# Effect of Sunlight and Shade on Norisoprenoid Levels in Maturing Weisser Riesling and Chenin blanc Grapes and Weisser Riesling Wines*

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**The effect of sunlight, shade and degree of ripeness on potentially volatile $C_{13}$-norisoprenoid concentrations in Weisser Riesling grapes and wines and in Chenin blanc grapes, was investigated. Norisoprenoids were released from their bound forms by acid and enzymatic hydrolysis. With few exceptions, norisoprenoid concentrations were significantly higher in sun-exposed grapes than in shaded grapes. Significant increases in norisoprenoid concentrations were observed with an increase in ripeness. Microclimatic conditions during grape ripening for the production of Weisser Riesling wine with a potential to form lower concentrations of TDN during ageing are proposed.**

The effect of climate and soil on the performance of the vine and on grape and wine quality represents a complicated interaction between light intensity, temperature, water supply, wind and physiological processes (Saayman, 1981). The microclimate within the canopy also has an important effect on grape development and composition (Smart *et al.*, 1985).

Sugar accumulation within the berries during ripening is mainly dependent on leaf photosynthesis, which in turn is dependent on light and temperature (Iland, 1989a). Photosynthesis ceases in the absence of light (Ashton & Admiraal, 1990) or may be greatly delayed at temperatures lower or higher than the optimum temperature range (Iland, 1989a). The interaction of light and temperature may cause increases or decreases in pH, acidity and the production of anthocyanins and phenolics (Iland, 1989b).

Differences between the effects of artificially shaded leaf and cluster treatments on Cabernet Sauvignon berry composition were reported by Rojas-Lara & Morrison (1989). Leaf shading delayed berry growth and sugar accumulation, while cluster shading had little effect on these phenomena. Cluster shading significantly affected anthocyanin accumulation, while leaf shading had little effect in this respect. These findings were confirmed in studies on naturally shaded Cabernet Sauvignon grapes (Morrison & Noble, 1990). Sensory evaluation of the grape juice and wine samples revealed significant differences in quality between the control (grapes totally exposed to sunlight) and the shaded treatments, but no significant differences between the different degrees of shade.

Few studies report on the effect of shade or sunlight exposure on the concentration of flavour compounds. Higher levels of free volatile (FVT) and potentially volatile (glycosidically bound) terpenes (PVT) were reported in sun-exposed Frontignac grapes than in shaded grapes during ripening (Williams *et al.*, 1985). Similarly, higher concentrations of PVT were found in fully exposed Gewürztraminer grapes than in partially or totally shaded grapes sampled between véraison and harvest (Reynolds & Wardle, 1988).

The effect of sunlight on $C_{13}$-norisoprenoid concentrations in grapes and wines has not yet received attention. Like monoterpenes, norisoprenoids constitute an important part of the aroma compounds of grapes and wines. Williams, Sefton & Wilson (1989) demonstrated the sensory significance of acid hydrolysates, which contained a great number of norisoprenoids, in varietal flavours of grapes and wines of Chardonnay, Sauvignon Blanc and Semillon. The importance of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), the vitispiranes and beta-damascenone in the aroma of wines is well known (Strauss *et al.*, 1987b). 1,1,6-Trimethyl-1,2-dihydronaphthalene is of special importance, since it is associated with the kerosene-like flavour which, almost exclusively, develops

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in Weisser Riesling wine during ageing (Simpson, 1978a; Simpson & Miller, 1983). It is generally accepted that this flavour becomes undesirable when present at high intensities, especially in wines produced in hot regions.

The existence of monoterpene and $C_{13}$-norisoprenoids in free and glycosidically bound forms in grapes was demonstrated by Williams *et al.* (1982). The techniques used to isolate these glycosides from grapes include selective retention of the precursors on $C_{18}$ reversed-phase adsorbent (Williams *et al.*, 1982) or on Amberlite XAD-2 resin (Gunata *et al.*, 1985a), and separation by droplet countercurrent chromatography (Strauss *et al.*, 1987a). The liberation of the aglycons by enzymatic and acid catalysed hydrolysis produced a variety of volatile compounds of which $C_{13}$-norisoprenoids formed an important part. Sefton *et al.* (1989) reported the identification and possible origins of 24 $C_{13}$-norisoprenoids in Chardonnay, Semillon and Sauvignon blanc juices. Winterhalter, Sefton & Williams (1990) identified 27 monoterpenes, 20 shikimate metabolites and 40 $C_{13}$-norisoprenoids in Weisser Riesling wine.

The purpose of this investigation was to evaluate the effect of sunlight and shade on potentially volatile TDN and other norisoprenoid concentrations in Weisser Riesling and Chenin blanc grapes and Weisser Riesling wines. Chenin blanc was included for purposes of comparison, since the wines of this cultivar are normally neutral and do not present kerosene characteristics. Special attention was paid to the recognition of viticultural practices, which could limit the TDN development potential of a wine during ageing, with a possible enhancement of the quality of aged Weisser Riesling wines.

## MATERIALS AND METHODS

**Sampling and harvesting of grapes:** Grapes of two *Vitis vinifera* L. cv. Weisser Riesling clones, namely 37 and W1, and grapes of Chenin blanc (clone Montpellier) from the Stellenbosch region [Nietvoorbij Institute for Viticulture and Oenology (Nietvoorbij) vineyards] were used during the 1991 vintage. Weisser Riesling vines (approximately 500 per block) were vertically trained (hedge system) and spaced 1,2 m apart in east-west orientated rows 2,75 m apart. Chenin blanc vines were trained and spaced similarly, but only one row of 50 vines was used.

Four duplicate samples of each of the naturally sun-exposed and shaded grapes from two Weisser Riesling clones were picked weekly over a period of three weeks. Sun-exposed grapes received direct sunlight in the morning on the southern side of the rows and in the afternoon on the northern side of the rows. Shaded grapes were shielded by leaves from direct exposure to sunlight. Approximately 2 kg of grapes per sample were collected as whole clusters on a representative basis. Chenin blanc was sampled similarly, but not in duplicate.

In addition, 120 kg of grapes of each Weisser Riesling clone, and from both sun and shady conditions, were harvested at the last two sampling stages. Wines were produced from these grapes according to standard white winemaking practices. Grapes were crushed, the juice left in contact with the skins for six hours, separated and then settled overnight with the aid of commercial Pectinex enzyme. Each clear juice was divided into two equal parts, each of which was fermented using the same yeast strain. Wines were not produced from Chenin blanc grapes.

**Isolation, liberation and extraction of aroma compounds:** Grape samples were crushed by hand and filtered through cheese cloth by applying slight pressure. Juice samples (50 ml) were treated with 10 ml 10% bentonite suspension, left for 10 minutes at 0°C, and centrifuged at 3000 rpm for 10 minutes. Glycosidically bound compounds in the clarified juices and wines were adsorbed on Amberlite XAD-2 resin, according to the technique of Gunata *et al.* (1985a), as adapted by Versini *et al.* (1987). The bound fraction was divided into two equal parts. Norisoprenoids were liberated from the first part by enzymatic hydrolysis (Rohapect C) at pH 5 in a waterbath (40°C, 15 hrs), and by acid hydrolysis at pH 1 in a waterbath (50°C, 4 hrs) from the second part. Liberated norisoprenoids were extracted with pentane/dichloromethane (2:1), the extracts concentrated and kept at 0°C until analysis by gas chromatography. Norisoprenoids were determined in the Weisser Riesling grapes and wines and in the Chenin blanc grapes.

**Gas chromatography and mass spectrometry:** The gas chromatographic analyses were performed, using a Hewlett Packard 5880A instrument with automatic dual integrators. The capillary columns and gas chromatographic conditions used, were:

1. Supelcowax 10 fused silica (60 m x 0,32 mm i.d., film thickness: 0,25 µm); temperature programme: 10 min. at 60°C, 1°C/min. up to 190°C, 30 min. at 190°C; detector: FID; detector temperature: 250°C; injection temperature: 200°C; carrier gas: helium; column flow rate: 1,5 ml/min; split ratio: 60:1.

2. SPB-5 fused silica (60 m x 0,32 mm i.d., film thickness: 0,25 µm); temperature programme: 5 min. at 80°C, 1°C/min. up to 250°C, 20 min. at 250°C; detector temperature: 300°C; other parameters as under 1.

Norisoprenoid concentrations were expressed as relative concentrations in relation to an internal standard (3-decanol). The identities of the norisoprenoids were confirmed, either by comparing their mass spectra and retention times with those of authentic standards, which were analysed under similar conditions and on similar columns, using a Finnigan 4600 mass spectrometer or tentatively by comparing their mass spectra with those reported in the literature.

**Statistical analyses:** The statistical significance of the effects of sunlight, shade and degree of ripeness on acid- and enzyme-released norisoprenoid concentrations in Weisser Riesling grapes and wines was determined by means of standard analysis of variance methods (Snedecor & Cochran, 1980). Since the effects of sunlight, shade and degree of ripeness between Weisser Riesling clones 37 and W1 were consistent, their data were combined for the calculation of these effects. The general impression obtained from the data was one of proportionality. The data were therefore simplified when transformed to the log scale. No statistical analyses were performed on Chenin blanc grape data.

## RESULTS AND DISCUSSION

The liberated $C_{13}$-norisoprenoids analysed in the grape and wine samples and their mass spectral data are listed in Table 1 and their structures given in Figure 1. As in previous studies (Sefton *et al.*, 1989) some $C_{13}$-norisoprenoids were released from their bound forms by acid, and others by enzymatic hydrolysis. The compounds analysed were grouped according to this phenomenon (Table 1). Compound **6**, called OH-TDN, was included on account of the relatively high concentrations in which its bound form occurred in grapes and wines. According to its mass spectral data it appeared to be a hydroxylated derivative of TDN (Table 1).

TABLE 1
Identities and mass spectral data of $C_{13}$-norisoprenoids liberated by acid and enzymatic hydrolysis from grapes and wines.

| Norisoprenoid | Mass spectral data (m/z,%) | Reference | Evidence for assignment |
|---|---|---|---|
| *Acid hydrolysis* | | | |
| (**1**) trans- and cis-Vitispirane | 192(70), 177(35), 163(5), 149(22), 136(42), 135(24), 121(35), 109(13), 108(12), 107(26), 105(14), 95(12), 94(15), **93(100),** 91(38), 79(25), 77(30), 67(17), 65(18), 55(30), 53(17), 43(38), 41(50), 39(27) | a | A |
| (**2**) 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) | 172(25), 158(13), **157(100),** 143(5), 142(55), 141(28), 129(7), 128(12), 127(3), 115(17), 77(13), 76(4), 71(3), 63(5), 51(4), 39(4) | b | A |
| (**3**) Megastigma-3,5,8-trien-7-one (beta-Damascenone) | 190(5), 121(43), 105(18), 91(10), 79(7), 77(8), **69(100),** 41(35), 39(12) | c | A |
| (**4**) 2,2,6-Trimethyl-8-(1-hydroxy) ethyl-7-oxabicyclo (4.3.0) nona-4,9-dienes (Actinidols) | 193(1), 164(6), **163(100),** 149(6), 145(10), 130(4), 121(12), 119(7), 107(8), 105(12), 93(19), 91(19), 81(5), 79(8), 77(12), 69(6), 55(6), 45(18), 43(57) | d | B |
| (**5**) 9-Hydroxymegastigm-7-en-3-one | 112(19), 110(8), 108(7), 97(25), 95(18), 94(11), 83(8), 79(7), 69(12), 55(15), 45(28), **43(100),** 41(27) | e | B |
| (**6**) OH-TDN (unknown) | 190(4), 172(30), 157(69), 147(6), 142(6), 133(21), **132(100),** 119(12), 117(20), 115(18), 105(22), 91(14), 77(18), 65(6), 57(6), 51(10), 45(41), 44(40), 43(45) | | |
| *Enzymatic hydrolysis* | | | |
| (**7**) Megastigma-4,8-dien-3,7-dione (3-Oxo-alpha-damascone) | 138(18), 123(22), 79(3), 77(6), **69(100),** 41(26) | f | B |
| (**8**) (E)-9-Hydroxymegastigma-4,7-dien-3-one (3-Oxo-alpha-ionol) | 194(5), 152(13), 137(22), 134(8), 107(12), 109(25), **108(100),** 95(11), 91(11), 81(8), 79(9), 45(20), 43(25) | f, i | A |
| (**9**) 3,9-Dihydroxymegastigm-5-en (3-Hydroxy-7,8-dihydro-beta-ionol) | 212(2), 194(3), 179(4), 161(25), 153(8), 137(29), 136(45), 123(14), **121(100),** 119(80), 109(20), 107(19), 105(30), 95(26), 93(48), 91(18), 81(29), 79(21), 77(13), 69(24), 67(28), 55(34), 45(17), 43(48), 41(39) | g, k | B |
| (**10**) (E)-9-Hydroxymegastigma-5,7-dien-4-one (4-Oxo-beta-ionol) | 208(4), 193(15), 175(3), **165(100),** 147(6), 137(35), 123(58), 107(34), 91(34), 77(24), 69(24), 55(39), 43(80) | h, j | B |
| (**11**) 9-Hydroxymegastigm-5-en-4-one (4-Oxo-7,8-dihydro-beta-ionol) | 210(4), 195(26), 165(33), 154(58), 152(70), 137(82), 135(50), 121(48), 108(57), 107(50), **109(100),** 95(45), 93(55), 79(47), 67(55), 55(61), 43(85), 41(65) | h, j | B |
| (**12**) 3,5-Dihydroxymegastigma-6,7-dien-9-one (Grasshopper ketone) | 209(10), 163(13), 151(4), 149(8), 131(5), 125(10), 123(27), 121(10), 109(9), 107(10), 105(5), 91(5), 85(4), 81(5), 79(10), 77(9), 55(4), 53(5), **43(100)** | e | B |
| (**13**) 6,9-Dihydroxymegastigma-4,7-dien-3-one (Vomifoliol) | 206(3), 168(6), 150(6), 135(6), 125(10), **124(100),** 111(8), 107(5), 94(4), 79(11), 69(5), 55(7), 43(23), 41(10) | g, i | B |
| (**14**) 6,9-Dihydroxymegastigm-4-en-3-one (7,8-Dihydrovomifoliol) | 170(20), 153(25), 152(41), 125(17), 123(13), 111(53), **110(100),** 108(30), 96(23), 68(18), 55(20), 43(40), 41(23) | g, e | B |

[a] Simpson, Strauss & Williams (1977); [b] Simpson (1978a); [c] Schreier & Drawert (1974); [d] Dimitriadis *et al.* (1985); [e] Sefton *et al.* (1989); [f] Strauss *et al.* (1987a); [g] Winterhalter & Schreier (1988); [h] Winterhalter (1990); [i] Strauss, Wilson & Williams (1987); [j] Winterhalter, Sefton & Williams (1990); [k] Sefton & Williams (1991). Apart from references g (quince) and h (passion fruit), all others refer to grapes and wine. A = Mass spectra and retention times were identical to those of authentic compounds; B = Mass spectra were consistent with those of published data.

TABLE 2
The effect of sunlight, shade and degree of ripeness on the relative concentrations of acid-released $C_{13}$-norisoprenoids in the grapes and wines of Weisser Riesling.[a]

| Norisoprenoid | | Grapes | | | | | Wine | |
|---|---|---|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S4 | R | S3 | S4 |
| trans-Vitispirane (**1**) | S | 6,74a | 8,99a | 19,24a | 25,06a | ** | 22,77a | 23,60a |
| | Sh | 2,80b | 2,55b | 9,32b | 12,50b | | 14,03b | 15,77b |
| cis-Vitispirane (**1**) | S | 4,94a | 6,72a | 15,24a | 18,67a | ** | 18,86a | 19,59a |
| | Sh | 1,97b | 1,97b | 7,49b | 11,24a | | 12,02b | 14,05b |
| 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) (**2**) | S | 12,52a | 19,24a | 53,35a | 61,32a | ** | 40,48a | 42,10a |
| | Sh | 5,13b | 5,30b | 23,24b | 29,14b | | 22,53b | 30,86b |
| beta-Damascenone (**3**) | S | 5,62a | 6,16a | 6,07a | 7,10a | ** | 1,98a | 2,71a |
| | Sh | 5,34a | 5,26a | 7,53a | 7,54a | | 2,28a | 3,18a |
| Actinidol 1 (**4**) | S | 1,30a | 1,69a | 3,75a | 3,56a | ** | 4,10a | 4,58a |
| | Sh | 0,50b | 0,91b | 2,01b | 2,12b | | 2,66b | 3,00b |
| Actinidol 2 (**4**) | S | 2,35a | 2,93a | 6,97a | 7,12a | ** | 6,27a | 6,67a |
| | Sh | 1,15b | 1,24b | 4,41a | 5,00a | | 4,14b | 4,89b |
| 9-Hydroxymegastigm-7-en-3-one (**5**) | S | 1,46a | 2,23a | 3,85a | 3,24a | ** | 2,09a | 2,93a |
| | Sh | 0,77b | 1,44b | 2,27b | 2,86a | | 1,43b | 2,21b |
| OH-TDN (unknown) (**6**) | S | 9,81a | 15,02a | 42,83a | 39,73a | ** | 23,73a | 31,74a |
| | Sh | 3,30b | 3,92b | 18,57b | 21,32b | | 14,04b | 20,02b |

[a] Statistical analysis was performed on log-transformed data. The F-test was followed by the LSD-test.

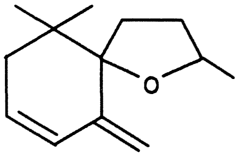
S1-S4 = Sampling stages of grapes.
S = Sunlight-exposed grapes.
Sh = Shaded grapes.
R = Level of significance for the increase in norisoprenoid concentrations in grapes over the sampling period (data for sun-light and shade combined).
** = Highly significant ($p \leq 0{,}01$).
Values between sunlight-exposed and shaded grapes at each sampling stage designated by the same symbol do not differ significantly ($p \leq 0{,}05$).

### Effect of sunlight and shade on $C_{13}$-norisoprenoid concentrations in grapes and wine

*Acid-hydrolysed $C_{13}$-norisoprenoids:* The effect of sunlight and shade on the relative concentrations of the acid-released norisoprenoids in Weisser Riesling grapes and wines is given in Table 2. These effects on the relative concentrations of TDN (**2**) and beta-damascenone (**3**) are also illustrated in Figures 2 and 3 (data for clones 37 and W1 combined).

With few exceptions, notably beta-damascenone (**3**) (Fig. 3), the norisoprenoid levels were significantly higher in grapes exposed to sunlight than in shaded grapes (Table 2). This phenomenon occurred at almost all sampling stages (Table 2) and even at the fourth stage where sugar accumulation reached the same level (Fig. 2). Similar tendencies occurred in the wines made from grapes harvested at the third and fourth sampling stages (Table 2). Differences in norisoprenoid levels between sunlight and shade were generally slightly smaller in the wines than in the corresponding grape samples (Fig. 2). This can possibly be explained by the difference in grape handling. The sampling of sun-exposed and shaded grapes for analyses could be performed more precisely, while less strict selection occurred when grapes were picked for wine production by a group of harvesters.

1,1,6-Trimethyl-1,2-dihydronaphthalene (**2**) (Fig. 2), which makes a major contribution to the typical kerosene-like bottle-aged character of aged Weisser Riesling wines is of special importance (Simpson, 1978a; Simpson & Miller, 1983). A threshold of 20 ppb in wine was reported by Simpson (1978a). Generally, it is accepted that this kerosene-like character becomes undesirable when present in high intensities, especially in wines produced in hot regions. The compound, called OH-TDN (**6**), showed similar concentration changes as TDN (Table 2). Whether this compound contributes to the aroma of wine is not known. Di Stefano (1985) mentioned a similar compound and suggested the structure to be that of 4-hydroxy-1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene. Winterhalter (1991) tentatively identified this compound as 6-hydroxy-1,1,6-trimethyl-1,2,5,6-tetrahydronaphthalene and sug-



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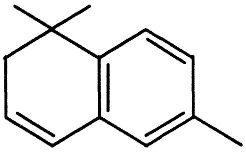

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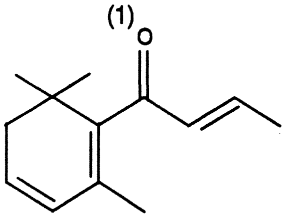

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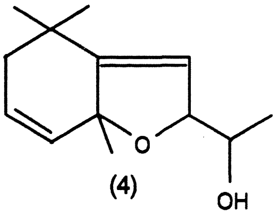

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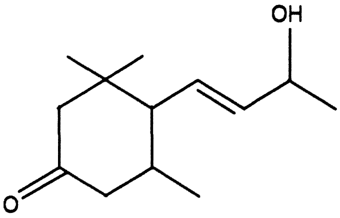

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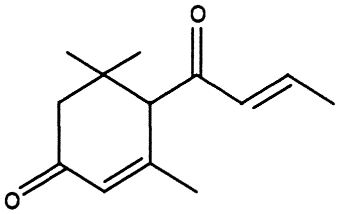

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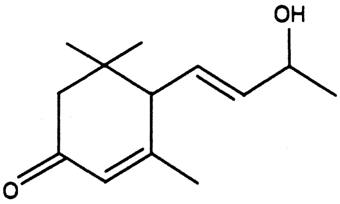

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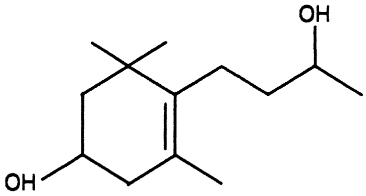

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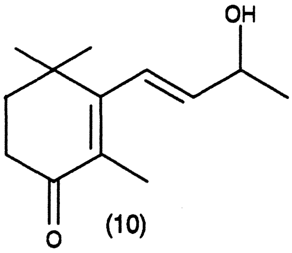

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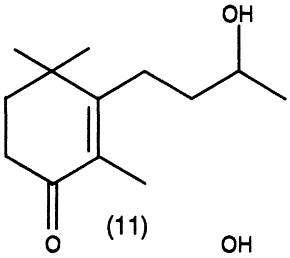

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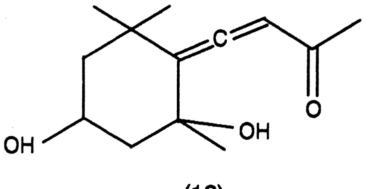

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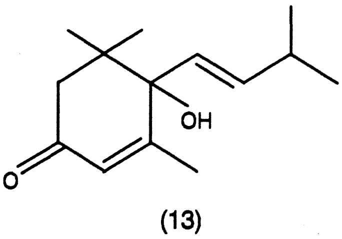

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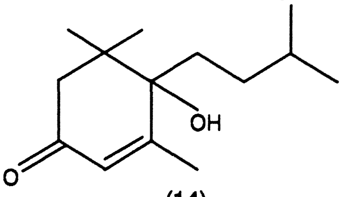

(14)

FIGURE 1
Structures of $C_{13}$-norisoprenoids (referred to in this work).

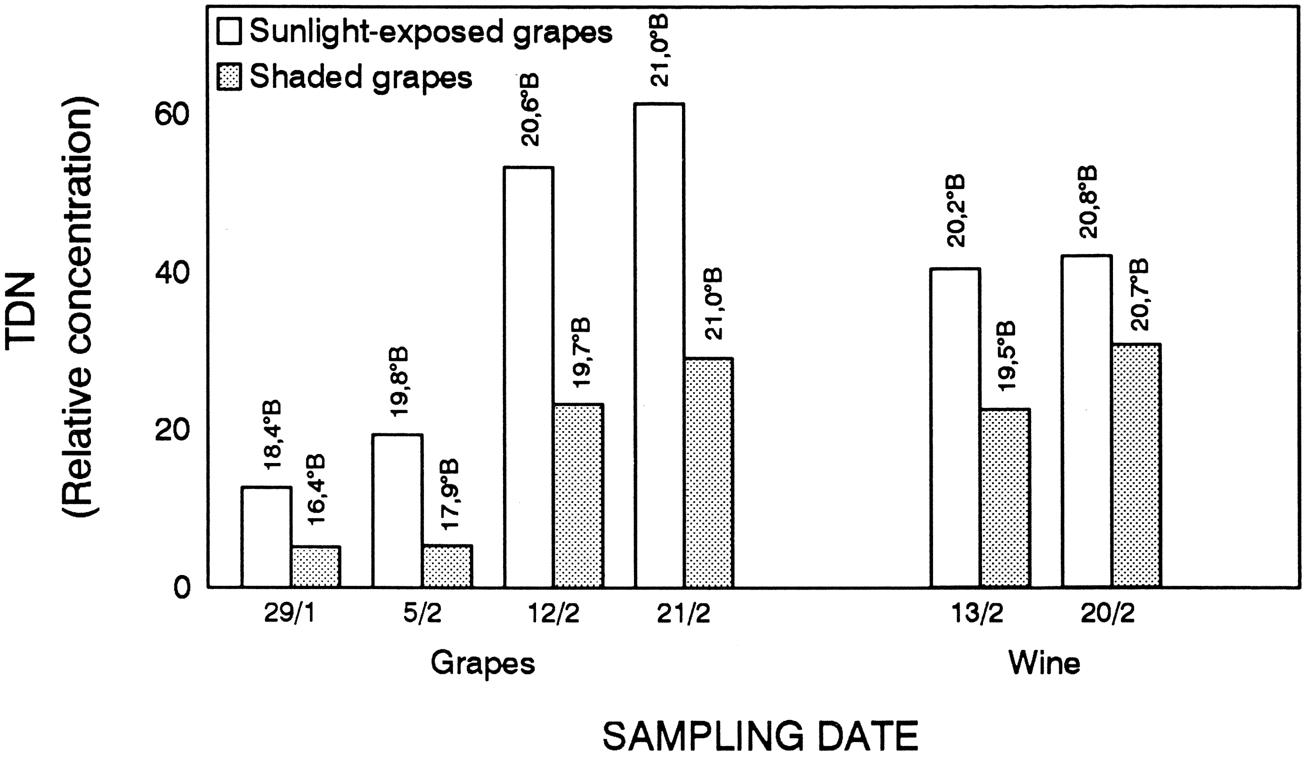

FIGURE 2
The effect of sunlight, shade and degree of ripeness on the relative concentrations of acid-released 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) in the grapes and wines of Weisser Riesling (data for clones 37 and W1 combined). Sugar concentration (°B) is indicated at each sampling date.

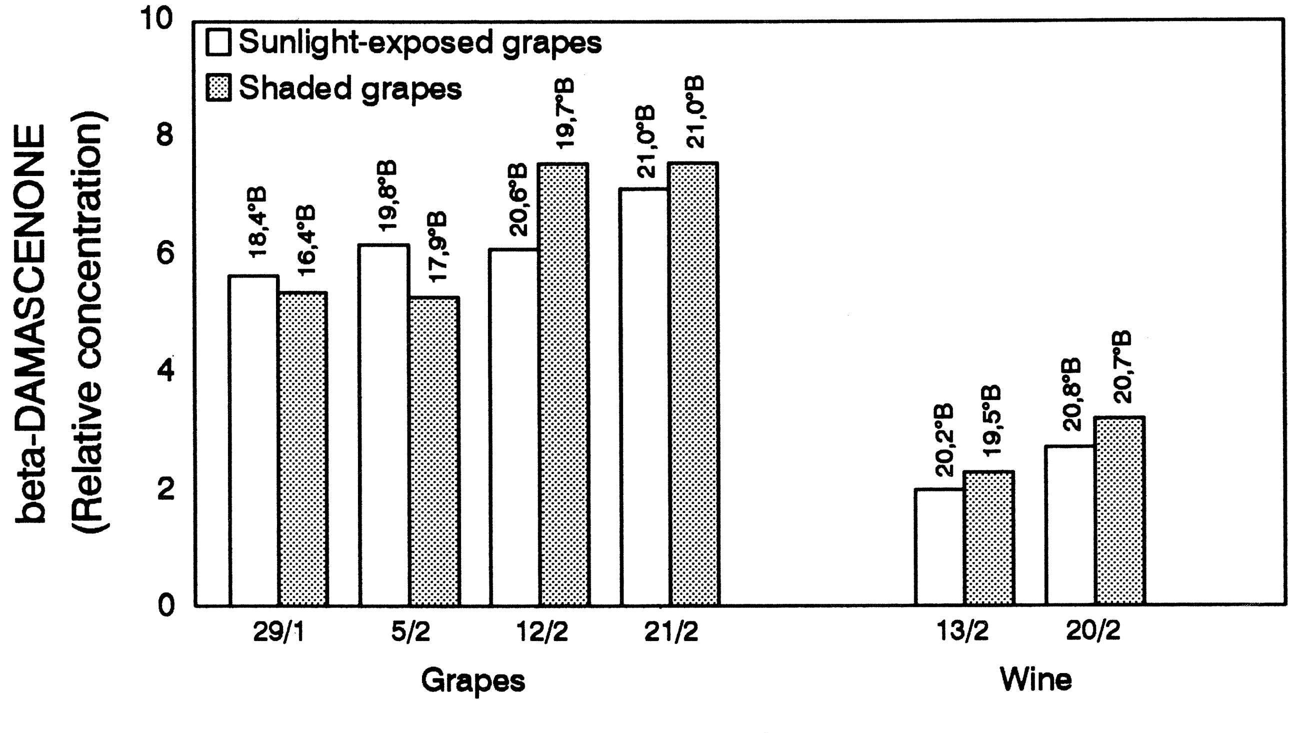

FIGURE 3
The effect of sunlight, shade and degree of ripeness on the relative concentrations of acid-released beta-damascenone in the grapes and wines of Weisser Riesling (data for clones 37 and W1 combined). Sugar concentration (°B) is indicated at each sampling date.

gested it to be an intermediate between the TDN-precursor and TDN.

The beta-damascenone (**3**) concentration (Fig. 3) was not significantly affected by exposure to sunlight (Table 2). Therefore, if shaded grapes are used for wine production, the contribution of beta-damascenone to aroma will, in contrast to that of other norisoprenoids, not necessarily be diminished. The marked decrease in beta-damascenone concentrations from grapes to wine (Fig. 3) cannot be explained. This compound is regarded as an important contributor to the typical character of young Weisser Riesling wines (Chisholm, 1990). Ohloff (1978) described the odour of beta-damascenone as reminiscent of exotic flowers with a heavy fruity undertone and reported its flavour threshold in water to be 9 x $10^{-3}$ ppb. Buttery, Teranishi & Ling (1988) reported a threshold in water of 2 x $10^{-3}$ ppb.

Apart from TDN and beta-damascenone, the sensory significance of only a few other norisoprenoids in wine is known. For example, the vitispiranes (**1**), which have a camphoraceous or eucalyptus odour (Simpson, 1978a; Simpson, Strauss & Williams, 1977), were reported to possess a flavour threshold in wine of 800 ppb (Simpson, 1978b). Like TDN, the vitispiranes also increase in concentration during ageing of wine and may affect wine quality as well (Simpson & Miller, 1983; Rapp & Güntert, 1985). The actinidols (**4**) also possess a camphoraceous odour, but no threshold value has been reported (Dimitriadis *et al.*, 1985). Williams *et al.* (1989) demonstrated the importance of acid hydrolysates of precursor fractions in the varietal flavours of grapes and wines. When added to a neutral wine medium, these hydrolysates of Sauvignon Blanc, Chardonnay and Semillon juices had a highly significant effect on aroma. Whether the great number of $C_{13}$-norisoprenoids, which formed a part of these hydrolysates, contributed to this phenomenon was not reported.

Chenin blanc grapes contained very low concentrations of trans- and cis-vitispirane (**1**), TDN (**2**), beta-damascenone (**3**), 9-hydroxymegastigm-7-en-3-one (**5**) and OH-TDN (**6**), and these results are therefore not given. The actinidols (**4**) showed similar tendencies in Chenin blanc grapes exposed to sunlight and shade as observed in Weisser Riesling grapes and were much higher in concentration in the former (Fig. 4). Since changes in the concentrations of actinidol 1 and 2 were similar, only the former is illustrated. Chenin blanc is a neutral cultivar and the character of its wines is highly dependent on the contribution of fermentation-produced volatiles. The flavour threshold values of the actinidols are not known and it is possible that the high concentrations in which they occur in Chenin blanc grapes could still be lower than these values. The actinidols, therefore, appear to be of less importance as fragrant norisoprenoids in Chenin blanc or Weisser Riesling grapes and wines.

*Enzymatically hydrolysed $C_{13}$-norisoprenoids:* The effect of sunlight and shade on the relative concentrations of the enzymatically liberated norisoprenoids in Weisser Riesling grapes and wines is given in Table 3.

As in the case of acid hydrolysis, norisoprenoid levels in Weisser Riesling were mostly significantly higher in grapes exposed to sunlight than shaded grapes (Table 3). An exception was megastigma-4,8-dien-3,7-dione (**7**). Similar tendencies occurred in the wines made from grapes harvested at the third and fourth sampling stages (Table 3). Chenin blanc grapes also showed relatively high levels of some norisoprenoids and the effect of sunlight and shade on their concentrations was similar to that of Weisser Riesling. An example for comparison between Chenin blanc and Weisser Riesling is given in Figure 5.

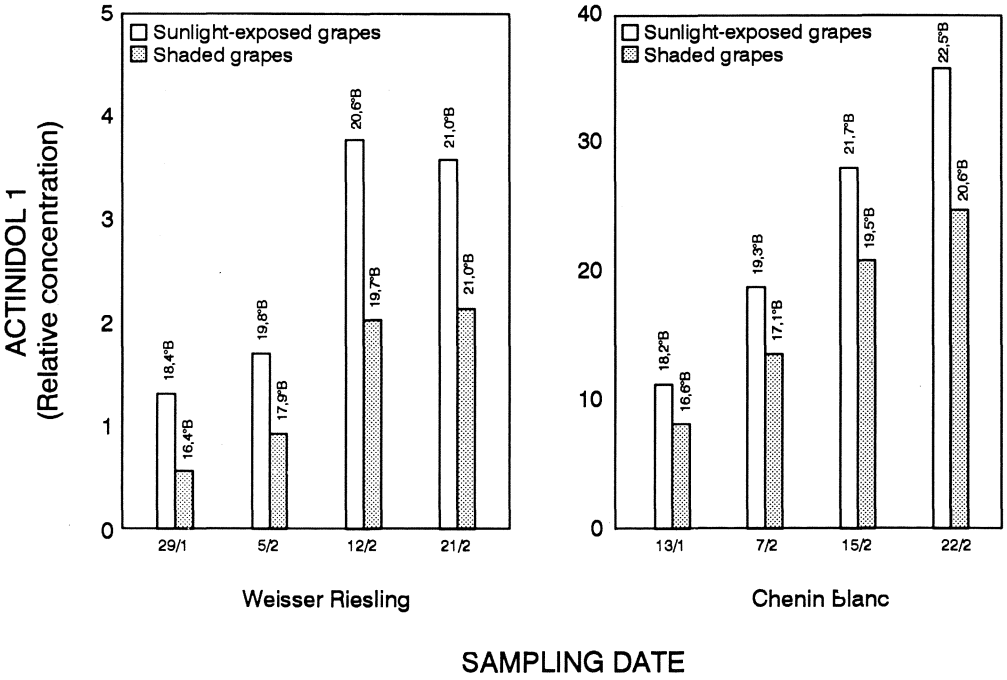

FIGURE 4

The effect of sunlight, shade and degree of ripeness on the relative concentrations of acid-released actinidol 1 in the grapes of Weisser Riesling (data for clones 37 and W1 combined) and Chenin blanc (clone Montpellier). Sugar concentration (°B) is indicated at each sampling date.

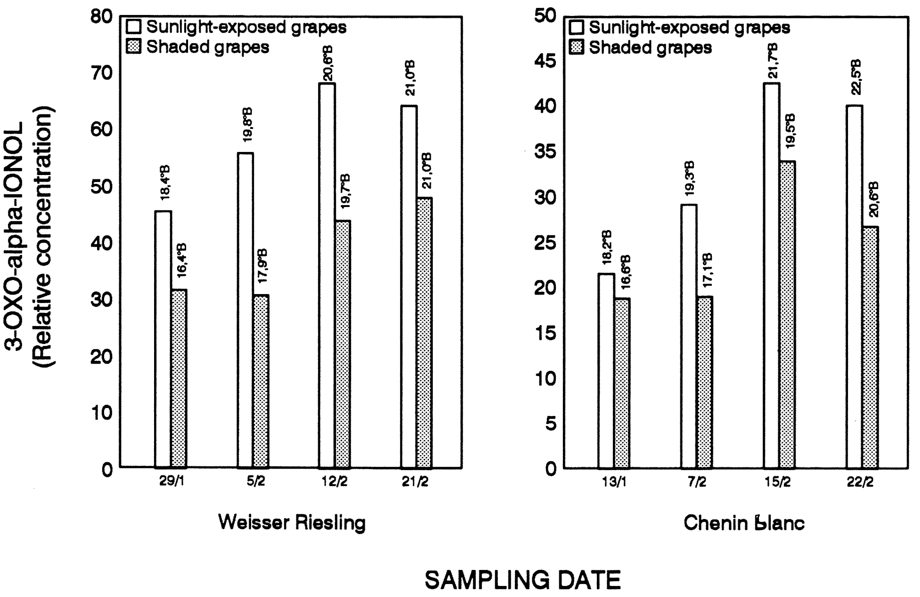

FIGURE 5

The effect of sunlight, shade and degree of ripeness on the relative concentrations of enzyme-released 3-oxo-alpha-ionol in the grapes of Weisser Riesling (data for clones 37 and W1 combined) and Chenin blanc (clone Montpellier). Sugar concentration (°B) is indicated at each sampling date.

TABLE 3

The effect of sunlight, shade and degree of ripeness on the relative concentrations of enzyme-released $C_{13}$-norisoprenoids in the grapes and wines of Weisser Riesling.[a]

| Norisoprenoid | | Grapes | | | | | Wine | |
|---|---|---|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S4 | R | S3 | S4 |
| Megastigma-4,8-dien-3,7-dione (**7**) | S | 11,7a | 21,0a | 18,2a | 14,1a | NS | 61,0a | 60,9a |
| | Sh | 13,5a | 19,3a | 20,6a | 15,2a | | 65,5a | 67,2a |
| 3-Oxo-alpha-ionol (**8**) | S | 45,4a | 55,5a | 67,8a | 63,9a | ** | 56,5a | 60,8a |
| | Sh | 31,4b | 30,5b | 43,8b | 47,8b | | 39,3b | 46,6b |
| 3-Hydroxy-7,8-dihydro-beta-ionol (**9**) | S | 3,6a | 2,4a | 2,3a | 2,4a | * | 3,3a | 5,1a |
| | Sh | 1,4b | 0,8b | 0,6b | 7,4b | | 1,6b | 2,6b |
| 4-Oxo-beta-ionol (**10**) | S | 7,0a | 8,0a | 8,3a | 4,2a | NS | 6,7a | 6,7a |
| | Sh | 5,0b | 3,6b | 5,1b | 4,2b | | 4,2b | 5,3a |
| 4-Oxo-7,8-dihydro-beta-ionol (**11**) | S | 13,3a | 17,8a | 20,3a | 17,6a | ** | 20,8a | 20,0a |
| | Sh | 9,3b | 9,0b | 11,2a | 12,7a | | 14,9b | 16,5a |
| Grasshopper ketone (**12**) | S | 46,4a | 85,9a | 115,4a | 140,5a | ** | 112,1a | 133,1a |
| | Sh | 42,2a | 77,9b | 119,7b | 110,6b | | 95,7b | 108,0b |
| Vomifoliol (**13**) | S | 32,4a | 69,1a | 177,3a | 148,9a | ** | 117,2a | 139,3a |
| | Sh | 23,2b | 51,9b | 128,2b | 112,2b | | 106,2b | 109,0b |
| 7,8-Dihydrovomifoliol (**14**) | S | 14,9a | 22,3a | 33,4a | 26,2a | ** | 27,8a | 31,0a |
| | Sh | 9,9b | 14,9b | 18,2b | 16,8b | | 21,6b | 21,1b |

[a] Statistical analysis was performed on log-transformed data. The F-test was followed by the LSD-test.

S1-S4 = Sampling stages of grapes.
S = Sunlight-exposed grapes.
Sh = Shaded grapes.
R = Level of significance for the increase in norisoprenoid concentrations in grapes over the sampling period (data for sunlight and shade combined).
NS = Not significant.
* = Significant ($p \leq 0,05$).
** = Highly significant ($p \leq 0,01$).

Values between sunlight-exposed and shaded grapes at each sampling stage designated by the same symbol do not differ significantly ($p \leq 0,05$).

The use of acid versus enzymatically catalysed reactions, as employed in this study, could be considered as a means to determine the potential development of aromatic volatiles such as norisoprenoids in wine. Acid hydrolysis products such as the vitispiranes, actinidols, beta-damascenone and TDN are quite commonly observed in wines. It has been stated that enzymatic liberation of potentially volatile compounds is a more natural process during which the natural composition of flavour compounds is less disturbed (Williams, Strauss & Wilson, 1983). However, $C_{13}$-norisoprenoids, such as 3-oxo-alpha-ionol (**8**), the grasshopper ketone (**12**) and vomifoliol (**13**), which were produced at relatively high concentrations by enzymatic hydrolysis, have as yet not been reported in wines. Winterhalter *et al.* (1990) identified 40 enzyme-released $C_{13}$-norisoprenoids in Weisser Riesling wine. With a total concentration of over 1300 ppb, these compounds were the most abundant volatile aglycons. To what extent they are released naturally and contribute to wine aroma is not known. It appears that these norisoprenoids are either not fragrant compounds or they occur naturally in very low concentrations. The transformation of enzyme-released norisoprenoids to other compounds during ageing of wine should also be considered.

Various macro- and microclimatic factors may affect the development of free and bound flavour compounds in grapes. The role of light in mechanisms of flavour biosynthesis appears to be of particular importance. Morrison & Noble (1990) claimed that the changes in berry composition as a result of leaf and cluster shading are more closely related to the effect of light than to that of temperature. Future studies should be conducted on the role of light in the biosynthesis of bound $C_{13}$-norisoprenoids.

**Effect of degree of ripeness on norisoprenoid concentrations in grapes and wine:** The effect of degree of ripeness on the relative concentrations of the acid- and enzymatically released norisoprenoids in Weisser Riesling grapes is given in Tables 2 and 3. Significant increases in acid-released norisoprenoid levels were observed in Weisser Riesling grapes with an increase in ripeness. Similar tendencies were found in most of the corresponding wines (Table 2). Strauss *et al.* (1987b) reported increases in the concentrations of vitispirane, TDN and beta-damascenone during ripening. These compounds were liberated from their bound precursors through heating of Weisser Riesling grape juice. In the present investigation, significant increases in enzymatically released norisoprenoid concentrations in grapes were mostly followed by slight decreases after the third sampling stage (Table 3). Norisoprenoid concentrations in Chenin blanc grapes showed similar changes during ripening as in Weisser Riesling grapes (Figs. 4 and 5). Similarly, increases and decreases in the concentrations of some free and bound monoterpenes during ripening were reported previously (Wilson, Strauss & Williams, 1984; Gunata *et al.*, 1985b; Marais, 1987).

Sugar accumulation was slower in shaded grapes than in sun-exposed grapes, but became equivalent at the fourth sampling stage of Weisser Riesling (Fig. 2). Most norisoprenoid concentrations differed greatly between sun-exposed and shaded grapes at this sampling stage (Tables 2 and 3), which indicates that the bound precursor development did not completely coincide with sugar accumulation. For instance, the TDN (**2**) concentration increases were 79,6% in the sun-exposed grapes [30,6% per degree Balling (°B)] and 82,4% in the shaded grapes (17,9% per °B) (Fig. 2). Therefore, it appears that the biosynthesis of TDN precursors was not parallel to sugar accumulation during ripening, which is in accordance with the suggestions of Wilson *et al.* (1984) on monoterpene glycoside biosynthesis.

## CONCLUSIONS

Potentially volatile $C_{13}$-norisoprenoids may be released from their bound forms in grapes or during wine ageing by acid and probably also by enzymatic hydrolysis. The development of these norisoprenoid precursors in grapes is significantly affected by sunlight, shade and degree of ripeness. Consequently, the composition and quality of wine will depend on, amongst other things, whether the grapes are matured in sunlight or shady conditions, and when the grapes are harvested during the grape ripening period. These factors may be considered in the production of Weisser Riesling wines from grapes with a lower potential to develop TDN and its typical kerosene-like aroma. In fact, the practice of harvesting grapes at an earlier ripening stage is already successfully applied on a limited scale in the South African wine industry.

Too much shade may result in rot and low concentrations of quality-enhancing aroma compounds, while overexposure to sunlight may lead to unacceptably high concentrations of phenolic and other undesirable compounds such as TDN, and sunburn even may occur. Therefore a canopy management system which would provide an effective shade/sunlight combination and produce wines with a low potential to develop TDN, but still with sufficient aroma, could be selected for Weisser Riesling.

The Amberlite XAD-2 isolation technique combined with acid hydrolysis offers the possibility of predicting the development of TDN in wine and, therefore, to some extent the potential quality of aged Weisser Riesling wines. However, it needs to be determined whether there is a correlation between naturally developed free TDN levels in aged wine and TDN levels obtained after acid hydrolysis of its bound forms in grapes or young wine. More work on the effects of the abovementioned factors on Weisser Riesling wine aroma and quality is needed.

## LITERATURE CITED

ASHTON, K. & ADMIRAAL, S., 1990. Effect of shading on vine physiology. *Aust. Grapegrower & Winemaker* **320,** 10-11.

BUTTERY, R.G., TERANISHI, R. & LING, L.C., 1988. Identification of damascenone in tomato volatiles. *Chem. Ind.* 7, 238.

CHISHOLM, M.G., 1990. Comparison of the most intense odour-active compounds formed during the fermentation of *Vitis vinifera* and *Vitis* spp. grape vine. In: Bioflavour '90. Proc. Int. Conf., 26-28 September, Glasgow, Scotland (In press).

DIMITRIADIS, E., STRAUSS, C.R., WILSON, B. & WILLIAMS, P.J., 1985. The actinidols: nor-isoprenoid compounds in grapes, wines and spirits. *Phytochemistry* **24,** 767-770.

DI STEFANO, R., 1985. Presenza di caratteri organolettici favorevoli in vini bianchi lungamente invecchiati. Indagine sui composti volatile e su alcuni parametri chimici e fisici di Riesling prodotti in Germania. *Riv. Vitic. Enol.* **4,** 228-241.

GUNATA, Y.Z., BAYONOVE, C.L., BAUMES, R.L. & CORDONNIER, R.E., 1985a. The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *J. Chromatogr.* **331,** 83-90.

GUNATA, Y.Z., BAYONOVE, C.L., BAUMES, R.L. & CORDONNIER, R.E., 1985b. The aroma of grapes. Localisation and evolution of free and bound fractions of some aroma components c.v. Muscat during first development and maturation. *J. Sci. Food Agric.* **36,** 857-862.

ILAND, P., 1989a. Grape berry composition - the influence of environmental and viticultural factors. Part 1. Temperature. *Aust. Grapegrower & Winemaker* **326,** 13-15.

ILAND, P., 1989b. Grape berry composition - the influence of environmental and viticultural factors. Part 2. Solar radiation. *Aust. Grapegrower & Winemaker* **328,** 74-76.

MARAIS, J., 1987. Total terpene concentrations in defining optimum grape maturity. In: BOOYSEN, J.H., DEIST, J. & VAN WYK, C.J. (eds.). 8th Int. Oenol. Symp., 27-29 April, Cape Town, Republic of South Africa. pp. 400-418.

MORRISON, J.C. & NOBLE, A.C., 1990. The effects of leaf and cluster shading on the composition of Cabernet Sauvignon grapes and on fruit and wine sensory properties. *Am. J. Enol. Vitic.* **41,** 193-200.

OHLOFF, G., 1978. Importance of minor components in flavors and fragrances. *Perfumer and Flavorist* **3,** 11-22.

RAPP, A. & GÜNTERT, M., 1985. Changes in aroma substances during the storage of white wines in bottles. In: CHARALAMBOUS, G. (ed.). The shelf life of foods and beverages. Proc. 4th Int. Flavor Conf., 23-26 July, Rhodes, Greece. pp. 141-167.

REYNOLDS, A.G. & WARDLE, D.A., 1988. Canopy microclimate of Gewürztraminer and monoterpene levels. In: SMART, R.E., THORNTON, R.J., RODRIGUEZ, S.B. & YOUNG, J.E. (eds.). Proc. 2nd Int. Cool Climate. Vitic. Oenol. Symp., 11-15 Jan., Auckland, New Zealand. pp. 116-122.

ROJAS-LARA, B.A. & MORRISON, J., 1989. Differential effects of shading fruit or foliage on the development and composition of grape berries. *Vitis* **28,** 199-208.

SAAYMAN, D., 1981. Klimaat, grond en wingerdbougebiede. In: BURGER, J. & DEIST, J. (eds.). Wingerdbou in Suid-Afrika. VORI, Stellenbosch, Republic of South Africa. pp. 48-66.

SCHREIER, P. & DRAWERT, F., 1974. Gaschromatographisch-massenspektrometrische Untersuchung flüchtiger Inhaltsstoffe des Weines. I. Unpolare Verbindungen des Weinaromas. *Z. Lebensm. Unters.-Forsch.* **154,** 273-278.

SEFTON, M.A. & WILLIAMS, P.J., 1991. Generation of oxidation artifacts during the hydrolysis of norisoprenoid glycosides by fungal enzyme preparations. *J. Agric. Food Chem.* **39,** 1994-1997.

SEFTON, M.A., SKOUROUMOUNIS, G.K., MASSY-WESTROPP, R.A. & WILLIAMS, P.J., 1989. Norisoprenoids in *Vitis vinifera* white wine grapes and the identification of a precursor of damascenone in these fruits. *Aust. J. Chem.* **42,** 2071-2084.

SIMPSON, R.F., 1978a. 1,1,6-Trimethyl-1,2-dihydronaphthalene: an important contributor to the bottle aged bouquet of wine. *Chem. Ind.* **1,** 37.

SIMPSON, R.F., 1978b. Aroma and compositional changes in wine with oxidation, storage and ageing. *Vitis* **17,** 274-287.

SIMPSON, R.F. & MILLER, G.C., 1983. Aroma composition of aged Riesling wine. *Vitis* **22,** 51-63.

SIMPSON, R.F., STRAUSS, C.R. & WILLIAMS, P.J., 1977. Vitispirane: a $C_{13}$ spiro-ether in the aroma volatiles of grape juice, wines and distilled grape spirits. *Chem. Ind.* **15,** 663-664.

SMART, R.E., ROBINSON, J.B., DUE, G.R. & BRIEN, C.J., 1985. Canopy microclimate modification for the cultivar Shiraz. I. Definition of canopy microclimate. *Vitis* **24,** 17-31.

SNEDECOR, G.W. & COCHRAN, W.G., 1980. Statistical methods (7th Edition). The Iowa State University Press, Ames, Iowa, U.S.A.

STRAUSS, C.R., WILSON, B. & WILLIAMS, P.J., 1987. 3-Oxo-alpha-ionol, vomifoliol and roseoside in *Vitis vinifera* fruit. *Phytochemistry* **26,** 1995-1997.

STRAUSS, C.R., GOOLEY, P.R., WILSON, B. & WILLIAMS, P.J., 1987a. Application of droplet countercurrent chromatography to the analysis of conjugated forms of terpenoids, phenols, and other constituents of grape juice. *J. Agric. Food Chem.* **35,** 519-524.

STRAUSS, C.R., WILSON, B., ANDERSON, R. & WILLIAMS, P.J., 1987b. Development of precursors of $C_{13}$ nor-isoprenoid flavorants in Riesling grapes. *Am. J. Enol. Vitic.* **38,** 23-27.

VERSINI, G., DALLA SERRA, A., DELL'EVA, M., SCIENZA, A. & RAPP, A., 1987. Evidence of some glycosidically bound new monoterpenes and norisoprenoids in grapes. In: SCHREIER, P. (ed.). Bioflavour '87. Analysis. Biochemistry. Biotechnology. Proc. Int. Conf., 29-30 September, Würzburg, Germany. pp. 161-170.

WILLIAMS, P. J., STRAUSS, C. R. & WILSON, B., 1983. Recent developments in grape flavour research. *Aust. Grapegrower & Winemaker* **232,** 20-24.

WILLIAMS, P.J., SEFTON, M. A. & WILSON, B., 1989. Non-volatile conjugates of secondary metabolites as precursors of varietal grape flavor components. In: TERANISHI, R., BUTTERY, R.G. & SHAHIDI, F. (eds.). Flavor chemistry: trends and developments. ACS Symposium series no. 388, American Chemical Society. Washington, D.C., U.S.A. pp. 35-48.

WILLIAMS, P.J., STRAUSS, C.R., WILSON, B. & MASSY-WESTROPP, R.A., 1982. Use of $C_{18}$ reversed-phase liquid chromatography for the isolation of monoterpene glycosides and nor-isoprenoid precursors from grape juice and wines. *J. Chromatogr.* **235,** 471-480.

WILLIAMS, P.J., STRAUSS, C.R., WILSON, B. & DIMITRIADIS, E., 1985. Origins of some volatile monoterpenes and nor-isoprenoids in grapes and wines - Biosynthetic and biogenetic considerations. In: BERGER, R.G., NITZ, S. & SCHREIER, P. (eds.). Topics in flavour research. Proc. Int. Conf., 1-2 April, Freising - Weihenstephan, Germany. pp. 335-352.

WILSON, B., STRAUSS, C.R. & WILLIAMS, P.J., 1984. Changes in free and glycosidically bound monoterpenes in developing muscat grapes. *J. Agric. Food Chem.* **32,** 919-924.

WINTERHALTER, P., 1990. Bound terpenoids in the juice of the purple passion fruit (*Passiflora edulis* Sims). *J. Agric. Food Chem.* **38,** 452-455.

WINTERHALTER, P., 1991. 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) formation in wine. I. Studies on the hydrolysis of 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol rationalizing the origin of TDN and related $C_{13}$-norisoprenoids in Riesling wine. *J. Agric. Food Chem.* **39,** 1825-1829.

WINTERHALTER, P. & SCHREIER, P., 1988. Free and bound $C_{13}$ norisoprenoids in quince (*Cydonia oblonga,* Mill.) fruit. *J. Agric. Food Chem.* **36,** 1251-1256.

WINTERHALTER, P., SEFTON, M.A. & WILLIAMS, P.J., 1990. Two-dimensional GC- DCCC analysis of the glycoconjugates of monoterpenes, norisoprenoids and shikimate-derived metabolites from Riesling wine. *J. Agric. Food Chem.* **38,** 1041-1048.