1.4 times more geraniol than unripe grapes. These researchers also reported that citral and citronellol occurred in nearly equal concentrations in the unripe and ripe grapes. Marais and van Wyk (1986) showed that maximum monoterpene concentration was reached prior to commercial harvest (22°Brix), and that beyond this level of maturity, the monoterpene concentration began to decrease. The conflicts reported in the literature may stem from the vastly different macroclimates used in these investigations, as well as isolation and analysis techniques.

9. Grapevine Climate and Monoterpenes

Canopy microclimate factors include radiation, temperature, humidity and evaporation with grapevine leaves being the major cause of microclimate variations within the canopy (Smart, 1985). The presence of fruit, shoots, stems and permanent vine parts play only a minor role in microclimatic variation within the canopy. Grape berries maturing in densely shaded canopy compared with open or exposed canopies are generally associated with the following: 1) low soluble solids, 2) high titratable acidity, 3) high malate concentrations, 4) elevated pH, 5) high potassium, 6) low fruit proline, 7) high arginine, 8) low total phenols, and 9) low anthocyanin concentration in red and high chlorophyll vs. flavonoid pigments in white cultivars (Kliewer and Lider, 1968; Smart, 1976l; Kliewer, 1980; Smart, 1985; Morrison and Noble, 1990; Zoecklein et al., 1992). The cause of these differences in fruit chemistry is due primarily to fruit exposure to sunlight and heat (Smart, 1985).

Monoterpenes can be influenced by various environmental factors such as soil and climate (Clark and Menary, 1980; Lawrence, 1986; Rapp, 1988). According to Fluck (1963), the most important extrinsic factors affecting essential oil production in plants are climate (temperature and light) and soil (nutrients and water). Manipulation of grapevine canopy climate may affect photosynthate movement within the grapevine. Carbohydrates are translocated in the phloem of the vine principally in the form of sucrose, while in the xylem fluid, relatively large amounts of amino acids, organic acids, inorganic nutrients and small amounts of sugars are transported (Winkler et al., 1974). Koblet (1977) studied the movement of CO_2 in grapevines and showed that not only leaves of the main shoot, but also those of laterals, begin to export photosynthates when they had reached about 30% of full size. Shaded leaves, on the other hand, showed practically no export of carbohydrates. Koblet (1977) also showed that the lowest leaf on a shoot exported most of its carbohydrates basipetally.

Until the blossom stage, the direction of translocation from the leaves in the middle of the shoot is bi-directional. Upper leaves export their assimilates mainly to the shoot tips. As growth continues, the predominant movement of photosynthates from higher leaves on a shoot is basipetally, principally into the berries. At the end of berry ripening, there is almost no export of assimilate from basal leaves. Defoliation of the upper part of a shoot induces upward movement of assimilates from lower leaves.

Research indicates that monoterpenes are important contributors to the aroma of certain grapes and wines. It seems possible that vineyard management factors which change the grapevine microclimate may influence the monoterpene concentration and therefore potential wine quality. By practicing 'Viticultural winemaking', that is, growing grapes to maximize the aroma and flavor potential, higher quality wines and more specific styles may be produced.

10. Fermentation and Monoterpenes

The aroma of grape juice is dependent upon a small group of free secondary metabolites that have escaped oxidation to polyols or conjugation. Glycosylation and oxidative steps such as hydroxylation are natural processes that remove components as direct contributors to aroma. While the studies performed by Zoecklein (1992) demonstrated increases in potentially volatile terpenes (PVT) and specific bound components as a result of canopy manipulation, oxidative products themselves have no direct aroma value. There is, however, some reclamation of aroma available through acid catalyzed hydrolysis of polyols and/or enzymatic hydrolysis of glycosides. Hydrolysis can produce a wide range of volatiles that may broaden and enhance the complexity of juices and wines. The use of yeast species and strains with improved hydrolytic activity could help to 'unlock' potential aroma components produced by canopy

modification. Currently, there are several yeast strains of *Saccharomyces cerevisiae* marketed as 'enhancers' of grape varietal expression. The implication is that such yeasts have increased ability to hydrolyze conjugated aroma precursors, improving the aroma and aroma intensity of the resultant wine.

The grapevine canopy manipulations discussed in Chapters 2 and 3 had a limited influence on free aroma compounds, but increased the concentration of oxidation products. Thus, these experiments suggest that environmental factors influence monoterpenes in grapes to an extent that can be measured analytically. Increases in PVT could be explained by increase in sunlight or thermal differences between control and treated vines, and or by changes in the sink - source relationship. The increase in conjugated aroma components can have a practical influence on wine character if PVT hydrolysis occurs.

The aroma of wines is a function of the components produced during juice processing, fermentation and aging and have been characterized by Rapp (1988) as follows: 1) primary grape aromas, compounds as they are found in undamaged plant cells; 2) secondary grape aromas, aroma compounds formed during crushing and pressing and by chemical, enzymatic-chemical and thermal reactions in grape juice; 3) fermentation bouquet, aroma compounds formed during the alcoholic fermentation; and 4) maturation bouquet: caused by chemical reactions during maturation of bottled wines. Of these sources of aroma, the primary grape aroma is of critical importance to wine quality (Amerine et al., 1980).

In grapes such as White Riesling, about 85% of the total monoterpene content is tied up as conjugated products or polyols. This leaves only about 15% as free to contribute to aroma. Research suggests that aroma and components of grapes are not end products of synthesis. Oxidative pathways leading to odorless compounds such as polyols and glycosides are

active. The presence of ß-rutinosides (6-O-α-L- rhamnopyranosyl-ß-glucopyranosides) and 6-O-α-L arabinofuranosyl-ß-D-glycopyranosides of Muscat grapes has been established (Williams et al. 1982b). However, glycosidic non-volatile precursors can give rise to aroma enhancing aglycones only as a result of hydrolysis. Acid hydrolysis of grape glycosides has been studied as a means of releasing bound monoterpenes with a view to enhancing the aroma of grape juice through the formation of free volatiles (Williams et al., 1982c, Abbott et al., 1993). Some hydrolysis would be expected during alcoholic fermentation as a result of the production of Kreb cycle acids, heat generation and the result of glycosidases produced by yeasts. However, acid hydrolysis alone promoted by excessive heat (>50°C) causes extensive rearrangement of monoterpenes (Williams et al., 1982c).

As an alternative, enzymatic hydrolysis would seem to be an attractive method for releasing the flavor potential of PVT's. Aryan et al. (1987) showed that the native β-glucosidases of grapes have a ph optimum of 5.0 and are inhibited by glucose. These two aspects suggest that in grape juice at natural pH (3.0-3.8) the ß-glycosidases activity is low.

Use of exogenous enzymes for aroma and flavor enhancement has been studied (Park and Noble, 1993). It is established that enzymes for flavor and aroma enhancement must contain β-glucosidase, α-arabinosidase, α-rhamnosidase, β-xylanosidase, and β-apiosidase activity. Additionally, these glycosidases should be capable of hydrolyzing both mono and diglycosides of primary and tertiary alcohols, norisoprenoids and shikimic acid derivatives. While enzymatic hydrolysis is less destructive than acid hydrolysis, commercially available products provide only limited activity in grape juice and wines. Most enzymes are reported to be relatively non-selective with pH optima between 5.0-6.0. Their pH optima

and glucose and alcohol sensitivity currently limits application. Additionally, plants are known to employ sugars with both ester and glycosidic linkages and several non-sugar moieties for conjugation (Betz and Koster, 1981). Thus, considering glucosidases hydrolysis alone may not give a complete picture of the bound aroma/components. These limitations coupled with FDA/BATF regulations regarding additives suggest the possible need for alternative methods of un-locking the potential of bound aroma constituents.

Several experiments have demonstrated the hydrolytic action of yeasts on monoterpene glycosides during fermentation (Cordonnier et al., 1975; Gunata et al., 1986). Total free monoterpenes in Muscat of Alexandria grapes have been shown to increase with a corresponding reduction in the total bound monoterpenes during fermentation and storage (Park and Noble, 1993). These changes were the result of hydrolysis occurring in an acidic media.

Gunata et al. (1986) reported the changes in the major free monoterpenes in Muscat of Alexandria grapes during fermentation with an unspecified strain of *Saccharomyces cerevisiae*. Geraniol, present initially at 49 μg/L, decreased to 5 μg/L immediately after fermentation. The free geraniol concentration slowly increased during wine aging as a result of hydrolysis of bound geraniol. In the same study, free α-terpineol initially present as a minor terpene in Muscat of Alexandria grapes (2 μg/L) increased slowly during fermentation to 5 μg/L and further increased to 70 μg/L in wines stored at 10°C following fermentation. However, in the above mentioned study, the majority of the terpene glycosides (from 77% to 89%) were not hydrolyzed during fermentation. Gunata et al. (1994) examined several enological yeasts and reported only limited influence on the release of volatiles from glycosides. It may be that yeast cells do not excrete ß-glucosidase activity and that glycosides

are not transported across the cell wall. Leighton (1994), suggests that carbon catalyzed repression of α-glucosides occurs during fermentation. Post fermentation lees contact in the absence of glucose, however, does show α-glucosidase to be active. With standard processing techniques, a large percentage of the aroma potential of the grape remains unused.

The biochemical mechanisms utilized for monoterpene production in White Riesling are also present in non-terpene varieties. Non-terpene varieties also derive their flavor and aroma from free volatiles while the majority of the potential is locked up in the form of conjugation products. Thus, the study of changes in grape monoterpenes resulting from vineyard management and research on the influence of fermentation on White Riesling aroma compounds may have direct relevance to other grape varieties.

MATERIALS AND METHODS

I. FRUIT COMPOSITION: Grape samples were maintained at -25°C until analyzed, except fruit used for sensory evaluation at harvest. In preparation for analysis, berry samples were warmed to 1°C, macerated and placed for one minute in a Tekmar model 400 laboratory stomacher. Standard AOAC analysis procedures were used, as described by Zoecklein et al. (1990), unless otherwise noted. Soluble solids (°Brix) and sugar per berry were determined with an American Optical temperature-compensating, hand-held refractometer, and pH and titratable acidity with a Fisher model 815 pH meter. Succinic, malic and tartaric acids were determined by HPLC using a BioRad isocratic system model 1306 uv detector at 210 nm, and Aminex ion exclusion HPX-87H (300mm x 7.8mm) column. Fruit potassium was determined by segregation of a 50g subsample from each replicate following the procedures of Mattick (1983) or Morrison and Noble (1990).

II. AROMA COMPONENT ANALYSIS

A. Analysis of FVT and PVT.

A method developed by Attaway et al. (1967), and modified by Dimitraidis and Williams (1984), was further modified to determine the FVT and PVT terpene fractions in various juice samples as follows:

1. Sample preparation. Berries stored at -25°C were thawed to approximately 1°C, destemmed and weighed. Grapes with visible signs of rot were eliminated. A 500 gram sample was then homogenized in a laboratory blender for 30 seconds at high speed, and placed in a laboratory stomacher (Tekmar model 400) for one minute, separating the solids from the juice. The pH of the juice was then adjusted to 6.8 by dropwise addition of 20% NaOH while mixing.

2. Distillation. 100 mL of the near-neutral juice was placed in the inner tube of a double boiler steam distillation apparatus in which distilled water in the outside flask generated steam to assist the separation of the free volatile monoterpenes. Exactly 25 mL of the condensate (FVT) was collected, and either saved for spectrophotometric analysis, or frozen at -25°C for further extraction and gas chromatographic analysis.

Without interrupting the steam flow, the juice was acidified with 50% (v/v) H_3PO_4 delivered from the side arm funnel into the still. Exactly 40 mL of the PVT distillate was collected, and either frozen at -25°C for subsequent extraction and gas chromatographic analysis, or taken for spectrophotometric development.

The volume of phosphoric acid required to obtain a consistent pH of 1.7 was determined for each fruit lot. This was necessary due to differences in the tartrate/malate ratio resulting from vineyard treatment and maturity within treatments. The buffering capacity, and therefore the volume of H_3PO_4 required to obtain a pH of 1.7, was determined by the following method: A 100 mL sample of juice was diluted to 150 mL with distilled water, and then neutralized to pH 6.8 with 20% NaOH. The volume of 50% (v/v) phosphoric acid required to reduce the pH of the diluted, near neutral sample was noted.

3. Spectrophotometric determination of the distillates. A stock solution of linalool (*ca.* 1 mg/mL) was prepared by weighing 50 mg of linalool (Aldrich Chemical Co., Milwaudee, WI.) into a 50 mL volumetric flask, dissolving in 10 mL of ethanol and making to volume with distilled water. Standard solutions were then prepared by 1 in 10 dilution with distilled water. A standard curve was prepared by using the following volumes of the linalool standard solution: 0.2 mL (20 μg), 1.0 mL (100 μg), 1.5 mL (150 μg), and 2.0 mL (200 μg), each made to 10 mL using

distilled water. A sixth sample containing only water was established as a blank.

A solution of 2% (v/v) vanillin in concentrated H_2SO_4 was prepared and stored in a ground glass-jointed bottle at 0-4°C until needed. Standard samples and distillates (10 mL) were placed in an ice bath for 20 minutes. Five mL of 2% vanillin-sulfuric acid solution was slowly added to each tube to avoid heat build-up. Samples were thoroughly agitated and further cooled in the ice bath for 5 minutes.

Following reaction with the vanillin-sulfuric acid reagent, the color was developed by heating the tubes in a water bath at 60°C $\pm$ 1°C for 20 minutes. After cooling at 25° C for 5 minutes, the optical density (OD) of the blank and standards was read at 608 nm using a Bausch and Lomb model 21 spectrophotometer and 1 cm disposable plastic cuvettes. Optical density vs. concentration was used for establishment of standard curves. The following formula provided terpene quantitation:

$$\text{FVT or PVT (mg/L)} = \frac{\mu g \text{ linalool x volume distillate collected}}{\text{volume of juice x 10}}$$

B. Determination of Free and Bound Monoterpenes and Aromatic Alcohols.

A sample of Amberlite XAD-2 (Rohm and Hass, Philadelphia, PA, 50 mesh) was poured into a 48 x 2.5 mm Soxhlet exactor column and refluxed with methanol, acetonitrile and dimethyl ether each for eight hours. The resulting resin was then dried and stored under methanol.

Grape samples stored at -25°C were warmed to 1°C, and a 100 or 500 gram sample placed into a laboratory mixer and ground for 30 seconds at high speed. Crushed grapes were then placed into a Tekmar model 400 laboratory stomacher for one minute. Sulfur dioxide (50 mg/L) and ascorbic acid (200 mg/L) were added to the juice samples to help minimize

oxidative rearrangement and the samples were cold centrifuged at 1°C for 20 minutes at 10,000 RPM.

1. Fractionation of Free Monoterpenes and Aromatic Alcohols

The pretreated Amberlite XAD-2 resin (10 cm^3) was placed into a 35 x 10 cm liquid chromatographic column fitted with a PTFE tap and glass wool plug on the top and bottom. Two 25 mL volumes of methanol followed by 25 mL of dimethyl ether and water were run through the column.

Ten μl of a 0.1% solution of 2-octanol in ethanol was used as an internal standard. The juice sample was mixed and added to the column at a flow rate of 2.5 mL/minute, and followed with 50 mL of glass distilled water, passed through an Amberite XAD-2 column to remove sugars, acids and other water soluble compounds. The free aroma compounds were then extracted with 50 mL of pentane at a flow rate of 2.0 to 2.5 mL/minute. Pentane was used for the elution of the free fraction because it provides adequate solubility without removing the glycosides remaining on the column. The pentane extract was then dried with anhydrous $MgSO_4$ and concentrated to a final volume of 50 μL at 40°C. Samples were stored at -25°C pending gas chromatographic analysis.

Free terpene diol were isolated on XAD-2 by using a modified solvent consisting of a mixture of pentane: dichloromethane (2:1) as suggested by Versini et al. (1987).

2. Fractionation of Bound Monoterpenes and Aromatic Alcohols

The bound terpenoid compounds and aromatic alcohols were then fractionated by eluting the column with 50 mL of ethyl acetate at a flow rate of 2.5 mL/minute. The glycosidic fraction was dried over magnesium sulfate, filtered and concentrated to 1 mL under vacuum at 40°C. The samples were then taken to dryness at 45°C using a stream of nitrogen gas.

a. Enzyme Hydrolysis of the Bound Fraction

Phosphate buffer (0.1 mL of 2.10^{-1} M citrate-phosphate) at pH 5.0 was added to the glycoside mixture and rinsed four times with 0.1 mL of pentane to remove any free or unbound terpenes. Samples were agitated using a laboratory vortex between rinses.

One of three commercial pectinolytic enzymes (Rohm-Tech Inc., Rohapect VS Super, Rohapect 7104, or Rohapect C) was added (0.4 mg/L) in 0.1 mL citrate-phosphate buffer. The sample was then mixed again, sealed and placed in a water bath for 24 hours at 40°C. Following incubation, the mixture was rinsed into 50 mL of citrate-phosphate buffer, pH 5.0, and 10 μL of a 0.1% solution of 2-octanol in ethanol added. The solution was then extracted with 5x5 mL Freon 11 and concentrated to 50 μL and stored at -25°C pending gas chromatographic analysis.

b. Acid Hydrolysis of the Bound Fraction.

Monoterpene glycoside samples (250 μL) were each dissolved in 3-5 mL of tartrate buffer at pH 3.2. The solution was extracted with cold Freon F11 (2x10 mL) to ensure the removal of volatile components prior to acid hydrolysis, and heated over a steam bath for 15 minutes, cooled and re-extracted with Freon F11 (3x12 mL). The solvent extract of the hydrolysis was made to 40 mL. A 5 mL portion of this solution was then concentrated to 50 μL by distillation through a glass column at 35°C. The residue was cooled and used for GC-MS analysis.

C. Gas Chromatography - Mass Spectrometry

Gas Chromatography was accomplished on a Hewlett-Packard gas chromatograph (model 5890A) with a split-mode capillary injection port and a flame ionization detector using a DB-5 bonded phase silica capillary column, (95%) dimethyl (5%) diphenyl polysiloxane - J&W Scientific) under the conditions outlined in Table 1. Volatile compounds were identified by comparing their mass spectra and retention times with published spectra

(Cornell Libary) and authentic standards (Aldrich Chemical Co., Milwaukee.

Table 1.

Conditions for Gas-Liquid Chromatography

Carrier gas flow rate (He)	1.30 mL/min
H_2 flow rate	30 mL/min
Air flow rate	350 mL/min
Make-up gas flow rate	30 mL/min
Column[a]	DB-5 (.25μm x 30m)
Split ratio	90:1
Injection size	1 μL
Injection temperature	250°C
Detector temperature	250°C
Oven temperature program	40°C/3 min175°C/ 3 min. to 21°C for 20 min.

[a]J&W Scientific

WI). Mass spectra for the compounds of interest are given in Table 2. Data was taken from mass spectra of known substances with the numbers in italics being the base peaks of those compounds. The common names, chemical names and structures of the compounds of interest are given in Table 3.

Gas chromatography mass spectrometry was performed using a Hewlett-Packard Model 5790 GC, V. G. Analytical Inc. Model 7070E election impact mass spectrometer, 70 eV scanning upwards from m/2 50 to m/2 500 at a rate of 2 sec/decade.

Table 2.

Mass spectra data of aroma components under study.

		m/e (%)
1.	rose oxide-cis	55(40) 69(65) 83(32) 97(4) 139(**100**) 154(16)
2.	trans - furan linalool oxide	55(40) 69(65) 83(32) 97(4) 139(**100**) 154(16)
3.	cis - furan linalool oxide	43(49) 59(**100**) 68(33) 81(20) 94(40) 111(30)
4.	nerol oxide	41(18) 53(13) 55(15) 67(66) 68(**100**) 69(13) 83(72) 96(11)
5.	linalool	55(12) 65(12) 68(20) 77(35) 93(**100**) 105(11) 121(35) 136(25)
6.	nerol oxide	53(35) 67(70) 68(**100**) 83(82) 85(30) 91(10) 96(10) 109(10) 152(10)
7.	hotrienol	41(14) 43(52) 53(7) 55(9) 67(48) 71(**100**) 79(8) 82(70)
8.	α-terpineol	55(15) 65(19) 67(35) 68(35) 77(40) 79(39) 91(43) 93(**100**) 107(15) 121(45) 136(30)
9.	trans - pyran linalool oxide	41(26) 43(50) 59(80) 87(53) 68(**100**) 79(20) 94(63)
10.	cis - pyran linalool oxide	41(26) 43(50) 59(80) 87(53) 68(**100**) 79(20) 94(63)
11.	nerol	55(10) 69(**100**) 80(10) 84(18) 93(27) 97(5) 111(5) 121(10) 154(5)
12.	geraniol	55(12) 69(**100**) 93(15) 111(5) 123(10)
13.	v-terpinene	27(30) 39(29) 51(15) 65(14) 77(35) 93(**100**) 105(10) 119(38) 136(30)
14.	phenethyl alcohol	39(10) 51(8) 65(15) 78(5) 91(**100**) 92(55) 104(18) 122(26)
15.	benzyl alcohol	27(16) 39(22) 51(40) 77(70) 79(**100**) 91(22) 107(70) 108(90)
16.	diol I	27(27) 39(17) 41(24) 43(86) 53(10) 55(23) 67(52) 71(93) 82(**100**) 85(10)
17.	diol II	18(18) 27(12) 41(21) 43(51) 67(**100**) 68(33) 69(18) 71(84) 82(47) 109(10)

Table 3.

Common and chemical names of compounds under study

Common Name	Chemical Name
trans-Furan linalool oxide	5-Ethenyltetrahydro-∝,∝,5-trimethyl-2-furanmethanol, trans
cis-Furan linalool oxide	5-Ethenyltetrahydro-∝,∝,5-trimethyl-2-furanmethanol, cis
nerol oxide	3,6-Dihydro-4-methyl-2-(2-methyl-1-propenyl-2H-pyran
linalool	3,7-Dimethyl-1,6-octadien-3-ol
hotrienol	3,7-Dimethyl-1,5,7-octadien-3-ol
∝-terpineol	∝,∝,4-Trimethyl-3-cyclohexene-1-methanol
trans-Pyran linalool oxide	6-Ethenyltetrahydro-2,2,6-trimethyl-2H-pyran-3-ol, trans
cis-Pyran linalool oxide	6-Ethenyltetrahydro-2,2,6-trimethyl-2H-pyran-3-ol, cis
nerol	3,7-Dimethyl-2,6-octadien-1-o1,Z
geraniol	3,7-Dimethyl-2,6-octadien-1-ol,E
diol I	3,7-Dimethylocta-1,5-dien-3,7-diol
diol II	3,7-Diemthylocta-1,7-dien-3,6-diol
diol III	3,7-Dimethyloct-1-en-3,6,7-triol
diol IV	3,7-Dimethyloct-1-en-3,7-diol 2,6-Dimethyl-27,7-octadiene-1,6-diol

CHAPTER I

Recovery Studies of Monoterpene Alcohols, Hydrocarbons, Oxides and Aromatic Alcohols

Abstract. A polymeric styrene resin (XAD-2) was evaluated for its ability to adsorb and desorb five monoterpene alcohols, three monoterpene hydrocarbons, four monoterpene oxides, two aromatic alcohols and a glucopyranoside from grape juice at two pH's. The recovery of free aroma compounds and the efficiency of XAD-2 was compared to that of a Freon 11 continuous extraction system. Percent recovery varied with each compound and averaged 90% or greater for both systems. Coefficients of variation was less than 4% with XAD-2 and 9% for the continuous system for all compounds. Optimum procedures for the desorption from XAD-2 and concentration of the compounds of interest were established.

Introduction

Sample preparation is a critical step in the analysis of aroma compounds. Volatiles are frequently intracellular and compartmentalized, requiring cellular disruption creating a complex matrix of water, lipids, phenols, carbohydrates, proteins, and insoluble materials. Such a matrix precludes direct chromatographic analysis. Cellular disruption can also lead to instability and chemical rearrangement of aroma compounds (Jenning and Rapp, 1983). Differences in the chemical complexity and variations in volatility add an additional measure of difficulty to accurate analysis. The concentration of aroma components are frequently low (ppm or less), thus requiring not only isolation of the volatile materials but also concentration of those materials by several orders of magnitude.

There is no universal technique for the isolation and preparation of all biological samples under all conditions. Techniques for the isolation of volatile compounds in juice and wine include the use of solvents such as trichlorofloromethane (Freon 11), carbon disulfide, dimethyl chloride, and pentane (Rapp et al., 1978). The use of Freon 11 in the isolation of juice and wine volatiles was investigated by Rapp et al. (1978) and found to be suitable for monoterpenes, including polyhydroxylated linalool derivatives. Marais (1986) proposed a continuous extraction technique for the isolation of grape monoterpenes using Freon 11 requiring an extraction period of several days. Aside from the time involved, a disadvantage of lengthy solvent extraction includes possible degradation and/or rearrangement (Jennings and Rapp, 1983).

Junk et al. (1974) proposed the use of porous macroreticular resins such as XAD-2 for concentrating a large number of compounds (in the range of 0.02 to 50,000 ppb). Zygmunt et al. (1983) showed that adsorption on XAD-2 is a feasible technique for the analysis of selected pollutants in industrial waste water. Hawthorne et al. (1987) reported the use of XAD-2

resin for the adsorption of volatile components in beer, while Edwards and Beelman (1990) utilized XAD-2 resins for such analysis in wines. Gunata et al. (1985b) reported the successful use of XAD-2 resins for retaining both free and bound grape and wine monoterpenes, but did not provide complete recovery data.

The XAD resins are nonpolar, agglomerated microspheres which expose a large surface area of adsorbent to solute molecules. The highly macroreticular structure allows rapid diffusion of solvents and solutes throughout the resin (Hawthorne et al., 1987). Adsorption of the solutes on the resin is determined by the hydrophobicity of the solute. The more hydrophobic a sorbate, the more firmly it is bound. This resin displays extraction capacities similar to those of coated silica (Di Stefanco and Guidoni, 1989). Strongly ionized compounds are not retained, and weakly ionic compounds may or may not be retained, depending upon the degree of ionization (Burnham et al., 1972). Retention efficiency is higher for aromatic compounds and lower for low molecular weight aliphatics (Jennings and Rapp, 1983).

The aroma components of grapes are present in either the free state or bound to sugars usually in the form of glycosides. The existence of a non-volatile, bound fraction has been shown and characterized (Cordonnier and Bayonove, 1974; Williams et al., 1982a). The bound aroma fraction of Muscat grapes consists of the disacchoride glycosides α,L-arabinofuransyl-ß,D-glucopyranoside or α,L-rhamnofuranosyl-ß,D-glucopyranoside. The aglycone of these compounds can be a terpenol, a terpene diol, 2 phenylethanol or benzyl alcohol (Williams et al., 1982a; Williams et al., 1982b; and Wilson et al., 1984).

This study was undertaken to evaluate and compare the percent recovery of aroma components using XAD-2 and a Freon 11 continuous extraction system. Procedures for desorption from XAD-2 and concentration

were established.

Materials and Methods

Amberlite XAD-2 (20-50 mesh-300m^2/g active surface area - 90A average pore diameter) was obtained from Rohm-Haas (Philadelphia, PA). Fines were removed by decanting after slurring in water, and the resin washed in a Soxhlet extractor sequentially with 200 mL each of methanol, acetonitrate, diethyl ether and then methanol again using an 8-hour extraction period for each solvent. The purified resin was stored under methanol in glass.

A 25 mL volume of methanol-slurried XAD-2 resin (6g wet weight) was poured into glass columns (30mm x 1mm) fitted with sylilated glass wool, and glass beads. The resin was washed sequentially with 25 mL of methanol, 25 mL of ether and 25 mL of purified water. Water used in column rinse and standard preparation was deionized, glass distilled and passed through purified XAD-2 resin.

A. Recovery of Free Monoterpenes and Aromatic Alcohols on XAD-2 Resin

1. Blank Analysis. To evaluate resin impurities, water (100 mL) was passed through a glass column containing 6 grams of XAD-2 resin prepared as previously described. The water was then extracted with 3x5 mL pentane, reduced to a volume of 50 μL and a 1 μL sample utilized for GC-MS analysis.

2. Recovery of free monoterpenes and aromatic alcohols. A 500 gram sample of White Riesling grapes stored at -25°C was thawed to 1°C, placed into a laboratory blender and homogenized for 30 second at high speed. The homogenate was placed in a Tekmar model 400 laboratory stomacher for one minute. Sulfur dioxide (50 mg/L) and ascorbic acid (200 mg/L) were added to the sample. The juice was centrifuged at 10,000 rpm for 20 minutes at 1°C and decanted. Ten μL of a 0.1% solution of 2-octanol in ethanol and 0.177 mg of n-octyl ß-D glucopyranoside (Sigma Chemical Co., St. Louis, MO) was added to juice as free and glycosidically bound

internal standards. Additionally, to one lot was added a standard solution containing known concentrations of monoterpenes and aromatic alcohols (listed in table 1) prepared in 5 mL of ethanol (40% v/v). Each juice lot was poured into separate columns and passed through XAD-2 resin at a rate of 2.0 to 2.5 mL/min. Free compounds were eluted from the resin with 50 mL pentane at a rate of 2.0 to 2.5 mL minute and dried over $MnSO_4$, at 45°C using a Vigreux column. The sample was concentrated to 50 μL and used for GC analysis. Recovery analysis was performed in triplicate.

3. Recovery of bound monoterpense and aromatic alcohols. Elution of the glycosides was accomplished using 50 mL of ethyl acetate at a rate of 2.0-2.5 mL/min. Ethyl acetate was purged from the resin column using nitrogen gas. The ethyl acetate was dried with anhydrous $MgSO_4$, filtered and concentrated to 1 mL under vacuum at 40°C.

A citrate-phosphate buffer -pH 5.0 (0.1 mL of $2x10^{-1}$M) was added to the glycoside extract. A pectinol solution (Rohm-Tech, Rotapech-C) was prepared by hydrating 0.4mg of pectinol in 0.1 mL of $2x10^{-1}$M citrate-phosphate buffer -pH 5 as described by Williams et al. (1983) and Gunata et al. (1985b). The enzyme solution was added to each glycoside extract, strongly agitated several times, hermetically sealed and placed in water-bath for 24 hours at 40°C. Following incubation, each reaction vessel was cooled to 5°C, hydrated with 50 mL citrate-phosphate buffer and extracted with Freon 11 (5x5mL). The solvent was eliminated by rectificaiton at 35°C to a final concentration of 50 μL. Recovery analysis was performed in triplicate.

B. Adsoprtion and Desorption Study Using XAD-2 Resin

1. Adsorption. A 100 mL sample of the spiked juice was passed through one column containing XAD-2 resin under conditions previously described, collected and then added to another separate column. Pentane (50 mL) was used to elute each column at a rate of 2.0 mL/min. The

pentane eluated from each column was analyzed separately by first drying and concentrating as previously described. Glucoside adsorption was evaluated using a model solution consisting of 0.177 mg of n-octyl ß-D-glycopyranoside (Sigma Chemical Co., St. Louis, MO) added to 100 mL of deionized-distilled water (pretreated by passage through a column of XAD-2 resin). The solution was then passed through a glass column as described above.

2. Desorption. A 100 mL sample of spiked juice was passed through an XAD-2 column (prepared as previously described) followed by 50 mL of purified water. Both water and juice were extracted with pentane (5x5mL), dried and concentrated for gas chromatographic analysis. Elution of the model glycoside (described under item 1 above) was accomplished using 50 mL of ethyl acetate at a rate of 2.0-2.5 mL/min. Ethyl acetate was purged from the resin column using nitrogen gas, dried with anhydrous $MgSO_4$, filtered, and concentrated as previously described.

3. Evaporation. A standard solution of known concentrations of monoterpenes was prepared in pentane. The solvent volume was then reduced to 50 μL using the concentration methods previously described. A 1.0 μL sample of the concentrated extract was used for GC injection.

4. pH effect. Solutions of known composition were prepared at two pH values, pH 3.5 with concentrate H_2SO_4 and pH using 9N KOH. Each solution was adsorbed, desorbed and concentrated in triplicate as previously described.

C. Continuous Extraction of Free Aroma Components

Continuous extraction of juice using Freon 11 was accomplished with a modified continuous extraction apparatus originally reported by Rapp et al. (1976).

1. Calibration standards and relative recovery. Frozen grapes (500g) were thawed to 1°C, and blended for 30 seconds with NaCl (360g).

The homogenate was then placed in a Tekmar model 400 laboratory stomacher for 1 minute, separated from the solids and titrated with 1M KOH to a pH of 7-8, and centrifuged at 10,000 rpm at 5°C for 20 minutes, with the supernatant decanted and retained. The residue was resuspended in pH 7 phosphate buffer, centrifuged, decanted and combined with the original supernatant. The clarified juice was again saturated with NaCL, and the pH rechecked to assure a value greater than 7.5.

One-octanol (0.077 mg) and n-octyl ß-D glucopyranoside (0.354 mg, Sigma Chemical Co.) were added as free and glycosidically bound internal standards. The juice was divided into two lots. A standard solution containing known concentrations of monoterpenes and aromatic alcohols were prepared in 5 mL of ethanol (40% v/v) and dissolved in one juice lot. Freon 11 (20 mL) was poured into each reaction vessel and a tuft of sylilated glass wool placed on the Freon surface to prevent emulsification. The chilled samples were carefully poured into separate extraction apparatuses, and the bottom 50 mm of each unit placed in ice. A condenser was added to the top of each extraction system with circulated water-glycol solution at -5°C.

A 60 mL pear shaped collected flask containing 20 mL of Freon 11 was fitted to each extraction unit, and immersed in a water bath at 35°C. The extraction proceeded over 72 hours with daily solvent changes. Pooled solvent extracts were dried with magnesium sulfate and stored at -25°C. Each Freon 11 extract (10 mL) was treated with pyridine (10 mL of a 1 μL/mL solution in Freon 11), concentrated in a sharply taped flask to a volume of 50 μl and stored at -25°C pending GC/MS analysis. The solvent stripped juiced from each reactor was passed through a separate column containing XAD-2 for the analysis of glycosides as described in section A. All glassware was prerinsed with pyridine (1μL/mL of solvent) to help bind trace acids.

D. Gas Chromatography and Mass Spectrometry

Gas Chromatography was accomplished on a Hewlett-Packard gas chromatograph (model 5890A) with a split-mode capillary injection port, flame ionization detector, a DB-5 bonded phase silica capillary column (95% dimethy (5%) diphenyl polysiloxane - J&W Scientific) under the conditions outlined in section D of Material and Methods.

Results

The percent recovery varied with each compound but averaged 90% or greater for both systems. Coefficients of variation was less than 4% with the XAD-2 and 9% for the continuous system for all compounds (Table 1).

Discussion

Blank analysis. A concern in using XAD-2 resin is that the resin can fracture, releasing impurities. GC-MS analysis suggested that the major contaminants noted in the blank were naphthaline, ethyl benzene and benzoic acid. Although the resin was cleaned in a Soxhlet extractor, minor contaminants remained. These results were consistent with James et al. (1981) and Wigilius et al. (1987). It is likely that the major GC peaks, and some minor unidentified peaks, were compounds trapped interstitially within the resin during polymer bead formation. Wigilius et al. (1987) noted that the contamination from XAD resins were reduced if all methanol was removed by ether prior to the introduction of water. Although this procedure was used, background peaks remained. Retention time for each contaminate was different enough from the compounds under study not to influence the analysis.

Relative Recovery. Relative recoveries of the volatile compounds extracted from White Riesling juice and isolated either on XAD-2 resin or by continuous extraction are shown in Table 1. Typical chromatograms of

TABLE 1.
Percent relative recoveries and coefficients of variation for recovery of volatile compounds from White Riesling *(Vitis vinifera L.)* juice using XAD-2 resin and a continuous extraction system[a]

Peak ID	Ret. Time Min.	Compound	XAD-2		Continuous Extraction	
			Relative Recovery %[b]	cv,%	Relative Recovery %	cv,%
A	13.60	p-cymene	49	<0.75	86	5.01
B	13.77	benzyl alcohol	87	1.29	88	6.03
C	14.63	v-terpinene	91	>0.95	90	4.11
D	14.99	trans-furan linalool oxide	99	1.74	93	2.95
E	15.44	cis-furan linalool oxide	107	>0.48	98	7.98
F	15.74	linalool	101	1.45	87	3.48
G	16.12	2 phenylethanol	89	3.14	81	5.80
H	16.55	rose oxide	96	3.00	89	7.51
I	16.90	nerol oxide	99	2.05	93	8.37
J	18.00	4-carvomethanol	103	2.02	95	6.89
K	18.31	α-terpineol	98	2.38	96	4.10
L	19.25	nerol	97	2.43	92	5.95
M	19.89	geraniol	98	>0.94	85	10.47

[a]Means of three replications.

[b]where % relative recovery equals: $[([C]_{sj} - [C]_j] / [C]_s \times 100$ where $[C]_{sj}$ is the concentration in the spiked juice, $[c]_j$ is the concentration in juice, and $[C]_s$ is the concentration added to spiked juice.

White Riesling extracts from both systems showed good resolution and peak symmetry. Recoveries with the XAD-2 resin were generally found to be acceptable, in the range of 90% or greater, the exception being p-cymene with a recovery of 49%.

The precision of the XAD-2 extraction technique, as measured by the coefficients of variation (cv) was less than or equal to 3% (Table 1). The relative percent recovery using Freon 11, continuous extration was generally not as good as that attained using the XAD-2 resin. The coefficient of variation for most compounds, was greater with the continuous system (Table 1). Difficulty was encountered with the continuous system as a result of the variation in Freon volume dripping in the juice. Variations in the liquid level due to slight changes in temperature and/or Freon condensation influenced bubble streaming, and presumably extraction. Marais (1986) recommended that extraction run continuously for 72 hours to maximize recovery from wine. It seems likely that in juice hydrolysis and rearrangement of monoterpenes can occur during this time, influencing both recovery and reproducibility.

Attempts to minimize rearrangement and degradation included salting out native glucosidases and pH adjustment.

XAD-2 Recovery steps. Three steps involved in using adsorbents for isolation and enrichment are adsorption, desorption and solvent evaporation. Each of these processes may give rise to losses, rearrangements and/or contamination. Each was evaluated to assure that optimum laboratory procedures were utilized.

Adsorption. Results of the adsorption study showed that all compounds were effectively adsorbed on the XAD-2 resin of the first column. Analysis of the pentane extracts of the second column showed the same chromatographic pattern as the blank. Therefore, the volume of resin

utilized was sufficient to adsorb the compounds under study, and adequate adsorption did occur.

Desorption. GC analysis of each pentane fraction demonstrated that all of the compounds under study were recovered from the first 50 mL pentane extract, and that the recovery percentages were similar to those noted in the adsorption study.

The desorption of free and bound aroma components on XAD-2 resin appeared to be complete and was confirmed as follows. The spent juice was pH adjusted to 3.0, an internal standard added and the juice distilled. The distillate (200 mL) was collected, extracted with pentane (5x5mL), dehydrated and concentrated as previously described. GC analysis demonstrate no peak retention at retention times of the compounds under study.

Evaporation. After eluting from the XAD-2 column, further concentration is necessary prior to GC analysis. Evaporation of the solvent may be performed by several different procedures. Wilgilius et al. (1987) reports that the recovery of volatile components following evaporation is dependent upon the volatility of each compound and the final volume of the extract. This study was performed to determine the extent of loss occurring at this step. Analysis performed in triplicate indicated an average recovery of greater than 90%. Terpene recovery, with the exception of v-terpine and p-cymene, was 96.5%.

Quantification of Glycoside Recovery. Monoterpene glycosides of Muscat varieties consist of mainly geraniol, nerol, linalool and α-terpineol (Williams et al., 1982a). Isolation procedures for grape glycosides must ensure separation of these compounds (present in μg/L quantities) from the bulk of the polar organic (fruit) constituents.

The 1-octanol ($C_8H_{18}O$ MWT 130.22) cleaved as a result of hydrolysis was determined by GC-MS. The recovery data from the hydrolysis of the

model glycoside-n-octyl-ß-D glucopyranoside averaged 89%.

pH Effect. It has been suggested that the rapid hydrolysis of monoterpenes, glycosides and polyols at a pH less than 4.0 necessitates adjustment of juice to near neutrality prior to solvent extraction (Wilson et al., 1984). A study of the effect of pH on the recovery of the compounds of interest demonstrated similar relative recovery percentages between the two pH values (native pH vs. pH 7.5). Some difference in the percent relative recovery for acid and phenolic compounds at different pH levels could be detected. However, monoterpenes, terpene oxides and aromatic alcohols used in this study have relatively low dissociation constants, which do not appear to affect adsorption or desorption.

Conclusion

Two methods were evaluated for the determination of free volatile components in grape juice. Both appear to be viable analytical procedures. The use of XAD-2 resin allowed for the separation and concentration of large quantities of grape aroma components by using inert solvents in a short time and at a low cost. The XAD-2 isolation procedure provided acceptable relative recoveries and low coefficients of variation. Optimum procedures for the utilization of XAD-2 were established.

CHAPTER II

Effects of Shoot Topping and Ethephon on Aroma Components of White Riesling (*Vitis vinifera L.*) Grapes

Abstract. Grapevine canopies were modified by shoot topping (control, 10 and 20 nodes per shoot retained) or ethephon application to shoot tips (600 mg/L) to examine the effects on grape aroma components in a warm, humid grape-growing region on Pendelbogen-trained White Riesling grapevines. The percentage of sunlight penetration into the canopy fruiting zone was least in control vines and similar among shoot-topped and ethephon-treated vines. At harvest, free volatile terpenes (FVT) increased as a result of ethephon treatment in two of three seasons and in shoot-topped vines in one season. Potentially volatile terpenes (PVT) and bound linalool, nerol, geraniol and α-terpineol were elevated in fruit from shoot-topped vines at one of three harvest dates.

Introduction

Wine quality is dependent upon the chemical constituents of the fruit and the agent(s) involved in the bioconversion into wine (Amerine et al., 1980). With advances in technology, winemakers can exercise considerable control over the microbiological aspects of fermentation. Accordingly, wines can be produced with a desired fermentation bouquet and no off-flavor development, illustrating the importance of fruit chemistry in producing highly palatable products.

Much of the aromatic quality of White Riesling wines is known to result from the presence of monoterpenes, important grape constituents (Ribereau-Gayon et al., 1975). Free monoterpenes, potent odorous compounds present as 10-carbon compounds derived from acyclic precursors, include alcohols, ethers, aldehydes, hydrocarbons and poly-functional derivatives. Dimitriadis and Williams (1984) and Ewart et al. (1984) demonstrated increases in monoterpenes with maturity and established a relationship between the concentration of monoterpenes in Muscat grapes and wine quality. Therefore, measuring monoterpene levels during grape ripening may help to identify optimum harvest dates.

Monoterpenes are present in grapes as odorous free volatile terpenes, free nonodorous polyhydroxylated linalool derivatives (polyols) and nonvolatile glycosides. Glycosidically bound terpenes are referred to as potentially volatile terpenes (PVT). Monoterpene glycosides have been elucidated as mixtures of disaccharide glucosides of several monoterpene alcohols, oxides and polyols. Additionally, the aromatic alcohols, 2-phenylethanol and benzyl alcohol, are also present in a glycosidically bound form in grapes (Williams et al., 1983). Acid or enzyme catalyzed hydrolysis of glycosides can yield free volatile aroma compounds or, alternatively, odorless polyols, which can undergo acid hydrolysis to form aroma components. Vineyard management and processing activities which

increase the FVT and/or the concentration and hydrolysis of PVT could enhance varietal character, thereby improving wine quality.

Dense, shaded canopies with inadequate light exposure produce fruit with low sugar, high malate, high potassium and high pH compared to grapes produced in sparse canopies (Kliewer and Lider, 1968; Smart, 1976; Carbonneau et al., 1978; Kliewer, 1980). Additionally, fruit quality can be reduced by rots promoted by poor canopy ventilation and reduced pesticide penetration into dense canopy interiors (Boniface and Dumartin, 1977; Gubler et al., 1987; English et al., 1989). Maturing fruit is particularly prone to fruit decay as a result of the hot and humid growing conditions of the southeast US.

Smart (1985) suggested that varying shoot numbers or adopting training and trellising systems that divide canopies into separate curtains of foliage are desirable methods of improving the grapevine microclimate. Each of these efforts has been used to improve the photosynthetic photon flux density (PPFD) of grapevine canopy interiord and grape quality (Carbonneau et al., 1978; Smart, 1985; Bledsoe et al., 1988). Mechanical shoot topping and growth regulators have also been used to improve the light penetration. Topping, however, has been reported to stimulate the growth of lateral buds although such growth is dependent upon growing conditions (Lavee, 1987). The combined leaf area of laterals can be greater than that of the primary shoot, and act as new photosynthetic sinks to compete with developing fruit for carbohydrates (Lavee, 1987).

The use of growth regulators to control and modify the development of grapevines has been widely studied (Winkler et al., 1974). Ethephon, an ethylene releasing growth regulator is capable of inhibiting both extension growth and lateral bud outgrowth in grapes (Lavee, 1987; Patterson and Zoecklein, 1990). On ethephon treated shoots the bunches

remain the only rapidly growing organ of the shoot (Lavee, 1984). Thus, ethephon can effect the source-sink relationship in grapes by directing the metabolic flow towards the fruit (Lavee, 1987).

A clear relationship between grapevine canopy microclimate, general fruit chemistry and rot incidence has been established. The few studies that have examined the effects of grapevine canopy management on aroma components include Ewart et al. (1984) and Reynolds and Wardle (1989). Both studies demonstrated a relationship between increased solar exposure and PVT formation in Muscat and Gewurztraminer grapes.

Because of excessive plant growth and canopy density in the mid-Atlantic region, research concerning canopy manipulation is of great importance. The objective of this study was to evaluate the effects of canopy modification by shoot topping or the application of ethephon on aroma components of White Riesling grapes grown in Virginia.

Materials and Methods

This research was conducted for three years (1986-1988) using White Riesling vines grown at Prince Michel Vineyards in Leon, Virginia. Vines were grafted to rootstock cv. SO-4 and planted in 1983 at a spacing of 1.1 m apart in rows 2.7 m wide. Vines were cane-pruned and trained to a Pendelbogen system to a head height of *ca*. 1 m. Pairs of movable catch wires were attached to the trellis to promote an upright canopy. Full-bloom occurred on 1 June $\pm$ 4 days in each of the three years of study.

Treatments: Treatments consisted of the following: 1) control, no canopy modification other than shoot positioning, 2) shoot topping to retain 10 nodes (10 leaves) per shoot, 3) shoot topping to retain 20 nodes (20 leaves) per shoot, and 4) application of 600 mg/L ethephon and surfactant (Ethrel^{R}, Union Carbide, Inc., Research Triangle Park, NC 27709) to shoot tips. Shoots were topped when they averaged 20 nodes, and

retopped once (twice in 1986) to maintain the desired node number. Initial shoot topping and retopping were done *ca.* 30 and 60 days after bloom, respectively. Ethephon was applied twice per season using a hand sprayer to the terminal five to 10 leaves of shoots at approximately the same time shoots were topped and retopped.

Treatments were arranged in a randomized complete block design that consisted of six replicates of five-vine plots. Shoots were adjusted following budbreak to approximately 16 shoots per vine in 1986 and 20 shoots per vine in 1987 and 1988. The crop was adjusted to two clusters per shoot in 1986, but was not regulated in 1987 or 1988.

Characterization of fruit microclimate: Photosynthetic photon flux density (PPFD) was measured inside and outside the canopy using a Licor^{R} line quantum sensor (model LI-191SB) inserted into the canopy, parallel to the row, at an average mid-point of the fruiting zone. Measurements were also made of exterior PPFD (above canopy). The ratio of interior to exterior PPFD was expressed as the percentage of available photosynthetically active radiation penetrating the canopy. Five measurements were made per treatment replicate.

Fruit composition and fruit harvest: A minimum of 100 berries were collected per treatment replication during ripening. Samples used for FVT, PVT and GC-MS analysis were stored at -25°C until analyzed. Berry samples were warmed to 1°C, macerated and placed for one minute in a Tekmar (model 400) laboratory stomacher. Standard AOAC procedures were used, unless otherwise noted, as described by Zoecklein et al. (1990), in Section I of Materials and Methods.

Fruit was harvested by vine on 8, 15, and 16 September in 1986, 1987 and 1988, respectively. The total number of clusters and the total fruit weight per vine were recorded at harvest.

Distillation of aroma components: A method developed by Attaway et

al. (1967), modified by Dimitraidis and Williams (1984), was further modified to determine the FVT and PVT fractions in various juice samples as outlined in section II of Materials and Methods.

Free and bound monoterpenes and aromatic alcohols: The methods used for the isolation and concentration of free and bound aroma components is outlined in Section II-B of Materials and Methods.

Gas chromatography and mass spectrometry: Volatile components were analyzed on a Hewlett-Packard gas chromatograph (model 5890A) equipped with a split-mode capillary injection port and a flame ionization detector using a DB-5 bonded phase silica capillary column (dimethyl (95%) diphenyl polysiloxane (5%) - J&W Scientific) under the conditions outlined in Section II-D and Table 1 of Materials and Methods. Component identification was confirmed using a Hewlett-Packard model 5790 GC, V. G. Analytical Inc., model 7070E electron impact mass spectrometer, 70 eV.

Sensory evaluation: Juice aroma was sensorially evaluated once by 14 individual judges using duo-trio difference testing, a modified paired-sample test in which a reference sample is identified, presented and followed by two randomly ordered coded samples, one of which is identical to the reference sample (Amerine and Roessler, 1983). A randomly selected sample of grapes was crushed, and a one liter sample of the juice was immediately treated with SO_2 (30 mg/L), enzyme (200 mg/L, Pectinol V.R., Rohm Tech) and ascorbic acid (30 mg/L). The juices (22°C $\pm$ 2°C) were placed in a Tekmar (model 400) laboratory stomacher for one minute and transferred to covered wine glasses for evaluation.

Statistical analysis: Data were analyzed for variance using SAS (SAS Institute, Cary, NC) general linear model (GLM) programs. Non-orthogonal contrasts were made of pre-selected treatment means using GLM procedures. Sensory responses were evaluated by a one-sided t-test according to Amerine and Roessler (1983).

Results

Fruit microclimate: Shoot topping increased the photosynthetic photon flux density in the canopies relative to control vines each season (Table 1). Ethephon effectively limited vegetative growth resulting in an increase in PPFD relative to control vines in 1986 and 1987. There was no difference in the PPFD between vines topped to 10 vs. 20 nodes retained.

Fruit yields: The weight of harvested fruit was lower for control vines than for shoot-topped vines in 1986 and 1987 (Table 2). Topping to retain 10 leaves per shoot reduced fruit yields relative to vines topped to 20 leaves per shoot in both 1987 and 1988. The 10-node topping also lowered berry weight in 1986 and cluster weight in 1988 relative to the 20-node treatment. Ethephon-treated vines had lower berry weights at harvest in 1986 and more clusters in 1988 relative to control vines.

TABLE 1.

Percent of available Photosynthetic Photon Flux Density (PPFD) penetrating fruit zones of White Riesling grapevines as a function of shoot topping to retain 10 or 20 nodes or foliar application of ethephon (600 mg/L).[y]

Canopy treatment	Percent penetration of PPFD[z] into fruiting zone		
	1986	1987	1988
Control (cont)	7.0[y]	1.2	4.1
10 nodes (10)	9.4	4.3	12.5
20 nodes (20)	12.1	3.4	8.0
Ethephon (Ethp)	12.9	3.2	8.6
Contrast:	prob. > F[z]		
Cont vs. 10, 20	**	***	**
10 vs. 20	ns	ns	ns
Cont vs. Ethp	***	***	ns

[y]Data are arithmetic means of percentage of PPFD penetration in the fruit zone, measured in μmol $m^{-2}s^{-1}$, taken before veraison (5° Brix) each season and square root transformed, although express here not transformed.

[z]Contrasts either not significant (ns) or significant at 1% (**) or 0.1% (***) level.

TABLE 2.
Fruit weight, number of clusters, average cluster weight, and average berry weight of White Riesling grapevines at the end of three growing seasons as influenced by shoot topping to retain 10 or 20 nodes, or foliar application of ethephon (600 mg/L).

Canopy treatment	Total fruit wt/vine (kg)			Total clusters/vine			Average cluster wt (g)			Average berry wt (g)		
	1986	1987	1988	1986	1987	1988	1986	1987	1988	1986	1987[y]	1988
Control (cont)	1.32	3.36	1.45	24	52	25	55	64	59	1.48	1.31	1.49
10 nodes (10)	1.50	3.63	1.09	25	58	22	59	64	50	1.45	1.29	1.39
20 nodes (20)	1.63	4.45	1.59	27	65	26	59	68	59	1.59	1.33	1.46
Ethephon (Ethp)	1.45	3.95	1.73	26	60	30	54	68	59	1.31	1.36	1.47
Contrast:	Prob. > F[z]											
Cont vs. 10, 20	*	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
10 vs. 20	ns	*	*	ns	ns	ns	ns	ns	*	*	ns	ns
Cont vs. Ethp	ns	ns	ns	ns	ns	*	ns	ns	ns	*	ns	ns

[y]Average berry weight 10 days before fruit harvest.

[z]Contrasts either not significant (ns) or significant at 5% (*) level.

General fruit composition: Shoot topping resulted in greater °Brix than observed in control fruit at harvest in 1987 and 1988 (Tables 3). In 1986 and 1987, topping to 10 nodes depressed °Brix relative to the 20-node treatment. Topping to 10 nodes frequently delayed the accumulation of soluble solids in the preharvest samplings (data not shown), but did not cause a lower sugar level than the control at harvest. Fruit from ethephon treated vines had greater °Brix at harvest than control fruit in 1987 and 1988. Treatment effects on pH and fruit potassium were not significant (fruit potassium data not shown).

Titratable acidity was consistently higher in fruit from control vines relative to both topping treatments (Table 3). Shoot topping to 10 nodes reduced the titratable acidity relative to the 20-node treatment in 1988. Ethephon application resulted in lower titratable acidity compared to control fruit in 1987 and 1988. Malic acid was reduced in the shoot-topped and ethephon treated vines in 1987 and 1988 relative to control fruit (Table 4). Shoot topping to 10 nodes resulted in more malic acid than was present in the 20-node treatment in 1987 and less tartaric acid in 1988. Tartaric acid was also lower in the control fruit than in both shoot topping treatments in 1987 and greater in ethephon treated fruit in 1988. In 1988, topping to 10-nodes resulted in lower tartaric acid relative to the 20-node treatment.

Free volatile terpenes: At harvest, ethephon increased FVT in 1986, while FVT from both shoot-topped and ethephon treated vines were increased in 1988 (Table 5). FVT was also expressed as a ratio of FVT to °Brix to allow comparisons among treatments while adjusting for differences in fruit maturity. Treatment effects were the same as reported in Table 5.

Potential volatile terpenes: PVT was higher in the shoot-topped vines at harvest only in 1988 (Table 5). Data in 1987 showed the opposite trend, higher PVT in fruit from control vines. A preharvest sample taken

TABLE 3.
°Brix, pH and titratable acidity of White Riesling grapes at harvest as influenced by shoot topping to retain 10 or 20 nodes or foliar application of ethephon (600 mg/L) for three growing seasons.[y]

Canopy Treatment	1986			1987			1988		
	°Brix	pH	Titratable Acidity (g/L)	°Brix	pH	Titratable Acidity (g/L)	°Brix	pH	Titratable Acidity (g/L)
Control (cont.)	17.6	3.32	8.91	15.6	3.26	11.70	17.9	3.31	9.22
10 Nodes (10)	17.7	3.40	7.89	15.6	3.21	8.13	18.3	3.37	7.99
20 Nodes (20)	18.4	3.37	8.45	16.6	3.26	8.12	18.7	3.28	8.65
Ethephon (Ethp)	17.6	3.41	9.40	16.3	3.25	9.72	19.5	3.26	8.39
Contrast:	Prob. > F[z]								
Cont. vs. 10, 20	ns	ns	*	*	ns	***	*	ns	**
10 vs. 20	*	ns	ns	***	ns	ns	ns	ns	*
Cont. vs. Ethp.	ns	ns	ns	**	ns	***	***	ns	**

[y]Data are means derived from analysis of total crop yield of each replicate.

[z]Contrasts either not significant (ns) or significant at 5%(*), 1% (**), or 0.1% (***) level.

TABLE 4.
Tartaric acid and malic acid (g/L) of White Riesling grapes as influenced by shoot topping to retain 10 or 20 nodes or foliar application of ethephon (600 mg/L) at harvest for three growing seasons.[y]

	Acids (mg/L)					
	1986		1987		1988	
Canopy Treatment	Tartaric	Malic	Tartaric	Malic	Tartaric	Malic
Control (cont)	3.42	2.68	2.08	2.91	3.72	3.57
10 nodes (10)	3.34	2.67	4.38	2.58	3.50	3.22
20 nodes (20)	3.46	2.52	3.65	2.35	3.86	3.12
Ethephon (Ethp)	3.21	2.44	2.68	2.46	4.46	3.88
Contrast:	Prob. > F[z]					
Cont. vs. 10,20	ns	ns	***	***	ns	*
10 vs. 20	ns	ns	ns	*	*	ns
Cont. vs. Ethp	ns	ns	ns	***	*	*

[y]Data are means derived from the analysis of each replicate.

[z]Contrasts either not significant (ns) or significant at 5% (*), or 0.1% (***) level.

TABLE 5.
FVT and PVT of White Riesling grapes at harvest in response to shoot topping to retain 10 or 20 nodes or foliar application of ethephon (600 mg/L) for three growing seasons.[y]

Canopy Treatment	FVT (mg/L)			PVT (mg/L)		
	9/08/86	9/15/87	9/16/88	9/08/86	9/15/87	9/16/88
Control (cont.)	.52	.57	.29	2.49	1.16	1.86
10 Nodes (10)	.56	.40	.49	2.32	.63	2.64
20 Nodes (20)	.58	.30	.50	2.83	.90	2.29
Ethephon (Ethp)	.68	.47	.56	2.06	1.12	2.14
Contrast:	Prob. > F^z					
Cont. vs. 10, 20	ns	ns	***	ns	*	*
10 vs. 20	ns	ns	ns	ns	ns	ns
Cont. vs. Ethp.	*	ns	***	ns	ns	ns

[y]Data are means derived from analysis of each replicate.

[z]Contrasts either not significant (ns) or significant at 5% (*), or 0.1% (***) level.

one week earlier in 1987, however, showed a higher PVT in shoot-topped relative to control vines when adjusted for differences in maturity (data not shown).

Shoot topping increased the total terpene concentration (FVT plus PVT) at harvest in 1988 and reduced the total terpene concentration in 1987 (Table 6). The PVT to FVT ratio, an indication of the portion of the terpene pool which are bound divided by the terpenes contributing directly to aroma, was decreased by ethephon treatment in 1986 and by both ethephon and shoot topping in 1988 (Table 6).

Free aroma components: There were no treatment effects on FVT at harvest in 1987, therefore, analysis of individual aroma components was not undertaken for that year. In 1986, the sum of the free monoterpene alcohols (geraniol, nerol, linalool and α-terpineol) was lower in the fruit from the 10-node treatment relative to all other treatments (Table 7) and greatest in the ethephon treatment in 1988 (Table 8). The sum of the aromatic alcohols was highest in the ethephon treated vines in 1986 and in the 10-node treatment 1988.

Bound aroma components: Increases in potentially volatile terpenes (PVT) as a result of treatment were not observed in 1986 or 1987. Additionally, the incidence of fruit rot was not different among treatments in 1988. Therefore, GC-MS analysis of glycosidically bound components was conducted only on the 1988 fruit. In 1988, the sum of the bound monoterpene alcohols (geraniol, nerol, α-terpineol and linalool) followed the same pattern noted for the PVT, higher in the 10-node treatment (Table 9). The sum of the bound aromatic alcohols, benzyl alcohol and 2-phenylethanol, was also numerically higher in the 10-node treatment.

Fruit rot: Fruit rot was severe in 1986 and 1987 as a result of frequent summer rains. Fruit rot was greatest in the control vines in

TABLE 6.
Total terpene concentration (FVT plus PVT) and terpene ratios (PVT/FVT) of White Riesling grapes at harvest in response to shoot topping to retain 10 or 20 nodes or foliar application of ethephon (600 mg/L) for three growing seasons.
of ethephon (600 mg/L) for three growing seasons.[y]

	FVT (mg/L)			PVT (mg/L)		
Canopy Treatment	9/08/86	9/15/87	9/16/88	9/08/86	9/15/87	9/16/88
Control (cont.)	3.01	1.73	2.15	5.44	2.65	7.72
10 Nodes (10)	2.88	1.03	3.13	4.41	1.85	5.39
20 Nodes (20)	3.41	1.20	2.79	5.00	3.73	4.66
Ethephon (Ethp)	2.74	1.59	2.70	3.26	2.37	4.80
Contrast:	Prob. > F[z]					
Cont. vs. 10, 20	ns	*	*	ns	ns	*
10 vs. 20	ns	ns	ns	ns	ns	ns
Cont. vs. Ethp.	ns	ns	ns	*	ns	ns

[z]Contrasts either not significant (ns) or significant at 5% (*), 1% level.

Table 7.
Influence of shoot-topping to retain 10 or 20 nodes or foliar application of ethephon (600 mg/L) on selected free aroma components of White Riesling grapes at harvest in 1986.

Compound (μg/L)[y]	17.0 °Brix[z] Control	17.7 °Brix 10 Node	18.4 °Brix 20 Node	17.6 °Brix Ethephon
benzyl alcohol	28.0	39.1	43.4	65.6
linalool	10.5	8.0	13.4	9.3
2-phenylethanol	59.5	72.0	158.1	202.9
α-terpineol	0.6	1.0	0.7	0.7
nerol	4.8	2.0	4.2	3.7
geraniol	26.4	10.7	25.0	23.5
Sum of aromatic alcohols: benzyl alcohol and 2-phenylethanol	87.5	111.1	201.5	268.5
Sum of monoterpene alcohols: geraniol, nerol, linalool and α-terpineol	42.4	21.7	43.3	37.2

[y]Compounds listed in order of increased retension time.

[z]Values denote maturity (°Brix) of pooled subsamples.

Table 8.
Influence of shoot-topping to retain 10 or 20 nodes or foliar application of ethephon (600 mg/L) on selected free aroma components of White Riesling grapes at harvest in 1988.

Compound (μg/L)[y]	17.9 °Brix[z] Control	18.3 °Brix 10 Node	18.7 °Brix 20 Node	19.5 °Brix Ethephon
benzyl alcohol	33.2	61.1	40.0	65.6
linalool	11.5	11.0	5.1	9.3
2-phenylethanol	26.0	50.3	28.6	202.9
α-terpineol	2.4	5.5	0.9	0.7
nerol	2.0	nd	2.0	3.7
geraniol	15.0	20.7	19.1	23.5
Sum of aromatic alcohols: benzyl alcohol and 2-phenylethanol	59.2	116.4	68.6	105.6
Sum of monoterpene alcohols: geraniol, nerol, linalool and α-terpineol	30.9	36.5	27.1	41.4

[y]Compounds listed in order of increased retension time.

[z]Values denote maturity (°Brix) of pooled subsamples.

Table 9.
Concentrations of bound, geraniol, nerol, α-terpineol, linalool, benzyl alcohol and 2-phenylelthanol in White Riesling grapes at harvest, as influenced by shoot topping to retain 10 to 20 nodes or foliar application of ethephon (600 mg/L) in 1988.

Compound (μg/L)[y]	17.9 °Brix[z] Control	18.3 °Brix 10 Node	18.7 °Brix 20 Node	19.5 °Brix Ethephon
geraniol	47.2	88.5	63.6	31.0
nerol	12.0	18.0	14.6	13.0
α-terpineol	119.3	148.6	96.0	42.0
linalool	91.0	191.4	141.1	112.0
benzyl alcohol	311.8	385.6	363.2	200.7
2-phenylethanol	237.4	281.2	228.6	217.1
Sum of bound monoterpenes: geraniol, nerol, α-terpineol and linalool	269.5	445.5	315.3	198.0
Sum of bound aromatic alcohols: benzyl alcohol and 2-phenylethanol	549.2	666.8	591.8	417.8

[y]Value denotes maturity (° Brix) of pooled subsamples.

1986 and 1987 (Zoecklein, 1993).

Sensory evaluation: Sensory evaluation of the juice aroma was not performed in 1986 due to excessive fruit rot. Sensory analysis demonstrated differences between the control and shoot-topped vines in 1987 and 1988 (Table 10). The sensory evaluation in 1987 may have been confounded by fruit rot. There were no differences between the control and ethephon treatment or between shoot topping to 10 or 20 nodes.

TABLE 10.
Summary of duo-trio aroma difference test on White Riesling juice at harvest as influenced by shoot topping to retain 10 or 20 nodes or foliar application of ethephon (600 mg/L) during two growing seasons.

	Correct Responses	
Comparison	1987	1988
Control vs. 10,20	12**	11*
10 vs. 20	8 ns	6 ns
Control vs. Ethep	7 ns	5 ns

*Either not significant (ns) or significant difference at 5% (*) and 1% (**). n = 14, minimum correct judgements to establish difference at 5%, 11 and at 1%, 12.

Discussion

The greatest difference in mean PPFD in the canopy fruiting zone was between control and shoot-topped vines, although differences were not large. Few canopy gaps were visible in the control vines, which typically had shoots that had elongated by veraison (approximately 5° Brix) and drooped over the trellis top, partially obstructing the original canopy. Lateral shoot growth resulted from shoot topping but was confined to several terminal nodes. Ethephon successfully controlled lateral development which is consistent with other reports (Lavee, 1987; Patterson and

Zoecklein, 1990).

The basis for the lower crop in the 10-compared to the 20-node treated vines in 1987 and 1988 may have been due to slight, but frequently insignificantly lower berry and cluster weights and clusters per vine. Yield reduction is dependent upon the amount of foliage retained (Kliewer, 1971). Reynolds and Wardle (1989) noted that hedging Gewurztraminer vines to retain 15 nodes per shoot also resulted in reductions in cluster and berry weights. Topping vines to 10 nodes delayed soluble solids accumulation and resulted in lower °Brix at harvest than in the 20-node treatment (in 1986 and 1987), with reductions comparable to those reported by and Kliewer and Bledsoe (1987). Leaf area retained was not quantified in this study, although others have suggested that between $7cm^2$ and $12cm^2$ of exposed leaf area are required to ripen each gram of fruit in terms of soluble solids accumulation (Kliewer and Antcliff, 1970). Thus, topping to 10 nodes per shoot may be considered too severe if the primary harvest criterion is °Brix. The increased °Brix of the ethephon compared to the control fruit in 1987 and 1988 is consistent with Lavee (1984), who suggested that ethephon changes the source-sink relationship in grapes by limiting vegetative growth, thus directing the metabolic flow towards the fruit.

Reductions in malic acid in shoot-topped vines occurred at harvest in 1987 and 1988 suggesting differences in the thermal environment of the fruit relative to control fruit. Kliewer (1971) showed that the amount of malic and tartaric acid in grapes is strongly and negatively correlated to temperature during the ripening period, but relatively independent of light intensity. The malic acid content and the percentage of the titratable acidity due to malic acid is greater under cool temperatures (Winkler et al., 1974).

The lower tartaric acid content of the control fruit in 1987 likely

reflects the high (80%) incidence of fruit rot. Davis and Reeves (1988) reported that tartaric acid is easily metabolized by fruit rots such as *Botrytis cinerea*.

FVT showed a limited response to treatment, possibly reflecting the limited, yet significant difference in canopy microclimate (PPFD) between control, shoot-topped and ethephon treatments. Reynolds and Wardle (1989) reported that the highest FVT in Gewurztraminer grapes was found in fruit exposed to greater than 35% of the ambient solar radiation. In 1986, ethephon treatment resulted in the maximum PPFD in the canopy fruit zone (only 12.9% of the ambient radiation) while increasing FVT. In 1987, ethephon increased light interception (by 3.2% of ambient) but not FVT. The lack of treatment effect on FVT in 1987 may be the result of a limited solar and/or thermal response or may reflect dilution due to the more than 20 cm of rainfall which fell the week preceding harvest. Ethephon treatment increased FVT in 1988 despite the insignificant difference in light interception (4.5%) between control and ethephon treated vines. The higher FVT in the ethephon treated fruit does not simply reflect advanced maturity; increases were also noted with ethephon treatment following adjustments for differences in maturity. Possible changes in the source-sink relationship caused by ethephon (Lavee, 1984) could conceivably influence the concentration of secondary metabolites such as monoterpenes, and may be responsible for the increased FVT.

The PVT to FVT ratio was decreased by ethephon in 1986 and 1988 as a result of elevated FVT, possibly reflecting differences in glycosidase activity. Ethephon did not, however, influence the PVT or the total terpene concentration at harvest. The effect of ethephon on PVT may have been muted by excessive fruit rot, a characteristic of the compound (Lavee, 1984). As stated, in 1987 more than 20 cm of rainfall fell the week preceding harvest, contributing to both dilution and excessive rot.

PVT concentration averaged 2.78 mg/L lower than the previous sampling for all treatments with a paralleled reduction in °Brix (from an average of 18.0 to 16.0 for all treatments), reflecting dilution. The incidence of fruit rot in 1987 for all treatments averaged 48% (Zoecklein, 1993). This fact likely contributed to the breakdown of grape monoterpenes as suggested by Rapp et al. (1986).

The sum of the major free monoterpene alcohols, e.g., linalool, geraniol, nerol and α-terpineol, was numerically lowest in the 10-node in 1986. The lower concentration in 1986 possibly reflects the delay in sugar accumulation noted in the preharvest samplings of the 10-node treatment. In 1988, the sum of the terpene alcohols was highest in the ethephon treatment, possibly the result of greater maturity (°Brix). Free, 2-phenylethanol was present in greater concentration than free benzyl alcohol in 1986, and in slightly lower concentration in 1988. These differences and the greater concentration of aromatic alcohols in 1986 may stem from a higher incidence of rot in 1986 than in 1988. Sponholz (1991) reported 2-phenylethanol to be an important metabolite in spontaneous yeast fermentations. Glycoside hydrolysis is a major pathway for the liberation of free aroma compounds (Williams et al., 1982c), which could also explain the observed enrichment of aromatic alcohols. Bound aromatic alcohols are not readily liberated from their glycoside by mild acid hydrolysis, unlike monoterpenes, but are reportedly cleaved by the action of both native enzymes and those produced by molds (Rapp et al., 1986).

Analysis of the 1988 bound monoterpenes demonstrated a pattern similar to the PVT, that is, elevated concentrations in the shoot-topped relative to control fruit. These results with White Riesling supports those of Strauss et al. (1986), who suggested that the principle bound monoterpene glycosides of Muscat grapes are linalool, geraniol, nerol and traces of α-terpineol. The alcohols produced from hydrolysis of gly-

cosides of White Riesling grapes were dominated by linalool and α-terpineol, with geraniol and nerol consistently less abundant.

Sensory evaluation: The changes in monoterpene concentration as a result of canopy manipulation were not always reflected in aroma evaluations of the juice. The sensory differences noted in 1987 between control and shoot-topped treatments likely reflect the high incidence of rot in the control fruit (80%) and the significant difference between rot incidence of control and shoot-topped vines (Zoecklein, 1993).

In 1988, the concentration of the four monoterpene alcohols at harvest averaged 307 μg/L and 34 μg/L for the bound and free forms for all treatments. The highest bound linalool concentration was in the 10-node treatment; 100 μg/L higher than in the control fruit. This difference is near the sensory threshold of linalool (Meilgaard, 1975). Increases in several monoterpenes in the 1988 shoot-topped vines may have corresponded to slight increases in the "terpene-like" character reported in the juice. Juice produced from both shoot-topped vines were distinguished from control but not from each other.

Shoot topping increased PVT relative to control vines only in 1988. Several confounding factors may have influenced PVT, thus limiting treatment response. Eschenbrush et al. (1987) and Reynolds and Wardle (1989) reported increases in PVT as a result of improvement in grapevine microclimate. However, both studies noted significant seasonal variations in response to canopy treatment. There are extreme seasonal variations in Virginia. Harvest dates for White Riesling represent a compromise between predicted rot severity, forecast precipitation and acceptable fruit chemistry. In both 1986 and 1987 excessive fruit rot necessitated harvest of relatively immature fruit. For example, the average soluble solids concentration of all treatments in 1986 and 1987 was 16.9 °Brix compared to 18.6 °Brix in 1988. Dimitriadis and Williams (1984), McCarthy and Coombe

(1985), and Gunata et al. (1985a) reported that glycosidically bound terpenes of Muscat Alexandria grapes increase with grape maturity (°Brix). It is possible that treatment differences were obscured in 1986 and 1987 due to limited fruit maturity.

The extensive growth of lateral shoots may have influenced the source-sink relationship in the shoot-topped vines. Shoot topping has been demonstrated to stimulate the outgrowth of lateral buds below the cut (Orpfer and Gonssard, 1980; Patterson and Zoecklein, 1990) producing new photosynthetic sinks that can compete with developing fruit for carbohydrates. In the current study, lateral shoot growth resulted from shoot topping and appeared to be much more extensive during the two wet seasons of 1986 and 1987 where PVT was not elevated by treatment.

During berry development, translocation of photosynthates from a newly expanding leaf is upward towards the shoot tip (Winkler et al., 1974). Actively growing shoot tips and laterals, therefore, are photosynthetic sinks. The direction of photosynthate movement is reversed, however, by shoot topping (Quinlan and Weaver, 1970). Increases in PVT as a result of canopy manipulation may have resulted from changes in the pattern of assimilate movement. Shoot topping without lateral development might result in changes in the source-sink relationship by minimizing intravine competition for carbohydrates (Quinlan and Weaver, 1970). Lateral shoots in the shoot-topped vines, which were particularly evident in the two wet seasons of 1986 and 1987, may have helped to mute the PVT response to treatment by influencing the sink-source relationship.

An additional explanation of the results of this study may stem from the extent of solar radiation received by the grapevine canopy interiors. Reynolds and Wardle (1989) reported an increase in the concentration of PVT in Gewurztraminer grapes exposed to solar radiation. In their study, shaded fruit received between 1.2 and 7.6% of the incoming solar radia-

tion, exposed fruit from 36 to 100%. In the current study, the maximum difference in light interception within the fruiting zone between control and treated vines was 5% of ambient. Thus, the limited treatment effect on PVT may have resulted from limited differences in thermal and/or solar radiation exposure. The largest difference in the percentage of light interception between control and treated vines occurred in the 10-node treatment in 1988 which also showed a higher PVT and PVT-to-°Brix ratio than noted for the two previous seasons. Reductions in malic acid in shoot-topped vines occurred at harvest in 1987 and 1988 suggesting differences in the thermal environment of the fruit relative to control fruit. This may help explain the increase in shoot-topped PVT in 1988 while the confounding influence of berry hydration and rot may have obscured such influences in 1987.

PVT may have also been confounded by seasonal differences in yield. Total fruit weight per vine was higher in shoot-topped compared with control vines in both 1986 and 1987. McCarthy (1986) and Eschenbrush et al. (1987) noted an inverse relationship between fruit yield and the concentration of grape monoterpenes. Elevated fruit weights in the shoot-topped vines may have helped to depress PVT in 1986 and 1987.

Conclusion

The effects of shoot topping to 10 or 20 nodes and the foliar application of ethephon were evaluated for their influence on the aroma components of White Riesling grapes. The results of this three year study highlight the extreme seasonal variation in fruit composition in Virginia. At harvest, shoot topping enhanced free volatile terpenes (FVT) and potential volatile terpenes (PVT) in one of three seasons. Ethephon had no influence on PVT, but increased FVT at harvest in two seasons. Shoot topping to 10 nodes increased PVT in one season and showed the highest

total concentration of bound geraniol, nerol, α-terpineol and linalool. The treatment effect on potentially volatile terpenes may have been affected by both environmental and biological factors. Juices produced from shoot-topped vines were distinguished from juices produced from control vines, but not from each other. Sensory responses were confounded by excessive fruit rot in 1987. Reductions in fruit rots by shoot topping were probably due to the interactions of several factors, such as improved canopy ventilation (Zoecklein, 1993). PVT concentration may be considered an important grape quality factor since the possibility of liberation of these terpenes from their bound forms exists throughout the winemaking process.

CHAPTER III

Effects of Fruit Zone Leaf Removal on Aroma Components in White Riesling *(Vitis vinifera L.)*

Abstract. Selective leaf removal from fruit zones of mature White Riesling grapevines grown in two vineyards was valuated for three seasons as a means of influencing grape aroma components. Two to four leaves per shoot were removed three weeks after bloom from around fruit clusters grown on either a high (1.8m above ground) bilateral cordon at one vineyard or a low (1.2m) bilateral cordon at the other. Leaf removal increased the percentage of sunlight penetration into the canopy fruit zone in four of six measurements in the three years of the study. FVT (free volatile terpenes) were generally unaffected by treatment. PVT (potentially volatile terpenes) were frequently higher in the fruit from leaf-pulled vines during maturation, including four of six commercial harvest dates. The sum of the bound geraniol, nerol, linalool, α-terpineol including hotrienol was higher in the leaf-pulled fruit at the end of each of the three growing seasons.

Introduction

White Riesling is an aromatic grape (*Vitis vinifera L.*) cultivar widely grown in cool wine producing regions such as the Alsace in France, Germany and Northern Italy. This cultivar has become widely planted in warm wine producing areas such as the mid-Atlantic region of the US. However, the lack of sufficient and characteristic aromas in some white wine cultivars such as White Riesling is believed to be caused, in part, by high average temperatures during the growing season (Marais, 1987). While macroclimate is an important factor affecting grape aroma components, mesoclimate, and particularly, microclimate are also important. Unlike macro and mesoclimate, the viticulturist has significant control over microclimate through grapevine canopy management.

Monoterpenes contribute to the floral and varietal aromas of Muscat, Gewurtztraminer and White Riesling grapes. Dimitriadis and Williams (1984) and Ewart et al. (1984) established a relationship between the concentration of monoterpenes in juice and wine quality. Therefore, measuring monoterpene levels in juice during ripening may help identify the optimum time of harvest, desirable growing conditions, and provide a means of objective assessment of grape quality.

In addition to the free volatile terpenes (FVT), there exists nonodorous terpene precursors referred to as potentially volatile terpenes (PVT). Williams et al. (1980) and Rapp et al.(1980) characterized the nonvolatile water-soluble precursor compounds as mixtures of disaccharide glycosides of several monoterpene alcohols and aromatic alcohols (Williams et al., 1982c). Acid or enzyme hydrolysis of glycosides can yield free volatile aroma components or odorless polyhydroxylated linalool derivatives (polyols) that can undergo acid hydrolysis to form aroma components. In White Riesling, about 85% of the total monoterpenes are glycosides and polyols, the non-aromatic components. This leaves only about 15% of the

total terpenes contributing to grape and wine aroma (Williams et al., 1981).

Sunlight can affect vine and grape physiology through photosynthetic and thermal responses. The amount of solar radiation reaching the interior canopy leaves and fruit has been shown to decrease geometrically as the number of leaf layers increases (Smart, 1985). The resulting low photosynthetic photon flux density (PPFD) causes a reduction in photosynthetic rates (Winkler et al., 1974). Varying shoot numbers, reducing vine vigor, or adopting training and trellising systems that divide canopies into separate curtains of foliage are desirable methods of improving grapevine microclimate (Smart, 1985; Bledsoe et al., 1988). These efforts have been used to improve the PPFD of grapevine canopies and enhance grape quality. For vineyards already planted to conventional trellis designs, however, alternative methods of decreasing canopy shade may be desired.

Several researchers have reported improvements in the grapevine canopy microclimate as a result of selected fruit zone leaf removal. Kliewer and Bledsoe (1987) and Bledsoe et al. (1988) found that leaf removal increased soluble solids and reduced titratable acidity, malic acid, pH, potassium, and improved wine quality.

Fruit zone defoliation studies have suggested potential benefits that include improved wine sensory properties even when primary grape metabolites were not affected (Smith et al., 1988; Hunter et al., 1991). Additionally, control of bunch rot due to several microclimate factors such as increased spray penetration and desiccation have been reported (Koblet, 1987; Rosenquist and Morrison, 1989; Zoecklein et al., 1992). Few studies, however, have determined the influence of leaf removal on grape aroma components. Reynolds and Wardle (1989) reported increases in Gerwurztraminer PVT as a result of selective leaf removal, but did not

examine individual aroma compounds.

The objective of this investigation was to determine the influence of selective leaf removal on White Riesling FVT, PVT and individual aroma components and their glycosidically bound precursors.

Materials and Methods

This experiment was conducted in northern Virginia over a three-year period (1987-1989) using two vineyard sites, 20 km apart, each with a specific training system (high or low-trained) utilizing White Riesling grapes. Both vineyards were grafted to cv 'Teleki 5C' rootstock, and cordon trained, although the height of the cordon and direction of shoot growth were different. Vines at the "high-trained" vineyard were planted in 1978 to east-west rows and trained to a high-wire (1.8m) bilateral cordon comparable to the Hudson River Umbrella system. Fruit clusters were borne from 1.0 to 1.8m off the ground. Vines were spaced 2.1m apart in rows 3.0m wide. Shoots were repeatedly positioned downward during the growing season and the vines were not hedged.

The "low-trained" vineyard was planted in 1979 to north-south rows and trained to a low wire (1.2m) bilateral cordon. Vines were spaced 1.8m apart in rows 3.7m wide. Fruit was borne within a discreet region just above the cordons. A pair of foliage catch wires 60 cm apart were placed 40 cm above the cordon wire. Shoots were positioned through and tied to the catch wires to produce a "V" arrangement of the canopy. Shoots were hedged in July, and once again in mid-August each year, retaining 14 to 16 leaves per shoot to prevent the shoots from shading the original canopy.

Treatments: Treatments were arranged in a completely randomized design that consisted of 10 replicates of three-vine plots. Treatments were 1) control, or no leaf removal, and 2) removal of two to four leaves per shoot, and small lateral shoots arising from the basal four to five

nodes of primary shoots. Generally, leaves were removed only from around the fruit clusters. At the high-trained vineyard, however, some mid-shoot leaves at the canopy exterior that shaded clusters within the canopy interior were removed. Leaves were removed by hand two to three weeks after full-bloom (between 6 and 12 June each year), and additional laterals were removed about 30 days later.

Cane pruning weights and shoot adjustment: Individual vine pruning weight was used to adjust shoot number following bud break. In both vineyards twenty shoots were retained for each 0.45 kg. of pruned canes. A maximum of 60 and 46 shoots per vine was retained at the high and low-trained vineyards respectively, with a minimum of 20 shoots per vine.

Canopy Isolation and density: PPFD was measured inside and outside the canopies using LiCor^{R}(Li-Cor, Lincoln, NE) line quantum sensor (model LI-191SBO). PPFD was measured at least once per season at, or shortly after *veraison* (approximately 5 °Brix), between 1000 and 1500 hours EDT. The sensor was inserted into the canopy, parallel to the row, at an average mid-point of the fruiting zone; thus, the sensor was held parallel to and just below the cordon at the high-trained vineyard and parallel to and just above the cordon at the low-trained vineyard. Three measurements were made per treatment replicate. Each canopy interior PPFD measurement at the high-trained vineyard consisted of three separate readings: one with the sensing face oriented vertically upright or skyward (0°), a second with the sensor angled 45° right of vertical, and a third with the sensor angled 45° left of the vertical. Those three interior readings were averaged and divided by a single reading taken above the canopy to determine the percentage of available PPFD that penetrated canopies. Canopy interior PPFD measurements at the low-trained vineyard consisted of two separate readings were averaged and divided by a single reading taken above the canopy. The slightly different procedures utilized at the high

and low-trained vineyards reflect the differences in training systems and the desire to measure maximum light interception.

Fruit composition and fruit harvest: A minimum of 100 berries were randomly collected from each treatment replicate at three, four or five ripening stages in 1987, 1988 and 1989 respectively. In 1988 and 1989, some fruit from each treatment replicate remained for an additional sampling period following commercial harvest (recorded as final harvest). Sampling began at approximately 13°Brix. All samples were maintained at -25°C until analyzed. Standard AOAC procedures were used as described by Zoecklein et al. (1990) unless otherwise noted and are described in Section 1 of Materials and Methods. At the commercial harvest season, the total number of clusters and total fruit weight per vine were recorded. Additionally, at harvest grape clusters with five percent or greater rot-affected berries were separated and counted as reported by Zoecklein et al. (1992).

Free and bound monoterpenes and aromatic alcohols: The methods used for the isolation and concentration of free and bound aroma components is outlined in Section II A and B of Materials and Methods.

Gas chromatography-mass spectrometry: Gas chromatography was accomplished on a Hewlett-Packard gas chromatograph (model 5890A) with a split-mode capillary injection port and flame ionization detector using a DB-5 bonded phase silica capillary column under the conditions outlined in Table 1 of Materials and Methods.

Gas chromatography-mass spectrometry was performed using a Hewlett-Packard model 5790 GC, V.G. Analytical Inc., model 7070E election impact mass spectrometer, 70 eV.

Sensory evaluation: In 1988 and 1989 harvested fruit was crushed and a two liter sample of each lot was immediately treated with SO_2(30mg/L), pectinolytic enzyme (200 mg/L, Ultrazyme 100, Rohm Tech) and

ascorbic acid (30 mg/L). The juice was placed in a Tekmar (model 400) laboratory stomacher for one minute and 15 mL transferred to covered wine glasses. Juices were sensorially evaluated twice by 11 untrained judges for differences in aroma between the control and the leaf-pulled fruit using duo-trio differences testing (Amerine and Roessler, 1983). The temperature of the juice samples was 22°C ± 2°C. In 1989, juice samples were also treated with H_2SO_4 to determine the effect of monoterpenes hydrolysis on juice aroma. The pH was adjusted to 2.5, held for 2 hours, adjusted to native pH with KOH, and re-evaluated. All evaluations were conducted in isolated booths under red light.

Data were analyzed using general linear model (GLM) programs (SAS Institute, Cary, NC 27511). Sensory data was evaluated using a one-sided t-test as described by Amerine and Roessler (1983).

Results

Canopy insolation: Leaf removal did not affect vine size as measured by cane pruning weights and within each vineyard, shoot density was comparable between treatments (data not shown). Leaf removal, designated as "leaf pull" or "LP", increased the average PPFD of canopy fruiting zones with two exceptions, the high-trained vineyard in 1987 and the low-trained vineyard in 1989 (Table 1). Vines at the high-trained vineyard produced a wider distribution of available PPFD than vines at the low-trained vineyard (data not shown).

Fruit yield: Fruit weight per vine did not differ between treatments in 1988 or 1989, but was greater for the leaf-pulled vines at both vineyards in 1987 (Table 2). Average cluster weights were comparable between treatments with two exceptions: leaf-pulled vines had greater cluster weights than control vines at the low-trained vineyard in 1987 and at the high-trained vineyard in one of the two years measured,

TABLE 1.
Influence of selective leaf removal on percentage of ambient Photosynthetic Photon Flux Density (PPFD) in the fruit zone of White Riesling grapevines at two locations for three seasons.[y]

	Percentage of PPFD in Fruit Zone		
Vineyard	1987	1988	1989
High-Trained			
Leaf pull	16.0	14.0	8.7
Control	16.3	6.5	2.7
t-test sig.[z]	ns	**	***
Low-Trained			
Leaf pull	4.3	4.7	3.7
Control	1.1	2.3	2.4
t-test sig.[z]	***	**	ns

[y]Data are the arithmetic means of percentage PPFD penetration in μMol $m^{-2}S^{-1}$. For statistical analysis, means were subjected to square root transformation.

[z]Significance of t-test of treatment means: ns denotes non-significance, ** and *** denote significant treatment differences at $p \leq 0.01$ and 0.001 levels respectively.

1989. The number of clusters per vine were comparable between treatments. Differences in berry weights were not observed (data not shown).

General fruit composition: Sugar per berry was generally not affected by treatment (data not shown). Leaf removal influenced °Brix at three of six commercial harvest dates but showed no consistent pattern (Table 3). °Brix accumulation was delayed in the leaf-pulled fruit at the low-trained vineyard in 1987 and the high-trained vineyard in 1989, while leaf removal increased °Brix at the low-trained vineyard in 1989. Fruit pH was unaffected by leaf removal (Table 3). Titratable acidity was lower in fruit from leaf-pulled vines in three of six commercial harvest dates, the low-trained vineyard in 1987 and 1988 and the high-trained vineyard in

TABLE 2.
Fruit weight per vine, cluster weight, clusters per shoot, and clusters per vine of White Riesling grapes as affected by selective leaf removal for three seasons in two vineyards.

		Fruit wt. per vine (kg)			Cluster wt (g)			Clusters per shoot			Clusters per vine		
Vineyard	Treatment	1987	1988	1989	1987	1988	1989	1987	1988	1989	1987	1988	1989
High-trained	Control	8.23	8.32	8.64	nd	90.9	68.2	nd	1.87	2.36	nd	88	121
	Leaf pull	9.14	9.32	10.73	nd	95.5	81.8	nd	1.92	2.39	nd	94	129
	t-test sig.[1]	*	ns	ns	--	ns	*		ns	ns	--	ns	ns
Low-trained	Control	7.23	6.05	5.91	77.3	90.9	81.8	nd	1.52	2.18	93	64	71
	Leaf pull	8.95	6.64	5.82	100.0	109.1	81.8	nd	1.56	1.89	92	67	68
	t-test sig.[1]	*	ns	ns	*	ns	ns		ns	**	ns	ns	ns

nd denotes not determined.

[1]Significance of t-test of treatment means: ns denotes non-significance, whereas *, and ** denote significant treatment differences at $p \leq 0.05$, and 0.01 levels, respectively.

TABLE 3.
Influence of selective leaf removal on °Brix, pH and titratable acidity of White Riesling grapes at harvest for three seasons and two vineyard sites.

	°Brix			pH			Titratable acidity (g/L)[y]		
	1987	1988	1989	1987	1988	1989	1987	1988	1989
High-Trained									
Leaf pull	18.2	17.2	17.0	3.19	3.64	3.56	7.08	6.24	7.87
Control	18.3	17.3	17.5	3.17	3.64	3.61	8.28	6.71	7.89
T-test sig.[z]	ns	ns	*	ns	ns	ns	*	ns	ns
Low-Trained									
Leaf pull	17.0	21.1	19.3	3.17	3.00	3.02	7.93	7.72	8.01
Control	17.9	20.9	19.0	3.14	3.02	3.05	11.79	8.27	8.54
T-test sig.[z]	*	ns	*	ns	ns	ns	***	*	ns

[y]Titratable acidity expressed as tartaric acid.

[z]Significance of t-test of treatment means: ns denotes non-significance, *, and *** denote significant treatment differences at $p \leq 0.05$, and 0.001 levels, respectively.

1989. The number of clusters per vine were comparable between treatments. Differences in berry weights were not observed (data not shown).

General fruit composition: Sugar per berry was generally not affected by treatment (data not shown). Leaf removal influenced °Brix at three of six commercial harvest dates but showed no consistent pattern (Table 3). °Brix accumulation was delayed in the leaf-pulled fruit at the low-trained vineyard in 1987 and the high-trained vineyard in 1989, while leaf removal increased °Brix at the low-trained vineyard in 1989. Fruit pH was unaffected by leaf removal (Table 3). Titratable acidity was lower in fruit from leaf-pulled vines in three of six commercial harvest dates, the low-trained vineyard in 1987 and 1988 and the high-trained vineyard in 1987 (Table 3). The malic acid content was consistently lowered by leaf removal at the low-trained vineyard only (Table 4). Selective leaf

TABLE 4.
Influence of selective leaf removal on malic acid of White Riesling grapes at harvest for three seasons and two vineyard sites.

	Malic Acid (g/L)		
Treatment	1987	1988	1989
High-Trained			
Leaf pull	2.37	2.93	2.20
Control	2.81	3.32	2.63
t-test sig.[z]	ns	ns	ns
Low-Trained			
Leaf pull	2.54	2.01	2.25
Control	3.37	2.47	2.65
t-test sig.[z]	***	**	***

[z]Significance of t-test of treatment means: ns denotes non-significance, **, and *** denote significance at $p \leq 0.01$, and 0.001 levels, respectively.

removal did not influence fruit potassium (data not shown).

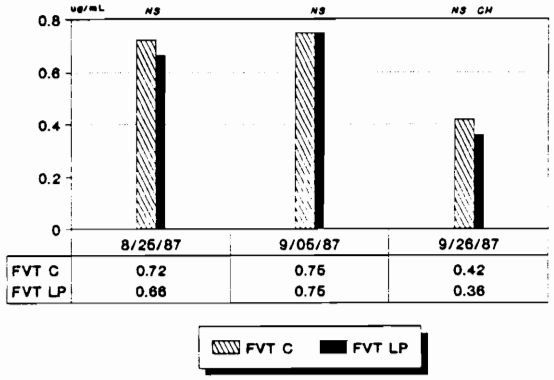
The incidence of fruit rot was typically lower in the leaf-pulled vines, with the exception of the low-trained vineyard in 1988 and the high-trained vineyard in 1989 where no treatment effects were noted (Zoecklein et al., 1992). Fruit rot metabolites were elevated in 1987 and 1988 and frequently showed a high positive correlation with the incidence of observed rot.

Free volatile terpenes: FVT were unaffected by treatment, with the exception of two sampling dates at the low-trained vineyard (commercial harvest in 1988 and mid season in 1989) when leaf removal increased FVT (Figures 1 and 2).

Free aroma components: A determination of free aroma components was undertaken for one year only (1989) due to the frequently insignificant differences between control and treated FVT. The sum of the free monoterpene alcohols, geraniol, nerol, linalool and α-terpineol was higher in the leaf-pulled fruit at the low-trained vineyard at each stage of maturity (Table 5). A slightly different pattern was observed at the high-trained vineyard where the sum of these alcohols were higher in the leaf-pulled fruit except at the first and final samplings (Table 6). The free diol I (3,7 dimethyloca-1, 5 dien-3,7-diol) concentration was greater in the leaf-pulled fruit at each sampling period at both vineyards. At the high-trained vineyard, the sum of the free aromatic alcohols (benzyl alcohol and 2-phenylethanol) was greater in the fruit from control vines at each sample date while the low-trained vineyard had a greater sum in the control fruit except at the last sampling. The sum of the free monoterpene oxide concentration was greater in the leaf-pulled fruit at the low-trained vineyard (Table 5) while the high-trained vineyard showed no consistent pattern (Table 6).

Potentially volatile terpenes: Leaf removal increased PVT at four

LOW-TRAINED VINEYARD 1987

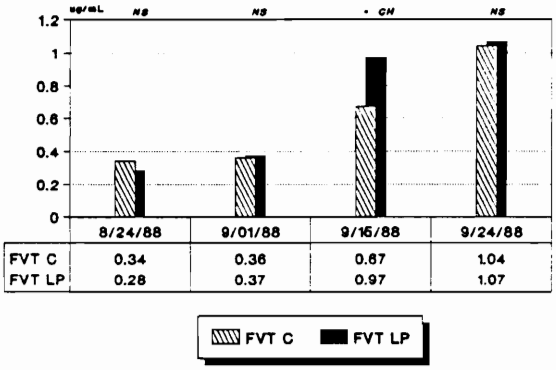


	8/26/87	9/05/87	9/26/87
FVT C	0.72	0.75	0.42
FVT LP	0.66	0.75	0.36

FVT C FVT LP

(NS) Not significant
(CH) Commercial harvest date

LOW-TRAINED VINEYARD 1988

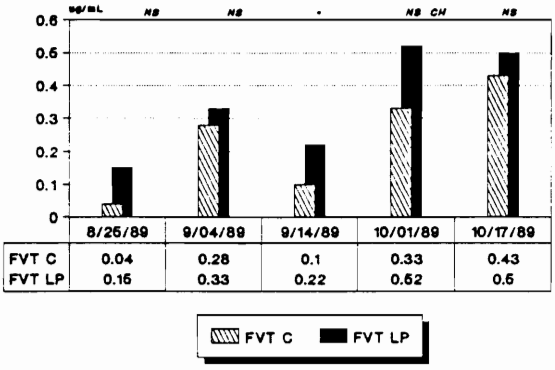


	8/24/88	9/01/88	9/15/88	9/24/88
FVT C	0.34	0.36	0.67	1.04
FVT LP	0.28	0.37	0.97	1.07

FVT C FVT LP

(NS) Not significant
(*) Significant at 5%
(CH) Commercial harvest date

LOW-TRAINED VINEYARD 1989



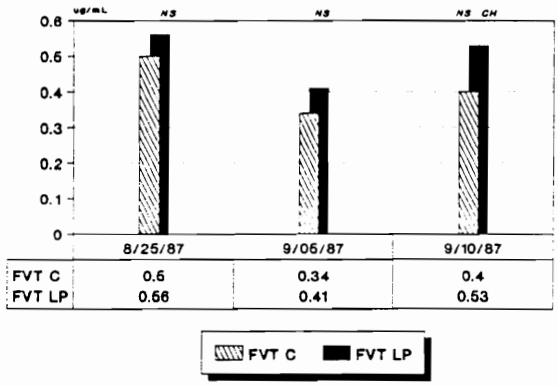
	8/25/89	9/04/89	9/14/89	10/01/89	10/17/89
FVT C	0.04	0.28	0.1	0.33	0.43
FVT LP	0.15	0.33	0.22	0.52	0.5

FVT C FVT LP

(NS) Not significant
(**) Significant at 1%
(CH) Commercial harvest date

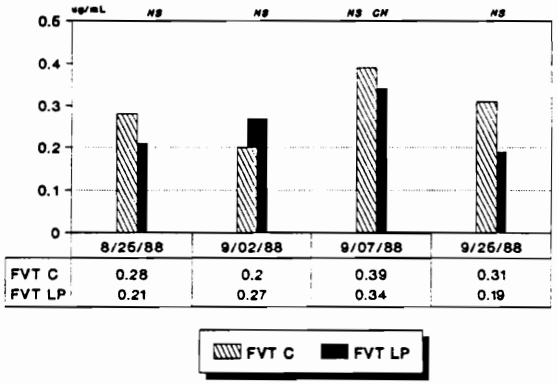
Figure 1. Effect of leaf removal on FVT of White Riesling grapes at the low-trained vineyard for three seasons

HIGH-TRAINED VINEYARD 1987



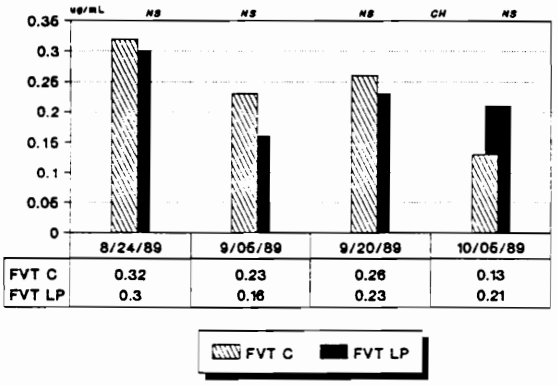
(NS) Not significant
(CH) Commercial harvest date

HIGH-TRAINED VINEYARD 1988



(NS) Not significant
(CH) Commercial harvest date

HIGH-TRAINED VINEYARD 1989



(NS) Not significant
(CH) Commercial harvest date

Figure 2. Effect of leaf removal on FVT of White Riesling grapes at the high-trained vineyard for three seasons

TABLE 5.
Effect of maturity and selected leaf removal on free aroma components of White Riesling grapes at the low-trained vineyard in 1989.

	Harvest Dates							
	8/25		9/04		10/05		10/17	
	°Brix[y]		°Brix		°Brix		°Brix	
Compound (μg/L)[x]	13.2	12.6	16.2	15.8	17.6	17.4	19.2	19.2
	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control
benzyl alcohol	6.5	14.0	23.0	32.2	23.5	27.6	37.7	30.4
t-furan linalool oxide	1.7	1.3	2.3	1.8	2.0	1.4	4.8	2.1
c-furan linalool oxide	nd	nd	0.7	0.4	0.3	0.7	0.9	2.1
linalool	4.5	3.0	15.6	9.5	27.5	4.7	39.4	20.2
2-phenylethanol	11.9	13.4	26.7	34.5	29.4	27.0	33.1	26.4
rose oxide	nd	nd	nd	nd	nd	nd	nd	nd
nerol oxide	nd	nd	nd	0.5	12.9	5.0	14.5	10.2
α-terpineol	4.0	nd	2.0	2.5	nd	2.8	4.4	3.7
nerol	5.5	1.2	1.7	0.9	2.9	1.9	3.0	2.3
geraniol	4.9	6.1	9.2	7.3	13.0	9.0	7.0	4.3
diol I[z]	2.9	2.6	13.7	11.3	22.8	18.4	40.8	31.4

TABLE 5. (Continued)
Effect of maturity and selected leaf removal on free aroma components of White Riesling grapes at the low-trained vineyard in 1989.

	Harvest Dates							
	8/25		9/04		10/05		10/17	
	°Brix[2]		°Brix		°Brix		°Brix	
Compound (μg/L)[1]	13.2	12.6	16.2	15.8	17.6	17.4	19.2	19.2
	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control
sum of monoterpene alcohols: geraniol, nerol linalool and α-terpinol	18.9	10.3	28.5	20.2	43.4	18.4	53.8	30.5
sum of aromatic alcohols: benzyl alcohol and 2-1 phenylethanol	18.4	27.4	47.9	66.7	52.9	54.6	70.8	56.8
sum of terpene oxides	1.7	1.3	3.0	2.7	15.2	7.1	20.2	12.8

[1]Compounds listed in order of increased retention time.

[2]Values denote maturity (°Brix) of pooled samples in chronological order.
nd denotes not found.

[3]Estimated value from mass spectrometer intensities.

TABLE 6.
Effect of maturity and selected leaf removal on free aroma components of White Riesling grapes at the high-trained vineyard in 1989.

Compound (µg/L)[x]	Harvest Dates							
	8/24		9/05		9/20		10/05	
	°Brix[y]		°Brix		°Brix		°Brix	
	13.4	14.0	16.8	16.8	17.0	17.6	17.6	18.2
	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control
benzyl alcohol	24.0	22.5	13.0	31.0	16.0	30.1	31.1	46.5
t-furan linalool oxide	1.3	2.8	2.3	2.9	2.2	2.1	6.3	7.4
c-furan linalool oxide	1.7	2.0	1.7	1.8	1.2	1.1	2.3	2.7
linalool	3.8	3.6	15.9	15.0	6.2	5.0	4.5	4.7
2-phenylethanol	25.0	28.7	36.8	36.8	21.0	30.0	38.9	56.8
rose oxide	nd	nd	nd	nd	nd	nd	nd	nd
nerol oxide	nd	2.9	6.4	6.1	2.3	2.1	3.4	5.0
∝-terpineol	0.3	2.7	13.0	1.8	1.7	1.0	1.7	2.0
nerol	2.7	1.1	8.7	1.7	1.0	1.2	1.0	2.2
geraniol	3.9	6.3	17.8	12.5	11.1	10.0	13.1	22.0
diol I[z]	9.9	8.8	23.2	13.5	27.3	20.1	37.2	26.1

TABLE 6. (Continued)
Effect of maturity and selected leaf removal on free aroma components of White Riesling grapes at the high-trained vineyard in 1989.

Compound (μg/L)[1]	Harvest Dates							
	8/24		9/05		9/20		10/05	
	°Brix[2]		°Brix		°Brix		°Brix	
	13.4	14.0	16.8	16.8	17.0	17.6	17.6	18.2
	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control
sum of monoterpene alcohols: geraniol, nerol linalool and α-terpinol	10.7	13.7	55.4	31.0	20.0	17.2	20.3	30.9
sum of aromatic alcohols: benzyl alcohol and 2-phenylethanol	49.0	51.2	49.8	67.8	37.0	60.1	70.0	103.3
sum of terpene oxides	3.0	7.7	10.4	10.8	5.7	5.3	12.0	15.1

[1]Compounds listed in order of increased retention time.

[2]Values denote maturity (°Brix) of pooled samples in chronological order.
nd denotes not found.

[3]Estimated value from mass spectrometer intensities.

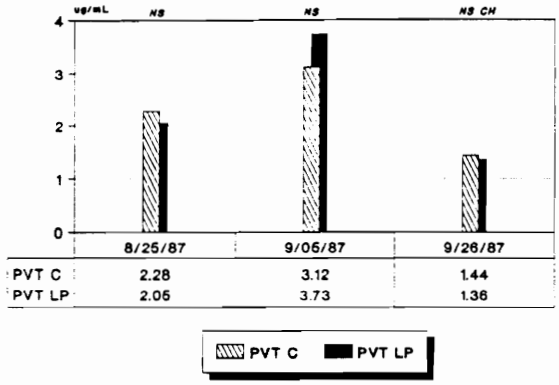
of six commercial harvest dates, at the low-trained vineyard in 1988 and 1989 and the high-trained vineyard in 1987 and 1989 (Figures 3 and 4). Treatment influenced PVT seven of twelve sampling periods at the high-trained and three of twelve at the low-trained vineyard. The effect of leaf removal on PVT was not evident at the initial sampling each year, with the exception of the high-trained vineyard in 1988. Some fruit was kept on the vine following commercial harvest at the high-trained vineyard in 1988 and both vineyards in 1989. This additional fruit maturity resulted in a variable effect on PVT. The high-trained vineyard demonstrated increased PVT in the leaf-pulled fruit in 1988. In 1989, the additional sampling period resulted in the loss of treatment effect.

Bound aroma components: Glycosidically bound aromatic alcohols and monoterpenes were measured in 1988 and 1989 and generally were numerically greater as a result of leaf removal (Tables 7-10). Analysis was not performed on the 1987 fruit due to the high incidence of rot. The sum of the bound monoterpene alcohols (geraniol, nerol, linalool, α-terpinol and including hotrienol) was frequently numerically higher in fruit from leaf-pulled vines. At most sampling dates the bound monoterpene alcohols were in greater concentration than the free form.

At most samplings, the sum of bound 2-phenylethanol and benzyl alcohol was greater in the leaf-pulled fruit. At all sampling dates, bound 2-phenylethanol was more abundant than bound benzyl alcohol.

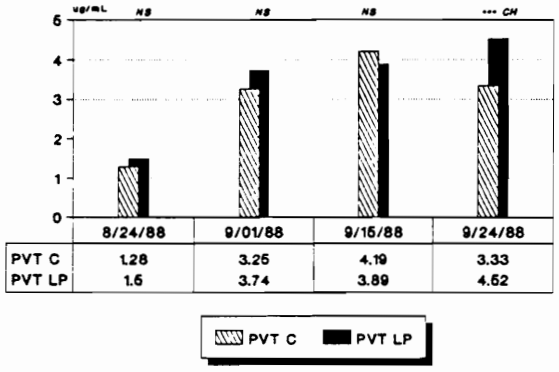
The sum of the bound monoterpene oxides was numerically greater in fruit from leaf-pulled vines at most samplings. An imbalance in the distribution of steroisomeric forms of furan linalool oxides was noted with the trans form always present in greater concentrations (Table 7-10). The concentrations of the stereoisomeric forms of the pyran linalool oxide glycosides were similar. These ratios did not appear to change with treatment. The cis form of rose oxide was present in greater concentra-

LOW-TRAINED VINEYARD 1987



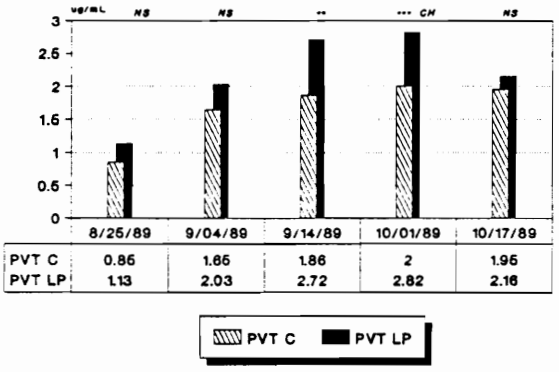
(NS) Not significant
(CH) Commercial harvest date

LOW-TRAINED VINEYARD 1988



(NS) Not significant
(***) Significant at 0.1%
(CH) Commercial harvest date

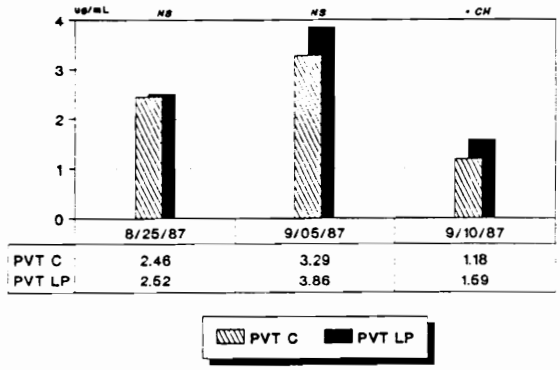
LOW-TRAINED VINEYARD 1989



(NS) Not significant
*(**, ***) Significant at 1%, 0.1%*
(CH) Commercial harvest date

Figure 3. Effect of leaf removal on PVT of White Riesling grapes at the low-trained vineyard for three seasons

HIGH-TRAINED VINEYARD 1987

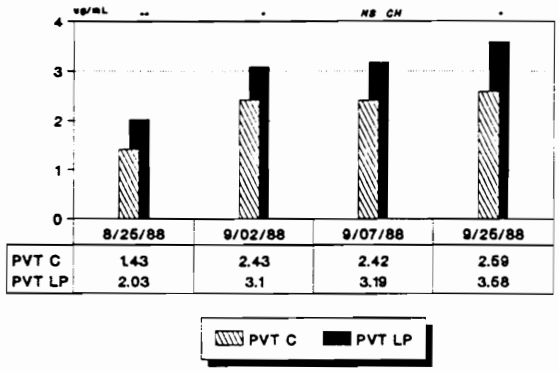


	8/25/87	9/05/87	9/10/87
PVT C	2.46	3.29	1.18
PVT LP	2.52	3.86	1.59

PVT C ■ PVT LP

(NS) Not significant
(•) Significant at 5%
(CH) Commercial harvest date

HIGH-TRAINED VINEYARD 1988

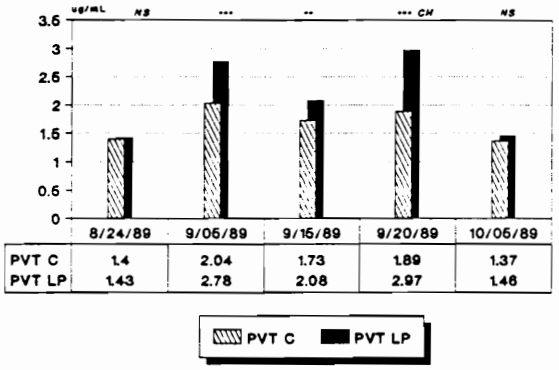


	8/26/88	9/02/88	9/07/88	9/26/88
PVT C	1.43	2.43	2.42	2.59
PVT LP	2.03	3.1	3.19	3.68

PVT C ■ PVT LP

(NS) Not significant
(•, ••) Significant at 5%, 1%
(CH) Commercial harvest date

HIGH-TRAINED VINEYARD 1989



	8/24/89	9/05/89	9/15/89	9/20/89	10/05/89
PVT C	1.4	2.04	1.73	1.89	1.37
PVT LP	1.43	2.78	2.08	2.97	1.46

PVT C ■ PVT LP

(NS) Not significant
(••, •••) Significant at 1%, 0.1%
(CH) Commercial harvest date

Figure 4. Effect of leaf removal on PVT of White Riesling grapes at the high-trained vineyard for three seasons

TABLE 7.
Effect of maturity and selected leaf removal on bound aromatic components of White Riesling grapes at the low-trained vineyard in 1988.

Compound (μg/L)[1]	Harvest Dates							
	8/24		9/01		9/15		9/24	
	°Brix[2]		°Brix		°Brix		°Brix	
	13.4	13.8	15.7	15.5	18.9	19.1	21.1	20.9
	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control
benzyl alcohol	1.6	1.5	2.9	7.1	2.6	2.3	4.3	2.7
t-furan linalool oxide	16.9	16.6	25.5	18.2	22.8	24.2	36.9	27.6
cis-furan linalool oxide	7.7	8.2	12.8	9.6	12.3	11.9	20.5	14.1
linalool	16.3	14.0	45.0	26.6	115.2	81.8	126.1	111.2
2-phenylethanol	70.7	69.0	109.8	84.1	160.7	155.4	226.3	176.7
t-rose oxide	0.4	0.4	0.6	0.6	0.4	0.5	1.0	0.6
nerol oxide	1.7	1.1	4.8	2.6	4.9	4.7	7.6	5.3
cis-rose oxide	2.2	2.1	5.4	3.9	6.9	5.3	7.8	51.4
hotrienol[3]	11.9	11.3	34.6	20.8	36.9	31.5	51.4	41.4
∝-terpineol	101.1	88.1	99.0	83.4	146.3	143.4	161.3	164.9
t-pyran linalool oxide[4]	2.2	3.1	5.4	3.8	8.2	8.1	14.8	10.1
cis-pyran linalool oxide[4]	3.0	3.7	5.1	3.4	7.7	7.8	13.4	9.7

TABLE 7. (Continued)
Effect of maturity and selected leaf removal on bound aromatic components of White Riesling grapes at the low-trained vineyard in 1988.

	Harvest Dates							
	8/24		9/01		9/15		9/24	
	°Brix[y]		°Brix		°Brix		°Brix	
Compound (μg/L)[x]	13.4	13.8	15.7	15.5	18.9	19.1	21.1	20.9
	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control
nerol	4.0	3.4	4.3	2.8	9.7	11.6	20.4	11.6
geraniol	7.8	8.9	13.5	8.9	34.2	28.6	60.2	42.6
sum of monoterpene alcohols: geraniol, nerol, linalool, α-terpineol, hotrienol	141.1	125.8	196.4	142.5	344.2	295.0	419.4	317.7
sum of aromatic alcohols: benzyl alcohol and 2-phenylethanol	72.3	70.5	112.7	91.2	163.3	157.7	230.6	179.4
sum of terpene oxides	34.1	35.2	59.6	42.1	63.2	62.5	103.9	75.2

[x]Compounds listed in order of increased retention time.

[y]Values denote maturity (°Brix) of pooled samples in chronological order.

[z]Estimated values from mass spectrometer intensities.

TABLE 8.
Effect of maturity and selected leaf removal on bound aromatic components of White Riesling grapes at the high-trained vineyard in 1988.

Compound (μg/L)[x]	Harvest Dates							
	8/25		9/02		9/07		9/25	
	°Brix[y]		°Brix		°Brix		°Brix	
	13.7	14.2	16.5	16.5	17.4	17.2	18.0	18.8
	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control
benzyl alcohol	1.9	1.4	2.6	2.9	4.6	3.9	7.2	5.7
t-furan linalool oxide	26.3	19.2	25.8	19.8	41.1	37.8	72.9	57.6
cis-furan linalool oxide	16.1	11.5	16.7	11.5	30.2	20.1	45.0	33.4
linalool	55.8	24.8	108.8	57.9	94.3	71.4	130.1	109.2
2-phenylethanol	118.2	71.1	134.2	91.8	177.4	176.9	309.7	270.8
t-rose oxide	0.3	0.2	0.1	0.5	0.4	0.5	0.7	0.7
nerol oxide	1.8	4.0	3.6	2.4	5.2	7.3	9.7	10.7
cis-rose oxide	4.3	2.6	5.8	3.9	8.7	6.2	14.0	10.6
hotrienol[z]	22.9	13.6	30.2	20.8	40.5	38.0	44.7	36.6
α-terpineol	83.1	86.3	169.3	91.2	215.0	200.2	234.1	212.6
t-pyran linalool oxide[z]	2.7	3.0	6.1	3.9	4.1	3.8	9.2	8.7
cis-pyran linalool oxide[z]	3.4	3.8	5.2	3.4	5.5	2.9	8.8	7.9

TABLE 8. (Continued)
Effect of maturity and selected leaf removal on bound aromatic components of White Riesling grapes at the low-trained vineyard in 1988.

Compound (µg/L)[x]	Harvest Dates							
	8/25		9/02		9/07		9/25	
	°Brix[y]		°Brix		°Brix		°Brix	
	13.7	14.2	16.5	16.5	17.4	17.2	18.0	18.8
	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control
nerol	5.6	3.3	9.4	3.7	14.8	12.5	28.3	18.9
geraniol	18.4	10.0	31.1	13.6	29.9	35.3	60.6	50.1
sum of monoterpene alcohols: geraniol, nerol, linalool, α-terpineol, hotrienol	185.8	138.0	348.8	187.2	394.5	357.4	497.8	427.4
sum of aromatic alcohols: benzyl alcohol and 2-phenylethanol	120.1	72.5	136.8	94.7	182.0	180.8	316.9	276.5
sum of terpene oxides	54.9	44.3	63.3	45.4	95.2	78.6	160.3	129.6

[x]Compounds listed in order of increased retention time.

[y]Values denote maturity (°Brix) of pooled samples in chronological order.

[z]Estimated values from mass spectrometer intensities.

TABLE 9.
Effect of maturity and selected leaf removal on bound aromatic components of White Riesling grapes at the low-trained vineyard in 1989.

	Harvest Dates									
	8/25		9/04		9/14		10/01		10/17	
	°Brix[y]		°Brix		°Brix		°Brix		°Brix	
Compound (μg/L)[x]	13.1	12.7	16.3	15.8	16.7	17.3	17.6	17.4	19.2	19.2
	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control
benzyl alcohol	2.0	3.4	8.5	1.9	0.5	0.3	1.2	0.9	1.4	0.8
t-furan linalool oxide	6.3	7.0	9.9	6.7	22.3	28.0	35.0	22.9	31.6	32.0
cis-furan linalool oxide	4.9	3.5	5.2	3.9	12.0	12.0	18.7	12.8	18.2	16.0
linalool	16.9	10.3	14.3	16.0	69.0	71.5	89.2	68.3	51.3	41.4
2-phenylethanol	88.4	59.4	99.7	96.3	140.0	116.0	167.0	184.0	142.0	136.0
t-rose oxide	1.3	nd	0.1	0.1	0.2	0.1	0.2	0.1	1.6	1.3
nerol oxide	6.3	4.8	0.9	1.3	0.6	0.5	1.0	1.0	3.5	9.2
cis-rose oxide	3.3	2.1	0.3	0.5	0.3	0.5	1.6	1.0	1.9	1.8
hotrienol[z]	17.8	11.3	1.6	2.8	3.2	3.0	8.3	5.6	9.6	5.7
α-terpineol	50.8	31.9	128.9	88.9	144.0	121.7	117.3	87.9	83.3	66.3
t-pyran linalool oxide[z]	4.1	2.2	0.9	1.0	1.2	4.0	4.0	2.3	5.4	4.2

TABLE 9. (Continued)
Effect of maturity and selected leaf removal on bound aromatic components of White Riesling grapes at the low-trained vineyard in 1989.

	Harvest Dates									
	8/25		9/04		9/14		10/01		10/17	
	°Brix[y]		°Brix		°Brix		°Brix		°Brix	
Compound (μg/L)[z]	13.1	12.7	16.3	15.8	16.7	17.3	17.6	17.4	19.2	19.2
	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control
cis-pyran linalool oxide[x]	4.2	2.2	0.6	0.7	1.0	0.8	3.0	2.1	4.0	3.0
nerol	5.4	2.9	6.1	9.1	3.4	4.5	2.8	2.0	3.6	3.2
geraniol	4.8	nd	25.2	18.0	55.0	16.7	68.7	53.4	20.2	18.1
sum of monoterpene alcohols: geraniol, nerol, linalool, α-terpineol, hotrienol	95.7	56.4	176.1	134.8	274.6	217.4	286.3	217.2	168.0	134.7
sum of aromatic alcohols: benzyl alcohol and 2-phenylethanol	90.4	62.8	108.2	98.2	140.5	116.3	168.2	184.9	143.4	136.8
sum of terpene oxides	33.6	21.8	17.9	14.2	37.6	43.1	63.5	42.2	66.2	67.5

[x]Compounds listed in order of increased retention time.

[y]Values denote maturity (°Brix) of pooled samples in chronological order.

[z]Estimated values from mass spectrometer intensities.

TABLE 10.
Effect of maturity and selected leaf removal on bound aromatic components of White Riesling grapes at the high-trained vineyard in 1989.

	Harvest Dates									
	8/24		9/05		9/15		9/20		10/05	
	°Brix[y]		°Brix		°Brix		°Brix		°Brix	
Compound (μg/L)[z]	13.4	14.0	16.8	16.8	17.6	18.3	17.0	17.5	17.6	18.2
	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control
benzyl alcohol	1.3	0.9	2.7	2.0	2.0	1.1	2.5	1.0	2.8	2.7
t-furan linalool oxide	17.9	18.3	28.0	23.5	26.2	14.5	53.2	30.5	38.6	25.2
cis-furan linalool oxide	7.9	9.9	15.0	12.4	16.4	11.0	31.3	18.9	25.4	13.7
linalool	30.6	11.4	83.4	55.3	73.0	41.6	100.1	139.7	78.0	42.4
2-phenylethanol	60.0	70.0	144.8	119.9	114.0	69.0	94.5	53.1	64.8	78.5
t-rose oxide	nd	nd	0.5	0.5	0.3	0.3	0.5	0.4	0.6	0.7
nerol oxide	3.8	2.8	3.6	2.7	4.2	3.7	5.9	nd	3.8	3.4
cis-rose oxide	5.3	2.4	5.1	4.2	4.9	3.2	8.9	7.5	4.6	2.7
hotrienol[x]	nd	nd	26.9	22.6	25.9	17.4	25.1	16.7	24.5	14.4
α-terpineol	93.5	53.7	137.0	107.5	145.6	81.4	214.9	125.5	181.3	101.8
t-pyran linalool oxide[x]	nd	nd	6.6	5.0	6.6	4.9	7.6	4.7	9.4	4.8

TABLE 10. (Continued)
Effect of maturity and selected leaf removal on bound aromatic components of White Riesling grapes at the low-trained vineyard in 1989.

	Harvest Dates									
	8/25		9/04		9/14		10/01		10/17	
	°Brix[y]		°Brix		°Brix		°Brix		°Brix	
Compound (μg/L)[x]	13.1	12.7	16.3	15.8	16.7	17.3	17.6	17.4	19.2	19.2
	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control	Leaf Pull	Control
cis-pyran linalool oxide[x]	nd	nd	6.6	5.0	6.6	4.9	7.6	4.7	9.4	4.8
nerol	1.6	0.8	8.5	5.9	9.2	5.8	17.0	12.6	10.3	6.2
geraniol	7.4	3.3	24.6	16.3	23.7	15.0	36.0	29.5	24.4	12.9
sum of monoterpene alcohols: geraniol, nerol, linalool, α-terpineol, hotrienol	133.1	69.2	280.4	207.6	277.4	161.2	393.1	324.0	318.5	177.7
sum of aromatic alcohols: benzyl alcohol and 2-phenylethanol	61.3	33.4	64.7	53.8	65.6	42.3	115.8	66.8	94.7	55.3
sum of terpene oxides	33.6	21.8	17.9	14.2	37.6	43.1	63.5	42.2	66.2	67.5

[x]Compounds listed in order of increased retention time.

[y]Values denote maturity (°Brix) of pooled samples in chronological order. ns denotes not found.

[z]Estimated values from mass spectrometer intensities.

tion than the trans isomer and in numerically greater concentration in the leaf-pulled fruit. Bound diol 1 is not readily liberated from its glycone by enzyme hydrolysis and therefore was not measured.

Sensory evaluation: Sensory evaluations were conducted in 1988 and 1989 comparing juice aromas. Analysis was not conducted in 1987 due to the extremely high incidence of fruit rot. The aroma of the control juice differed from leaf-pulled juice from the low-trained vineyard in 1988 (Table 11). Aroma differences were not perceived in 1989 in untreated juice. Significient differences, however, were noted in the aroma of acid hydrolyzed juice at both vineyards in 1989.

Discussion

The macroclimate of the two vineyard sites in Northern Virginia typifies much of the mid-Atlantic region, with precipitation during the summer averaging about 10 cm per month, and the mean temperature in July at 24°C. The abundant moisture and heat contribute to excessive vine size

TABLE 11.
Effect of selective leaf removal on the duo-trio difference tests for White Riesling juice aroma at the high-trained and low-trained 1988 and 1989.

	High-Trained	Low-Trained
COMPARISON	AROMA	AROMA
1988 Control vs. Leaf Pull	11[ns]	16.
1989 Control vs. Leaf Pull	14[ns]	13[ns]
1989 Control vs. Leaf Pull (Acid hydrolyzed juice)	16[*]	16.

[y]Numbers indicate correct responses out of twenty-two responses. Significance paired t-test (nd), and (*) indicate no significant difference and significance at $p \leq 0.05$. Sixteen correct responses needed for significances at $p \leq 0.05$ level.

resulting in leaf and cluster shading. Although significant differences in the PPFD were noted between control and treated vines, these differences were not large. Shading was particularly evident at the low-trained vineyard where low PPFD values were recorded in the fruit zone even in the leaf-pulled treatments. At the low-trained vineyard in 1987, for example, the fruiting zone of the control vines received about 1% of the ambient PPFD, while less than 5% was received by the treated vines. At the low-trained vineyard the training system encouraged upward growth, forming a "roof" over the fruiting zone that helped to reduce canopy isolation. Alternatively, the high-trained vineyard, with its open canopy and downward growth, allowed for more light in the fruit zone than the low-trained vineyard.

Differences in fruit weight per vine in 1987 at both vineyards and the average cluster weight at the low-trained vineyard may reflect loss of fruit weight in control vines due to rot and subsequent dehydration, rather than improved yield components as a result of leaf removal. For example, the incidence of fruit rot in 1987 was 82% and 73% for control vines compared with 62% and 54% for the leaf-pulled fruit at the low and high-trained vineyards, respectively.

The lower sugar content (°Brix) of the low-trained leaf-pulled grapes in 1987 and the high-trained leaf-pulled fruit in 1989 could suggest insufficient leaf area to mature the crop. Several defoliation studies have also reported reductions in °Brix (Kliewer and Antcliff, 1970; May et al., 1976; Kingston and Van Epenhuijsen, 1989). However, Bledsoe et al. (1988), working with Sauvignon blanc grapes, reported increases in °Brix as a result of selective leaf removal, similar to the increase noted only once in the current study (the low-trained vineyard in 1989). An increase in °Brix may be due to a lower water content of sun exposed berries, as suggested by Crippen and Morrison (1986). It also seems likely that

variations in the °Brix response to leaf removal could be the result of cultivar and macroclimate differences. Working in a cool grape growing region in California, Bledsoe et al. (1988) found that basal leaf removal increased the degree-day heat accumulation in the cluster region by as much as 2.7%. The influence of leaf removal on sugar accumulation may be enough to advance maturation.

Titratable acidity was lower in the leaf-pulled fruit (3 of 6 commercial harvest dates), likely the result of differences in solar exposure and the incidence of fruit rot. Kliewer and Lider (1968) and Smart (1982) demonstrated that clusters exposed to solar radiation have low malic acid concentrations compared to shaded clusters. Reduced malic acid in the low-trained, leaf-pulled vines helps to explain the reduction in titratable acidity. Leaf removal frequently reduced the incidence of fruit rot, possibly as a result of the interaction of factors such as evaporative potential, temperature, humidity, increased spray penetration, and solar exposure (Zoecklein et al., 1992).

FVT were generally unresponsive to canopy modification, which is consistent with Eschenbrush et al. (1987). FVT appeared to decline in 1987 at the low-trained vineyard at the final sampling. This may have resulted from turnover mechanisms characteristic of secondary metabolites, including curtailment of synthesis, volatilization, and/or oxidative degradation. Croteau (1984), for example, reported a 50% transformation of monoterpenes in peppermint including both losses and glycoside formation. Zoecklein et al. (1992) reported White Riesling berry temperature to be 11.5 $\pm$ 4.8°C and 3.1 $\pm$ 3.0°C warmer than the ambient daytime air temperature for exposed and shaded fruit, respectively. At the end of each growing season, both control and leaf-pulled grapevine canopies at the low-trained vineyard had a high percentage of senescent leaves, possibly increasing fruit exposure to direct radiant energy. Day

time temperatures at the end of the 1987 growing season were frequently greater than 30°C with warm nighttime temperatures, possibly contributing to lower photosynthetic activity and a reduction in terpene synthesis.

Strauss et al. (1986) reported that the principle free volatile monoterpenes in Muscat varieties are linalool, geraniol, nerol, linalool oxides, α-terpineol, and citronellol. With the exception of citronellol, these compounds were present and, often in greater concentration in fruit from the leaf-pulled White Riesling vines. The concentration of free monoterpene alcohols and oxides underwent several fluctuations during fruit maturation, particularly at the high-trained vineyard, where free linalool, α-terpeniol, declined and frequently peaked again. There was no obvious climatic variable to account for this flux.

Free diol I increased rapidly and dominated the free terpene distribution. Diol I concentrations were numerically higher in fruit from leaf-pulled vines. The concentration approximated or exceeded the sum of the free monoterpene alcohols at the final two samplings at both vineyards. Wilson et al. (1984), working with Muscat grapes, also reported the concentration of free diol I to be greater than the sum of all other free monoterpenes in ripe fruit. Polyhydroxylated compounds such as diol I are extremely labile and easily lost in extraction and analysis, rearranging even under mild acid conditions to produce a wide array of volatile terpenes (Williams et al., 1980). Efforts to minimize breakdown included adjustment of juice pH to neutrality prior to solvent extraction and the removal of trace acids from glassware and solvents with pyridine prior to analysis. Due to possible losses in the extraction procedures and the fact that the diol I estimations are based upon mass spectrometry intensities, data must be interpreted with caution. The elevation in diol I may not have been reflected in the FVT analysis performed by distillation, because of the labile nature of this compound.

The free linalool oxide oxidation state compounds appeared to be more abundant in the riper leaf-pulled fruit at the low-trained vineyard. Monoterpene oxides have been reported to be the result of polyol hydrolysis (Williams et al., 1980). It is possible that the elevated free nerol oxide concentrations reflect the increase in free diol I and subsequent hydrolysis. The concentration of oxidized monoterpenes and the transformation of these compounds in the fruit are of possible interest in search of minor, yet possibly important, aroma components.

Although differences in FVT were generally not observed, qualitative as well as quantitative changes in the free aroma components may be sensorially significant. Linalool and geraniol, for example, are much more potent and pleasantly fruity than α-terpineol and the monoterpene oxides. Linalool and geraniol have aroma thresholds in water of 100 and 130 μg/L respectively, while the linalool oxides aroma thresholds are in the 3000-5000 μg/L range (Marais, 1983). One monoterpene can decrease the aroma threshold of another, and a mixture (including oxides) can become more aromatic than the single most aromatic component of that mixture (Ribereau-Gayon et al., 1975). However, the maximum difference in the concentration of individual free monoterpenes between leaf-pulled and control fruit was below the sensory thresholds reported for each compound at every sampling period.

Leaf removal influenced PVT at a greater number of samplings than FVT. In 1987, however, leaf-pulled PVT was greater than control PVT at only one sampling period, commercial harvest at the high-trained vineyard. In 1987, treatment may have been confounded by both yield and fruit rot. Fruit yield was greater for the leaf-pulled vines at both vineyards in 1987. McCarthy (1986) and Eschenbrush et al. (1987) demonstrated that higher fruit yields reduced PVT in Muscat grapes. Additionally, fruit rot was extreme in 1987, with the incidence of fruit rot at both vineyards

averaging 78% in control and 58% for leaf-pulled fruit. Rot may have led to the apparent reductions in both FVT and PVT at the low-trained vineyard and PVT at the high-trained vineyard as suggested by Boidron (1978) and Morin and Richard (1985).

PVT appeared to decline by the final sample dates at both vineyards in 1987 and 1989 while °Brix increased. Gunata et al. (1985a) reported increases in the glycosidically bound terpenes of Muscat Alexandria after commercial ripeness (22 °Brix), while others have reported the opposite trend (Ewart et al., 1984; Wilson et al., 1984). The PVT concentrations in the later sampling dates may reflect metabolic decline and/or breakdown in ripe or overripe fruit. However, reductions in glycosidically bound compounds cannot simply be explained by increases in FVT or individual hydrolysis products measured. With the exception of the high-trained vineyard in 1987, the FVT content also showed some reduction at the end of the 1987 and 1989 seasons.

The bound monoterpene alcohols were dominated by linalool and α-terpineol, with geraniol and nerol frequently less abundant. The sum of the concentration of these glycosides plus hotrienol was higher in the leaf-pulled fruit at each sampling even when sugar concentration (e.g. °Brix) in control fruit was greater. This may suggest that glycoside accumulation is independent of sugar translocation or concentration.

Changes in the concentration of bound monoterpene alcohols with maturity appeared more irregular in 1989 than in 1988, particularly at the high-trained vineyard. This may reflect the wider distribution of available PPFD noted at the high-trained vineyard, particularly in 1989. Increases in secondary metabolites would be expected to accompany periods of high metabolic activity (Croteau, 1984). However, glycosides of monoterpene alcohols appeared to have declined at both vineyards by the last sampling date in 1989 (paralleling the change in PVT) even though °Brix

increased. Despite apparent reductions, monoterpene alcohols in the leaf-pulled fruit averaged 87 μg/L more than the control fruit for both vineyards at the last sampling. The high-trained leaf-pulled fruit had a terpene alcohol concentration which was 141 μg/L more than the control fruit, a difference above the sensory threshold of linalool (100 μg/L) and geraniol (140 μg/L) in water (Marais, 1983).

A slightly different pattern was noted in 1988, where monoterpene alcohols appeared to show a steady increase with increased maturity. The higher concentration of monoterpene alcohols in 1988 compared to 1989 may reflect advanced maturity in the 1988 fruit at harvest.

The sum of the bound aromatic alcohols followed the same general pattern noted for the terpene alcohols; that is they appeared to increase with maturity. The exception was the high-trained vineyard in 1989 where concentrations peaked at the second sampling. The level of benzyl alcohol was consistently lower than that of 2-phenylethanol and lower than reported elsewhere for White Riesling grapes (Gunata et al., 1985b). This may be the result of clonal variation which has been shown to influence grape aroma components (Rapp, 1988).

The sum of the bound monoterpene oxides increased numerically with maturity and was frequently higher in leaf-pulled fruit. Oxides were dominated by trans-furan linalool oxides which is consistent with Wilson et al. (1984). Increases in terpene alcohols are of greater interest than oxides due to differences in their sensory thresholds. For example, the sensory thresholds of the furan oxides are perceptible in water only at concentrations greater than 6000 μg/L (Marias, 1983).

Monoterpenes can undergo considerable fluctuations as a result of isomerization, and/or breakdown (Croteau, 1984). Such changes were reflected in this study. An inherent difficulty in the analysis of grape monoterpenes, including polyols, stems from their labile nature. For

example, hydrolysis would be expected to convert at least a portion of the labile diol I to both hotrienol and nerol oxide (Williams et al., 1980). Hotrienol was generally found in higher concentration in the fruit from leaf-pulled vines yet the irregular pattern demonstrated in the low-trained vineyard in 1989, for example, may have resulted from the unavoidable breakdown of diol I. Trio 3(3,7-dimethyloct-1-en 3,6,7 triol) was not detected, although this very labile compound has been reported in White Riesling grapes (Williams et al., 1980). Elevated levels of linalool oxides may have resulted, in part, from the hydrolysis of trio 3 (Williams et al., 1980). It is also possible that some change in the concentration of individual monoterpenes could have resulted from biochemical rearrangement in addition to hydrolysis of polyols. For example, linalool may be formed from nerol and/or geraniol, while α-terpineol may be generated by linalool, nerol and/or geraniol (Banthorpe et al., 1978; Wilson et al., 1984).

Several explanations can be offered for the increase in PVT in fruit from leaf-pulled vines. For example, Allard (1944) and Burbott and Loomis (1967) have suggested that increased photosynthesis resulting from increased light interception may explain enhanced monoterpene production in peppermint. Grapevine modification studies which increase solar radiation into the canopy have also reported to increase photosynthetic efficiency of the remaining leaves (Buttrose, 1966; Kriedemann and Lenz, 1972; Hofacker, 1978). Possible increases in photosynthesis were not consistently reflected in increased °Brix or sugar per berry in this study. However, leaf removal had the most frequent influence on PVT at the high-trained vineyard where differences between the treated and control PPFD were frequently the greatest.

PVT could have been influenced by changes in the pattern of assimilate movement. Williams et al. (1981) suggested that the grape

hypodermal cells are the sites for both biosynthesis and storage of terpenes such as gerianol, with precursor materials translocated from the leaves. The source-sink relationship in fruit crops involves the leaves (source of photosynthesis products) and fruit (usually the sink for photosynthesis products) and the movement between them. Older basal leaves, which were removed, have photosynthesis rates 1/3 of recently expanded leaves (Winkler et al., 1974). Kapps (1984) noted that shaded leaves on the basal portion of grapevine shoots do not contribute to either yield or fruit soluble solids. Treatment probably resulted in the removal of leaves not contributing to assimilate production, and may have caused changes in the source-sink relationship. Quinlan and Weaver (1970), for example, were able to alter the pattern of assimilate movement toward the clusters by various treatments, including the removal of basal leaves. Additionally, it is known that grape berries have a high sink strength (Johnson et al., 1982). Coombe et al. (1987) showed a four-fold increase in grape dry mass during six weeks of growth, with little change in the dry mass of any other part of the plant. Basal leaf removal may have minimized intravine competition for carbohydrates going to the fruit by the elimination of superfluous sinks.

Sensory evaluation: Evaluators were able to distinguish leaf-pulled from control juices from the low-trained vineyard in 1988. However, differences were not necessarily based on typical terpene-type aroma "notes". The relatively high levels of fruit rot metabolites may have added an unavoidable confounding influence. The 1989 leaf-pulled and control juice samples provided the same sensory results. However, the addition of a hydrolyzing agent (H_2SO_4) to lower the pH to 2.5 and the readjustment back to native pH resulted in significant aroma response. Hydrolyzed, leaf-pulled juices in 1989 were characterized as having tropical fruit aromas. A higher concentration of bound polyols in the

leaf-pulled fruit could help explain the difference between the initial and subsequent sensory analysis of the 1989 samples. Polyols are sufficiently labile to break down hydrolytically, particularly at low pH, yielding a vast array of volatile aroma compounds (Williams et al., 1980). However, due to the differences in the incidence of fruit rot at the low-trained vineyard and the severity at both vineyards, it is possible that rot was also a concluding influence in 1989.

Conclusion

The effect of selective fruit zone leaf removal on aroma components of White Riesling grapes grown at two sites, on one of two training systems for three seasons, was investigated. Generally, leaf removal did not influence FVT and had a limited influence on the individual free grape aroma components. Leaf removal increased the PVT in three of twelve and seven of twelve samplings for the low and high-trained vineyard respectively, over the three years of study. The sum of the glycosidically bound monoterpene alcohols was often numerically higher in fruit from the leaf-pulled vines. Differences in juice aromas were frequently not observed. Sensory analysis appeared to be confounded by extreme fruit rot which occurred in both control and, to a lesser extent, leaf-pulled vines. The inability to reduce rot further may be due to the complex nature of fruit rots in Virginia.

Varietal grape aroma and aroma intensity are needed for high quality White Riesling wines. Most of the volatile flavorants in grapes are alcohols which are removed from direct contribution to flavor by biochemical oxidation. After synthesis, monoterpenes are biochemically directed toward reducing flavor as a result of additional hydroxyls and/or by glycosylation. Once a monoterpene is directed to an oxidative pathway to form a polyhydroxylated compound or glycoside, it is no longer a grape

flavorant. While this study demonstrated the influence of leaf removal on increasing PVT, these products themselves have no direct and immediate flavor value. However, there is some reclamation of flavor by hydrolysis of glycosides and polyols. Therefore, the increase in glycosidically bound aroma components as a result of selective leaf removal represents an increase in the pool of potential aroma components.

CHAPTER IV

Effect of Selected Strains of *Saccharomyces cerevisiae* on Free and Bound Monoterpenes of White Riesling (*Vitis vinifera L.*) During Fermentation and Aging

Abstract. Four strains of *Saccharomyces cerevisiae* were evaluated for their influences on free and conjugated aroma components of White Riesling grapes, immediately following and 45 days post-fermentation on the lees (*sur lie*). The potentially volatile terpene (PVT) concentrations were similar among wines following fermentation, the exception being those produced by the Fermiblanc (FB) yeast strain. Fermentation resulted in a decrease in the sum of the bound terpenes from 7-29%. Hydrolysis of bound compounds occurred following storage *sur lie*, the exception being the FB fermented wines. Fermentation reduced free terpenes, except for α-terpeniol, hotrienol, citronellol, furanic and pyranic linalool oxides. Fermentation also increased both free benzyl alcohol and 2-phenylethanol. In newly fermented and aged wines the concentrations of free volatiles differed among yeasts, but differences were always below the sensory threshold for each compound.

Introduction

The aroma of wines is a function of grape components and those produced during processing, fermentation and aging. They have been characterized by Rapp (1988) as: 1) primary grape aroma compounds found in undamaged plant cells; 2) secondary grape aroma compounds formed during crushing and pressing and by chemical, enzymatic-chemical and thermal reactions in grape juice; 3) fermentation bouquet, aroma compounds formed during alcoholic fermentation; 4) maturation bouquet caused by chemical reactions during maturation of bottled wines.

Yeasts have a strong influence on the sensory character of wines. Investigations on the effect of yeasts on wine aroma confirm the diversity of strains with respect to the production of higher alcohols and esters (Houtman and du Plessis, 1986). Indeed, it has been suggested that yeast strain selection be established for specific varieties and styles of wines (Giudici et al., 1985). The microbiological "quality" of a yeast is easily determined; however, suitability of a particular strain for stylistic winemaking depends on alcohol tolerance, the ability to ferment completely at low temperatures, resistance to sulfur dioxide, minimal lag phase, malic acid degradation, the production of glycerol, the killer factor and β-glucosidases (Fleet, 1993).

Secondary grape metabolites are responsible for the principle flavor compounds of juice and provide the basis of "varietal" character in wines (Schreier, 1984). While there are numerous reports on yeast metabolites and their sensory influences, relatively little research has been directed toward understanding the effect of yeasts on grape aroma components and their precursors.

In White Riesling, an important aromatic grape variety, terpenols are important aroma constituents. These compounds are present as free volatile terpenes (FVT) and as conjugated or bound, nonvolatile

precursors -potentially volatile terpenes (PVT). The FVT group contains both free, odorous compounds and non-odorous polyhydroxylated derivatives (polyols). Conjugated components (mainly glycosides) can undergo acid or enzyme hydrolysis to yield free volatiles or odorless polyols, which can undergo acid hydrolysis to form aroma components. This same scheme applies to benzyl and 2-phenylethanol, the two aromatic alcohols also found in White Riesling grapes.

Hydrolysis of glycosides has been studied as a means of enhancing the aroma of grape juice through the formation of free volatiles (Williams et al., 1982c; Abbott et al., 1993). For complete enzymatic hydrolysis of conjugated grape monoterpenes to occur, β-glucosidase, α-arabinosidase, α-rhamnosidase, β-xylanosidase, and β-apiosidase activity must be present (Gunata et al., 1994). Initially, the 1-6 linkage of the sugar must be cleaved by either α-arabinofuranosidase or α-L-rhamnosidase. Arabinose, rhamnose and the corresponding monoterpenyl β-D-glucoside are released. The liberation of the monoterpenol then can take place after the action of β-D-glucosidase (Gunata et al., 1988).

In order to fully enhance grape aroma, glycosidases must be capable of hydrolyzing both mono and diglycosides of primary and tertiary alcohols, norisoprenoids and shikimic acid derivatives (Park and Noble, 1993). Additionally, grapes are known to employ sugars with both ester and glycosidic linkages and several non-sugar moieties for conjugation, further restricting the effectiveness of enzymes (Betz and Koster, 1981). These limiting factors have added to speculation that yeasts play only a minor role in unlocking the flavor potential of bound aroma components. Hock et al. (1984) showed *S. cerevisiae* (unnamed strain) produced only traces of farnesol during fermentation. This suggests the terpene pattern of various grape cultivars used for varietal classification is not influenced by yeast fermentation. However, several experiments have also

demonstrated hydrolysis of monoterpene glycosides during fermentation (Cordonnier et al., 1975; Gunata et al., 1986). Gunata et al.(1986) and Park and Noble (1993) showed free monoterpenes of Muscat of Alexandria increased with reductions in the conjugate forms during fermentation and storage. However, these changes resulted from hydrolysis occurring in an acidic media (wine) and may not have been influenced directly by the yeast.

Although *Saccharomyces cerevisiae* produces the enzymes needed for glycoside hydrolysis with standard winemaking techniques, a large percentage of the aroma potential of the grape remains in the conjugated form and is, non-volatile (Gunata et al., 1994). It has been suggested that yeast cells may not excrete ß-glucosidase and/or that glycosides are not transported across the cell wall (Williams, 1994). Leighton (1994) proposed carbon catalyzed repression of β-glucoside occurs during yeast fermentation which prevents enzymatic hydrolysis of glycosides. However, post-fermentation lees contact in the absence of glucose showed active β-glucosidase.

The use of yeast species and strains with improved hydrolytic activity could help to 'unlock' potential aroma components, and be of broad-based significance to the wine industry because the biochemical mechanisms utilized for mono-terpene production are also present in non-terpene varieties. Non-terpene varieties also derive their aromas from free volatiles while the majority of the grape potential is locked up in the form of conjugated products.

Currently, several strains of *Saccharomyces cerevisiae* are marketed as "enhancers of varietal expression." The implication is these yeasts have increased ability to hydrolyze conjugated aroma precursors, improving wine aroma and aroma intensity. Comparing chromatograms of grape juice and corresponding wines can yield useful information about the changes in

aroma composition as a result of fermentation and whether components originate in the fruit or are formed during fermentation. This study evaluated the influence of four strains of *Saccharomyces cerevisiae* on White Riesling monoterpenes during and following fermentation.

Materials and Methods

White Riesling grapes (750 pounds) were harvested from Barboursville Vineyards in 1994 at 17.1 °Brix, chilled to 9°C for 12 hours and crushed. Skin contact occurred for four hours at 9°C followed by pressing. The free run and press juice were combined and cold settled at 1°C for 24 hours, racked, warmed to 18°C and divided into sixteen 3-gallon carboys, with 1.94 gallons of juice per container.

Treatments: Four carboys were randomly selected and inoculated with a 3% (v/v) actively growing culture of a single strain of *Saccharomyces cerevisiae* grown from 5 grams of dry active yeast rehydrated in a 5% dextrose solution at 45°C. Each culture was microscopically examined for percent budding and culture viability as described by Zoecklein et al. (1990).

The cultured yeasts examined included the following high "varietal expression" strains:

VL-1, and F47 (Lallemand, Inc., Montreal, Canada) and

Fermiblanc, FB, (Gist-brocades, Inc., Cedex, France).

Prise de Mousse PDM, (UCD 796, Universal Foods Corp., Milwaukee, Wisconsin), a standard yeast strain.

Pre-fermentation and following fermentation each wine lot, was analyzed for °Brix, alcohol % (v/v), pH, titratable acidity, tartaric, and malic acids, potentially volatile terpenes (PVT), and selected free and conjugated monoterpenes as described by Zoecklein et al. (1990). Fermentations were analyzed for the above mentioned parameters at dryness (less than 2.0g/L reducing sugar). Additionally, dry wines were analyzed

for glucose, fructose, succinic and acetic acids and glycerol.

Fermentation rates were determined for each lot from 5-50% of sugar consumption and zero to 99% sugar utilization. Selected free and bound aroma components were isolated before, during and following fermentation using Amberlite XAD-2 as described in the Material and Methods section. The hydrolysis procedures were as follows: phosphate buffer (0.1 mL of 0.2 M citrate-phosphate) at pH 5.0 was added to the conjugate mixture and rinsed four times with 0.1 mL of pentane to remove free or unbound compounds. Samples were agitated using a laboratory vortex between rinses. A commercial pectinolytic enzyme (Rohapect C, Rohm-Tech Inc., New York, NY) was added (0.4 mg) in 0.2 mL citrate-phosphate buffer (0.2 m) at pH 5.0 and incubated for 24 hours. Ten μL of a 0.1% solution of 2-octanol in ethanol was added as an internal standard. The solution was extracted (5x5mL) with trichlorofluromethane (Freon 11), concentrated to 50 μg/L and stored at -25°C (for less than 4 days) until gas chromatographic analysis.

Following fermentation a portion of each wine lot was stored *sur lie*. Forty-five days post-fermentation, free and conjugated fractions were isolated from the *sur lie* wines as described above.

Gas chromatographic analysis was performed as described in the Material and Methods section.

Statistical analysis: One-way analysis of variance and Least Significant Difference (LSD) comparison tests of SAS (SAS Institute, Cary, NC) were used to statistically interpret differences in means, if any. Analysis was conducted at the 95% confidence level.

Results

Fermentation rate: The fermentation rate between 5 and 50% sugar utilization differed among treatments (Table 1). The fermentation rate for the entire period (0-99% sugar utilization) also differed among

treatments with PDM the most efficient (Table 1).

Table 1.

Effect of *Saccharomyces cerevisiae* strains on fermentation rate (°Brix per day) of White Riesling at two sugar ranges.

	Yeast Strains			
Sugar range	PDM	D47	FB	VL1
5 - 50%	1.46[a]	1.30[ab]	1.45[a]	1.20[b]
0 - 99%	0.97[a]	0.75[b]	0.64[c]	0.72[bc]

Significance of LSD test of treatment means at $P \leq 0.05$
Means with the same letter are not significantly different

Table 2.

Effect of *Saccharomyces cerevisiae* strains on White Riesling wine chemistry.

	Yeast Strains			
Components	PDM	D47	FB	VL1
Ethanol (%)	9.80[a]	9.39[a]	9.24[a]	9.34[a]
pH	3.11[a]	3.14[b]	3.15[b]	3.11[a]
Titratable acidity (g/L)	7.48[a]	7.44[a]	7.53[a]	7.21[b]
Tartaric acid (g/L)	4.24[a]	4.67[b]	4.33[a]	4.30[a]
Malic acid (g/L)	3.40[a]	3.43[a]	3.29[a]	3.31[a]
Succinic acid (g/100 mL)	0.12[a]	0.11[ab]	0.11[ab]	0.09[b]
Glucose (g/100 mL)	0.49[b]	0.56[a]	0.14[c]	0.52[ab]
Fructose (g/100 mL)	nd	nd	0.19	nd
Glycerol (g/100 mL)	0.57[a]	0.47[c]	0.61[a]	0.52[b]

Significance of LSD test of treatment means at $P \leq 0.05$
Means with the same letter are not significantly different
nd denotes non detectable

Wine chemistry: There were no differences in ethanol among the yeasts, although glycerol, a trihydroxylic alcohol, differed significantly, ranging form 0.47 to 0.61 g/L for the D47 and FB respectively (Table 2).

Titratable acidity, organic acid profile and pH differed slightly among treatments (Table 2). VL-1 had a lower titratable acidity and along with PDM the lowest pH. Minor differences in the concentration of tartaric and succinic acids did not strongly correlate with either pH or titratable acidity. Acetic acid did not vary among strains (data not shown).

The FB yeast demonstrated a unique pattern of carbohydrate utilization with the lowest glucose and highest fructose remaining following fermentation (Table 2).

Aroma components: There were no significant differences in PVTs among treatments immediately following fermentation, the exception being FB (Table 3). Post-fermentation lees contact reduced the PVT content for each treatment, the exception being FB. The largest reduction occurred with PDM.

Table 3.

Effect of strains of *Saccharomyces cerevisiae* on potencially volatile terpenes (PVT, mg/L) of White Riesling following fermentation and 45 days *sur lie*.

	Yeast Strains			
	PDM	D47	FB	VL1
Fermentation	0.425[a]	0.428[a]	0.342[c]	0.424[a]
45 days *sur lie*	0.339[c]	0.378[b]	0.349[c]	0.362[bc]

Significance of LSD test of treatment means at $P \leq 0.05$
Values in rows designated by the same letter do not differ significantly

Monoterpene glycoside concentration decreased by 7 to 29% from juice to the completion of fermentation. The reduction of bound terpene alcohols (geraniol, nerol, α-terpineol and linalool) and aromatic alcohols (benzyl and 2-phenylethanol) was greatest with FB (Figure 1).

Changes in the concentration of free aroma components were variable depending on the particular compound (Figure 2). In many cases free terpenes were not matched directly with decreases in the conjugated forms.

Discussion

Fermentation Rate: Fermentation increases the chemical and flavor complexity of juice by assisting in extraction of grape components, modifying some grape derived compounds, and producing yeast metabolites. PDM was the most rapid fermenter, confirming one of its touted attributes. Mean days required for the completion of fermentation ranged from 17 (0.97° Brix/day) for the PDM to 29 (0.64° Brix/day) for the FB. The fermentation rate between 5-50% sugar utilization provided a comparable image of the rate during the proliferation phase of yeast growth by ignoring the lag phase (Houtman and du Plessis, 1986). Differences within this range were not large, confirming that rate reduction occurred primarily toward the end of fermentation. Sugar transport is the primary means of fermentation rate control. Variation in any of the three irreversible steps of glycolysis, sugar phosphorylation, phosphofructokinase and/or pyruvate kinase impacts the activity of glucose transport and is believed responsible for rate variation among yeasts (Bisson, 1993).

Wine Chemistry: The major volatiles of enological importance resulting from direct yeast metabolism are ethanol, acetic acid and glycerol (Amerine et al., 1980). Glycerol was the only primary volatile that differed among treatments. Radler and Schutz (1982) reported substantial variation in the glycerol content among strains of *Saccharomyces cerevisiae* as a result of differences in their ability to

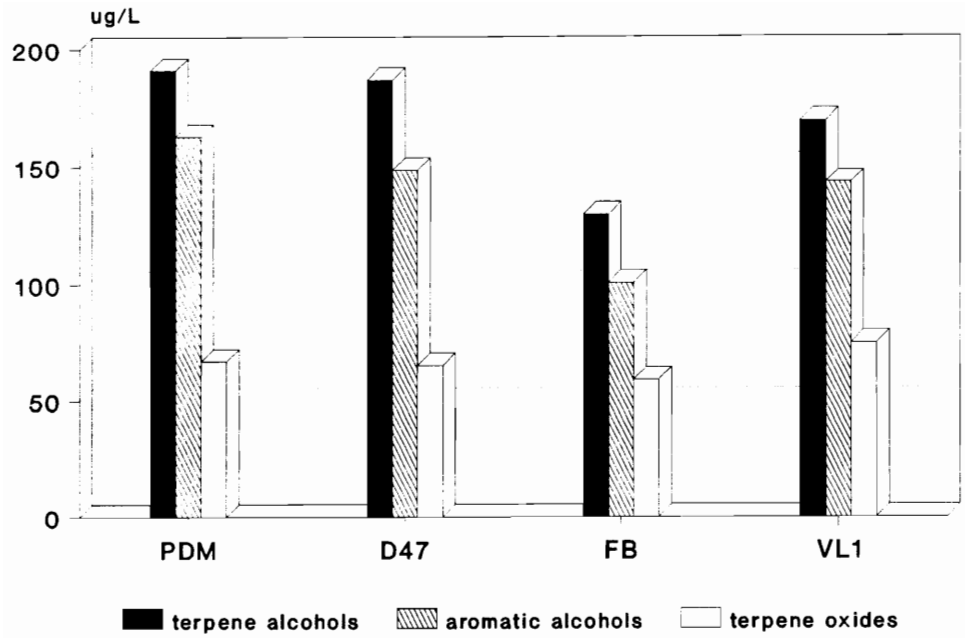


Figure 1. Effect of S. cerevisiae strains on bound aroma components of White Riesling following fermentation

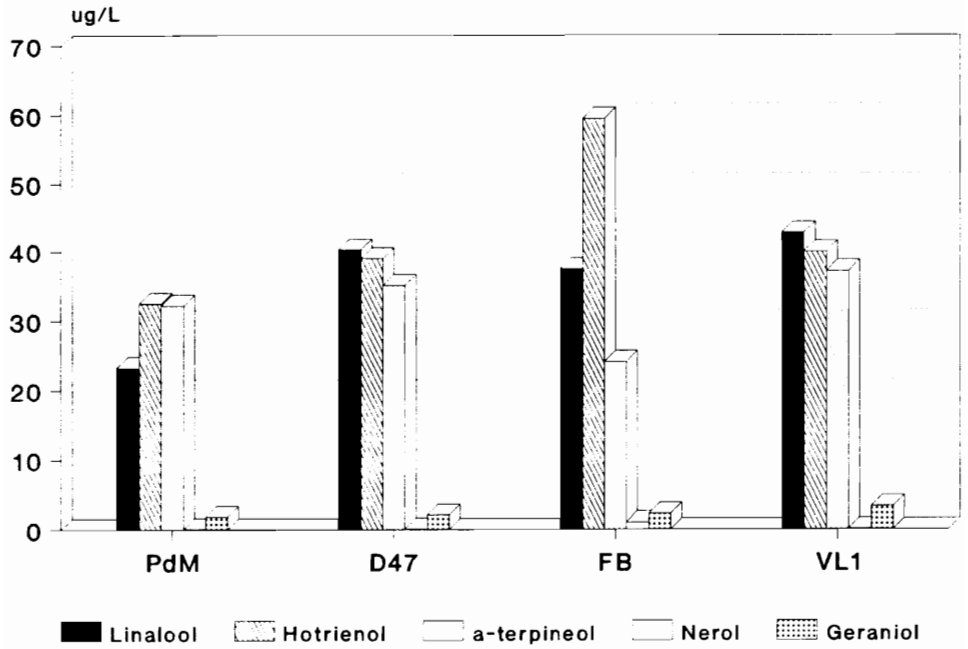


Figure 2. Effect of S. cerevisiae strains on free volatiles following fermentation

reduce dihydroxyacetone phosphate. Because of its relatively high specific gravity, glycerol is believed to contribute to wine body and may be an important attribute of both PDM and FB.

Organic acids contribute significantly to wine taste through contributions from the grape and the influences of the biochemistry of fermentation. Tartaric acid is the most stable organic acid found in wine; not metabolized by yeast (Sponholz, 1993). However, free tartaric acid can easily precipitate in the form of potassium bitartrate, likely responsible for variations among yeasts noted in this study. Although yeast strains can show as much as 40% variation in their ability to ferment malic acid (Rodriguez and Thornton, 1990), no significant differences were noted.

Aroma Components: In juice and wines the majority of the terpenes and aromatic alcohols were present as sugar conjugates. Fermentation resulted in a decrease of 7 to 29% in the sum of the bound terpenes under study. The greatest decrease occurred with FB. Following fermentation, the FB also had the lowest PVT concentration suggesting enhanced hydrolytic activity of the yeast. The extent of hydrolysis of individual glycosides was variable, although much greater with alcohols than oxides for each yeast strain. Enzyme hydrolysis of monoterpene glycosides is greatly influenced by the structure of the aglycone (Williams et al., 1982c). The tertiary structure of monoterpene alcohols such as linalool and α-terpineol appears to be a determinant factor in hydrolytic efficiency. The rate of β-glucosidase hydrolysis of primary alcohols, for example, is higher than tertiary alcohols while the opposite trend occurs with acid hydrolysis (Gunata et al., 1994). The lower the media pH, the greater the rate of acid catalyzed hydrolysis (Williams et al., 1982c). The PDM and VL-1 had the lowest pHs. However, wines produced from these yeasts did not have lower PVTs but did show the greatest reduction after

lees storage.

The sum of the bound aromatic alcohols followed the same general pattern noted for the terpene alcohols. That is, they decreased by an average of 25% following fermentation. The greatest reduction (36%) occurred with the FB. The concentration of bound benzyl alcohol was consistently lower than 2-phenylethanol with each yeast.

The principle free monoterpene alcohols found in White Riesling juice are linalool, α-terpineol, geraniol and nerol. The average concentration of linalool remained almost constant, while nerol and geraniol decreased as a result of fermentation. This is the opposite trend reported by Diaz-Cervantes (1979) in Muscat juice. Free α-terpineol increased by an average of 430% of the initial juice concentration, the greatest increase with FB.

The level of free 2-phenylethanol also increased considerably as a result of fermentation, an average of 190%. The increase can be attributed to the production by yeast and the hydrolysis of the bound form (Williams et al., 1982c; Gunata et al., 1988).

Furanic and pyranic linalool oxides, α-terpineol, citronellol and hotrieol increased with all yeasts. The oxides, and α-terpineol have been identified among enzymatically released volatiles from grape glycosides (Strauss et al., 1986; Voirin et al., 1992).

It was difficult to correlate, in all cases, increases in the concentration of a particular free monoterpene solely to enzyme or acid hydrolysis of its corresponding glycoside. This may be due to isomerization and/or breakdown (Croteau, 1984), and the metabolism and absorption of some monoterpenes by wine yeast (Di Stefano et al., 1992).

Some changes in individual free monoterpene concentration could also have resulted from the biochemical rearrangement. For example, increases in citronellol and hotrienol are likely not due to hydrolysis of the

corresponding glycoside since no conjugated form has been identified in grapes (Voirin et al., 1992). Both are likely the result of acid catalyzed dehydration of diol 1 (Park and Nobel, 1993). Additionally, linalool may be formed from nerol and/or geraniol, while α-terpineol may be generated from linalool, nerol and/or geraniol (Wilson et al., 1984). Williams et al. (1980) demonstrated that hotrienol under acid conditions rearranges to nerol oxide. This is consistent with the moderately low levels of hotrienol seen and the presence of oxides. It has also been established that 3,7-dimethyloct-1-en-3,6,7-triol can rearrange in an acid media to produce mainly the trans form of furan linalool oxide. Furthermore, both geraniol and nerol can be reduced by yeasts during fermentation (Di Stefano et al., 1992).

Diol 1 increased as a result of fermentation and, the concentration varied notably among yeasts. Polyhydroxylated compounds such as diol 1 are extremely labile, rearranging even under mild acid conditions to produce a wide array of volatile compounds (Williams et al., 1980). While diol 1 does not contribute directly to wine aroma, hydrolysis would be expected to convert at least some of this polyol to two aroma components, hotrienol and nerol oxide (Williams et al., 1980). Indeed, the yeast with the greatest concentration of diol 1 (FB) had the greatest concentration of nerol oxide.

Post-Fermentation Lees Contact: Aging involves the slow release of conjugated aroma components as a result of acid hydrolysis (Williams et al., 1982c). Post-fermentation lees contact generally lowered PVTs compared to both newly fermented wines (except the FB) and those stored for the same time period in the absence of lees. Differences presumably were the result of yeast autolysis.

Yeast autolysis results in the loss of intercellular organization and the release of cellular proteins, lipids, nucleic acids and

polysaccharides into the environment (Hough and Maddox, 1970). The rate of autolysis is a function of time, the conditions of the media and the yeast species and strain (Amerine et al., 1980). Sensory changes as a result of autolysis are thought to be due directly to the release of cellular components and indirectly to enzyme activity. Dubourdieu et al. (1988) reported β-glucosidases excreted into the media as a result of yeast autolysis but noted limited hydrolytic activity. Leighton (1994) suggested that yeast glycosidases are inhibited by even minor concentrations of glucose, and are active only in wines with little or no residual glucose. It is interesting to note that the yeast associated with the greatest reduction in glycosides immediately following fermentation (FB) produced wines with glucose levels much lower than all others.

Sensory Considerations: Qualitative and quantitative changes in free aroma components may be sensorially significant. Linalool and geraniol, for example, are much more potent and pleasantly fruity than α-terpineol and monoterpene oxides. Linalool and geraniol have aroma thresholds in water of 100 and 130 μg/L respectively, while the linalool oxide aroma thresholds are in the 3000-5000 μg/L range (Marais, 1983). Additionally, one monoterpene can decrease the aroma threshold of another, and a mixture (including oxides) can become more aromatic than the single most aromatic component of that mixture (Ribereau-Gayon et al., 1975). However, the maximum difference in the concentration of individual free Monoterpenes between treatments was below the sensory thresholds reported for each compound.

The sum of the terpene oxides increased with lees contact with the increase similar among yeasts. Oxides were dominated by the trans-furan linalool oxides, consistent with Wilson et al. (1984). Increases in terpene alcohols are of greater interest than oxides due to differences in their sensory thresholds. For example, the sensory threholds of furan

oxides are perceptible in water only at concentrations greater than 6000 μg/L (Marais, 1983).

Conclusion

The aroma of wine is, in part, dependent upon a small group of secondary grape metabolites that have escaped oxidation to polyols or conjugation. Oxidative products themselves have no direct aroma value. There is, however, some reclamation of aroma though acid catalyzed hydrolysis of polyols and/or enzymatic hydrolysis of glycosides which can produce a wide range of volatile compounds.

This study demonstrated a slightly different pattern of White Riesling monoterpene conjugates among wines fermented with different yeasts. Differences in free terpenes, however, were consistently below the sensory threshold for each compound. It is apparent from this research that wine yeasts marketed as enhancers of varietal expression derive such distinction from metabolites other than monoterpenes. This research should be continued to evaluate the effect of yeast strain on other known conjugates such as aliphatic residues, norisoprenoids and shikimic acid metabolites.

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Vita

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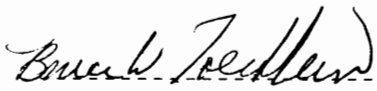

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