Effect of Cultural Practices and Environmental Factors on Fruit and Wine quality

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SUMMARY

Recent advances in solid phase micro extraction methods (SPME) enable fast and accurate aroma compound determination by GC–MS. Studies made using this method revealed specific effects of irrigation rates and timings on the aroma profile of musts and wines. The major effects of cultural practices are probably via their effect on crop load. Mineral fertilization which significantly affected the petiole and blade mineral content affected the aroma profile as well. Rootstock and environmental conditions had a significant effect on the concentration of free and bound monoterpenes and other aroma contributing compounds in the must and the wine. Training systems such as vertical shoot positioning and ballerina were found to significantly affect the aroma profile of the must, the pH, sugar and acid content were affected as well. Irrigation with moderate saline water was found to positively affect the aroma of wines and must of a few varieties. The concentration of sixteen aroma compounds identified by GC–MS analysis of *Cabernet Sauvignon* wine were found to be significantly affected by moderate saline irrigation treatments. A significant interaction between salinity and rootstock effect on must and wine aroma compound composition and maturity parameters was found in two experimental sites.

KEY WORDS

cultural practice, fertigation, fruit quality, irrigation, rootstock, salinity, training system, wine quality

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INTRODUCTION

In spite of the recent improvements in winemaking technologies, wine quality is determined mainly by the chemical composition of the fruit. Some of the chemical constituents of the fruit do not undergo changes during the fermentation process whereas others serve as precursors for newly formed compounds. Basic perceptions such as bitterness, sweetness sourness and astringency are elicited by groups of compounds including glycosides, dipeptides, sugars, acids, alkoloides and phenols characterized by low volatility. Volatile compounds in wine are numerous and originate in the grapes, during the crushing and the fermentation process and during the various maturation stages of the wine. This paper will focus on the aromatic properties of grapes and wines since these are perhaps the most important quality component as compared with other beverages. The odorous compounds are perceived by both the nose and the mouth and considerably affects the overall wine score.

Many odorous compounds are present in fruit and wines as free. aroma compounds as well as glycosidically bound and hydroxylated compounds Odorous aroma compounds are being released during fruit maturation or storage by enzyme or acid hydrolysis. The presence of these compounds in plants was first reported by Bourquelot and Bridel (1913) while their importance in grapes, must and wines aroma and quality was recognized 3 decades ago (Cordonnier and Bayonov, 1974). Many aroma compounds in musts and wines are present as free odorous or non odorous forms which undergo transitions via glycosilation, oxidation and hydrolysis processes. One of the major aroma components which contribute to the varietal character of aromatic or the so called floral varieties are known as terpenes (Marais, 1983, Rapp *et al.,* 1984, Strauss *et al.,* 1986). Non aromatic cultivars such as *Sauvignon blanc* and *Chardonnay* also contain monoterpenes at lower concentrations (Augustyn *et al.,* 1982, Simpson and Miller, 1984).

Terpenes are one of the most important groups of aroma compounds of grapes, must and wines. The various terpene compounds consist of two basic 5 carbon isoprene units - monoterpenes, three - sesquiterpenes, four - diterpenes and six - triterpenes. The monoterpenes are natural aroma compounds with very low sensing thresholds which are found as trace constituents in grapes particularly in aromatic cultivars such as various muscats (Gunata *et al.,* 1985).

Hydrolysis of glycosides yields equimolar proportions of various and highly diverse aglycones and sugar moieties (Siegel, 1990).

More than 200 different aglycones including aliphatic ,terpene, and sesquiterpene alcohols, hexanols, C_{13} norisoprenoides, acids, hydroxylacids, phenyl propanederivatives and related compounds, acetates and hemiacetales have been identified (Crouzet, 1997). The presence of glucosides of 2 important aromatic alcohols, 2- phenyl ethanol and benzyl alcohol as well as of few hexanols were reported as well (Williams *et al.,* 1983, Siegel, 1990). Monoterpene glycosides are present in the fruit as well as in the wines and undergo very slight changes if at all during the *Saccharomyces cerevisiae* fermentation.

Hardie and O'brien (1988) proposed that some of the volatile constituents of grape perform functions arising from the close dependence of wild vitis species upon avian agents of dispersal. This created a selection pressure for insect mediated pollination which insures more effective pollen fertilization compared to wind alone. Terpenes may have evolved in grapes as attractants of insect pollinators. The name "Muscat" is derived from an ancient usage that signified the ability to attract bees (Hardie and O'brien, 1988) and the free terpenes released may serve in the faecal pellet as attractant of secondary agents of dispersal, e.g. ants, or as inhibitors of competitive plant species.

Terpenes in other plant species have been assigned the following roles: Attraction ofpollinators, (Rodriques and Levin, 1976), defenders by repulsion of insects, fungi, browsing animals and microorganisms (Luckner, 1984) and phytotoxins released into the soil to inhibit the germination and growth of other plants (Gant and Clebsch, 1975).

Terpenes are secondary products of the photosynthetic process and are formed in photosynthethic active tissues via the mevalonic acid pathway (Bantherop *et al.,* 1972, Cori 1983, Croteau, 1984, Shoseyov, 1988).

Both ^{14}C labeled mevalonic acid as well as gaseous labeled $^{14}CO_2$ fed to illuminated leaves were incorporated into grapes within 72 hours (Shoseyov,1988) and *in vitro* grown grape berries showed capability of glycosidic biosynthesis (Bravdo *et al.*, 1989). The roll of Photosynthetic tissues in monoterpene biosynthesis as well as that of the petioles in passing water soluble glycosides formed in the blades to the fruit was well established (Croteau and Sood, 1985, Gunata *et al.,* 1986, Wilson, 1986). Biochemical transformations between the various terpenoide forms seem to be the consequences of evolutionary mechanism aimed at enabling the mobilization of the insoluble monoterpene aglycones from their major production site in photosynthetic tissues to target sites in organs such as flowers, fruits, shoots and roots. The conversion of odorous hydrophobic terpenes into water soluble non odourus glycosides via glycosilation at the production site and hydrolyzing them by glucosidases at the target organs might be considered as an important evolutionary trait.

The varietal flavor characteristics are dependent on the relative concentration and the threshold perception level of individual flavorants which undergo primary and reversal secondary transformations. These biochemical transformations are affected by physiological and environmental conditions in the vineyard as well as by technological winemaking variables and thereby open ways for enormous variability quality and perception of wines made from a single cultivar.

Monoterpenes are secondary metabolites synthesized in the leaves and partially in developing fruit (Bravdo et al. 1989). It is well established that the precursor mevalonic acid undergoes incorporation into monoterpenes. (Hefendehl et al. 1967, Banthorp et al. 1972). It has been demonstrated that three forms of monoterpenes are present in grape juice and wines; free monoterpenes, polyhydroxylated monoterpenes (polyols) and glycosidically bound monoterpenes, out of which only the free monoterpenes are odorous (Cordonnier and Bayonov, 1974, Williams et al. 1981, Williams et al. 1982, Williams et al. 1983).

The monoterpene glycosides are water soluble and can easily be transported from the leaves to the fruit whereas the free monoterpenes are hydrophobic and are being transported as glycosides after undergoing a glycosilation process. Glycosidically bound monoterpenes can undergo enzymatic hydrolysis by native b glucosidase present in the berries (Bayonov et al. 1974).

One of the major factors determining the varietal character of the fruit is the aroma profile, formed by the free aroma copmpounds and the transformations the various forms of free, bound and hydroxylated forms(Williams, 1983) though the mechanism of the profile formation is not clear. The glycosides profile formed by a glycosilation process in the leaves does not resemble the profile found in the fruit (Gunata et al.1986). Terpenes are formed in the blades and are found in both their free and bound forms in the blades and in the petioles, the petioles are relatively richer in free terpene forms (Gunata *et al.,* 1986). The same researchers also suggested that the petioles play a role of filter, retaining free terpenols and letting through the more mobile bound terpenols. Comparison of the terpenol composition of three grape varieties, *Cabernet Sauvignon*, *Sauvignon blanc* and *Muscat of Alexandria* showed a greater profile similarity in the leaves than in the fruit (Siegle, 1990).

Winkler et al. (1974) showed that muscat inflorescence grafted onto non Muscat vines retained the muscat flavor whereas non muscat inflorescence grafted on Muscat vines retained their variatal character. It seems therefore that the mechanism of glycoside selection functions in either the petiole or the fruit itself.

The bound aroma compounds content is higher than that of the free aroma in most fruits (Bravdo and Shoseyov, 2000). The bound aroma compounds are not necessarily monoterpenes and many other bound aroma compounds are normally present in must and wines (Siegle, 1990).

The release of free terpenes and other volatiles from glycosides in the berries is performed by acid catalyzed reactions, and endogenous -β-glycosidases, however these enzymes do not hydrolyze many glycosides and have an optimum pH of 5.0 which means that most of their activity is done at the latest stages of ripening namely after the time of normal commercial harvest (Bayonov *et al.,* 1984, Arian *et al.,* 1987, Gunata *et al.,* 1998).

RECENT

GC-MS analysis of aroma compounds went through revolutionary breakthroughs in the last few years. The invention of SPME (Solid Phase Microextraction) by Pawliszyn (1997) and its commercialization in the last few years (Yang and Peppard, 1994) enables a fast solvent free extraction. This highly effective technique can be used to concentrate volatile organic compounds from liquid samples (immersion SPME) or from the vapor space above a liquid or solid sample (headspace SPME). Instead of the former rather long procedure of liquid liquid extraction which required 24 hours per wine or juice sample of 500ml, the SPME technology needs 5-15 minutes for equilibrating each sample of 5ml. The SPME technique offers a fast way to analyze the original composition of flavor compounds in the gas phase i.e. the olfactory impression perceived by the nose is analytically detected. Identification of peaks by a computer library containing about 280,000 compounds and software capable of copying the peak's area or height into excel electronic spreadsheets and then run various statistical programs.

EFFECT OF CULTURAL PRACTICES

Cultural practices affect fruit and wine quality both directly and indirectly. Most cultural practices such as irrigation, fertilization, training systems and canopy management alter the vegetative to reproductive growth ratio namely the drop load and thereby the quality. While the traditional view associate crop level with fruit quality, studies made during the last 3 decades showed that crop load rather crop level is a more appropriate term for assessing the effect of cropping on fruit quality.

Crop level is expressed in terms of as the weight of fruit per unit land i.e. tons per ha. Crop load is defined as the weight of fruit per unit of vegetative growth namely kg fruit per kg pruning weight or per cm^2 leaf area, or their reciprocals (Bravdo et al. 1984, 1985, Kliewer and Weaver, 1971). Crop load is expressed either as the reciprocal of yield per unit of leaf area (kaps and Cahoon, 1992, Kliewer and weaver, 1971) or as yield to prunning weight ratio (Bravdo et al, 1984,1985, Jackson and Lombard, 1993). Pruning weight is well correlated with leaf area (Smart at al. 1985) and therefore may serve as a practical means for crop load calculations. Leaf are however is hard to be measured by growers.

Crop level is primarily determined by the number of clusters per ha whereas crop load is associated with the vegetative to reproductive relationships (Bravdo and Hepner, 1987, Jackson and Lombard, 1993). The size of the cluster premordia decreased with increasing crop load (Bravdo et al. 1984).

EFFECT OF IRRIGATION

Irrigation of winegrapes is prohibited in many European countries due to a traditional believe that it reduces wine quality. However since the introduction of drip irrigation during the last three decades and the expanding viticulture to many arid areas to countries and states (such as Australia, South Africa, Israel and a few US states - California, Arizona, Texas etc.) it becomes more obvious that excellent wines with very high quality can be obtained under different irrigation regimes. Moreover, the European authorities has recently approved irrigation of vineyards on slopes exceeding 30% steepness as well as vineyards in Portugal with special permits from the national wine Institute. Irrigation, particularly drip, enables to control soil water availability and thereby the vine water potential at various vegetative and reproductive stages. Incorporating fertilizer injection into the water, namely fertigation, provides additional means for direct and indirect control of physiological processes associated with production and quality. Drip irrigation is now common in many temperate wine production regions all over the world. Under these conditions, irrigation is applied only during periods of insufficient precipitation.

It seems that the only direct effect of irrigation is on the turgor which controls the vegetative as well as the berry growth. The control of turgor during the growing season enables to regulate the crop load. Post veraison rains may subject the grapes to berry splitting, and bunch rot and thereby reduced quality. Drip irrigation hardly affects the microclimate and

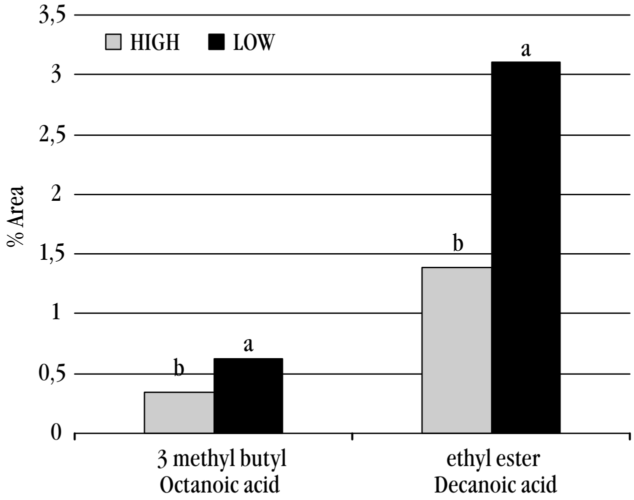

FIGURE 1. Effect of drip irrigation treatments on two aroma compounds of Cabernet sauvignon wine. Rehovot 1998. High and low irrigation – Continuous maintenance of 12 and 18 kPa water potentials respectively at 50 and 20cm vertical and horizontal distances respectively from the dripper. Computerized irrigation and electrotensiometers were used for controlling soil water potential. % area relates to the percentage of area of each peak relative to the total peak area.

therefore less likely to increase diseases. Conflicting data regarding the effect of water stress on wine quality were reported by various authors (Bravdo et al. 1985, Bravdo and Hepner ,1987, Jackson and Lombard, 1983, long, 1987, Naor et al. 1993, Matthews and Anderson, 1988). No solid evidences for the commonly belief associating water deficit with wine quality. It is quite obvious that excessive post veraison vegetative growth reduces fruit quality due to sink competition between the growing shoots tips and the rapid stage III growing fruits. It seems that the most

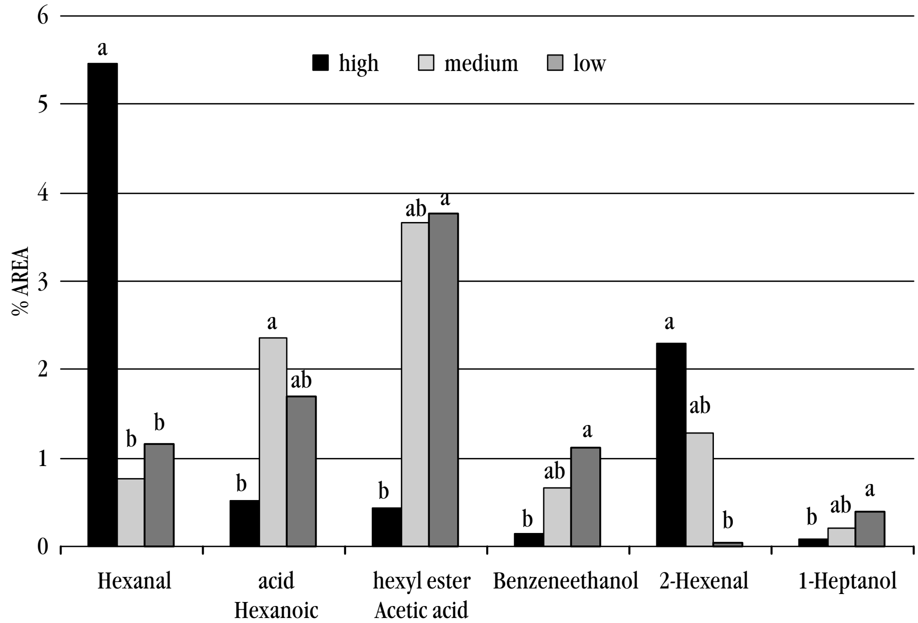

FIGURE 2. Effect of three fertigation levels on five aroma content of Cabernet sauvignon must. Rehovot, 1998. The fertigation levels were 50, 100 and 150 kg/ha N using a 7-3-7 NPK liquid fertilizer. The ratio 7-3-7 represents Pure N, P_2O_5, and K_2O. % area relates to the percentage of area of each peak relative to the total peak area.

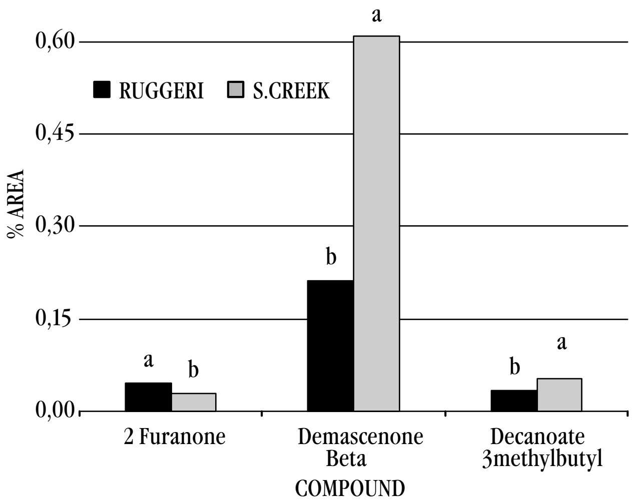

FIGURE 3. Affects of two rootstocks; 140 Ruggeri and Salt creek on a few aroma compounds in Cabernet sauvignon wine, Sde Boker, 1998. % area relates to the percentage of area of each peak relative to the total peak area.

appropriate growth phase for cutting back irrigation depends on the vigor of the vineyard and the environment, namely the soil and the meso climate. In extremely vigorous vineyards on fertile soils it is essential to cut back the irrigation right at the beginning of the season and maintain moderate irrigation in order to complete the desired leaf area before veraison and enable the ripening process to proceed under conditions of vegetative growth. On the other hand, severe post veraison water stress may reduce quality and the best results were obtained at a moderate stress sufficient to eliminate vegetative growth (Bravdo et al. 1985, Naor et al. 1993). GC-MS analyses of *Cabernet Sauvignon* must and wine showed significant increase in two aroma compounds –3- methyl butyl Octanoc acid and ethyl ester Decanoic acid by lowering the irrigation rate (Fig1.) while the other 45 compounds detected by the GC-MS were not affected. No significant effect of the irrigation level on the wine quality were detected by the sensory evaluation panel.

The high irrigation consisted on continuous maintenance of a water potential of 12kPa at 50 and 20 cm vertical and horizontal distance from the dripper by a computerized irrigation while the low irrigation consisted of 18 kPa.

EFFECT OF FERTIGATION

It is hard to separate between the specific effect of mineral fertilization and the indirect effect via the alteration of the crop load. Nitrogen fertilization normally stimulates vegetative growth without enhancing the nitrogen content of the must.

However in cases where Nitrogen fertilization have increased the N content of cv *Thompson seedless* must as well as the content of amino acids such as arginin and proline, a significant increase in the wine volatile content was apparent (Bell et al.1979).

Phosphorous fertilization of cv *Cabernet sauvignon* vineyard was found to triple the petiole P content, decrease berry size and increase the monoterpene content of the wine as well as the sensory evaluation score.

High NPK fertigation level has also enhanced the Hexanal and 2 Hexanal content of Cabernet Sauvignon must (Fig. 2) – these compounds are known to enhance the herbaceous character of the must and wine. The lower fertigation levels have enhanced the content of Hexanoic acid, Hexyl ester of Acetic acid, Benzeneethanol and 1- Heptanol of the

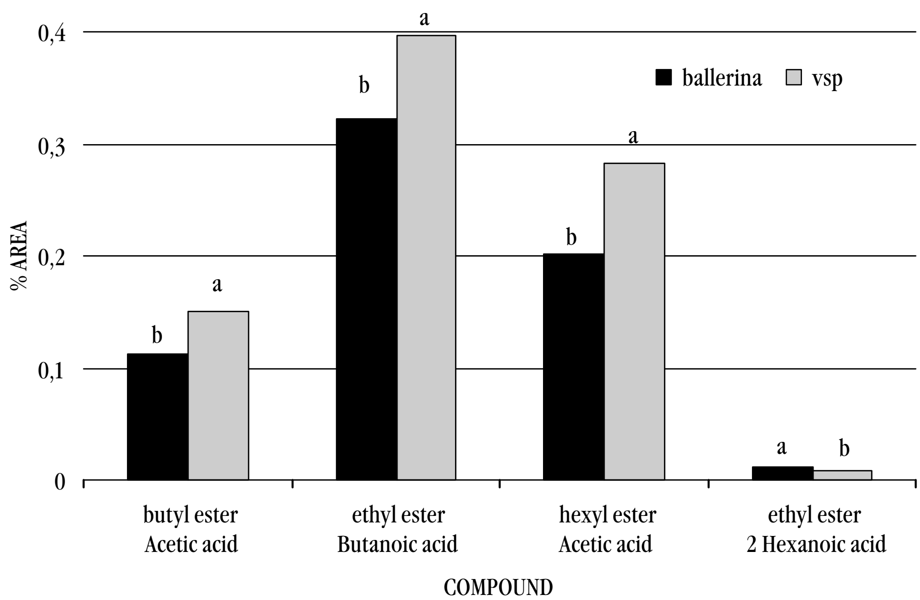

FIGURE 4. Effect of two training systems, VSP-Vertical Shoot Positioning and Ballerina on four aroma compounds of Cabernet sauvignon, El Rom, 1998. % area relates to the percentage of area of each peak relative to the total peak area.

must (Fig. 2). No significant effects of the fertigation levels on wine quality were detected by the sensory evaluation panel.

EFFECT OF ROOTSTOCKS

In an experiment conducted in the Ramat Negev area of Israel in a slightly saline soil irrigated by fresh water, wine of Cabernet sauvignon was found to have higher beta Demascanone and Decanoate 3 methyl butyl ester on Salt creek, whereas the 2 furanone content was higher in the wine made from grapes on the 140 Ruggerie rootstock (Fig. 3).

EFFECT OF TRAINING SYSTEM

Not much data was ever published on the effect of new training systems on wine quality. The balerina training system is a modified Scott Henry method proposed by Richard Smart (unpublished). This method, consist of bending and tying down part of the fruiting shoots in the green stage while the rest of the shoots are positioned vertically, thereby increasing the exposed surface area. The Scott Henry system relates to cane pruning whereas the ballerina method is practiced with both, spur and cane pruned vineyards.

Cabernet sauvignon wines from vertical positioned shoots (VSP) trained vines were higher in Butyl Ester Acetic acid, Ethyl ester Butanoic acid and Hexyl ester Acetic acid and lower in Ethyl ester 2 Hexanoic acid. These four compounds were significantly affected by the training system among some 60 compounds detected by the GC-MS analysis (Fig. 4).

EFFECT OF SALINITY

Irrigation with water salinity ranging between 2.7–6.0 dS/m is known to affect the quality of commercially grown tomatoes and melons grown in the Negev desert region of Israel (Mizrahi and Pasternak 1985). The high quality was expressed in sensory evaluation tests as well as by high glucose found by laboratory analyses. We have found by GC-MS analyses using solid phase microextraction (SPME) that salinity has considerably increased the free monoterpenes as well as other volatiles content. Interesting enough, many of the monoterpenes found in tomatoes are also present in winegrapes. The mechanism responsible for the effect of salinity on the aroma compound content is not yet clear.

Studies of Cabernet Sauvignon, Merlot, Sauvignon Blanc and Chardonnay wines made from experimental vineyards planted in the Negev region were found to be of very good quality. GC-MS analyses of Cabernet Sauvignon wines grown in the Negev region showed a significant effect on the concentration of 16 compounds out of 36 separated by the gas chromatograph (table 1). Sensory evaluation revealed significant differences between the saline and fresh water treatments by duo trio tests but no significant differences were found by quality score. It was agreed that the wines of the saline water irrigated plots had a distinct character but only part of the panelists had preferred them. The saline water irrigated plots in the experimental vineyard had produced so far six commercial crops. Leaf mineral analyses revealed gradual accumulation of chlorides and therefore the question of the life expectancy of the vines is still before us.

TABLE 1. List of sixteen volatiles (some of them unidentified) out of thirty-six compounds which were significantly affected by saline water irrigation treatments

Scan #	Order	Name	Ion trace
228	1*	1-butanol (?)	41
236	2*	Amyl acetate (iso or N)	70
265	3*	Oxirane (4 methyl 2,3 epoxy pentane)	41
308	4*	?	70
318	5	Hexanoic acid hexyl ester	144
342	6	Acetic acid hexyl ester	61
350	7	?	77
376	8	?	55 + 56
382	9	Propanoic acid 2 hydroxy ethyl ester	45 + 118
389	10	1 Hexanol	84
394	11	3 Methyl 1,4 Pentadiene	67
400	12*	?	58
427	13	Octanoic acid ethyl ester	172
434	14*	acetic acid	60 + 61
482	15*	?	56
486	16*	?	70
489	17	3 Methyl 2 butanol	73
495	18	1 Propoxy 2 propanol (?)	59
509	19*	?	87
513	20	Decanoic acid ethyl ester	156 + 199 + 200
527	21*	Butanoic acid diethyl ester	174
575	22*	?	87
580	23	Phenyl ethyl acetate	104
583	24	Beta Demascenone	121 + 190
590	25*	Nerol	68 + 69 + 93
600	26*	Benzyl alcohol	107 + 108
608	27*	Ethyl 3 ethylbutyl butanedioate	129
613	28	2 Phenyl ethanol	91
653	29	?	128 + 151
662	30	Octanoic acid	60
679	31	?	43
726	32	Pyranone (?)	144
738	33	?	93
762	34*	Butanedioic acid diethyl ester	101
841	35*	?	173
904	36	?	149

* = Significant

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