Effect of Region on Free and Bound Monoterpene and C_{13}-Norisoprenoid Concentrations in Weisser Riesling Wines*

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Free and bound monoterpene and C_{13}-norisoprenoid concentrations of Weisser Riesling wines of different vintages and from different regions in South Africa, Germany and Northern Italy were compared. The concentrations of some of these compounds differed between climatic regions. For example, older Weisser Riesling wines from South Africa displayed significantly higher 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), trans-vitispirane and trans-1,8-terpin concentrations than those from the cooler European countries. Canonical and stepwise discriminant analysis of the wine data allowed the successful classification of the wines according to origin. A comparison of meteorological data of the countries revealed that in the cooler wine regions of Germany and Northern Italy the average daily temperatures were lower, the average monthly rainfall higher and the daily sunshine hours fewer than in the warmer South African wine regions.

Vitis vinifera L. cv. Weisser Riesling, or Rhine Riesling, is one of the most important white grape cultivars in the world. Although the classical Weisser Riesling wines of the world are produced in relatively cool wine regions, like in Germany, high-quality wines are also produced in the warmer wine regions of South Africa. It is believed that the region and region-related factors, e.g. average daily temperature, rainfall and sunlight hours per day, may have a prominent effect on the chemical composition and character of the wine produced. A better understanding of such effects may facilitate the control of the development of aroma compounds detrimental to wine quality, such as 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN).

Free and bound terpenes and C_{13}-norisoprenoids are known to be important constituents of grape and wine aroma (Williams *et al.*, 1982; Rapp, Mandery & Güntert, 1984; Strauss *et al.*, 1986; Sefton *et al.*, 1989). Several C_{13}-norisoprenoids have been identified upon liberation from their precursors in Weisser Riesling grapes and wine specifically (Winterhalter, Sefton & Williams, 1990a; 1990b). Of particular interest is the identification of two glycosidically bound compounds suggested to be precursors of TDN (Humpf, Winterhalter & Schreier, 1991; Winterhalter, 1991). Rapp, Güntert & Heimann (1985) reported a characteristic varietal terpene profile for Weisser Riesling wines from various countries. They also reported that TDN developed at a later stage in German Weisser Riesling wines than in those from the other countries. In addition, the Weisser Riesling terpene profiles were distinctively different from those of Welschriesling (Austria, Yugoslavia, Italy), Cape Riesling (South Africa), Hunter Valley Riesling (Australia) and Emerald Riesling (California, USA).

Investigations on the effect of region on the aroma composition of wines of the same cultivar are often complicated by variations in climatic and viticultural conditions as well as by wine-making techniques. The present study should therefore be regarded as an exploratory investigation to compare Weisser Riesling wines of different vintages and climatic regions in South Africa, Germany and Northern Italy in terms of free and bound monoterpene and C_{13}-norisoprenoid concentrations.

MATERIALS AND METHODS

Commercially available Weisser Riesling wines of different vintages and climatic regions in South Africa, Germany and Northern Italy, classified according to Winkler *et al.* (1974) (Table 1), were compared in terms of certain free and bound monoterpenes and C_{13}-norisoprenoids, listed in Table 2. The free compounds were extracted with Freon 11 and the extracts analysed by gas chromatography (Marais, 1986). Glycosidically bound monoterpenes and C_{13}-norisoprenoids were isolated on Amberlite XAD-2 resin, according to the technique of Gunata *et al.* (1985), as adapted by Versini *et al* (1987). Compounds were liberated by enzymatic hydrolysis (Rohapect C) at pH 5 in a waterbath (40°C, 15 hrs). Liberated compounds were again transferred onto Amberlite XAD-2 resin, eluted with pentane/dichloromethane (2:1) and analysed by gas chromatography. The capillary columns and gas chromatographic conditions used were described by Marais, Van Wyk & Rapp (1992a). Compound concentrations were expressed as relative concentrations in relation to internal standards, i.e. 3-decanol (for enzymatically released volatiles) and 2-ethyl hexanol (for free volatiles). The identities of all compounds were confirmed by comparing their mass spectra and retention times with those of authentic standards.

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TABLE 1
Weisser Riesling wines of different vintages and regions in South Africa, Germany and Northern Italy.

Country	Vintage and wine producer	Region*
South Africa	1989 and 1986 Klein Constantia	Constantia, region II
	1989, 1986 and 1979 Simonsig	Stellenbosch, region III
	1989 and 1986 Nederburg	Grapes mainly from Stellenbosch, region III
	1989 and 1980 KWV	Grapes from Durbanville, region III
Germany	1989 and 1986 Geilweilerhof	Pfalz, region I
	1989 and 1986 Frankweiler	Pfalz, region I
	1989, 1986 and 1979 Deidesheim	Pfalz, region I
Northern Italy	1989 Laimburg (3 clones)	Trentino-South Tyrol, region II
	1989, 1986 and 1979 Hirschprunn	Trentino-South Tyrol, region II
	1989 and 1986 Cornaiano	Trentino-South Tyrol, region II
	1989 and 1986 Jermann	Friuli, region III

*Regions classified according to Winkler *et al.* (1974).

TABLE 2
Free and bound monoterpenes and C_{13}-norisoprenoids in Weisser Riesling wines.

Free	Bound
trans-Furan linalool oxide	trans-Furan linalool oxide
cis-Furan linalool oxide	cis-Furan linalool oxide
Nerol oxide	Linalool
Linalool	alpha-Terpineol
Hotrienol	trans-Pyran linalool oxide
alpha-Terpineol	cis-Pyran linalool oxide
trans-Pyran linalool oxide	Citronellol
cis-Pyran linalool oxide	Nerol
Citronellol	Geraniol
Nerol	2-Hydroxy-1,8-cineole
2,6-Dimethyl-3,7-octadien-2,6-diol (Diendiol-1)	trans-Geranic acid
2,6-Dimethyl-7-octen-2,6-diol (Hydroxylinalool)	2,6-Dimethyl-3,7-octadien-2,6-diol (Diendiol-1)
trans-1,8-Terpin	2,6-Dimethyl-1,7-octadien-3,6-diol (Diendiol-2)
trans-Vitispirane	3,7-Dimethyloctan-1,7-diol (Hydroxycitronellol)
1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN)	2,6-Dimethyl-7-octen-1,6-diol
	trans-2,6-Dimethyl-2,7-octadien-1,6-diol (8-Hydroxylinalool)
	cis-2,6-Dimethyl-2,7-octadien-1,6-diol (8-Hydroxylinalool)
	trans-3,7-Dimethyl-2-octen-1,7-diol (Hydroxygeraniol)
	Megastigma-5,8-dien-3,7-dione
	9-Hydroxymegastigma-4,7-dien-3-one

Meteorological information on average daily temperature (°C), average monthly rainfall (cm) and average sunshine hours per day was obtained for each region from the nearest meteorological station.

Canonical discriminant analysis was performed on the relative concentrations of the free compounds (Table 2) in all of the wines, using the SAS System (SAS Institute Inc., 1990). In the case of the bound compounds, the covariance matrix was singular, thereby preventing the analysis of the complete data set. After that a stepwise discriminant analysis by stepwise selection was performed on the relative concentrations of both free and bound compounds (Table 2). This was again followed by a canonical discriminant analysis of the selected variables. The significance of the effect of region on the mean relative concentrations of the selected variables (1989 and 1986 vintages) was obtained by using standard analysis of variance methods (Snedecor & Cochran, 1982). Students t least significant difference at the 5% level was used to compare treatment means.

RESULTS AND DISCUSSION

Comparison of wine aroma profiles: Commercially available Weisser Riesling wines from different regions in South Africa, Germany and Northern Italy (Table 1) were compared with respect to certain free and bound monoterpenes and C_{13}-norisoprenoids (Table 2). Employing the concentrations of all the free compounds in the discriminant analysis, a successful classification of the wines into

TABLE 3
Discriminant variables selected in order of importance for the classification of Weisser Riesling wines from South Africa, Germany and Northern Italy.

Free compound	F-value to enter	Bound compound	F-value to enter
trans-Vitispirane	12,729**	2-Hydroxy-1,8-cineole	16,319**
1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN)	8,967**	cis-Furan linalool oxide	5,946 *
alpha-Terpineol	7,878**	cis-Pyran linalool oxide	5,688 *
trans-1,8-Terpin	5,678 *	Megastigma-5,8-dien-3,7-dione	4,535 *
trans-Furan linalool oxide	3,779 *	cis-8-Hydroxylinalool	4,254 *
Diendiol-1	2,258 *	Diendiol-1	3,979 *

** = Highly significant ($p \leq 0{,}01$).
* = Significant ($p \leq 0{,}05$).

three distinct groups was obtained, each group containing wines of one country only (Fig. 1).

Subsequently, using stepwise discriminant analysis, six of each of the free and bound compounds with the highest discriminatory power (F-values) were selected for further classification (Table 3). This analysis permitted a classification of the wines into the same groups as before (Figs. 2 and 3). A very high accuracy of prediction of the wine origin was obtained in all three analyses (Table 4). This was possible despite the fact that the wines were of different ages and that different viticultural and wine-producing techniques could have been used during their production.

The effect of region on the mean relative concentrations of the selected free and bound monoterpenes and C_{13}-norisoprenoids (1989 and 1986 vintages) is given in Tables 5 and 6.

In the case of the variables listed in Table 5, no interactions occurred between the main effects, i.e. region and year, and therefore the data for the 1989 and 1986 vintages could be combined for each region. Apart from trans-furan linalool oxide, alpha-terpineol and diendiol-1, the mean concentrations of the other compounds were significantly higher in the South African wines than in the European wines (Table 5). However, in the case of three variables, listed in Table 6, interactions occurred between the main effects. The mean concentrations of TDN and trans-1,8-terpin were significantly higher in the 1986 South African wines, while that of megastigma-5,8-dien-3,7-dione was significantly higher in the 1989 Italian wines. Higher concentrations of TDN, trans-1,8-terpin and trans-vitispirane were previously reported in aged Weisser Riesling wines, and it has been suggested that these compounds contribute to the bottle-aged character of terpene-rich wines (Simpson & Miller, 1983; Rapp, Güntert & Ullemeyer, 1985). The higher levels of these compounds in aged South African Weisser Riesling wines in comparison to those of similar vintages from Germany and Italy confirm that the South African wines in general require a shorter time to develop a strong bottle-aged character. This may be ascribed to the presence of higher concentrations of the precursors of these compounds in the South African wines. Higher average storage temperatures also contributed to this phenomenon, as was clearly demonstrated in Weisser Riesling wines stored at 15°C and 30°C for four years and one year, respectively (Marais *et al.*, 1992a).

TABLE 4
Prediction matrix (%) for 15 and six variables, respectively.

Fifteen Variables (free compounds).

Region	South Africa	Germany	Northern Italy
South Africa	99,8	0,0	0,2
Germany	0,0	99,8	0,2
Northern Italy	0,1	0,0	99,9

Six Variables (free compounds).

Region	South Africa	Germany	Northern Italy
South Africa	97,5	0,3	2,2
Germany	0,0	93,9	6,1
Northern Italy	0,2	0,9	98,9

Six Variables (bound compounds).

Region	South Africa	Germany	Northern Italy
South Africa	96,7	3,3	0,0
Germany	0,0	99,5	0,5
Northern Italy	0,0	0,0	100,0

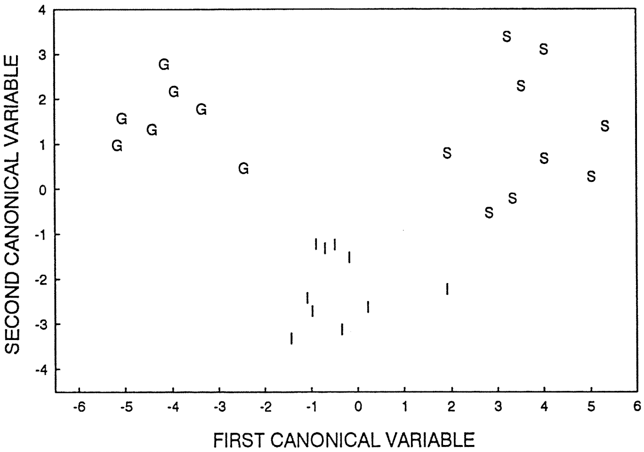


FIGURE 1

A canonical plot showing discrimination between Weisser Riesling wines from South Africa (3 climatic regions) (S), Germany (Pfalz) (G) and Northern Italy (2 climatic regions) (I) on the basis of 15 free monoterpenes and C_{13}-norisoprenoids.

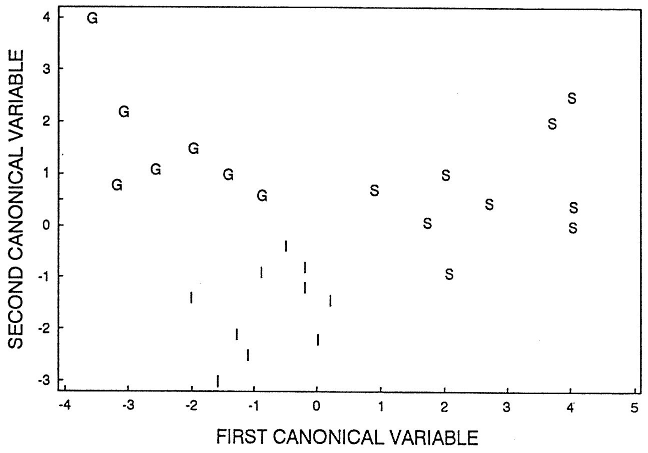


FIGURE 2

A canonical plot showing discrimination between Weisser Riesling wines from South Africa (3 climatic regions) (S), Germany (Pfalz) (G) and Northern Italy (2 climatic regions) (I) on the basis of 6 free monoterpenes and C_{13}-norisoprenoids.

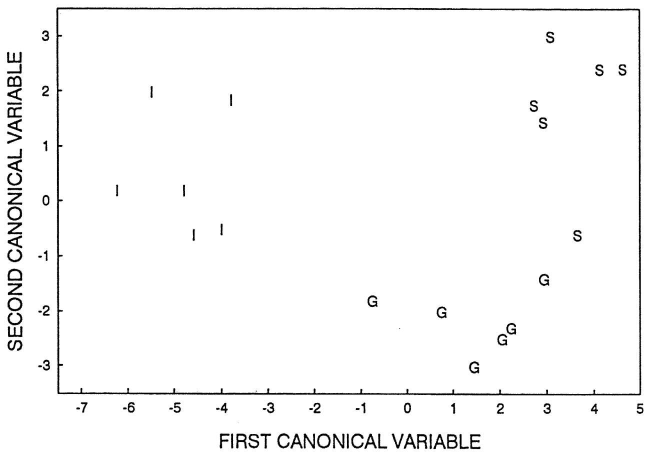


FIGURE 3

A canonical plot showing discrimination between Weisser Riesling wines from South Africa (3 climatic regions) (S), Germany (Pfalz) (G) and Northern Italy (2 climatic regions) (I) on the basis of 6 bound monoterpenes and C_{13}-norisoprenoids.

TABLE 5

The effect of region on the mean relative concentrations* of certain free and bound compounds in Weisser Riesling wines (1989 and 1986 vintages combined).

Compound	South Africa	Germany	Northern Italy	LSD-value
Free				
trans-Furan linalool oxide	38,46 a	31,36 a	27,75 a	14,214
alpha-Terpineol	105,12 a	63,38 a	59,75 a	50,749
Diendiol-1	33,92 b	78,07 a	42,92 ab	42,135
trans-Vitispirane	21,95 a	13,42 b	2,02 c	7,497
Bound				
cis-Furan linalool oxide	128,63 a	106,88 a	63,78 b	33,593
cis-Pyran linalool oxide	18,53 a	8,55 b	7,92 b	5,883
2-Hydroxy-1,8-cineole	18,12 a	13,67 b	9,25 c	3,145
Diendiol-1	28,27 a	54,70 a	64,85 a	38,304
cis-8-Hydroxylinalool	660,83 a	272,23 b	327,75 b	205,130

* Six measurements.

Values in rows designated by the same symbol do not differ significantly ($p \leq 0,05$).

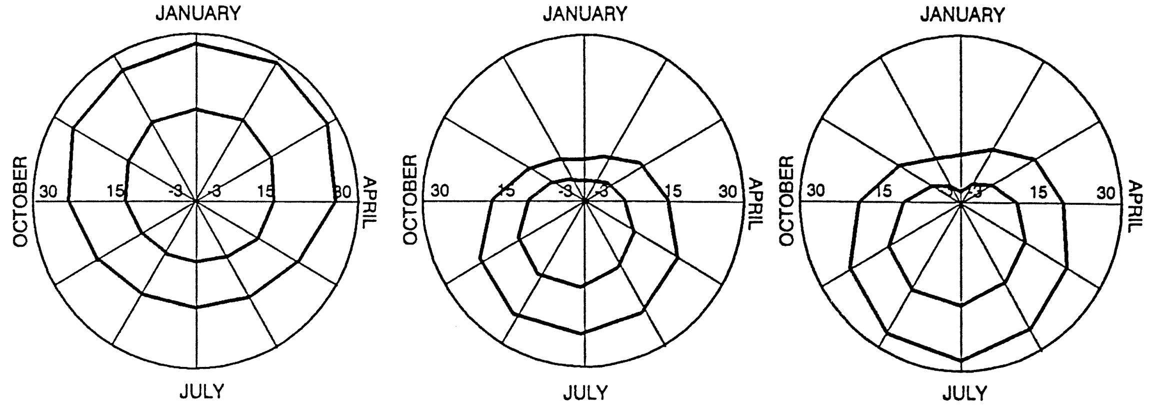
TABLE 6

The effect of region and vintage on the mean relative concentrations* of certain free and bound compounds in Weisser Riesling wines.

Compound	South Africa		Germany		Northern Italy		LSD-value
	1989	1986	1989	1986	1989	1986	
Free							
trans-1,8-Terpin	1,23 b	18,17 a	1,70 b	4,30 b	0,00 b	5,33 b	7,787
1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN)	11,07 b	33,03 a	2,03 b	7,33 b	1,20 b	3,20 b	10,365
Bound							
Megastigma-5,8-dien-3,7-dione	56,33 b	27,00 b	18,87 b	40,23 b	180,00 a	44,83 b	37,602

* Three measurements.

Values in rows designated by the same symbol do not differ significantly ($p \leq 0,05$).

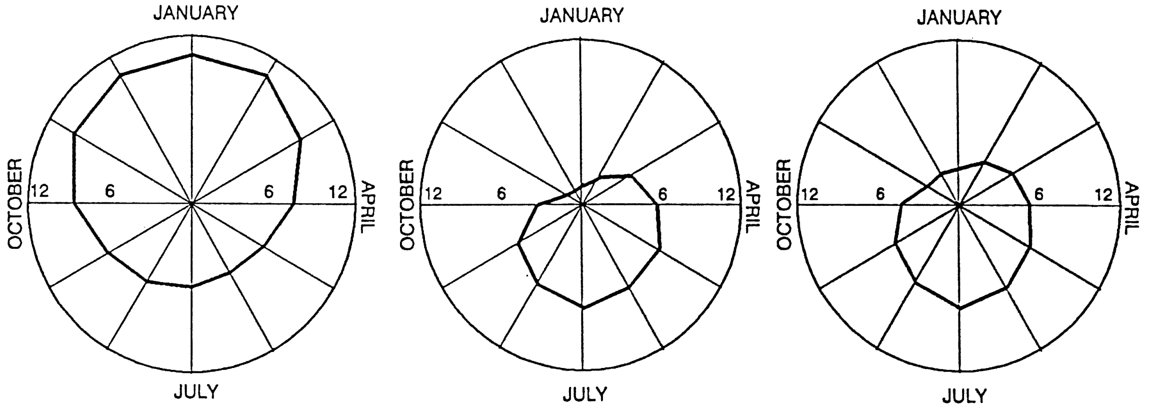


South Africa - Stellenbosch
(1903 - 1989)

Germany - Pfalz
(1951 - 1980)

Italy - Trentino - South Tyrol
(1959 - 1988)

Air Temperature (°C)

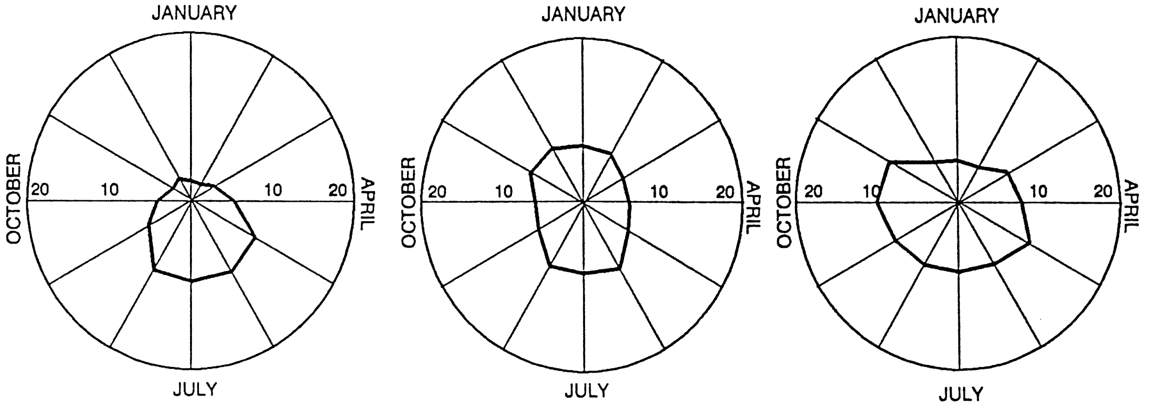


South Africa - Stellenbosch
(1972 - 1989)

Germany - Pfalz
(1951 - 1980)

Italy - Trentino - South Tyrol
(1959 - 1988)

Sun hours/day



South Africa - Stellenbosch
(1968 - 1989)

Germany - Pfalz
(1951 - 1980)

Italy - Trentino - South Tyrol
(1959 - 1988)

Precipitation (cm)

FIGURE 4
Average maximum and minimum daily temperatures (°C), average sunshine hours per day and average monthly rainfall (cm) for typical regions in South Africa (Stellenbosch), Germany (Pfalz) and Northern Italy (Trentino-South Tyrol).

Some of the free and bound compounds in Tables 5 and 6 are chemically related in the sense that linalool can be transformed to the furan and pyran linalool oxides (Ribéreau-Gayon, Boidron & Terrier, 1975; Strauss, Wilson & Williams, 1986), diendiol-1, trans-1,8-terpin via alpha-terpineol (Rapp *et al.*, 1984), cis- and trans-8-hydroxylinalool (Rapp, Mandery & Niebergall, 1986) and 2-hydroxy-1,8-cineole (Bitteur *et al.*, 1990). In addition, diendiol-1 may be transformed to hotrienol and nerol oxide (Williams, Strauss & Wilson, 1980). The abovementioned transformations may probably be enhanced in grapes and wines subjected to higher environmental temperatures, and may account for several of the trends in the observed levels of compounds in warm versus cool regions.

The selected variables, employed to discriminate between the wines from the three countries, are not necessarily responsible for differences in character or style of these wines, but could be judged as indicators of such differences. In this regard, the results of the enzymatically released compounds should be evaluated with caution, since it has not been proved that they are released naturally in grapes to the same extent as observed under the experimental conditions. The free compounds, on the other hand, could be more reliable parameters of wine character, since they occur naturally in wines.

Comparison of meteorological data: Average maximum and minimum daily temperatures, average monthly rainfall and average sunshine hours per day for typical regions in South Africa, Germany and Northern Italy are illustrated in Figure 4.

Average daily temperatures were clearly higher in the South African region than in the German and Northern Italian regions (Fig. 4). Grape ripening (February/March) in the South African region coincided with average daily temperatures varying between 20°C and 22°C, while in the German and Italian regions temperatures during this period (September/October) varied between 10°C and 14°C, and between 11°C and 17°C, respectively.

Sunlight may play an important role in the formation of various chemical compounds by means of photo-oxidative reactions. For example, it was found that the concentrations of C_{13}-norisoprenoids such as TDN and trans-vitispirane, released from their precursors by acid hydrolysis, were significantly higher in sun-exposed Weisser Riesling grapes than in shaded grapes (Marais, Van Wyk & Rapp, 1992b). Even though day length during the summer months is longer in Europe than in South Africa, it is evident that the number of sunshine hours per day for all seasons was much higher in South Africa (Fig. 4). For example, this varied between 9 and 11 for the Stellenbosch region, and between 4 and 6 for the European regions during the respective grape-ripening months. Rainfall was much higher in the German (51 to 58 mm) and Northern Italian (85 – 99 mm) regions than in the South African region (15 – 25 mm) during the same period (Fig. 4).

The abovementioned climatic factors cannot be evaluated in isolation because their effect on the performance of the vine and on grape and wine quality is complicated by an interaction with various other factors, such as viticultural practices. Furthermore, each vineyard site could have a mesoclimate slightly different from that of the nearest meteorological station. Nevertheless, sunlight and temperature may be selected as factors playing an important role in causing differences between wines from warmer regions (South Africa) and cooler regions (Germany and Northern Italy). For example, higher average temperatures and a higher number of sunshine hours per day coincided with significantly higher free TDN, trans-vitispirane and trans-1,8-terpin concentrations, probably derived from high precursor levels in the corresponding grapes, in South African Weisser Riesling wines (Tables 5 and 6). In addition, other monoterpene transformations, like those of linalool, may be accelerated in grapes and wines from warmer climatic regions. Furthermore, differences in aroma composition of wines may lead to differences in wine characters. For example, in Constantia, which is classified as region II (Table 1), more delicate and complex wines are produced than in the other regions. This may be ascribed to slightly lower temperatures and fewer sunshine hours per day, caused by the influence of the sea and the shadowing effect of the adjacent mountains.

CONCLUSIONS

The application of canonical discriminant analysis on the concentrations of only six monoterpenes and C_{13}-norisoprenoids of Weisser Riesling wines from South Africa, Germany and Northern Italy, which are known to be different in climate, permitted the prediction of origin with a high degree of accuracy. The fact that TDN was selected as one of the compounds with a highly significant discriminatory power is in support of claims that this compound with its pronounced kerosene-like aroma is in fact responsible for marked aged bouquet differences in Weisser Riesling wines from warm regions to those from cool regions.

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