Elucidation of Odour-potent Compounds and Sensory Profiles of Vidal blanc and Riesling Icewines from the Niagara Peninsula: Effect of Harvest Date and Crop Level

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Abstract

It was hypothesized that the freeze/thaw cycles endured by icewine grapes would change their chemical composition, resulting in unique chemical fingerprint and sensory properties, and would be affected by harvest date (HD) and crop level (CL). The objectives were: 1) to identify odour-active compounds using gas chromatographic and sensory analysis; 2) to determine the effect of CL and HD on these compounds; 3) to determine the icewine sensory profiles; 4) to correlate analytical and sensory results for an overall icewine profile.

CharmAnalysis™ determined the Top 15 odour-potent compounds in Vidal and Riesling icewine and table wines; 24 and 23 compounds, respectively. The majority of the compounds had the highest concentrations in the icewines compared to table wines. These compounds were used as the foundation for assessing differences in icewine chemical profiles from different HD and CL.

Vidal and Riesling icewine were made from grapes picked at different HD; H1: 19 December; H2: 29 December; H3: 18 January; H4: 11 February (Vidal only). H1 wines differed from H3 and H4 wines in both Vidal and Riesling for aroma compounds and sensory profiles.

Three CL [control (fully cropped), cluster thin at fruit set to one basal cluster/shoot (TFS), and cluster thin at veraison to one basal cluster/shoot (TV)] were evaluated for Riesling and Vidal cultivars over two seasons. Vidal icewines had the highest concentration of aroma compounds in the control and TV icewines in 2003 and in TFS icewines in 2004. In Riesling, most aroma compounds had the highest concentration in the TV icewines and the lowest concentration in the TFS wine for both years. The

thinned treatments were associated with almost all of the sensory attributes in both cultivars, both years.

HD and CL affected the chemical variables, aroma compounds and sensory properties of Vidal and Riesling icewines and freeze/thaw events changed their sensory profile. The most odour-potent compounds were β-damascenone, *cis*-rose oxide, 1-octen-3-ol, 4-vinylguaiacol, ethyl octanoate, and ethyl hexanoate. The role of β-damascenone as a marker compound for icewine requires further investigation. This research provides a strong foundation for the understanding the odour-active volatiles and sensory profiles important to icewine.

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Chapter 1
Introduction and Literature Review

1.1 Introduction

Icewine is a sweet late harvest dessert wine produced from grapes that have frozen naturally on the vine. The frozen grapes are pressed, leaving water behind as ice crystals, which results in a must concentrated with sugar, acids, aroma, and flavour compounds. Canadian icewine has become an internationally recognized wine product however there is very little research on this wine style.

The goal of this study was to characterize the effect of harvest date and crop level on odour-active compounds in Riesling and Vidal blanc icewines from the Niagara Peninsula. It was hypothesized, first, that the freeze and thaw cycles through which icewine grapes endure would lead to changes in their chemical composition, giving them a unique chemical fingerprint and sensory properties and, second, that these changes in chemical composition would be affected by harvest date and crop level. The objective of this study was four-fold: 1) to identify odour-active compounds which could be used to characterize Niagara icewine using gas chromatographic and sensory analysis; 2) to determine the effect of crop level and harvest date on these odour-active compounds; 3) to determine the sensory profiles of the wines through sensory analysis; and 4) to correlate the analytical and sensory results for an overall profile of the icewines.

To accomplish these objectives wines were made from grapes frozen naturally on the vine, pressed and fermented at the Cool Climate Oenology and Viticulture Institute at Brock University. The finished wines were subjected to basic chemical analysis to determine variables such as pH, titratable acidity, residual sugar, and ethanol

concentration. Sensory descriptive analysis was performed using a trained panel to determine the sensory profile of the wines and how these profiles differed due to harvest date and crop level. Gas chromatography-olfactometry-mass spectrometry (GC-O-MS) was used to determine and quantify the most odour potent compounds in the experimental wines extracted using stir bar sorptive extraction (SBSE). Multivariate statistical analysis was used to relate the results from the sensory descriptive analysis and the analytical compounds found through GC-O-MS.

Currently, there is limited research on the chemical or sensory composition of icewines, and none on the effect of harvest date or crop level. Therefore all information gathered from this research will be valuable and relevant to the Canadian and international grape and wine industry.

1.2 Icewine

Icewine is a sweet late harvest dessert wine produced from pressing grapes that have naturally frozen on the vine, leaving water behind as ice crystals. Frozen grapes are harvested and pressed at -7 °C, EU regulations, (OIV 2003) to -8 °C, Canada, (Ontario 1999) typically between December and January. The resultant must is concentrated in sugar, acids, and aroma and flavour compounds, giving the wine its rich, balanced and intense flavour profile.

Icewine was discovered accidentally, in 1794 in Franconia, Germany when an early frost froze Riesling grapes still hanging on the vine in late November. To salvage the grapes they were pressed frozen and the concentrated juice was fermented into wine to create the first *eiswein* (Schreiner 2001). Historically *eiswein* (or icewine) was made only in very small quantities when weather permitted and was cherished with family and

friends. Now icewine is produced in many countries around the world at the northern limits of grapegrowing where winter conditions are cold enough to allow the grapes to freeze on the vine before harvest. Canada is the world's largest producer of icewine but other countries including Germany, Austria, United States, Slovenia, Luxembourg, Croatia, the Czech Republic and Hungary produce icewine as well (Schreiner 2001).

Canada came onto the icewine scene in the late 1980's. With its reliably cold winters, icewine could be produced every year and production increased with demand. Now, Canada produces millions of cases of this specialty wine for local and international consumption every year. Icewine is made in all wine production regions of Canada spanning from Nova Scotia on the east coast to British Columbia on the west coast. The bulk of icewine production is in Ontario, principally the Niagara Peninsula, where warm summers and cold winters allow for optimal conditions to grow and harvest grapes for icewine. In 2007, 1.17 million litres of icewine were produced in Ontario (Ontario 2008).

It is believed, anecdotally, that the freeze and thaw cycles through which grapes destined for icewine are subjected give the wine its unique flavour profile and cannot be duplicated by freezing alone. The production of icewine is a very expensive, labour intensive and risky undertaking. Grapes are left hanging on the vine long past commercial harvest, which, results in the loss of yield from dehydration, rot, wind, as well as animal predation. The grapes destined for icewine must be netted to protect them from predators and to prevent loss from clusters falling on the ground. Since the water is left behind as ice crystals, icewine grapes yield only 15 to 20% that of table wine requiring substantially more acreage for the same yield (Pickering 2006). Icewine must is typically sold by the litre with one litre of Vidal icewine juice selling for $15.76 CDN and one litre of *Vitis*

vinifera icewine juice selling for $24.16 CDN at 35 °Brix (Ontario 2010). There is also always the risk that the grapes will rot before the temperatures are cold enough for an icewine harvest or that a mild winter will prevent icewine all together.

1.2.1 Icewine Regulations

Due to the specific climatic conditions which are required for icewine production there are strict regulations to adhere to in order to label a wine icewine. The International Organization of Vine and Wine (OIV) signed an agreement with Germany, Austria and Canada on 23 June 2000 which established international regulations for icewine production. A further amendment in 2003 by the OIV gave a definition of icewine and regulations that must be followed for its production (OIV 2003). This agreement states that icewine (*eiswein* or *vin de glace*) can only be made from grapes frozen in the vineyard at temperatures lower or equal to -7 °C. The frozen grapes must be harvested and pressed at these same temperatures and contain a minimum of 25.3 °Brix (110° *Oechsle*). The minimum alcoholic strength of the finished wines must be 5.5% by volume and the maximum limit of volatile acidity is 2.1 g/L expressed as acetic acid. It also stipulates that all grapes for icewine come from the same region. With the exception of the Mosel in Germany which follows these regulations, Germany and Austria require the minimum °Brix of the must for icewines be 29.6 (125° Oechsle in Germany or 27.0 Klosterneuburger in Austria) which is the same requirement as *Beerenauslese* (a sweet late harvest, botrytis-affected dessert wine) (OIV 2003).

Canada has its own, even more stringent, regulations set forth by the Vintners Quality Alliance (VQA) Act of Ontario(Government of Ontario 1999) in 2005 and similar rules in British Columbia (Agri-Food Choice and Quality Act 2005). In the VQA

Act, the term icewine is trademark protected and can only be used for wines made from grapes naturally frozen in the vineyard in specified viticultural areas, at temperatures $\leq$ -8 °C after the 15 November of the vintage year. The grapes must be harvested and pressed at these same temperatures. The minimum allowed soluble solids concentration of the juice must be 35 °Brix with no single pressing being less than 32 °Brix. The finished wine is required to have a residual sugar concentration of not less than 125 g/L (Ontario) or 100 g/L (British Columbia) and a titratable acidity of not less than 6.5g/L (expressed as tartaric acid). Other styles of dessert wines can be made from late harvest grapes with must concentrations less than 35 °Brix; late harvest, select late harvest, and special select late harvest have minimum soluble solids concentrations of 22, 26, 30 °Brix respectively (Ontario 1999).

1.2.2 Icewine: Viticultural Considerations

Riesling and Vidal blanc are the most common choices for icewine production in North America but other cultivars include Cabernet Franc, Gewürztraminer, Ehrenfelser, and Kerner. The choice of cultivar for icewine production is important as it will affect the sensory and chemical composition of the finished wine. Characteristics of a good icewine cultivar are that it has thick skin, be late maturing, posses a high natural acidity and be winter hardy (Nurgel et al. 2004).

First, it is important to have a thick skinned cultivar that can withstand rot, wind, rain and freeze-thaw events. A grape berry destined for icewine goes through a series of freeze and thaw cycles that impose physical and mechanical stress on its structure. Physical stress results from the perforation of the berry's cellular components, such as cell walls and vacuoles, by the jagged edges of ice crystals formed inside the berry when

subjected to freezing temperatures. It is believed that as the ambient temperature warms up, the ice crystals melt causing cellular components previously segregated to mix. The result is the integrity of the berry is compromised which can lead to enzymatic activity and changes in its chemical and sensory profile. Mechanical stress is exerted on the skin of the berry throughout and after the growing season in late harvested grapes, the result of water movement and expansion from heavy rains and ice formation. If the berry skin cannot support the expansion due to the influx of water or freezing, it splits, releasing its contents, and it becomes susceptible to oxidation and infection by a host of pathogens. Therefore, thick skinned cultivars are essential to maintain berry integrity enabling the juice to be released at pressing and not before.

Second, late maturing cultivars are advantageous as they have a shorter hang time from regular harvest to icewine harvest in December to January. The longer the hang time of grapes destined for icewine after they mature, the greater the potential for loss of yield due to dropped fruit, rot, desiccation, and predation. A cultivar with a long growing season, such as Riesling, which is typically harvested mid to late October, would in general hold up better and yield more juice than an early maturing cultivar such as Chardonnay, which is harvested mid- September. The strength of the actual cluster will also affect the ability of the cultivar to hang until icewine harvest. Having a strong peduncle and rachis will better enable the cluster to hang on the vine preventing loss of yield from dropped fruit during strong winds, and rain or snowstorms.

Third, the cultivar should posses a high natural acidity to balance the residual sweetness in the wine. The sensory profile of icewine often describes the acid-sugar balance as being an important determinant of the wine's quality. Without the acid

backbone, icewine can be perceived as too sweet, unbalanced or flabby and not age-worthy. As grapes mature, the sugar concentration increases and the acid concentration decreases; however, certain cultivars such as Riesling, Chenin blanc and Sauvignon blanc retain a high natural acidity at full maturity. In Canada, acidification is permitted in icewine up to 4.0 g/L, enabling icewine to be made from low acid cultivars like Gewürztraminer.

Fourth, the winter hardiness of the cultivar is crucial to ensure continued success. Due to the cold temperatures grape vines are subjected in order to make icewine, it is important that winter hardy cultivars are selected and planted; therefore, most icewine cultivars are of western European origin (*Proles Occidentalis*).

Riesling and Vidal blanc possess these four criteria and therefore are excellent cultivars for icewine production. Vidal blanc is a white French-American hybrid consisting of 75% *V. vinifera* genetic background, from a cross between Ugni blanc and Rayon d'Or (Seibel 4986) (Galet 1998). A key viticultural feature is its winter hardiness. The cultivar has large cylindrical clusters, with medium-sized, thick-skinned berries that are disease resistant (Galet 1998). It is a high acid cultivar prone to overcropping, which enables it to produce large yields for icewine production. Cluster thinning is essential for table wine production. Vidal blanc is a late maturing cultivar harvested usually in mid- to late October in eastern North America (Chisholm et al. 1994). The propensity of Vidal blanc to produce well balanced, concentrated icewines has pushed it to international acclaim and recognition.

Riesling is considered by many to be the best choice of cultivar for producing icewines of the highest quality and ageability due to its high natural acidity (Nurgel et al.

2004). It is the noble grape of Germany, known for producing a wide range of wine styles from bone dry to ultra sweet, both clean and Botrytis-affected. Riesling has all the characteristics of the ideal icewine grape; it is late maturing, high acid, thick skinned providing some disease resistant and winter hardiness (for *V. vinifera*).

1.2.3 Icewine: Oenological Considerations

The major concern during icewine fermentation is the high sugar concentration of the icewine must that can put commercial yeast strains under extreme hyperosmotic stress leading to metabolic changes that affect cell growth, fermentation ability and sensory profile. Hyperosmotic stress causes cell shrinkage, reduced peak cell concentration and biomass and elevated levels of glycerol and volatile acidity in icewines and may also lead to stuck or sluggish fermentations (Pitkin et al. 2002, Kontkanen et al. 2004, Nurgel et al. 2004). It has been shown that when yeast cells are under hyperosmotic stress they activate the high osmolarity glycerol response (HOG) causing the cell to produce glycerol to balance the high external osmotic pressure. The overproduction of glycerol during an icewine fermentation shifts sugar metabolism away from ethanol production and towards acetic acid production leading to elevated concentrations of volatile acidity and lower than desired ethanol concentration in the finished wine (Pigeau et al. 2007).

The initial soluble solids concentration of the icewine must will affect the fermentation kinetics; a must at 35 °Brix will place the yeast under less osmotic stress than a must at 42 °Brix, thereby resulting in less accumulation of acetic acid and glycerol in the former than the latter. Pigeau *et al.* (2007) found that increasing the soluble solids concentration of icewine must from 40 to 46 °Brix decreased yeast growth, sugar consumption rate, the total amount of sugar consumed, and the total concentration of

ethanol produced. Understanding the mechanism by which acetic acid is produced in icewine is important due to the relatively low sensory threshold of this compound and the legal restrictions for the maximum allowed concentrations of volatile acidity (mainly acetic acid) in icewines (2.1 g/L).

The major determinant of yeast strain selection for icewine is the ability of the yeast to ferment in a high sugar environment. For this reason commercial yeast strains are most commonly used for icewine fermentation. Factors to consider when selecting a yeast strain are alcohol tolerance of the yeast, osmotic stress tolerance of the yeast, optimal fermentation temperature, hydrogen sulphide and volatile acidity production, assimilable nitrogen and oxygen requirements. The impact of seven yeast strains on fermentation rate, acetic acid and glycerol production, and sensory characteristics during icewine fermentation in real and synthetic icewine must was studied (Erasmus et al. 2004). From this study, it was recommended that ST, N96 and EC1118 are the yeast strains most suitable for icewine fermentations.

In Canada, the most common yeast strains for icewine fermentation are K1-V1116 followed by EC-1118. It is for this reason that K1-V1116 was selected as the yeast strain for all experimental icewine fermentations for this thesis. Lalvin ICV K1V-V1116 is a *S. cerevisiae* yeast strain isolated in 1972 at INRA in Montpellier, France. This was the first competitive factor yeast to go into commercial production and has become one of the most popular dehydrated yeast strains. Relevant attributes for icewine production include its ability to start fermentation rapidly, alcohol tolerance to 18%, large fermentation temperature range (10 to 35°C) and ability to ferment at low temperatures, low production of hydrogen sulphide and volatile acidity, and low to average requirement

of yeast assimilable nitrogen. The yeast produces high concentration of floral esters such as isoamyl acetate, hexyl acetate and phenyl ethyl acetate that contribute to the aromas of the finished wines. It is known for its ability to ferment under difficult conditions and is recommended for the production of icewine. (Lallemand 2008).

Once the yeast strain has been selected it is also important to consider the amount of yeast to add to the fermentation. Since it is well documented that yeast in an icewine fermentation have smaller cell size, less biomass and a lower peak concentration than in a table wine fermentation, it seems intuitive that they would require a higher initial concentration of inoculum. Kontkanen *et al.* (2004) investigated the effect of inoculum rate on icewine fermentations at two concentrations, 20 and 50 g dry weight/hL of K1-V1116 yeast and the two methods of inoculation, direct and step-wise acclimation of the yeast cells to the high sugar concentration, over a 30-day period. Regardless of inoculation method, the 20 g dry weight/hL fermentations did not achieve the desired 10% alcohol, with only 7.8% and 8.1% alcohol (v/v) produced for the direct and step-wise acclimation methods, respectively. In contrast, at 50 g dry weight/hL, both inoculation methods finished fermentation with 12.1% and 10.5% alcohol (v/v) for the stepwise and direct inoculum, respectively. It is therefore recommended to inoculate icewine with a dose of 50 g dry weight/hL of must using a step-wise acclimation method to condition the yeast cells to the high soluble solids concentration. The step wise acclimatization resulted in higher cell biomass and viability of the yeast cells allowing it to ferment the icewine must to the desired alcohol concentration. For this study the step-wise acclimation method of Kontkanen et al. (2004) was used to re-hydrate and inoculate the yeast.

1.3 Wine Volatile Analysis

The volatile constituents of grape and wine were first investigated by those working with Concord grapes (Power and Chesnut 1921, Sale and Wilson 1926). They determined methyl anthranilate concentrations in juice and identified it as contributing to Concord's "foxy" aroma. In 1957, Webb and Kepner were the first to study Muscat of Alexandria; they found many esters and alcohols (Webb and Kepner 1957). Over the next 50 years many advances and research initiatives have greatly expanded this field. This section will discuss the background and pertinent research pertaining to the methods chosen for the volatile analysis of wine which can be applied to icewine.

1.3.1 Gas Chromatography

Gas chromatography (GC) has been used to identify, quantify, separate and analyze the important volatile components as these flavour and aroma compounds are sensitive to compositional changes in the matrix, and can be used as an indicator to variation in the product (Marsili 2002). GC is a physical method of separation in which the components to be separated are distributed between two phases, the stationary phase and the mobile phase (McNair and Miller 1998). The individual components are separated based on the amount of time they spend in the column, retention times, which depend on their interactions with the stationary and mobile phases. Once eluted from the column, the compounds pass through a detector and a peak is generated on the chromatogram (McNair and Miller 1998).

The technique of chromatography was developed at the start of the 20th century, when Tswett credited as the "father of chromatography" separated plant pigments by liquid chromatography (McNair and Miller 1998). The first work with GC was published

in 1952 by Martin and James. They reported the separation of fatty acids by partition chromatography using nitrogen as the mobile phase and stationary phase of silicone oil/stearic acid with a diatomaceous earth support (Bartle and Myers 2002). GC was quickly adopted for separation of petroleum products and within 10 years had expanded to many other research areas including food and flavour analysis (Bartle and Myers 2002).

In 1958, Bayer was the first to use GC to study aroma compounds in wines (Rapp and Mandery 1986). Since then > 800 volatile components have been identified in grapes and wine using GC (Nykanen, 1986, Schreier 1979). There are many different GC detectors used for the identification and quantification of grape and wine volatiles, such as flame ionization detectors (FID), mass spectrometry (MS), olfactometry (O), electron capture, or nitrogen phosphorous (Hayasaka et al. 2005). However, the two most important detectors coupled to the GC for odour active volatiles are gas chromatography – mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O).

Gas Chromatography- Mass Spectrometry

As previously mentioned, GC is a widely used technique to separate volatile compounds because of its speed of analysis, high resolution of complex mixtures, and its ability to quantify results, but it is unable to confirm the identity of a peak (McNair and Miller 1998). The purpose of MS is to identify compounds based on their composition and molecular weight, making it an ideal detector for GC. The sample is injected into the GC and separated into individual compounds, by their boiling points and polarity producing a retention time as it leaves the end of column and enters the MS. In the MS, compounds are bombarded with high energy electrons producing fragmented charged

ions; molecular ions and their fragmentation ions. These ions are introduced to the mass analyzer and separated based on their mass to charge ratio (*m/z*). The resulting mass spectrum, fragmentation pattern, is a plot of the ion abundance determined by the mass to charge ratio. The detection limits for MS are extremely low, in the range of 10^{-12}g (picograms) making it an invaluable method for the identification and quantification of trace volatiles in grapes and wines (Hayasaka et al. 2005).

The first report of GC-MS applied to grape and wine volatile analysis was by Stevens et al. (1966) to extract and identify 78 components from Muscat of Alexandria, of which 60 had been previously reported. Since then GC-MS has been used extensively in grape and wine analysis to expand research knowledge in areas such as; grape (Salinas et al. 2004), yeast (Erasmus et al. 2004), and fermentation derived volatiles (Kontkanen et al. 2004, Loscos et al. 2007), wine taints (Insa et al. 2005, Kotseridis et al. 2008), off-odours (Rapp 1998), impact odorants (Tominaga et al. 2000, Aznar et al. 2001, Lopez et al. 2003), and ageing effects of wine volatiles (Cullere et al. 2004, Hernandez-Orte et al. 2009).

Gas Chromatography - Olfactometry

Of the hundreds of volatile compounds found in wine many are not odor-active or are found in wine below their sensory threshold and therefore do not contribute to wine aroma (Guth 1997a, Ferreira et al. 2000). GC-O was developed to elucidate which of these numerous volatiles compounds do in fact contribute to wine aroma.

The first publication of smelling the effluent from a GC was in 1964 by Fuller of Colgate-Palmolive CO. after separation of volatiles on a packed column (Fuller et al. 1964). The technique was used in a qualitative and informal manner by flavorists and

perfumers to identify what contributed to the odour of a product. The major drawback of the technique was the instrumentation, as sniffers had the uncomfortable task of smelling the hot effluent over vents of non-destructive detectors (Acree 1997). Dravnieks and O'Donnell (1971) designed a GC-O that most resembles the current models, in it they had a sniff port through which humidified air passed along with the effluent as it exited the GC for more comfort and control during sensory evaluation.

Since the introduction of GC-O, different methods of analysis have been introduced to assess the aroma from the olfactory port: dilution analysis, detection frequency, posterior intensity and time-intensity. For this study dilution analysis, specifically CHARM, was used to elucidate the odour potent aroma compounds in Vidal and Riesling icewines and therefore will be discussed in the most detail.

Dilution analysis is based on the determination of threshold concentrations or odour potency of an aroma. The two methods of dilution analysis are CHARM (Acree et al. 1984) and AEDA, Aroma Extract Dilution Analysis (Ullrich and Grosch 1987). CharmAnalysis™ is a dilution technique where a sample is serially diluted until the odour is no longer detected. A computer program records when an aroma is detected, its perception and its duration. From this its aroma potency can be determined as well as which aromas contribute most to the overall smell of the product. The resultant Charm value is a dimensionless measure of odour intensity based on the dilution at which no odour is detected and the number of coincident responses at the lower dilutions (Acree et al. 1984). AEDA is similar to CharmAnalysis™ in that it uses dilutions to determine the odour activity of a compound and is based on the odor-detection threshold. The major difference being that AEDA does not take into account the duration of the odour event.

Therefore, the odour potency is recorded based on it flavor dilution (FD) that corresponds to the maximum dilution at which the odorant is perceived by at least one judge (Ferreira and Cacho 2009).

Detection frequency looks at the number of times a panel of judges detects an aroma over several replications of the effluents eluting from the olfactory port at the same concentration. The percentage of judges able to detect an odorant is called the Nasal Impact Frequency (NIF) and if the duration of the sensation is also recorded then it is called the Surface of Nasal Impact Frequency (SNIF) (Pollien et al. 1997). For every perceived odour a NIF value is assigned, one if an odor was detected and zero if it was not. The total NIF value for a compound is determined by the addition of all the NIF scores for each judge divided by the number of sniffing runs performed (Ferrari et al. 2004). Therefore the higher the NIF score, the more odor potent the compound.

Intensity measurement techniques include OSME and posterior intensity. OSME (smell in Greek) developed by McDaniel et al. (1989) which uses time-intensity to measure the perceived odor intensity of a compound from the GC effluent. The judge indicates the intensity of an eluting odor by moving a variable resistance knob as well as the start and end time of the aroma. The result is similar to a chromatogram where peak height is relative to intensity and the area under the peak to time and intensity (Miranda-Lopez et al. 1992). The posterior intensity method is a quantitative measure of the odorant intensity achieved by having the judge provide, on a simple line scale, a measurement of the intensity an eluting aroma (Ferreira et al. 2003b).

In the wine industry, GCO has been used extensively to determine key odorants and aroma profiles of many different cultivars, including Pinot noir (Moio et al. 1995,

Girard et al. 1997), Vidal blanc (Chisholm et al. 1994), Botrytis affected wines (Sarrazin et al. 2007), and icewine (Cliff et al. 2002) to name a few. Ferreira and Cacho (2009) have compiled an extensive summary table of papers published using GC-O to profile wines over the past 10 years.

1.3.2 Extraction of Grape and Wine Volatiles

In the past 15 years many new techniques have been introduced in the area of volatile analysis to increase recovery, minimize loss, reduce the time and expense of the extraction procedure, as well as ensure a representative sample is being analyzed. Solvent extraction or liquid-liquid extraction (LLX) is the most common conventional technique applied to the analysis of volatiles. A solvent is selected that has similar properties, such as polarity, to those of the analytes of interest and the volatiles will partition into the solvent based on their solubility with that solvent. It is a relatively easy technique that does not require sophisticated equipment and provides consistent and reproducible results. However, a major disadvantage of this technique is the cost related to the use and disposal of solvents, which can be harmful to the environment, toxic and carcinogenic and very expensive. Also many low boiling point volatiles can be lost during the concentration process by evaporation, which results in an inaccurate representation of the sample.

Arthur and Pawliszyn (1990), of the University of Waterloo, developed solid phase microextraction (SPME) to provide a rapid solvent free extraction technique for the analysis of volatiles. The major advantage of SPME is that is combines sampling, extraction, concentration, and sample introduction to the GC all in one step without the use of solvents. SPME looks like a modified syringe that consists of a fiber holder and

fiber assembly and a retractable SPME fiber (Vas and Vekey 2004). The fiber is a thin fused-silica optical fiber, which is coated with a thin polymer film. The polymer coating acts like a sponge and concentrates the analytes by absorption and adsorption processes when exposed to the sample either by direct immersion of the fiber or headspace sampling of the volatiles. The major disadvantage of SPME is the small surface area for diffusion of the analytes, which is only about 0.6 µl for a 100-µm PDMS fiber (Hayasaka et al. 2003). This affects the sensitivity of the method because the recovery of volatiles increases with the volume ratio of the PDMS phase to sample matrix ratio (Hayasaka et al. 2003, Vas and Vekey 2004).

A new technique marketed by Gerstel was developed in 1999 called stir bar sorptive extraction (SBSE), commercially known as Twister (Baltussen et al. 1999). This was the extraction method chosen for the analysis of all research icewines in the present study. SBSE is based on the same properties as SPME but makes up for its limited sampling capacity by using a stir bar, typically 10 mm in length, incorporated into a glass tube and coated with polydimethylsiloxane (PDMS). Coating the stir bar enables the volume of the PDMS phase to increase significantly to about 55 µl (range of 25 – 125 µl) compared to 0.6 µl previously mentioned for SPME. The stir bar is placed in the sample matrix and stirred with the liquid of interest or placed in the headspace above the sample. Stirring causes the analytes to partition between the matrix and the PDMS phase on the stir bar based on partition coefficients, this large phase volume increases the sensitivity of SBSE and allows for low detection and quantification limits (Hayasaka et al. 2003). This also improves the signal to noise and allows for superior detection on the scan mode of the MS. After stirring, the stir bar is transferred from the sample to a thermal desorption

unit mounted on a programmable temperature vaporization (PVT) injector on the GC, the analytes are cryo-focused and then thermally desorbed and run through the GC column (Hayasaka et al. 2003).

The main advantages of SBSE over other extraction techniques are due to its higher phase ratio for better recovery and sample capacity. SBSE is an order of magnitude more sensitive than conventional methods and it has shown its application to a wide range of analysis which include volatile aromatics, and odor compounds (Sánchez-Rojas et al. 2009). The major disadvantage of SBSE is the non polar nature of the PDMS coating making it less suitable for the extraction of very polar compounds without prior derivatizations (Sánchez-Rojas et al. 2009).

However, this technique is widely used in the grape and wine industry due to its low detection limits and it eliminates time consuming and expensive solvent extraction techniques for many different applications. SBSE has successfully been applied to the following wine research; the trace analysis of 2,4,6-tricholoroanisole (cork taint) (Hoffmann et al. 2000), the determination of dicarboximide fungicides (Sandra et al. 2001), volatile phenol analysis (Diez et al. 2004), the characterization of aroma profile of Madeira (Alves et al. 2005), determination of potent odorants in Chardonnay wines (Buettner 2004), to determine volatile compound evolution during ripening of *Vitis vinifera* (Salinas et al. 2004), and for flavour and compositional analysis (Hayasaka et al. 2003). It has also been used in the beverage industry for the analysis of volatiles of malt whisky (Demyttenaere et al. 2003) and the determination of the hoppy aroma in beer (David et al. 2001).

1.4 Wine Aroma Compounds

An aroma compound is a volatile compound that interacts with the olfactory epithelium to produce an odorous sensory response. The aroma and flavour of a wine is an important quality parameter and therefore a tremendous amount of research has focused in this area to understanding what aroma compounds are responsible or contribute to a wines aroma, how they are formed, and how they change over time. Wine aroma compounds come from a diverse group of chemical classes including; terpenes, norisoprenoids, volatile thiols, acids and phenol, esters, lactones, alcohols, aldehydes, ketones and methoxypyrazines.

1.4.1 Odour Activity Values (OAVs)

Odour activity values (OAVs) are used to determine the importance of a compounds aromatic contribution to the product. The OAV is calculated by dividing the concentration of the aroma compound by its sensory threshold concentration in a similar matrix to provide a unit less measure of the contribution of that odorant to the product (Grosch 1993). An aroma compound found above its threshold (OAV>1) is considered as having an odour impact. The greater OAV above threshold, the more the aroma compound is thought to contribute to overall aroma. OAVs are commonly utilized to interpret GC-O data because they are a useful way to determine the relative importance of an aroma compounds contribution to the product.

Many disadvantages of OAV have been discussed in the literature. The three main concerns are: 1) inaccuracy of the reported sensory threshold; 2) does not take into account interactions with other compounds; and 3) does not follow Stevens' Power Law.

First, the reported sensory threshold of an aroma compound can vary due to a variety of factors, including; number of individuals tested, rigors of testing and the matrix in which it was calculated, which could be air, water, a model wine solution or a neutral base wine. This can lead to an inflated OAV, which will incorrectly relate an aroma compounds importance to the wine (Francis and Newton 2005). It is therefore important to ensure that the sensory threshold used to calculate the OAV was determined in matrix closely matching the one under investigation, or to calculate it oneself for each compound.

Second, due to the nature of calculation of OAVs they do not take into consideration any interactions that may be occurring in the matrix and contributing to wine aroma. This is especially the case for OAVs below threshold (OAV<1) and those just above threshold. Research has shown that these aroma compounds with low OAVs do play a role in the overall aroma, by acting as aroma enhancers or aroma suppressors (Escudero et al. 2004) .

Third, Stevens Power Law was derived to explain the relationship between perceived intensity and concentration of a sensory stimulus (Stevens 1971). It is a logarithmic relationship effective in relating aroma intensity to its concentration for both sensory analysis and GC-O data of pure compounds over a range of concentrations (Kamadia et al. 2006). Whereas, OAVs are based on a linear relationship related to information gained from serial dilutions and therefore do not follow Stevens Law.

Regardless of the disadvantages, OAVs remain a popular method of expressing the potency of an aroma compound. The advantages are OAVs are their ease of calculation, especially with all published sensory threshold values found in the literature

and they are a useful way of providing insight into the potential contribution an aroma compound makes to the overall product. The limitations of OAVs can be overcome by pairing OAVs with results from other techniques like GC-O, reconstitution and omission studies . It is important to remember that OAVs and sensory thresholds need to be considered as rough estimations of an aroma compound's potency to a product such as wine. They are simply a guide, or tool, to provide insight into the complex nature of wine aroma.

1.4.2 Sources of Aroma Compounds

Wine aroma is a complex mixture of chemical compounds derived from the various stages of wine production. Aroma compounds end up in the wine through three main routes; they originate in the grapes, they are the result of fermentation, and from chemical reactions occurring in the finished wine during storage and ageing (Rapp and Mandery 1986).

Grape derived aroma compounds. These compounds are found in the berries at harvest as odor active volatiles (free form) or as non-volatile, odour-less precursors which are released into their odour active form during processing (Rapp and Mandery 1986). Monoterpenes and methoxypyrazines are examples of aroma compounds present in their free form in the grapes at harvest, while cysteine conjugated volatile thiols, and sugar glycosylated terpenes and norisprenoids must first be converted through chemical reactions into odour active compounds before they can contribute to wine aroma (Fischer 2007). Many of these grape derived aroma compounds are important contributors to varietal aroma and are considered impact odorant, a concept to be discussed later in the section.

Fermentation derived aroma compounds. Wine yeast produce a variety of compounds as a result of their metabolism during fermentation that contribute to the aroma of wine; such as esters, higher alcohols, ketones, aldehydes, fatty acids and acetates (Schreier 1979). The production of ethanol is a very important by-product of fermentation that affects the overall aroma of a wine. The concentration at which the sensory threshold of an aroma compound is determined will vary depending on the matrix of evaluation, whether it was tested in water versus an ethanol/water solution, for example (Fischer 2007). The presence of ethanol in the wine matrix increases the solubility of most aroma compounds; this in turn reduces their volatility, which results in an increase in the sensory threshold concentration (Ferreira et al. 2008). The sensory thresholds of 1-hexanol and ethyl benzoate were found to be 2500 and 60 μg/L in water, respectively (Buttery et al. 1988). In a model wine solution, the sensory threshold of 1-hexanol and ethyl benzoate were found to be much higher at 8000 and 575 μg/L, respectively (Ferreira et al. 2000). This large increase in the sensory thresholds demonstrates that the introduction of ethanol to the matrix changes the concentration at which an aroma compound can be perceived and is therefore an important consideration to wine aroma compounds. Ethanol has also been shown to mask or suppress esters (Escudero et al. 2007) and enhance other aroma compounds such as decanal (Ferreira et al. 2008).

Few esters are present in grapes, most are formed during fermentation and are found in the finished wine. Esters are the reaction product between alcohols and acids and are important fermentation derived aroma compounds. Esters in wines have two main origins; enzymatic esterification during the fermentation process and chemical

esterification during long-term aging. Acetic acid esters of higher alcohols, such as isoamyl acetate, have intense fruit odours that contribute to the overall aroma. Isoamyl acetate, with its strong banana aroma, is an ester found to contribute the most to the aroma profile of wines (Van Der Merwe and Van Wyk 1981) and is the one of the only esters along with ethyl phenylacetate, with its floral honey aroma, that have been identified as impact odorants (Ferreira et al. 2000, Tat et al. 2007).

Storage derived aroma compounds. These compounds develop in the wine as they age due to chemical reactions occurring from storage conditions of oak and bottle age. Examples of storage derived aroma compounds are (E) -whiskylactone, sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone), and TDN (1,1,6-trimethyl-1,2-dihydronaphthalene). Whiskylactone imparts a wood, toasty aroma to oak aged wines (Boidron et al. 1988). Sotolon, has a caramelized aroma and has been found to be an important aroma compound in botrytis affected wine (Masuda et al. 1984), Madeira (Camara et al. 2006), ports (Ferreira et al. 2003a) and Pedro Ximenez sherry (Martin et al. 1992). TDN results from the breakdown of carotenoids during bottle ageing in Riesling wines (Versini et al. 1996). TDN has an aroma of petroleum, kerosene and diesel and is associated with aged wines from warm climate viticultural areas such as South Africa and Australia (Marais et al. 1992).

1.4.3 Wine Impact Odourants

Cordonnier (1956) first identified that terpenes contribute to the characteristic aroma of the Muscat cultivars. Since then, many studies and review articles have focused on trying to determine what aroma compound contributes most to the characteristic aroma of a wine. This single compound, which can impart on a wine, its distinct aroma is

known as an impact odorant. Many impact odorants have since been identified; linalool in Muscat cultivars (Bayonove and Cordonnier, Bayonove and Cordonnier 1970), *cis*-rose oxide in Gewurztraminer (Ong and Acree 1999), methoxypyrazines (Allen et al. 1991) and volatile thiols (4-mercapto-4-methylpentan-2-one, 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate) (Darriet et al. 1995, Tominaga et al. 1996) in Sauvignon blanc and Cabernet Sauvignon, isoamyl acetate in wine made from Tempranillo (Ferreira et al. 2000) and Pinotage (Van Wyk et al. 1979), ethyl phenylacetate in Aglianico wines (Tat et al. 2007), rotundone in Shiraz wine (Siebert et al. 2008), and sotolon in many dessert wines (Masuda et al. 1984) to name of few examples. However, not all wines have impact odorants but rather a combination of aroma compounds that work together to create the characteristic aroma of that cultivar. The γ-lactones are a group of related chemical compounds that works as a 'family' to impart tropical fruit and coconut aroma to a wines aroma (Ferreira and Cacho 2009).

Aroma compounds can therefore be categorized by studying their overall contribution to its aroma. Through sensory reconstitution studies on wine aroma using model wine solutions, the exact role of an aroma compounds can be determined (Guth 1997b, Ferreira et al. 2002). That is to say, does it add to the aroma, take away from the aroma or have no effect on the aroma of the wine if it is omitted. From these studies aroma compounds can be classified based on their aromatic contribution as; impact odorant, contributor, secondary or subtle contributor, aroma enhancer or aroma depressor (Ferreira and Cacho 2009). It is important to note that an aroma compound could fit into several of these categories depending on its concentration in the wine in relation to its sensory threshold.

An impact odorant is a compound that contributes its distinct aroma to the wine giving it a characteristic aroma that can be identified. If an impact odorant is removed from the wine, it will no longer be identified as the same product.

Contributors can be individual or groups of aroma compounds with similar chemical characteristics that add to the overall aroma of the product. Depending on their concentration in the wine, contributors can vary in how they are perceived and what their aroma(s) contribute to the bouquet. At higher concentrations contributors can impart a distinct aroma nuance that can be identified, such as 'red fruit' or 'tropical'. Whereas, at lower concentrations they may simply add to the bouquet a more simplistic note of 'fruity'.

Subtle contributors are individual or groups of aroma compounds which are present above their sensory threshold but do not impart any specific aroma characteristics to the product. Their role is to add to the wines aromatic base, however if they were removed from the product they would have little effect on the sensory profile.

Aroma enhancers and depressors are compounds that do not impart a distinct aroma to the product but work by altering the perception of the aromatic nuance that are present. An aroma enhancer increases the perceived aroma; if the compound is removed from the wine, the odour perception on which it places emphasis will be reduced. The reverse is true for an aroma depressor; it acts to decrease the perception of an aroma in the wine, which will increase if it is removed.

It has also been shown that if a wine does not contain any impact odorants, as in the case of wines made from Maccabeo, then omission studies provide little insight into

the sensory relevance of its aromatic composition but does provide information into what makes up a basic wine (Escudero et al. 2004).

1.4.4 Sensory Analysis

Analytical chemical methods look at wine from an individualistic perspective, whereas sensory analysis looks at wine from a holistic approach. Sensory analysis takes all the components in the wine as a whole to evaluate the overall product. Sensory analysis is therefore a vital part of understanding the aroma profile of the wine because all the individual odour active compounds are evaluated together. Analytical analysis of wines, through methods such as GC, can provide information about the exact concentration of the individual compounds. However, it gives no information as to how the wines are perceived by people (consumers), at what concentrations differences can be detected, or how those differences affect the overall sensory perception of the product. A trained panel of people will always be required to notice and describe these differences. Since valuable information is gained from sensory analysis and instrumental analysis individually, it only seems natural to try and relate them using multivariate statistical analysis.

Common techniques for the evaluation of sensory differences in wine include discrimination tests, analytical intensity rating tests and consumer tests (Noble and Lesschaeve 2006). A common type of discrimination testing is difference testing. Difference tests, such as triangle tests, are used when the differences in the samples may be too difficult to describe. In a triangle test panelists are told to make a forced choice by choosing the sample that is different from the three samples presented to them, where two are the same product and one is different (O'mahony 1986). If the samples are found to

be different, the next step is often to run sensory descriptive analysis to describe and quantify the differences.

Analytical intensity rating tests are implemented to describe and quantify how the products differentiate from one another. Descriptive sensory analysis (DA) is one such technique, which allows a complete sensory description of the products and helps to identify variation in the products that contribute to the sensory differences (Lawless and Heymann 1999). In DA, the panelists develop a lexicon of sensory descriptors through consensus which describe the differences in the products. The panelists use this lexicon to rate the intensity of each individual descriptor for each of the products on a line scale (usually 15 cm), producing quantitative data that can be analyzed statistically. The sensory profiles of the products are shown by plotting the mean intensity rating for each attribute in each product and difference determined through analysis of variance (ANOVA). The two main requirements of DA to produce consistent and reliable flavor profiles are: 1) the lexicon must contain only terms that are non redundant and describe differences in the products and 2) the panelist receive extensive training in order to rate each attribute consistently and reproducibly. The data obtained through descriptive analysis can be related to preference ratings and instrumental results (Noble and Lesschaeve 2006).

1.5 Relating Sensory and Instrumental Data

With all of the information obtained from both sensory and instrumental analysis, it seems logical to want to relate them. Therefore, one of the aims of flavor chemistry is to create mathematical models that establish (predict) a relationship between the chemical composition of a product to its sensory attributes (Aznar et al. 2003). Before relating

sensory and instrumental data, a fundamental understanding of what is to be gained from combining the individual data set must be considered: 1) an understanding of the mechanisms through which chemical and physical properties of the product (wine) act to produce a sensory sensation; 2) an understanding of how a change in any aspect of the processing may affect the sensory properties; and 3) establishment of a relationship between sensory and instrumental data to detect changes at the same time in the sensory response (Qannari and Schlich 2006).

The current way of relating sensory and instrumental data is to use a succession of different statistical methods to provide information from different points of view, which gathered together depict the entire picture (Qannari and Schlich 2006). Statistical methods used in this approach include correlation analysis, principle component analysis (PCA), cluster analysis, canonical variant analysis (CVA) and partial least squares regression analysis (PLS). Multivariate statistical methods are required for interpreting the relationships between sensory and instrumental data, due to the complexity of the data sets (Noble and Lesschaeve 2006). It is important to note that these multivariate methods do not associate which aroma compound is responsible for a specific sensory attribute, but identify what aroma compounds may be involved and therefore provide focus for subsequent research (Noble and Ebeler 2002).

PLS is a recommended multivariate analysis method to study the relationship between sensory and instrumental analysis of wine and food (Noble and Ebeler 2002, Qannari and Schlich 2006). Advantages of this method are its ability to handle data sets where the number of variables is greater than the number of samples (wines), where there is a high degree of co-linearity among response variables (sensory) and predictor

variables (instrumental), and where there is a significant amount of random error in the data (Chien and Peppard 1993).

PLS relates sensory and analytical data through modeling of the linear combination of variables in one set of data (instrumental) to predict much of the variation in the another set of data (sensory). It indicates how well the variables in one data set can predict the variation in another data, but it cannot test the significance (Noble and Ebeler 2002). Studies looking at aged Spanish red wines (Aznar et al. 2003); California Chardonnay wines (Lee and Noble 2006); fresh strawberries (Schulbach et al. 2004); wine sensory properties related to grape cultivar (Campo et al. 2005); and vinification effects on Pinot noir (Girard et al. 2001) have all used PLS to relate sensory and analytical data.

1.6 Factors Affecting Wine Composition and Sensory Profiles

This section will discuss the various factors known to affect wine composition and sensory profiles which may be relevant to icewine. Since grapes destined for icewine hang on the vine long past regular commercial harvests, it is thought that they undergo chemical reactions analogous to wine oxidation and ageing because of freeze and thaw events. Ripe grapes are not harvested for several months once full maturity is achieved, therefore understanding the effect of harvest date, grape maturity and ripening in other wine styles and grape cultivars may provide insight to icewine chemical composition and sensory profiles. The effect of crop level on chemical and sensory properties in table grapes is well researched; however, we have no information on its effect in icewine.

1.6.1 Oxidation of grapes

Freeze and thaw events

Freezing and thawing of the grapes results in cellular disruption as cells are ruptured by ice crystals upon freezing. This allows for reactions to occur upon thawing that would not in an intact cell, as enzymes and substrates are no longer segregated. Oxidation is one such reaction; caftaric acid is the major phenol in grape must and pulp and is a substrate for browning reactions when exposed to polyphenoloxidases (PPO) (Romeyer et al. 1985). The caftaric acid is oxidized to caftaric acid o-quinones, which polymerize to brown pigments in must. However, in the presence of glutathione and oxygen, glutathione competes for the caftaric acid and is oxidized to s-glutathionyl caftaric acid by PPO (Cheynier et al. 1990). PPO cannot bind to s-glutathionyl caftaric acid becase it does not have a substrate for this enzyme therefore no further browning will occur (Singleton et al. 1984, Singleton et al. 1985a). PPO, caftaric acid and glutathione are all separately compartmentalized within the cells of healthy intact berries. Singleton et al. (1985b) showed that undamaged grapes allowed to dry in the dark, retained their levels of caftaric acid and their green colour as raisins. However, in the presence of sunlight caftaric acid concentrations decreased in the several days accompanied by the onset of browning, with no production of s-glutathionyl caftaric acid. Therefore, upon raisining browning is delayed until the physical barrier between the caftaric acid and PPO is breached (Singleton et al. 1985b), as would occur during freezing and thawing. This could explains why as the icewine grapes hang on the vine they change from green to brown, as enzymatic browning occurs when caftaric acid is oxidized by PPO.

Kilmartin et al. (2007) looked at the polyphenol content and browning in Canadian icewines. They found the concentration of total hydrocinnamates and total caffeic acids to substantially decrease over four harvest dates in both Riesling and Vidal blanc icewines. Total hydrocinnamates decreased from 75 mg/L for Vidal, 32 mg/L for Riesling at harvest 1 (22 November), to < 10 mg/L for both cultivars at harvest 4 (14 January). Total caffeic acids decreased from 60 mg/L for Vidal, 25 mg/L for Riesling to < 5 mg/L for both cultivars for the same harvest dates. Climatic events and/or hang time of the fruit in the later harvest dates is likely the explanation for the loss of polyphenols in reaction similar to hyperoxidation in white wines.

Tian et al., (2009) compared the concentration of 11 phenolic acids and 5 flavan-3-ols in natural and artificially frozen grapes. Naturally frozen Vidal grapes harvested in January, showed a 70% decrease in the concentration of phenolic acids compared to grapes harvested in October and frozen artificially. The authors concluded that the polyphenolic content of icewines, could be used as a marker to identify wines made by natural freezing versus wines made from freeze concentrated grapes (Tian et al. 2009).

Wine Ageing

The oxidation of berries from freeze and thaw cycles is analogous to wine aging, which is an oxidative process (Boulton et al. 1995). Therefore it is plausible to consider chemicals and their reactions associated with white wine aging to be present in grapes used for icewine. Riesling wines have been shown to develop from a light straw colour to a deep yellow with age along with changes to its sensory profile (Simpson and Miller 1983). Studies into white wine aging have shown that over time there is a decrease in linalool and geraniol but an increase in linalool oxides, nerol oxides and hotrienol in

Riesling and Vidal wines (Chisholm et al. 1994, Reynolds et al. 1994). β-damascenone also decreases with bottle age, whereas, other compounds, such as furfural and TDN, increase with age.

A study looking at the aging effects on Vidal blanc wine found that in young wines aromas of apple, citrus, fruity and floral were the predominant descriptors used by a sensory panel (Chisholm et al. 1994). In contrast aged wines were described by a loss of fruity character, and an increase in pungent, oxidized and vegetal aromas. GC-O on the same wines identified the dominant aromas in both young and aged wines to be fruity and floral however found a marked decrease in the concentration of these aromas in the aged wines. The levels of both β-damascenone and the monoterpene alcohols linalool and geraniol were lower in aged Vidal wines. The terpene alcohols are oxidized during storage to terpenes oxides which have higher sensory threshold and therefore are not perceived in the older wines.

Studies looking at the effect of aging on Riesling wine have found that the concentration of the monoterpene alcohols decrease in quantity, with a subsequent increase in the monoterpene oxides which have a higher sensory threshold, thus reducing their sensory perception. Another characteristic of aged Riesling is the production of TDN over time to above its sensory threshold. This is able to change the sensory profile of the wine, as it is not found in significant concentrations in young wines. The highest concentration of TDN was found to be produced at wine and juice pH, therefore with bottle age TDN concentrations increase as the precursors are hydrolyzed in the wine (Reynolds et al. 1994).

1.6.2 Consequences of Hang Time

Grape Desiccation

Desiccation of the icewine grapes is another consequence of extended hang time and should also be considered in addition to freeze and thaw events and oxidation. Drying of grapes results in water loss (Costantini et al. 2006), changes in aroma compound composition (Chkaiban et al. 2007, Genovese et al. 2007) (Moreno et al. 2008), sugar concentration (Bellincontro et al. 2009), cellular disruption and tissue softening (Ramos et al. 2004).

Bellincontro et al. (2004) found that dehydrating Trebbiano, Malvasia and Sangiovese grapes either through controlled dehydration or accelareated tunnel dehydration increased the sugar content, ethanol concentration and the concentration of esters and higher alcohols while decreasing the concentration of C_6-compounds such as 1-hexanol. Drying grapes in the sun of Jerez, Spain has also shown changes in the volatile composition of the grape must which had higher concentration of ethanol, phenylethanol, ethyl acetate, isoamyl alcohol and γ-butyrolactone (Franco et al. 2004). The volatile composition of Pinot noir wines made from dehydrated grapes had higher concentrations of guaiacol, terpenes (citronellol, geraniol, eugenol) and norisoprenoids (β-damascenone, β-ionone) compared to non-dried wines, (Moreno et al. 2008). The increase in concentration was greater than expected from dehydration alone and it was believed that dehydration of the grapes resulted in the production of important aroma compounds.

While it seems reasonable to assume changes due to desiccation of icewine grapes volatile composition would be similar, first the effect of climate and temperature need be

considered since both of the previously mentioned studies were conducted with off vine drying in hot climates with dehydration temperature of at least 20°C. Molecular differences in off-plant and on-plant withering response were identified using AFLP-transcriptional profiling of Corvina grapes (Zamboni et al. 2008). Since icewine grape desiccation occurs on the vine some differences in composition would be seen. Temperature also affects the amount of water loss during grape drying, which is related to volatile compound metabolism and production of volatile acidity (Bellincontro et al. 2009). Cesanese grapes (red *V.vinifera*) had different accumulation of volatile compounds when dried at 20°C and 10°C. At 10°C the grapes had increased formation of ethyl acetate and other acetate esters including isoamyl acetate, whereas at 20°C there was a higher concentration of C6-compounds.

Pathogen Infection

The species and population of wild yeasts, bacteria and moulds on the bloom of icewine have been investigated (Chamberlain et al. 1997, Subden et al. 2003). However, their effect on aroma compound concentration and sensory profiles has not been determined. It is know that pathogen infection of grapes may result in the development of aroma compounds with positive or negative effects on the sensory profile of the wine and final wine quality.

Metabolic activity of *Botrytis cinerea* resulted in an increase in the concentrations of whisky lactones, 1-octen-3-ol, and benzaldehyde and a decrease in concentration of ethyl esters and acetates in late harvested Botrytis affected sweet Fiano wines compared to non-Botrytis affected base wines (Genovese et al. 2007). Botrytis-affected wines from Bordeaux, France were characterized by higher concentration of homofuraneol®,

furaneol®, phenylacetaldehyde and methional than base wines and two unique odourant areas described as grapefruit and curry were idenfied through GC-O (Sarrazin et al. 2007). Tokaj Aszu wines from Hungary are characterized by γ-lactones that elicit aromas of coconut, chocolate, and peach, which result from the oxidizing influence of *B. cinera.* Sporulation of the *B.cinera* was observed at 5°C on strawberry leafs (Sosa-Alvarez et al. 1995). Therefore, the potential of Botrytis infection in icewine grapes is very likely and would result in changes in the chemical and sensory profiles of the wine.

Sour rot is a grapevine disease characterized by browning, disagregation of the cellular structure, pedicel detachment and a strong odour of ethyl acetate (Guerzoni and Marchetti 1987). Sour rot infection of Riesling grapes was shown to increase sugar concentration, glucose to fructose ratio, titrable acidity, glycerol and gluconic acid concentrations and reduce berry weight compared to 'clean' fruit (Zoecklein et al. 2001). Non-infected fruit had higher concentrations of geraniol, nerol and linalool, whereas sour rot infection increased the concentration of *trans*-furan linalool oxide, benzyl alcohol, 2-phenylethanol, 2-methyl-1-propanol and 3-methyl-1- butanol (Zoecklein et al. 2001). In the Niagara Peninsula the pathogenic organisms found and identified by PCR and gene sequencing to cause sour rot were *Hanseniaspora uvarum*, *Candida zemplinina*, *Gluconobacter cerinus* and *Gluconobacter frateurii* (Plant 2008)

1.6.3 Harvest date as a determinant of wine composition and sensory profile

Perhaps the most important harvest decision is choosing when to harvest the grapes. Icewine is one of the only wine styles for which the soluble solids concentration at maturity is irrelevant, because it is the harvest temperature that is critical to freezing the water inside the berry and concentrating all its components. Harvest temperature is

the most important variable that determines the °Brix value of the resultant icewine juice (Ziraldo and Kaiser 2007). The optimal harvest and pressing temperature range for icewine is -9 °C to -11 °C giving a soluble solids concentration in the must between 38 to 40 °Brix, titratable acidity between 10 to 12 g/L and a pH between 3.1 and 3.3 (Ziraldo and Kaiser 2007). If temperatures are higher, the water is not sufficiently frozen in the berry, in addition to the legal issues pertaining to harvesting and pressing at -8 °C or colder. If temperatures are considerably lower, the berry is too frozen, increasing the potential °Brix values and pressing time but decreasing the yield, profitability and difficutly for yeast to ferment.

As "hang time" increases for late harvest wines, desiccation of the fruit causes the berry to shrivel concentrating the acid and sugars in the berry. As a result, colder temperatures are required in order to freeze the berry to achieve the desired soluble solids concentration in the must later in the season. Higher yields of icewine juice are usually obtained in December because there is less desiccation of the fruit, therefore the air temperature does not have to be as cold to achieve the desired °Brix value. However, it is believed that the freeze and thaw cycles are critical to developing the sensory profile for which icewines are known. Some producers will have several icewine harvests from mid-December to late January to achieve a balance between flavour profile and yield.

Fenoll et al. (2009) used GC-MS to monitor changes in free and bound volatile compound concentration in Muscat Hamburg grapes during maturation over two vintages. In both years, geraniol was found to be the most abundant free volatile compound at the initial stage of maturations, with its concentration decreasing throughout ripening. Whereas, the concentrations of linalool of other free form volatiles increased

during ripening. At grape maturity, most volatile compounds showed higher concentration in the bound form compared to the free form, except for linalool and linalool oxides where the reverse was true. The OAVs for each free form volatile were calculated but only linalool, *cis*-rose oxide, citral and geraniol were found to contribute the aroma of Muscat Hamburg grapes.

Fernão-Pires grapes from northern Portugal were followed from veraison (onset of ripening) for five weeks and several variety- and pre-fermentation-related volatile compounds were screened to determine optimal harvest parameters (Coelho et al. 2007). All screened volatile compounds; 16 terpenes, two C_{13} norisoprenoids, five C_6 compounds and two aromatic alcohols, were found to increase in concentration to a maximum at day 20 after veraison. After day 20, the concentration of all volatiles was found to decrease. They concluded that while peak volatile composition occurred with the white grapes maturity (ratio of sugar/acidity), it was a short window before concentration began to drop off for varietal and pre-fermentation volatiles.

This thesis is the first time the effect of harvest date on icewine chemical and sensory profiles has been studied. However, the changes in the aroma composition and sensory profiles of grapes and other wines styles during maturation and ripening have been extensively studied (Marais and van Wyk 1986, Reynolds et al. 1993, Salinas et al. 2004, Coelho et al. 2007, Palomo et al. 2007, Oliveira et al. 2008, Fenoll et al. 2009). These studies provide knowledge of what may be occurring in icewines but unfortunately none of them track the volatile composition of grapes long past maturity as is the case with icewine, and how they may change.

1.6.4 Crop level as a determinant for wine composition and sensory profile

Yield calculations for icewine crops are based on October estimates for regular harvest (Ziraldo and Kaiser 2007) because it becomes more difficult to accurately estimate crop late in the season as the individual berries are at different stages of desiccation due to climatic conditions. Yield estimations of 150 L/tonne, or approximately 7 tonnes/acre for Vidal blanc, and 125 L/tonne or 5 tonnes/acre for Riesling are used by one of Ontario's largest icewine producers as a guideline for maximum yield and quality (Ziraldo and Kaiser 2007). However, anecdotally it is not uncommon for growers to crop vines up to 10 tonnes/acre to increase the volume of icewine juice they can sell to the wineries, especially for Vidal blanc.

Cluster thinning is a standard viticultural practice performed to keep the grapevine in balance, thus, preventing overcropping. The net effect of cluster thinning is to improve the quality of the grapes, which is achieved due to an increase in the ratio of leaf area to crop and maintain the health of the vine (Winkler et al. 1962). Reynolds (1989) found that cluster thinning changed the composition of the grapes with increases in sugar content and pH and a decrease in total acids. It also increased cluster weight, berries per cluster, and berry weight and also advanced maturation of the fruit.

Cluster thinning has been shown to improve fruit composition in French-hybrids such as De Chaunac and Seyval blanc as well as *V. vinifera* cultivars (Reynolds 1989). As of 2009, no research has focused on the effect of crop level on the composition of dessert wines. However, in table wines several studies have established a clear relationship between crop level and varietal characteristics in table wines. Reynolds et

al.(1996) found that cluster thinning produced Pinot noir wines which were rated by panelist as having less grassy and vegetative characteristics and were rated higher for descriptors such as black pepper, cherry, and currant. PCA results showed correlations between typical Pinot noir descriptors and cluster thinning.

Studies with Riesling have shown similar results; where cluster thinned grapes have a higher concentration of monoterpenes (McCarthy et al. 1985). The effect of cluster thinned vines to three different crop levels; 1, 1.5, and 2 clusters per shoot were studied to determine the effect of vineyard treatments on Riesling composition and sensory response (Reynolds et al. 1994). They found that monoterpene concentrations decreased with increasing number of clusters per shoot. Linalool was positively correlated with ripe fruit character and sweetness and negatively correlated with green-fruit flavor and cluster thinning was found to increase the perception of ripe fruit character in the wines. Monoterpenes such as linalool, linalool oxides, terpineol and citronellol were associated with lower crop levels and low to moderate shoot densities and were found to increase in concentration with age (Reynolds et al. 1994).

1.7 Conclusion

The production of icewine is influenced by viticultural, oenological, sensory and chemical factors which make it a unique wine style. This study tries to elucidate how harvest date and crop level affect the sensory profiles and responsible aroma compounds of Riesling and Vidal blanc icewines through sensory and instrumental analysis, SBSE-TDS-GC-O-MS.

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Chapter 2
Odour Potency of Aroma Compounds in Riesling and Vidal blanc Table Wine and Icewines by Gas Chromatography-Olfactometry-Mass Spectrometry

Amy J. Bowen and Andrew G. Reynolds

Abstract

This study aimed to elucidate the odour potency of aroma compounds in Riesling and Vidal blanc table wines and icewines from the Niagara Peninsula using stir bar sorptive extraction coupled with gas chromatography olfactometry mass spectrometry. CharmAnalysis™ was used to determine the most odour-potent compounds in Vidal blanc (syn. Vidal) and Riesling icewines and table wines from a commercial producer. The top 15 odour potent compounds in each wine by each judge were identified and quantified resulting in 23 and 24 compounds for Riesling and Vidal, respectively. The most odour-potent compounds determined by CharmAnalysis™ for Vidal and Riesling wines were β-damascenone, decanal, 1-hexanol, 1-octen-3-ol, 4-vinylguaiacol, ethyl hexanoate and ethyl 3-methylbutyrate. In general, icewines had higher concentrations of most aroma compounds compared to table wines. Through computation of odour activity values, the compounds with the highest odour activity for the icewines were β-damascenone, 1-octen-3-ol, ethyl octanoate, *cis*-rose oxide and ethyl hexanoate. In table wines the highest odour activity values were found for ethyl octanoate, β-damascenone, ethyl hexanoate, *cis*-rose oxide, ethyl 3-methylbutyrate and 4-vinylguaiacol. These finding can be used as a foundation to determine impact odorants in icewines and the effects of viticultural and oenological practices on wine aroma volatile composition.

Key words: wine aroma, volatile analysis, odour activity values, β-damascenone, esters

Introduction

Wine aroma is the result of the complex interaction of hundreds of volatile compounds that together form a matrix to produce a sensory response. The major criterion of a compound to be aromatic, or odour-active, is its volatility enabling it to reach the olfactory epithelium and elicit a sensory perception. As a result, odour-active compounds are generally low molecular weight with high volatility. Understanding what contributes to a wine aroma has been the goal of many research initiatives since the

1940's and continues today. The source of wine aroma compounds can be explained by their source of origin; 1) from the grape berry, 2) volatiles originating from grapes as non-volatile precursors released through processing and storage, 3) from yeast and/or bacterial metabolism, 4) from oak extraction and 5) from chemical reactions occurring during storage and ageing (Rapp and Mandery 1986, Francis and Newton 2005).

Wine aroma compounds can also be categorized based on their contribution to the overall aroma of the matrix (Ferreira et al. 2008) as: 1) an impact odorant; a compound that is able to impart its characteristic and identifiable aroma to the wine, such as linalool in Muscat wines or sotolon in certain dessert wines. 2) Impact groups or families are compounds with similar chemical structure and aroma that act together to contribute an identifiable aroma to the wine, such as γ-lactone and volatile phenols. 3) Subtle families or groups are not able to contribute an identifiable aroma but contribute nuances to the overall aroma, such as fruity. 4) Compounds that form the base of wine aroma are generally below their sensory threshold but can act on other compounds altering their perceived aromas as aroma enhancers and depressors. 5) Off-flavour compounds whose presence decreases the quality of the wine, such as trichloroanisoles responsible for cork taint.

Gas chromatography-mass spectrometry (GC-MS) is the most used analytical instrumentation to determine the concentration of volatile compounds and is widely used in wine aroma analysis. However, this method gives no indication of which compounds in the sample contribute to its aroma, only the amount present in the sample. A common measure to assess the contribution of an aroma compound to a product is through the use of odour activity values (OAVs), which are calculated by dividing the concentration of

the analyte by its sensory threshold. An OAV greater than one indicates the compound is found above its sensory threshold and contributes to the products aroma. The larger the OAV, the more potent the compound is thought to be. OAV are a good indication of the potential potency of an aroma compound in that matrix. However, there are several factors to consider when using OAV to assess odour activity: threshold determination and matrix effects (Francis and Newton 2005, Fischer 2007). OAV are most useful when the sensory threshold is determined in a similar matrix, as it is well established that aroma compounds have different threshold concentrations is air, water and wine (Francis and Newton 2005). OAVs also provide no information on matrix effects and interactions with other compounds which can result in aroma enhancement or depression. Finally, an OAV greater than one does not mean the aroma compound will be perceived in the wine.

Gas chromatography-olfactometry (GC-O) is an analytical method used to determine which odour-active compounds in a chromatographic run contribute to the wine aroma. This technique combines a traditional GC fitted with a non-destructive olfactory port enabling a person to smell the effluent. The odour description, when an odour-active compound elutes above its sensory threshold, is recorded along with its retention time to identify the odorant areas of the chromatogram.

GC-O methodologies have diverged into three main categories: dilution analysis, intensity ratings and frequency detection. Osme (McDaniel et al. 1989), finger-span method (Etievant et al. 1999) and simple intensity rating (Ferreira et al. 2003) are all intensity rating techniques that ask the sniff judge to rate the perceived intensity of the eluting odorant. Frequency detection methods, such as nasal impact frequency (NIF) (Pollien et al. 1997) use a panel of judges to smell GC effluent and compounds are ranked

based on how many times they are detected. Both of these methods provide useful information by identifying and rating the odour-active compounds, however only dilution analysis provides a quantitative measure of the odour potency. CharmAnalysis™ (Acree et al. 1984) and aroma extract dilution analysis (AEDA) (Ullrich and Grosch 1987) are the two quantitative dilution techniques. In dilution analysis, the sample is serially diluted and sniffed by a small panel of judges until no odour is detected. The compounds which are present in the highest dilutions are those which contribute most to the wine aroma, therefore are the most odour-potent compounds.

In CharmAnalysis™ the sniff judge uses a computer program to indicate the beginning and end of the odour by depressing the mouse button and describe the odour eluting based on developed lexicon. The charm value produced is based on the peak height (number of dilution detected) and length (duration of odour event) (Acree et al. 1984). AEDA uses the same principle to produce a flavour dilution (FD) value based on the dilution and only the start time of the odour event. The Charm dilution analysis was used in this study to determine the most odour-potent compounds in Vidal blanc (hereinafter referred to as Vidal) and Riesling icewine and table wines. CharmAnalysis™ has been previously used to characterize aroma compounds in lychee fruit (Ong and Acree 1998), Gewurztraminer wines (Ong and Acree 1999), Vidal and Riesling wines from Ohio (Chisholm et al. 1994), coffee extracts from different freeze drying techniques (Sagara et al. 2005), and in wine extracts from different extraction methods to determine the best method of aroma analysis by GC (Moio et al. 1995).

In order for the GC-O results to make sense, the extract sniffed by the judges must be a good representation of the original product, wine. Liquid-liquid extraction of a wine

sample using a solvent of similar chemical properties, such as polarity, is the traditional method for volatile analysis of the sample by GC and GC-O-MS. Disadvantages of this method include time consuming extraction procedures, costs associated with solvents and their safe disposal and the production of artifacts during extraction. In 1990, Pawliszyn came up with a solventless extraction technique known as solid phase microextraction (SPME) (Arthur and Pawliszyn 1990). Advantages of this technique are that it combines sampling, extraction, concentration and sampling to the GC all into one step using a polymer coated fiber contained in a modified syringe holder. The fiber is either directly immersed or suspended in the headspace of the analyte for the volatiles to adsorb before being injected into the GC inlet, eliminating the need for solvents. The major disadvantage of this technique is the small sampling capacity of the fiber, only about 0.6 µl for a 100-µm PDMS fiber, which effects the sensitivity of recovery (Hayasaka et al. 2003).

In 1999, a new technique based on the same principal as SPME but with several advantages was introduced known as stir bar sorptive extraction (SBSE), commercially sold as Twister (Baltussen et al. 1999). SBSE uses a 10 mm long glass encased magnetic stir bar coated with 0.5 mm thick layer of polydimethylsiloxane (PDMS). Analytes are extracted by placing a stir bar either directly in an aqueous mixture or suspending in its headspace. The volatiles partition onto the PDMS coating and are then thermally desorbed into the GC inlet which is cryo-cooled with liquid nitrogen. The main advantage of SBSE over SPME is the increase sampling capacity, 25 to 125 µL, provided by the PDMS coating, enabling improved signal to noise ratios, increased sensitivity, and low detection and quantitation limits (Hayasaka et al. 2003). It is for these reasons that SBSE

was used for the extraction of volatiles in this study. It is the first time to the authors' knowledge that SBSE has been used in conjunction with dilution analysis, CharmAnalysis™, to determine the odour potency of aroma compounds. However, SBSE has been used extensively for the volatile analysis of grapes (Salinas et al. 2004), wine (Alves et al. 2005, Zalacain et al. 2007, Tredoux et al. 2008) and other alcoholic beverages such as beer (David et al. 2001) and malt whisky (Demyttenaere et al. 2003).

Ontario is Canada's largest wine region and the Niagara Peninsula its largest appellation producing sparkling wines, table wines and dessert wine from mainly *Vitis vinifera* cultivars. Icewine is the wine style synonymous with the Canadian wine industry made from Vidal and Riesling with the bulk of its production from the Niagara Peninsula. In 2008 over 1 million litres of icewine were produced in Ontario, 75 and 12 percent of it was made from Vidal and Riesling cultivars, respectively (Ontario 2010). In 2009, almost 900 000 litres of icewine were produced in Ontario of that 75 and 6 percent were made from Vidal and Riesling, respectively (Ontario 2010).

Icewine is a sweet late harvest dessert wine made from grapes naturally frozen on the vine at -8°C or colder and pressed while frozen. The resultant wine is concentrated in sugar, acids and aroma/flavour compounds. In Ontario, Canada the production of icewine is strictly regulated by the Vintner's Quality Alliance (VQA) of Ontario. In order for a wine to labeled icewine under VQA regulations it must be made from grapes harvested after 15 November, with a harvest temperature $\leq$ -8°C, a must concentration of at least 35°Brix, with 125 g/L residual sugar and 6.5 g/L titratable acidity in the finished wine. Icewines are characterized by intense aromas of honey, peach, apricot and caramel with the palate displaying a balance of sweetness and acidity. While icewine is

internationally renowned and produced in many countries, very little is known about this unique wine style and its volatile composition.

A survey of icewine research in the literature shows studies that have looked at the sensory and chemical composition of icewine (Cliff et al. 2002, Nurgel et al. 2004); the impact of vintage and viticultural area on some chemical parameters (Soleas and Pickering 2007), the polyphenol content (Kilmartin et al. 2007, Tian et al. 2009); the impact of yeast strain (Subden et al. 2003, Erasmus et al. 2004), yeast inoculation method (Kontkanen et al. 2004) and yeast hyperosmotic stress response in icewine fermentations (Pigeau et al. 2007, Pigeau and Inglis 2007). However, there is limited research on the aroma volatiles composition of icewine, besides a preliminary study by Cliff et al. (2002) and qualitative profiling of icewine volatiles fractions using solid-phase microextraction-gas chromatographic-time-of-flight mass spectrometric methods (Giraudel et al. 2007, Setkova et al. 2007a, Setkova et al. 2007b).

Other regions have characterized their wines based on their aroma composition and the identification of impact odorants that impart to the wines a specific, unique and identifying aroma which is distinct to that wine style (Tominaga et al. 1996, Guth 1997a, Ferreira et al. 2002, Fretz et al. 2005). Our aim is to elucidate if such a compound can be found in Vidal and Riesling icewines using gas chromatography-olfactometry-mass spectrometry. The main objective of this study is to determine and quantify the most odour-potent compounds in Riesling and Vidal table wine and icewine from the Niagara Peninsula, Ontario, Canada. Much is known about the chemical composition of Vidal and Riesling table wines. Comparison of table wine and icewines will provide information as to how the odor potency changes and what compounds may be affected.

The grapes for the commercial table wines and icewines in this study came from the same vineyard block for both the Vidal and Riesling wines. Some of the grapes in the block were harvested at regular commercial harvest for table wines while the rest of the grapes in the block remained on the vine until temperatures permitted icewine harvest. Therefore difference in odour-potency and odour potent compounds can only be attributed to wine style and harvest date since all other parameters were the same. Since very little is known regarding icewine, the results of this study will aid in our understanding and the characterization of Canadian icewine, and the aroma compounds important to this wine style.

Materials and Methods

Wines. Four commercial wines from the 2004 vintage were donated by Coyotes Run Winery in Niagara-on-the-lake, ON for analysis. They consisted of 2004 Riesling icewine, 2004 dry Riesling table wine, 2004 Vidal icewine and 2004 off-dry Vidal table wine. All wines of the same cultivar were from the same vineyard but harvested at different times to reflect the two different wine styles: table wine and icewine.

Chemicals. Analytical standards (Table 2.1) were purchased from Aldrich (Oakville, ON, Canada), Sigma-Aldrich (Oakville, ON), Fluka (Oakville, ON), Bedoukian (CT, USA), Acros Organic (NJ, USA). β-Damascenone was a gift from Dr. T. Acree, Cornell University. Chemical standards were diluted in dichoromethane (Caledon; Georgetown, ON) and stored at -25°C.

Volatile extraction. Wine volatiles were extracted by stir bar sorptive extraction (SBSE), commercially known as Twister, using 10 mm stir bar (Gerstel, Baltimore, MD)

coated with polydimethylsiloxane (PDMS, 0.5 mm film thickness) in 10-mL extraction vials for 60 minutes at 1000 rpm. Stir bars were removed from the extraction vial, dried with a lint free tissue, rinsed with Milli-Q water (Millipore) and stored in a 4-mL amber vial at 4°C until analysis. Wines were used full strength for GC-MS quantification. For GC-O analysis, wines were diluted 10-fold with a model wine solution made to match the composition of the icewine and table wine. The icewine model wine solution contained 11.57 g/L tartaric acid (EMD Chemical Inc., Darmstadt, Germany), 153 g/L fructose (Caledon; Georgetown, ON), 11% (v/v) ethanol (Commercial Alcohols Inc.; Brampton, ON), with a pH of 3.61. The table wine model wine solution contained, 7.4 g/L tartaric acid, 5 g/L fructose, 12% (v/v) ethanol, with a pH of 3.33. All wines were spiked with an internal standard, 100 µg/L *n*-dodecanol (Sigma; Oakville, ON) in GC-grade dichloromethane.

Gas chromatography-mass spectrometry (GC-MS). Instrument: Agilent 6890N/5975B gas chromatograph mass spectrometer equipped with a Gerstel thermal desorption unit (TDS2; Gerstel, Baltimore, MD) and cooled injection system (CIS4; Gerstel, Baltimore, MD) programmable temperature vaporization (PTV) inlet and an olfactometry port (DATU, Geneva, NY). Analytical column: Agilent HP-5MS, 5% phenyl methyl siloxane, 30m length, 0.25 mm internal diameter and 0.25 µm film thickness. Carrier gas: 1.4 mL/min 5.0 purity helium (Praxair, Mississauga, ON). Oven program: initial temperature 35°C held for 3 min, increased by 6°C/min to 155°C, increased by 30°C/min to a final temperature 240°C. Thermal desorption: initial temperature 30°C, increased by 60°C/sec to 250°C and held for 3 min. TDS transfer line temperature 275°C connected to CIS4 inlet cryo-cooled to -70°C with liquid nitrogen in

solvent vent mode. After desorption, CIS4 inlet temperature was increased at 12°C/sec to 280°C and held for 5 min while analytes are released on the column. The column was attached to a splitter (Gerstel, Baltimore, MD), with equal proportions to the MS and the back inlet (olfactory port). The MS was run in scan mode, 30 to 400 Da for compound identification and in select ion monitoring (SIM) mode selecting for one quantitative ion and three qualitative ions for each compounds for quantification (Table 2.1).

GC-olfactometry (GC-O). All instrumental parameters are the same as listed for the GC-MS. The olfactory port connected through the back detector was heated to 250°C. The effluent from the column was supplemented with 45 mL/min nitrogen and was heated with humidified air to prevent drying and irritation while sniffing.

Judge reproducibility. Judges were given blind, four repetitions of the same wine over four days, to ensure they were detecting similar odour events, identifying the perception and were reproducible. An odour event was characterized by its odour perception, retention time and if it was detected in at least 3 of the 4 repetitions. Thirty and 26 odour events were detected by Judge 1 and 2 respectively, and of those 21 odour events were the same (same retention index and perception). Judges were therefore deemed reproducible.

Lexicon generation. The judges sniffed all wine at the initial concentration and generated a list of descriptors to describe the aroma perceptions eluting from the GC-O. The judges met to discuss the lexicon terms and through consensus generate the final lexicon. This lexicon was used for subsequent analysis (Table 2.2).

***CharmAnalysis*™.** GC-O data was collected by the dilution analysis method CharmAnalysis™ (DATU, Geneva, NY), a computer software program which records the retention time, linear retention index (based a series of *n*-alkanes C_6-C_{19}; Sigma Aldrich, Oakville, ON) and odour perception. Wine was diluted, in model wine, 10-fold for the initial concentration; all subsequent dilutions were 3-fold until no odour events were detected. Each wine at each dilution was extracted using SBSE, sniffed by two judges experienced in aroma recognition until no aroma was detected.

Top 15. Each wine was sorted by CHARM value for each judge, all erroneously identified odour events were removed. The top 15 odour events for each judge in each wine were retained and subsequently identified and quantified. Charm values were normalized into odour spectrum values (OSV) for comparison between judges and wines (Acree 1997; Table 2.3).

Identification and quantification. Compounds were identified by comparison of retention time, odour perception and mass spectra (Wiley7Nist05 library) to pure standards. Three-point calibration curves were run for each analyte in model wine solution to ensure linearity ($r^2 > 0.9$; Table 2.1). Standard curve concentrations and compound were quantified based on the ratio of the peak area of the compound relative to the peak area of the internal standard to determine the concentration of the analytes. Analysis was run in duplicate with relative standard deviation between replicates ranging from 0.5 and 12%.

Statistical analysis. Two tailed t-tests (Microsoft Excel) were used to determine differences between table wines and icewines from each cultivar at $p < 0.05$.

Results

GC-O. Icewines and table wines had similar odour-active compounds but differences in their odour potency (Table 2.3). For each of the judges, the top 15 odour-active compounds were retained, determined by descending Charm values. The Charm value were then converted to odour spectrum values (OSV) which is the odour potency normalized to the most potent odorant detected (Acree 1997) to enable comparison between judges, wine and cultivars. This is the same principal as in mass spectroscopy, where the ions fragments are normalized, and expressed as a percentage, to the most abundant ion fragment produced in a spectrometer.

In total, 32 odour events were identified by combining all compounds in each of the top 15 lists in Vidal and Riesling icewine and table wines (Table 2.3). Of these 32 compounds, 24 and 23 odour events were found in Vidal and Riesling wines, respectively. Four compounds identified in the top 15 of Vidal wines were not found in Riesling; ethyl 2-methylbutyrate, isoamyl acetate, ethyl valerate, and 1-heptanol. Similarly three odour-active volatiles; 2-phenylethyl acetate, ethyl cinnamate and β-ionone were identified in the Riesling top 15 which were not found in Vidal. The results do not imply that the above mentioned compounds are not present in Vidal and Riesling wines, because in most cases they are, only that they were not the most odour-potent and therefore were not listed in the top 15 odor events in any wine by either judge for the other cultivar.

Five odour events could not be identified because no compound was found by MS to associate with the odour peak. It is well known that the human nose is a more sensitive

detector than a GC-MS (Acree 1997); therefore it is not surprising or uncommon to have some unidentified odour events. These five unknowns, based on their linear retention index, were named by that number; unknown 1018, 1027, 1658, 1722 and 1761 (Table 2.3). Unknown 1722 detected in Vidal and Riesling wines, was described as smelling of black pepper. However, no compound could be detected by the SCAN mode of the GC-MS which matched the mass spectrum, odour perception and retention time. No further study was conducted to determine the identity of the five unknowns.

Only two odour events were found in all wines by both judges through CharmAnalysis™, decanal and β-damascenone. Both had high odour potency, listed in the top three odour-potent events in all cases. Decanal had the highest odour potency value (OSV=100) in Vidal and Riesling icewine by Judge 1 and Vidal and Riesling table wine by Judge 2. Decanal was described as having a petrol, vinyl/plastic, citrus, green aroma. β-damascenone had the highest odour potency in Riesling icewine by Judge 1 and table wine by Judge 2, it was described by a distinct pear aroma.

Ethyl 3-methylbutyrate, 1-hexanol, 1-octen-3-ol, ethyl hexanoate and 1-octanol were five compounds that were odor potent in almost all cases; they were missing from only one wine. 1-Hexanol was found to be the second most odor potent aroma in Vidal and Riesling icewine by Judge 1. 1-Octanol was the most odour-potent compound in Vidal table wine by Judge 1. Other odour-potent compounds found in most wines were *cis*-rose oxide, phenethyl alcohol, nerol oxide, ethyl phenylacetate and 4-vinylguaiacol (Table 2.3).

The fruity, sweet smelling esters, ethyl isobutyrate, ethyl butyrate and ethyl 2-methylbutyrate were found to have more odour potency in table wines than icewines for

both Vidal and Riesling. Ethyl-2-methylbutyrate was found to be the most odour-potent compound in Vidal table wine by Judge 1. Similarly, the clove-smelling 4-vinylguaiacol and floral-smelling geranyl acetone were more odour-potent in Riesling table wine than icewines. The reverse was found with acetophenone, which had higher odor potency in the icewines than table wines.

GC-MS. The odour-potent compounds determined through GC-O analysis were quantified and their odour activity values (OAVs) determined based on published sensory thresholds (Table 2.4).

Statistical analysis (t-test) found that Vidal icewine and table wines were different for 22 of 24 compounds, but ethyl isobutyrate and 1-hexanol were not different. Vidal icewine had a higher concentration of most compounds--15 of the 22 different compounds--than the table wine. Ethyl butyrate, isoamyl acetate, ethyl hexanoate, acetophenone, ethyl octanoate, decanal, and 4-vinylguaiacol had a higher concentration in the table wine than the icewine. No compounds were unique to either wine-style for Vidal.

Riesling icewine and table wine were different for 18 of 23 compounds quantified. Five compounds--ethyl isobutyrate, ethyl butyrate, ethyl 3-methylbutryate, 1-hexanol and acetophenone--were not different. Similar to Vidal, 14 of the 18 compounds had higher concentrations in icewine than table wine. Only ethyl hexanoate, ethyl octanoate, decanal, and geranyl acetone had higher concentrations in table wine. Riesling icewine had one unique compound, 1-octanol, which was not detected in the table wine.

Both Vidal and Riesling table wine had higher concentrations for ethyl hexanoate, ethyl octanoate and decanal than in icewine. The table wine concentrations were 82 and

21 % higher for ethyl hexanoate, 260 and 132 % higher for ethyl octanoate, and 1300 and 368 % higher for decanal in Vidal and Riesling, respectively. The reverse was for 1-octen-3-ol; its concentration were over 2500 and 300% higher in Vidal and Riesling icewines, respectively, than table wines.

β-Damascenone, linalool and *cis*-rose oxide have all been previously identified as odour-potent compounds in Vidal and Riesling wines (Chisholm et al. 1994). γ-Nonalactone, ethyl phenylacetate, and isoamyl acetate are important impact odorants identified in other wine styles and were found to be odour-potent in these commercial wines. With the exception of isoamyl acetate, which had 20% higher concentration in Vidal table wine, all these compounds had higher concentrations in the Vidal and Riesling icewine.

Large concentration differences were found between Vidal and Riesling icewines and table wines. Vidal showed larger concentration differences between table wine and icewine than Riesling (Table 2.4).

Odour activity values. The concentration of each compound was divided by its sensory threshold (Table 2.5) to determine its odour activity value (Table 2.4). Any OAV >1 is considered above its sensory threshold and is said to contribute to the aroma of the product. The higher the value > 1, the more potent or dominant a compound will be. Vidal icewines and table wine had 15 and 14 compounds, respectively, above their sensory threshold (OAV > 1). Linalool and ethyl 2-methylbutyrate were above their sensory threshold for icewine but not table wine and decanal was only found above its sensory threshold in Vidal table wine. Riesling ice wine and table wine were found above their sensory threshold for the same 12 compounds.

The highest OAVs in Vidal and Riesling icewines were determined for β-damascenone (902 and 186, respectively) and in table wine, ethyl octanoate had highest OAVs (533 and 205, respectively). β-damascenone had the second-highest OAV in both table wines (224 for Vidal; 78 for Riesling). Ethyl octanoate had the second-highest OAV in Riesling icewine (88) and third-highest in Vidal icewine (147). Other compounds with high OAVs for Vidal icewine were 1-octen-3-ol and *cis*-rose oxide, both with OAVs >100. Vidal was found to have higher OAVs than Riesling for the most odor potent compounds (Table 2.4).

Some compounds with high Charm or OSV value (and therefore deemed odour-potent through CharmAnlysis™ analysis) were not found to have OAVs > 1, and therefore are considered to be below their sensory thresholds. However, all compounds were detected as odour-active by the judges through GC-O analysis since they were identified by their aromas as they eluted from the column. Decanal, 1-hexanol and 1-octanol are three such compounds that were among the most odor-potent compounds found through GC-O but having low OAVs. Decanal was found above its sensory threshold (OAV = 6.8) only in Vidal table wine. 1-Hexanol and 1-octanol were not found above their sensory thresholds (OAV>1) in any wine.

Discussion

Comparison of GC-O to OAV results. The most potent odorants determined by CharmAnalysis™ were decanal, β-damascenone, and 1-hexanol in Vidal and Riesling icewines and table wines. Calculation of OAV gave a different pattern of odour potency. While β-damascenone had a high OAV and was the most odour-potent compound in

Vidal and Riesling icewines and the second-most potent compound in table wines, decanal, 1-hexanol and octanol all had OAVs < 1 with the exception of Vidal table wine, which had an OAV of 6 for decanal. Therefore based on OAV, those compounds found to be highly odour-potent by Charm are not considered to contribute to the aroma of the wine based on their calculated odour activity. Other potent odorants determined by CharmAnalysis™ were 1-octen-3-ol, *cis*-rose oxide, ethyl 2- and 3-methylbutyrate, ethyl hexanoate, nerol oxide, ethyl phenylacetate and 4-vinylguaicol. High OAVs in icewines were found for 1-octen-3-ol, ethyl octanoate, *cis*-rose oxide and ethyl hexanoate and in table wines for ethyl octanoate followed by β-damascenone, ethyl hexanoate, *cis*-rose oxide, and 4-vinylguaiacol.

Discrepancies between GC-O and OAV are not uncommon due to a variety of factors. Charm response will differ for the sensory response of the food because a compounds volatility in the GC effluent is 100% but in a food/wine matrix that could be quite different if the extraction was not conducted in the headspace of the wine (Acree et al. 1984). Dilution analysis methods such as CharmAnalysis are based on a linear correlation between odour intensity and concentration. It has been shown that this relationship is logarithmic and better explained by Stevens' Power Law (Stevens 1971) than dilution analysis (Kamadia et al. 2006). It is therefore considered necessary to compare quantitative results through conversion to OAV to determine real contribution of various compounds to the aroma (Sarrazin et al. 2007). OAV are thought to be more representative of a food (wine) matrix since they consider concentration and sensory threshold but this too has its shortcoming (Escudero et al. 2004, Francis and Newton 2005).

Whenever possible, the sensory thresholds were taken from the literature in a model wine solution. However, in some case this was not possible, which may explain some of the discrepancies between differences in odour potency determination by Charm and OAV. Since OAVs were determined from published sensory threshold and not conducted in this study, there could be matrix effects that affect the sensory detection threshold of a compound either inflating or suppressing its importance. The high sugar concentration of the icewine may be one of these matrix effects that affects how aromas are perceived. This is a potential area of future research. 1-Hexanol, which was found to be odour-potent through Charm but due to the published sensory threshold of 8000 μg/L in a 10% ethanol solution (Guth 1997b) was not found to have an OAV > 1. The concentration of 1-hexanol in the experimental wines ranged from 700 to 1700 μg/L, well below the published sensory threshold. Since the concentration of 1-hexanol was not found to differ between table wines and icewines it was not a compound that could be used to characterize either wine style.

Escudero et al. (2004) aimed to characterize the aroma of Maccabeo wine and found, similar to this study, that not all compounds with high flavour dilution (FD) were found to have OAV > 1. In fact, they found that having a high OAV is not necessary and does not mean that a compound will affect the aroma of a wine. The results of this study found the most potent odorants by GC-O AEDA and OAV were not able to elicit the same sensory perception in reconstitution studies nor were they found to be impact odorants by omission and addition studies. Two compounds with low FD and OAV values, 4-mercapto-4-methylpen-2-one and 2-methyl-3-furanthiol, contributed most to the aroma of the wine. They proposed the concept of an aroma buffer in wine comprised of

the mix of ethanol, esters, acids, volatile phenols, β-damascenone and fusel alcohols which can only be broken by a compound with very different aroma properties adding a new aroma perception to the wine. The same could be true for Vidal and Riesling wines but since reconstitution, omission and additional studies were not conducted on these wines, we cannot know for sure, but this could be an area of future research.

Decanal results from fatty acid degradation during fermentation and has not previously been identified as a potent odorant in either Vidal or Riesling wines. Decanal could have a low OAV due to bad extraction or quantification since it elutes very close to ethyl octanoate and therefore it odour potency is underestimated. Another explanation for the low OAV of decanal, even though it was found to be highly odor potent in experimental wine, may be due to its interaction with ethanol. Ethanol has the power to enhance the odour of some volatiles such as decanal (Ferreira et al. 2008). The aroma buffer previously mentioned could also account for these discrepancies.

Odour-potent compounds. The Charm values and OAVs of the icewines and table wines differed for many attributes. In general the icewines were found to have higher ratings than table wines for most compounds; this is not surprising considering icewines are characterized by their intense aroma profiles. In addition, pressing icewine grapes frozen would concentrate the volatile fraction of the must and wine leaving most of the water behind as ice. Genovese et al. (2007) found that sweet Fiano wines, made from later harvested (26 °Brix), semi-dried to 32 °Brix, 20 % Botrytis-infected grapes, had higher concentrations of terpenes, β-damascenone, lactones, aldehydes and ketones than wine made from base Fiano wine (22°B). They attributed the higher concentrations in the sweet, passito-style, wines to the overripeness and drying process of the grapes

resulting in the concentration of the aromatic compounds in the skins and facilitated an easier transfer to the must during winemaking. These conditions would be analogous to icewine grapes hanging throughout the fall before freeze events occur.

β-damascenone was a highly potent odorant in Vidal and Riesling icewine and table wine as determined by both Charm and OAV in this study. This is consistent with previous GC-O analysis of Vidal and Riesling table wines (Chisholm et al. 1994, Chisholm et al. 1995, Komes et al. 2006) however, β-damascenone was not previously identified in icewine (Cliff et al. 2002). The result of carotenoid breakdown, the C_{13} norisoprenoid, β-damascenone, is released through enzymatic or acid hydrolysis during fermentation and wine ageing from its grape glycoconjugate precursors. It has an extremely low sensory threshold (0.05 μg/L) and has been widely reported in natural products. Generally β-damascenone is not an impact odorant, it is found to have a high OAV but does not contribute a distinct character to the wine in sensory studies (Guth 1997b, Ong and Acree 1999, Escudero et al. 2004). While β-damascenone does possess a distinct aroma, it requires a large change in concentration in order to perceive a significant change in its intensity (Escudero et al. 2004). In red wine, β-damascenone has been shown to enhance the fruity character while suppressing the green, vegetal notes of methoxypyrazines (Pineau et al. 2007). The concentration of β-damascenone in Vidal icewine was 300% higher than in table wine, perhaps this concentration is great enough to break the aroma buffer proposed by Ferreira's research group. Only future research will be able to answer these questions.

Terpenes, like norisoprenoids, contribute to wine aroma as free odour-active compounds present in the grapes and as glycosidically-bound non-volatiles released

during processing and storage. Terpenes found to be odour-potent in this study are linalool and *cis*-rose oxide with high Charm values and OAV > 1. Nerol oxide was found in the top 15 compounds for Vidal and Riesling wines but had OAV < 0.01 due to its high sensory threshold reported in the literature. All three terpenes were found to have higher concentrations in icewine than table wines. *Cis*-rose oxide and linalool are both impact odorants in white wine cultivars Gewurztraminer (Guth 1997b, Ong and Acree 1999) and Muscat (Bayonove and Cordonnier 1970), respectively. The terpene composition of grape cultivars provides them with a 'fingerprint' by which they can be identified regardless of grape maturity, vintage or origin (Rapp 1998a). Although linalool has been previously reported as odour-potent through GC-O analysis (Chisholm et al. 1994, Komes et al. 2006) no specific impact compound has been related to either Vidal or Riesling wines.

Esters and alcohols are the main aroma compounds originating from yeast metabolism during fermentation. Esters, generally provide fruity and citrus aromas to the wine and are found to contribute to the base aroma of the wine and not as an impact odorant with the exception of isoamyl acetate and ethyl phenylacetate (Ferreira and Cacho 2009). Ethyl octanoate and ethyl hexanoate were the two most odour-potent esters in the experimental wines, with higher OAV and concentrations in table wines than icewines for Vidal and Riesling.

Ethyl hexanoate and 2-phenethyl alcohol were found in high levels in Gewurztraminer wines, likely a result of fermentation, and were found to vary among wine samples suggesting the differences in winemaking can affect their concentration (Ong and Acree 1999). These compounds were previously found to contribute to the

wine bouquet resulting from fermentation and therefore not suitable for the identification of wine origin or grape cultivar – not impact odorants (Rapp 1998b). Ethyl hexanoate, ethyl butyrate, ethyl octanoate, ethyl decanoate form a family of compounds produced from the same metabolic pathway and demonstrate similar aromas, together in a group our noses cannot differentiate one from another (Ferreira and Cacho 2009). As a result, it is not possible to determine their individual sensory impact, odour potency, in a wine matrix. Therefore even though ethyl octanoate was found to have the highest OAV in table wine by GC-O does not mean that removing it will alter the sensory perception of the wine since the other esters are still present in the aroma matrix of the product.

1-Octen-3-ol has a mushroom aroma and was found to have much higher concentrations and odour activity in icewine than table wine for Vidal and Riesling. 1-Octen-3-ol has been associated with Botrytis affected wines as an impact odorant (Rapp and Mandery 1986), with wines made from grapes infected with powdery mildew (*Uncinula necator*) (Darriet et al. 2002) and with fungal infections in grapes and musts (Pallotta et al. 1998). The concentration of 1-octen-3-ol is unaffected by fermentation unlike 1-octen-3-one, therefore if present in the must it will be present in the finished wine (Darriet et al. 2002). Its concentrations were 27 and 4 times greater in Vidal and Riesling icewines, respectively, with the second highest OAV in Vidal icewine and the fourth highest in Riesling icewine. The higher concentration and odour potency of this compound in icewines is best explained by the presence of Botrytis. A similar result was found comparing the concentration of sweet Fiano wine to base Fiano wines in the Campania region of Southern Italy, were the concentration of 1-octen-3-ol was over 5500 % higher in the sweet, passito-style wine (Genovese et al. 2007).

4-Vinylguaiacol is a volatile phenol with an influence on wine flavour formed from thermal or enzymatic decarboxylation of cinnamic acid, p-coumaric acid, ferulic acid. This volatile phenol has previously been shown to be odour-potent in Croatian Riesling table wines, contributing a smoky/spicy note (Komes et al. 2006).

In general, the most odour-potent compounds found through GC-O in this study, β-damascenone, ethyl octanoate, ethyl hexanoate, ethyl butyrate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, phenylethyl alcohol, 4-vinylguaiacol and linalool, have been previously identified in Vidal and Riesling table wines (Chisholm et al. 1994, Chisholm et al. 1995, Komes et al. 2006). To date only one study has performed GC-O on icewines, which identified 34 volatiles as odor active in Vidal and Riesling icewine, they concluded that no single impact compound was found and that icewine aroma was a complex interaction of the many volatiles present in the wine (Cliff et al. 2002). While our findings would support that research, the list of volatiles identified in this study as odour-active are quite different, in fact with the exception of several esters, no compounds are shared. This is most likely due to different extraction methods and GC-O methodologies. By using dilution analysis compared to frequency detection, only the most odour-potent compounds were identified in this study. Many of these compounds had low concentrations, for example β-damascenone, linalool, *cis*-rose oxide, and 1-octen-3-ol and eluted close to large acid and ester peaks which at the undiluted concentrations the aroma perceptions could have been combined with the larger peak on the mass spectra and misinterpreted during identification.

Conclusions

Through CharmAnalysis™ the top 15 odour events for Vidal and Riesling icewine and table wines were determined. The most odour-potent compounds in both wine styles were β-damascenone, decanal, 1-hexanol, 1-octen-3-ol, *cis*-rose oxide. Three compounds were odour-potent in table wine but not icewine: 4-vinylguaiacol, nerol oxide and geranyl acetone.

Vidal icewine was characterized by 15 odour-active compounds with OAVs > 1, the most odour-active were β-damascenone, 1-octen-3-ol, ethyl hexanoate, *cis*-rose and ethyl hexanoate. Vidal table wine was characterized by 14 odour-active compounds with OAVs > 1, the most odour-active were ethyl octanoate, β-damascenone, ethyl hexanoate, *cis*-rose oxide and 4-vinylguaiacol.

Riesling icewine was characterized by 12 odour-active compounds with OAV > 1; β-damascenone, ethyl octanoate, ethyl hexanoate, 1-octen-3-ol, and ethyl isobutyrate were the most odour-active. Riesling table wine was characterized by the same 12 compounds as Riesling icewines, however, the highest odour activity values were mostly associated with esters; ethyl octanoate, β-damascenone, ethyl hexanoate, ethyl isobutyrate, ethyl-3-methylbutyrate.

The role of decanal, 1-hexanol and 1-octanol should be further investigated to understand their contribution to the aroma of Vidal and Riesling table wines. All three were found to be very odour-potent through CharmAnalysis™ but not based on the calculation of OAVs, where they were all determined to be below their sensory

thresholds. Explanations could be linked to discrepancies between GC-O and OAV, inaccurate sensory threshold concentrations and matrix effects.

This study provides information regarding the volatile composition of Vidal and Riesling table wine and icewine, however we cannot conclude anything about impact odorants or odour importance only their relative potencies. Table wines had lower concentrations and OAV for most aroma compounds with the exception of 4-vinylguaiacol, decanal, ethyl octanoate and ethyl hexanoate. These results can be used as a foundation to determine any impact odorants in icewines through reconstitution and omission studies and could be used as markers to identify changes in the odour-active composition of wines related to viticultural and oenological practices.

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Table 2.1 Chemical standards, quantitative and qualitative ions, and calibrated intervals for Vidal and Riesling icewines and table wines.

			Quantitative	Qualitative	Calibrated interval ([µg/L])			
Analyte	Supplier	CAS no.	ion (m/z)	ions (m/z)	Vidal	r^2 value	Riesling	r^2 value
ethyl isobutyrate	Aldrich	97-62-1	43	71, 88, 116	50-150	0.977	150-600	0.923
ethyl butyrate	Aldrich	105-54-4	71	43, 88, 116	20-180	0.962	125-500	0.915
ethyl 2-methylbutyrate	Aldrich	7452-79-1	57	102, 74, 130	5-125	0.997	-	-
ethyl 3-methylbutyrate	Aldrich	108-64-5	88	41, 70, 130	15-135	0.993	15-135	0.973
1-hexanol	Sigma-Aldrich	111-27-3	56	43, 69, 84	1000-6250	0.964	300-4800	0.979
isoamyl acetate	Aldrich	123-92-2	43	70, 55, 87	150-600	0.935	-	-
ethyl valerate	Sigma-Aldrich	539-82-2	88	57, 101, 130	1.0-36	0.995	-	-
1-heptanol	Acros Organics	111-70-6	70	56, 83, 98	2-200	0.996	-	-
1-octen-3-ol	Aldrich	3391-86-4	57	72, 85, 99	1-400	0.999	1.0-400	0.904
ethyl hexanoate	Aldrich	123-66-0	88	99, 60, 144	300-1200	0.999	300-1200	0.993
acetophenone	Aldrich	98-86-2	105	77, 120, 51	1.0-25	0.985	1.0-25	0.979
1-octanol	Sigma-Aldrich	111-87-5	56	41, 69, 84	10.0-20	0.918	2.0-32	0.979
linalool	Sigma-Aldrich	78-70-6	71	93, 121, 154	5-125	0.972	20-500	0.989
cis rose oxide	Fluka	16409-43-1	139	69, 83, 154	3-108	0.998	0.5-24.5	0.998
phenethyl alcohol	Acros Organic	60-12-8	91	122, 65, 51	15000-135000	0.977	15000-60000	0.942
nerol oxide	Bedoukian	1786-08-9	68	83, 41, 152	5.0-80	0.997	20-80	0.993
ethyl benzoate	Aldrich	93-89-0	105	122, 77, 150	1.0-9	0.993	1.0-9	0.995
ethyl octanoate	Aldrich	106-32-1	88	101, 127, 172	400-1600	0.992	300-12000	0.975
decanal	Sigma-Aldrich	112-31-2	57	70, 82, 112	0.25-16	1.000	0.5-4.5	0.984
ethyl phenylacetate	Aldrich	101-97-3	91	164, 65, 136	2.0-50	0.984	2.0-50	0.987
2-phenethyl acetate	Aldrich	103-45-7	104	43, 91, 78	-	-	3.0-75	0.991
4-vinylguaiacol	Alfa Aesar	7786-61-0	150	135, 107, 77	20-320	0.944	25-1225	0.987
γ nonalactone	Aldrich	104-61-0	85	41, 114, 156	0.5-200	0.981	10-160	0.976
β-damascenone	Gift	23726-93-4	69	105, 121, 190	2.0-8	0.994	2.0-32	0.996
geranyl acetone	Alfa Aesar	3796-70-1	43	69, 151, 194	0.15-0.60	0.991	0.15-0.60	0.990
ethyl cinnamate	Aldrich	103-36-6	131	103, 176, 77	-	-	3.0-27	0.975
β-ionone	Aldrich	79-77-6	177	43, 135, 192	-	-	0.01-0.25	0.992

Table 2.2 Lexicon of terms used for GC-O to describe perception of odour events, developed by consensus of both 'sniff' judges.

Terms	
banana	honey
black pepper	mushroom
bread/yeast	musty
caramel/butterscotch/ burnt sugar	peach
citrus (grapefruit, lime, orange)	pear
clove	petrol
coconut	tropical fruit
coffee	vinegar
cotton candy	vinyl/plastic
dried fruit/raisin	walnut
earthy/green	wood
floral	other
fruity	oops

Table 2.3 Combined list of the top 15 aroma compounds determined through CharmAnalysis for Vidal and Riesling icewine (IW) and table wines (TW) sorted by linear retention index (LRI) converted to odour spectrum values (OSV) for comparison indicating odour perception and identification. No value indicates compound was not detected in the top 15 by that judge for that wine.

		VIDAL				Riesling				Odour	
NO	LRI	IW 1	IW 2	TW 1	TW 2	IW 1	IW 2	TW 1	TW 2	Perception	Compound
1	745			7	3			22		fruity	Ethyl isobutyrate
2	782			9	3			6	15	fruity	Ethyl butyrate
3	832			100	8					fruity	Ethyl 2-methylbutryrate
4	843	21	8		2	15	7	52	22	fruity, tropical	Ethyl 3-methylbutyrate
5	852	99	39		6	71	15	68	32	bread/yeast	1-hexanol
6	859			5						banana	Isoamyl acetate
7	905	3								coffee	Ethyl valerate
8	957	78								vinyl/plastic	1-heptanol
9	963	67	30	11	5	4	6	35		mushroom	1-octen-3-ol
10	981	44		29	6	1	6	49	16	tropical fruity	Ethyl hexanoate
11	1018			12						plastic, musty	Unknown 1018
12	1027			22						wood	Unknown 1027
13	1050	9	11				10			caramel	Acetophenone
14	1063	2	100	23	31	1		21	89	mushroom, musty	1-octanol
15	1090	39	6				5			fruity, floral	Linalool
16	1096	24	9		10	18	9		17	citrus, floral	*Cis*-rose oxide
17	1104		6		3	2	6	4		floral	Phenethyl alcohol
18	1127			43	13	2		47	12	wood	Nerol oxide
19	1165		60				5		6	floral, yeast	Ethyl benzoate
20	1176			22				16		green, citrus	Ethyl octanoate
21	1184	100	87	78	100	100	51	98	100	petrol	Decanal
22	1228	3	15			7		4	18	caramel	Ethyl phenylacetate
23	1240					2				floral	2-phenethyl acetate
24	1300	21	14	33	7			37	6	clove	4-vinylguaiacol
25	1350		8				4		7	coconut	γ- nonalactone
26	1372	85	84	58	63	59	100	100	59	pear	β-damascenone
27	1444		37	57				29	9	floral	Geranyl acetone
28	1455						5			fruity	Ethyl cinnamate
29	1481					1				floral	β-ionone
30	1658						2			floral	Unknown 1658
31	1722	44			1				6	black pepper	Unknown 1722
32	1761						4			floral	Unknown 1761

Table 2.4 Concentration and odour activity values of Vidal blanc (A) and Riesling (B) table wines and icewines, compounds in bold indicate found above its sensory threshold (OAV>1).

Compound	VIDAL					RIESLING				
	conc. (μg/L)		OAV			conc. (μg/L)		OAV		
	TW	IW	TW	IW	Significance	TW	IW	TW	IW	Significance
ethyl isobutyrate	72.3	71.3	**4.82**	**4.75**	**ns**	189	189	**12.6**	**12.6**	**ns**
ethyl butyrate	162	78.6	**8.09**	**3.93**	***	137	137	**6.87**	**6.86**	**ns**
ethyl 2-methylbutyrate	14.2	27.4	0.79	**1.52**	**					
ethyl 3-methylbutyrate	25.3	29.9	**8.43**	**9.97**	**	24.1	23.8	**8.03**	**7.92**	**ns**
1-hexanol	1707	1513	0.21	0.19	**ns**	727	773	0.09	0.10	**ns**
isoamyl acetate	205	173	**6.83**	**5.77**	*					
ethyl valerate	2.06	9.70	**1.38**	**6.47**	**					
1-heptanol	7.94	28.6	**2.65**	**9.55**	**					
1-octen-3-ol	6.97	188	**6.97**	**188**	***	4.58	18.5	**4.59**	**18.5**	*
ethyl hexanoate	878	480	**62.7**	**34.3**	**	461	381	**32.9**	**27.2**	*
acetophenone	1.84	1.50	0.03	0.02	**	1.94	1.93	0.03	0.03	**ns**
1-octanol	9.34	10.6	0.09	0.10	**	Nd	4.78		0.04	***
linalool	12.8	46.9	0.51	**1.88**	**	34.3	50.3	**1.37**	**2.01**	***
cis rose oxide	5.03	22.2	**25.2**	**111**	**	0.63	2.24	**3.16**	**11.2**	**
phenethyl alcohol	15274	20140	**1.09**	**1.44**	**	16102	16853	**1.15**	**1.20**	*
nerol oxide	6.98	25.8	0.002	0.01	**	18.3	30.9	0.01	0.01	**
ethyl benzoate	0.65	3.36	0.001	0.01	**	0.95	1.19	0.002	0.002	*
ethyl octanoate	2665	739	**533**	**148**	***	1025	441	**205**	**88.3**	**
decanal	13.7	0.95	**6.84**	0.48	***	1.59	0.34	0.80	0.17	**
ethyl phenylacetate	4.29	10.0	0.06	0.14	**	7.14	8.69	0.10	0.12	**
2-phenethyl acetate						4.79	9.21	0.02	0.04	***
p-vinylguaicol	111	81.2	**11.1**	**8.12**	**	79.1	82.4	**7.91**	**8.24**	**
γ- nonalactone	2.78	8.02	0.09	0.27	***	15.4	17.9	0.51	0.60	***
β-damascenone	11.3	45.1	**225**	**902**	***	3.89	9.30	**77.8**	**186**	**
geranyl acetone	0.26	0.30	0.004	0.01	**	0.33	0.23	0.01	0.004	*
ethyl cinnamate						4.71	5.99	**4.28**	**5.45**	*
β-ionone						0.03	0.06	0.33	0.71	*

Significance was determined by t-test between wine styles with ns, *, **, *** indicating not significant, significance at $p<0.05$, 0.01, 0.001 respectively.

Table 2.5 Odour perception and sensory threshold (μg/L) of aroma compounds used for compound identification and calculation of odour activity values (OAV).

Compound	Odour Perception	Odour threshold μg/L	Reference
Ethyl isobutyrate	sweet	15	3
Ethyl butyrate	apple	20	4
Ethyl 2-methylbutryrate	apple	18	3
Ethyl 3-methylbutyrate	fruit	3	3
1-hexanol	green, resin	8000	4
Isoamyl acetate	banana	30	4
Ethyl valerate	fruity	1.5	7
1-heptanol	nutty, green	3	2
1-octen-3-ol	mushroom	1	1
Ethyl hexanoate	apple, fruit	14	3
Acetophenone	flower, almond	65	1
1-octanol	chemical, burnt	110	1
Linalool	floral	25	3
Cis-rose oxide	lychee, rose	0.2	4
Phenethyl alcohol	honey, spice	14000	3
Nerol oxide	oil, flower	3000	6
Ethyl benzoate	floral, fruit	575	3
Ethyl octanoate	fruity	5	3
Decanal	soap, tallow	2	2
Ethyl phenylacetate	fruit, sweet	73	8
2-phenethyl acetate	rose, honey	250	4
4-vinylguaiacol	clove, curry	10	3
γ– nonalactone	coconut,peach	30	5
β- damascenone	apple, rose	0.05	4
Geranyl acetone	magnolia, green	60	1
Ethyl cinnamate	honey, spice	1.1	3
β– ionone	floral (violet)	0.09	3

1. Buttery et al. (1988)
2. Fazzalari et al. (1978)
3. Ferreira et al. (2000)
4. Guth (1997)
5. Nakamura et al. (1988)
6. Rapp (1990)
7. Takeoka et al. (1995)
8. Tat et al. (2007)

Chapter 3
The Effect of Harvest Date on Vidal blanc and Riesling Icewine from the Niagara Peninsula: I. Chemical Variables and Aroma Compounds

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Abstract

Icewine is a sweet dessert wine made from pressing grapes naturally frozen on the vines. In Ontario, the Vintners' Quality Alliance regulates icewine production and specifies that harvest must be after 15 November. We hypothesized that the freeze and thaw cycles endured by icewine grapes would change their chemical and sensory profiles due to climatic events. The objective of this study was to determine the influence of harvest date on icewine must and wine chemical variables and aroma compound profiles. Riesling and Vidal icewines were made from grapes picked between December 2004 and February 2005; Harvest 1 (H1): 19 December; Harvest 2 (H2): 29 December; Harvest 3 (H3): 18 January; Harvest 4 (H4): 11 February (Vidal only). Must analysis found icewines to differ for titratable acidity in both cultivars and for pH in Vidal icewines. Wine analysis found all attributes to differ. All aroma compounds differed ($p<0.05$) in Vidal and Riesling wines. The highest concentrations for most aroma compounds were found in the latest harvest date, 16 of 24 for Vidal (H4) and 17 of 23 for Riesling (H3). The latest harvest date had the highest concentrations of ethyl isobutyrate, ethyl 3-methylbutyrate, 1-hexanol, 1-octen-3-ol, 1-octanol, *cis*-rose oxide, nerol oxide, ethyl benzoate, ethyl phenylacetate, γ-nonalactone and β-damascenone in both Vidal and Riesling. H1 for both cultivars had the highest concentration for ethyl butyrate, ethyl hexanoate, linalool, 4-vinylguaiacol and ethyl octanoate. Odour activity values were calculated; the most odour-potent compounds were β-damascenone, *cis*-rose oxide, 1-octen-3-ol, ethyl octanoate, ethyl hexanoate, and 4-vinylguaiacol in both cultivars across harvest dates. Principal component analysis found most attributes with the last harvest date, with the exception of 4-vinylguaicol which was associated with H1. Harvest date was identified as a discriminating dimension using canonical variant analysis in Vidal and Riesling for volatile compounds.

Key words: gas chromatography-mass spectrometry, wine aroma, odour activity values

Introduction

Icewine is a sweet late harvest dessert wine produced from pressing grapes that have naturally frozen on the vine, leaving water behind as ice crystals. Frozen grapes are harvested and pressed at -8 °C or colder typically between December and January. The resultant must is concentrated in sugar, acids, and aroma and flavour compounds, giving

the wine its rich, balanced and intense flavour profile. Icewine is produced in many countries around the world at the northern limits of grapegrowing where winter conditions are cold enough to allow the grapes to freeze on the vine before harvest. Canada and Germany are the world's largest producer of icewine with other countries including Austria, United States, Slovenia, Luxembourg, Croatia, the Czech Republic and Hungary also producing icewines (Schreiner 2001).

Icewine is made in all wine production regions of Canada from Nova Scotia on the east coast to British Columbia on the west coast. However, the bulk of icewine production is in Ontario, principally the Niagara Peninsula, where warm summers and cold winters allow for optimal conditions to grow and harvest grapes for icewine. In Ontario, Canada icewine production is strictly regulated by the Vintners' Quality Alliance (VQA) (Government of Ontario 1999). In the VQA Act, the term Icewine is trademark protected and can only be used for wines made from grapes naturally frozen in the vineyard in specified viticultural areas, at temperatures $\leq$ -8 ^{o}C after the 15 November of the vintage year. The grapes must be harvested and pressed at these same temperatures. The minimum allowed soluble solids concentration of the must is 35 ^{o}Brix with no single pressing being less than 32 ^{o}Brix. The finished wine is required to have a residual sugar concentration of not less than 125 g/L and a titratable acidity of not less than 6.5g/L (expressed as tartaric acid). Other styles of dessert wines can be made from late harvest grapes with must concentrations less than 35 ^{o}Brix and would be labelled; late harvest, select late harvest, and special select late harvest wines with a minimum soluble solids concentrations of 22, 26, 30 ^{o}Brix respectively.

The main cultivars for icewine production in Ontario are Vidal blanc (syn. Vidal) and Riesling. Vidal is a white French-American hybrid consisting of 75% *V. vinifera* genetic background, from a cross between Ugni blanc and Rayon d'Or (Seibel 4986) (Galet 1998). A key viticultural feature is its winter hardiness. The cultivar has large cylindrical clusters, with medium-sized, thick-skinned berries that are disease resistant (Galet 1998). It is a high acid cultivar prone to overcropping, which enables it to produce large yields for icewine production. However, it is Riesling that is considered by many to be the best choice of cultivar for producing icewines of the highest quality and ageability due to its high natural acidity. It is the noble grape of Germany, known for producing a wide range of wine styles from bone dry to ultra sweet, both clean and Botrytis-affected. Riesling has all the characteristics of the ideal icewine grape; it is late maturing, high acid, thick skinned providing some disease resistance and winter hardiness (for *V. vinifera*).

The production of icewine is a very expensive, labor intensive and risky undertaking. Grapes are left hanging on the vine long past commercial harvest, which, results in the loss of yield from dehydration, rot, wind, as well as animal predation. The grapes destined for icewine must be netted to protect them from predators and to prevent loss from clusters falling on the ground. Since the water is left behind as ice crystals, icewine grapes yield only 15 to 20% that of table wine requiring substantially more acreage for the same yield (Pickering 2006). There is also always the risk that the grapes will rot before the temperatures are cold enough for an icewine harvest or that a mild winter will prevent icewine production all together.

All of the above conditions combined make the choice of when to harvest the grapes perhaps the most important harvest decision. The single most important variable that determines the °Brix value of the resultant icewine juice is harvest temperature. Icewine is one of the only wine styles for which the soluble solids concentration at maturity is irrelevant, because it is the temperature that is critical to freezing the water inside the berry and concentrating all its components. As "hang time" increases for late harvest wines, desiccation of the fruit causes the berry to shrivel concentrating the acid and sugars in the berry. As a result, colder temperatures are required to freeze the berry and achieve the desired soluble solids concentration in the must later in the season and thus reducing yields.

Higher yields of icewine juice are usually obtained in December since there is less desiccation of the fruit, and the air temperature does not have to be as cold to achieve the required 35 °Brix value. It is believed anecdotally that the freeze and thaw cycles are critical to developing the sensory profile for which icewines are known. As a result, some producers in Ontario will have several icewine harvests from mid- December to late January in order to achieve a balance between flavour profile and yield. Currently, very little is known regarding what effects the sensory properties of icewines and none regarding the effect of harvest date on icewine chemical and sensory properties.

The oxidation of berries from freeze and thaw cycles is analogous to wine aging, which is an oxidative process (Boulton et al. 1995). Therefore it is plausible to consider chemicals and their reactions associated with white wine aging to be present in grapes used for icewine. Riesling wines have been shown to develop from a light straw colour to a deep yellow with age along with changes to its sensory profile (Simpson and Miller

1983). Studies into white wine aging have shown that over time there is a decrease aroma compounds such as linalool and geraniol but an increase in linalool oxides, nerol oxides and hotrienol in Riesling and Vidal wines (Chisholm et al. 1994, Reynolds et al. 1994a). β-damascenone also decreases with bottle age, whereas, other compounds, such as furfural and TDN, increase with age.

A study looking at the aging effects on Vidal table wine found that in young wines aromas of apple, citrus, fruity and floral were the predominant descriptors and aged wines were described by a loss of fruity character, and an increase in pungent, oxidized and vegetal aromas by a sensory panel (Chisholm et al. 1994). In contrast GC-O on the same wines identified the dominant aromas in both young and aged wines to be fruity and floral however found a marked decrease in the concentration of these aromas in the aged wines. The terpene alcohols are oxidized during storage to terpenes oxides which have higher sensory threshold and therefore are not perceived in the older wines (Rapp et al. 1985).

In other wine styles the effects of varying harvest date have been studied and have shown differences in aroma compound and sensory profiles. Fenoll et al., (2009) used GC-MS to monitor changes in free and bound volatile compound concentration in Muscat Hamburg grapes during maturation over two vintages. In both years, geraniol was the most abundant free volatile compound at the initial stage of maturations, with its concentration decreasing throughout ripening. Whereas, the concentrations of linalool of other free form volatiles increased during ripening. At grape maturity, most volatile compounds showed higher concentration in the bound form compared to the free form, except for linalool and linalool oxides where the reverse was true. The OAVs for each

free form volatile were calculated but only linalool, *cis*-rose oxide, citral and geraniol were found to contribute the aroma of Muscat Hamburg grapes.

The effect of hang time on odour-active volatile compounds in Fernão-Pires grapes from northern Portugal were followed from veraison (onset of ripening) for five weeks and several variety- and pre-fermentation-related volatile compounds were screened to determine optimal harvest parameters (Coelho et al. 2007). All screened volatile compounds--16 terpenes, two C_{13} norisoprenoids, five C_6 compounds and two aromatic alcohols, increased in concentration to a maximum at day 20 after veraison. After day 20, the concentration of all volatiles decreased. They concluded that while peak volatile composition occurred with the white grapes maturity (ratio of sugar/acidity), it was a short window before concentration began to decrease for varietal and pre-fermentation volatiles.

These studies highlight some of the potential changes icewine grapes could be experiencing while "hanging" on the vine until cold enough temperatures to harvest. This study was designed to elucidate changes in odour-active volatile compounds based on four distinct harvest dates throughout the commercial icewine harvest period of December to February. The objectives of this study were to determine how Vidal and Riesling icewines made from four distinct harvest dates would affect the: 1) Standard chemical variables and; 2) aroma compound concentrations.

Materials and Methods

Chemicals. Analytical standards (Table 3.1) were purchased from Aldrich (Oakville, ON, Canada), Sigma-Aldrich (Oakville, ON), Fluka (Oakville, ON),

Bedoukian (Danbury, CT, USA), Acros organic (Geel, Belgium). β-Damascenone was a gift from Dr. T. Acree, Cornell University. Chemical standards were diluted in dichloromethane (Caledon; Georgetown, ON) and stored at -25°C.

Wines. Riesling and Vidal icewines were made from grapes harvested from Garphil Farms in west St. Catharines, enough to fill 100 bins (≈15 kg capacity) each over the four harvest dates (25 bins per harvest date). Grapes were harvested over the course of the icewine season. The harvests were picked as follows: Harvest 1 (H1) on 19 December 2004 at -10°C; harvest 2 (H2) 29 December 2004 at -1°C; harvest 3 (H3) on 18 January 2005 at -16°C; harvest 4 (H4) on 11 February 2005 at -4°C. There were only three harvest dates for Riesling due to bird predation following H3.

Grapes were pressed by variety and harvest date in the large membrane press (660kg capactity; Enoveneta PP12, Padova, Italy) at two bars until the must measured approximately 37 °Brix. The exact starting °Brix was measured on each pressing (harvest date), the must was then divided into three 20-L carboys for triplicate fermentations.

Fermentation. The must was inoculated with Lalvin® K1-V1116 *Saccharomyces cerevisiae* (Lallemand) as per the yeast rehydration procedure of Kontkanen et al. (2004) into 20-L carboys. The fermenting must was left at room temperature overnight and was then placed in an 18 °C fermentation chamber. Fermentation was stopped by addition of 75 mg/L potassium metabisulphite (Sigma, Oakville, ON) when the ethanol, determined by GC-FID (Agilent Technologies, Mississauga, ON), was 10 % v/v, after which the carboys were moved to the -2 °C chamber for cold stabilization. The icewines were left to settle for up to 2 weeks then racked into clean carboys to remove the lees.

Bottling. At bottling, icewines were brought to room temperature, 100 mg/L of potassium metabisulphite (Sigma, Oakville, ON) and 100 mg/L potassium sorbate (Sigma, Oakville, ON) were added to the wines. A 250-mL wine sample was taken and frozen at -25°C for future wine analysis. The icewine was filtered through a 1 µ pad filter (Scott Laboratories, Pickering, ON) and 0.45 µ membrane filter (Millipore, Bedford, MA). It was then bottled in 375-mL bottles, corked (Scott Laboratories, Pickering, ON) and put in the wine cellar for storage at 12 °C until analysis.

Must and wine analysis. Must samples were removed from a -25 °C freezer and thawed overnight at 4°C, samples were then placed in an 80 °C Isotemp 228 water bath (Fisher Scientific, Toronto, ON) for 1 hr to dissolve precipitated solids and allowed to cool to room temperature. A 25-mL sample was measured for titratable acidity (TA) to a pH endpoint with 0.1% NaOH using the PC titrate autotitrator (Man-Tech Associates Ltd., Guelph, ON). A Fisher 825 MP pH meter was used to measure must pH (Fisher Scientific, Ottawa, ON). °Brix was measured using a temperature-corrected Abbé benchtop refractometer (model 10450; American Optical Corp., Buffalo, NY).

Finished wines were measured for TA and pH using the same method described above for must analysis. Absorbance at 420nm was measured to determine degree of browning of the wines on an Ultrospec 2100 Pro UV/Visible spectrophotometer (Biochrom Ltd., Cambridge, England). Acetic acid (K-ACET, Megazyme, Bray, Ireland), glycerol (K-GCROL, Megazyme, Bray, Ireland) and glucose-fructose (K-FRUGL, Megazyme, Bray, Ireland) concentrations were determined based on the manufacture directions of the Megazyme enzyme kits. Ethanol (% v/v) was determined

with an Agilent 6890 GC-FID according the method of Nurgel et al. (2004). All analyses were conducted in duplicate for each fermentation replicate.

Volatile extraction. Wine volatiles were extracted by stir bar sorptive extraction (SBSE), commercially known as Twister, using 10-mm stir bar (Gerstel, Baltimore, MD) coated with polydimethylsiloxane (PDMS, 0.5 mm film thickness). A 10-mL sample of icewine was poured into a 10 mL extraction vial and spiked with an internal standard, 100 µg/L *n*-dodecanol (Sigma; Oakville, ON) in GC-grade dichloromethane. The stir bar was added to the wine and extracted for 60 minutes at 1000 rpm. The stir bar was removed from the extraction vial, dried with a lint free tissue, rinsed with Milli-Q water (Millipore, Bedford, MA) and stored in a 4-mL amber vial at 4 °C until analysis later the same day.

Gas chromatography-mass spectrometry (GC-MS). Instrument: Agilent 6890N/5975B gas chromatograph mass spectrometer equipped with a Gerstel thermal desorption unit (TDS2), cooled injection system (CIS4) and programmable temperature vaporization (PTV) inlet (Gerstel, Baltimore, MD). Analytical column: Agilent HP-5MS, 5% phenyl methyl siloxane, 30m length, 0.25 mm internal diameter and 0.25 µm film thickness. Carrier gas: 1.4 mL/min 5.0 purity helium (Praxair, Mississauga, ON). Oven program: initial temperature 35°C held for 3 min, increased by 4°C/min to 155°C, increased by 30°C/min to a final temperature 240°C. Thermal desorption: initial temperature 30°C, increased by 60°C/sec to 250°C and held for 3min. TDS transfer line temperature 275 °C connected to CIS4 inlet cryo-cooled to -70°C with liquid nitrogen in solvent vent mode. After desorption, CIS4 inlet temperature was increased at 12°C/sec to 280 °C and held for 5 min while analytes are released on the column. The MS was run in

scan mode, 30 to 400 Da for compound identification and in select ion monitoring (SIM) mode selecting for one quantitative ion and three qualitative ions for each compounds for quantification (Table 2.1).

Identification and quantification. The compounds were identified as the 'top 15' odour-potent volatiles by GC-O CharmAnalysis™ (Bowen and Reynolds 2010c) and are listed in Table 3.1. The compounds were identified by comparison of retention time, odour perception and mass spectra (Wiley library) to pure standards. Three-point calibration curves were run for each analyte in model icewine solution to ensure linearity ($r^2 > 0.9$) (Table 2.1). The icewine model wine solution contained 11.57 g/L tartaric acid (EMD Chemical Inc., Darmstadt, Germany), 153 g/L fructose (Caledon; Georgetown, ON), 11% (v/v) ethanol (Commercial Alcohols Inc.; Brampton, ON), with a pH 3.61. Standard curve concentrations and compounds were quantified based on the ratio of the peak area of the compound relative to the peak area of the internal standard to determine the concentration of the analytes. Analysis was run in duplicate and relative standard deviation between replicates was determined. Odour activity values (OAV) for the compounds in each wine were calculated by dividing the concentration by its sensory threshold, a value greater than one indicated the compound contributed to the aroma of the wine.

Statistical analysis. All statistical analysis was performed using XLSTAT (Addinsoft, Paris, France) statistical software. To determine if differences exist between harvest dates for must and wine chemical variables a two factor (treatment x rep) analysis of variance (ANOVA) was performed. Least significant difference (LSD) values were determined for significant attributes ($p < 0.05$). A three factor ANOVA (harvest date x

fermentation rep x GC rep) with two-way interactions was used to determine if differences existed between aroma compounds according to harvest date ($p < 0.05$). Mean scores and LSD were calculated for aroma compounds that differed by harvest date.

The mean concentration of the aroma compounds was analyzed by principal components analysis (PCA) for each fermentation rep using the correlation matrices to determine the compounds which best describe the variation in the harvest dates. Next, canonical variant analysis (CVA) using a stepwise forward model ($p < 0.05$) was used to determine if icewines from different harvest dates could be differentiated based on difference in the aroma compounds. Only those attributes found to differ in concentration by harvest date were used in the PCA and CVA.

Results

Must chemical variables. Chemical analysis completed on the must found there to be significant differences ($\alpha \leq 0.05$) between harvest dates for both Riesling and Vidal icewines, shown in Table 3.2. Riesling icewine musts were different in terms of TA. H1 had a higher TA (8.45 g/L) than either H2 (7.99 g/L) or H3 (7.21 g/L). Vidal icewines showed a similar pattern with TA, with H1 having the highest TA of 11.21 g/L and TA decreasing with each subsequent HD resulting in H4 having the lowest TA of 9.48 g/L. pH was also different for the Vidal icewines. H3 had higher pH (3.55) than either H1 and H4 (pH values of 3.47 and 3.48, respectively) or H2 (pH = 3.44).

Wine chemical variables. Differences were found between harvest dates for both Riesling and Vidal icewines for all attributes (Table 3.3). H2 icewines had higher A420 values for Vidal (0.615) and Riesling (0.645) than the other harvest dates icewines. Glycerol concentrations increased in icewines from both cultivars throughout the season. In Riesling icewines, the concentration increased from 9.99 g/L (H1) to 12.45 g/L (H3) and in Vidal icewines from 10.71 g/L (H1) to 15.20 g/L (H4).

Aroma compounds. ***Analysis of variance.*** All aroma compounds differed with respect to harvest date in both Vidal and Riesling icewines (Table 3.4). In Vidal icewines 16 of 24 aroma compounds were found to have the highest concentration in H4, while only six compounds had the highest concentration in H1. The compounds with the highest concentration in H1 were predominantly esters: ethyl butyrate, ethyl 3-methylbutyrate, isoamyl acetate, ethyl hexanoate, and ethyl octanoate. Linalool and 4-vinylguaiacol also had the highest concentration in the earliest harvest dates. H3 had the lowest concentrations for 14 compounds. The lowest concentration for 18 of the compounds was found in H2 and H3 (Table 3.4).

A similar trend was seen in Riesling icewines where 17 of 23 aroma compounds had the highest concentrations in H3 and only seven compounds had the highest concentrations in H1 (Table 3.4). The compounds in Riesling icewine with the highest concentration in H1--ethyl butryrate, ethyl hexanoate, ethyl octanoate, 4-vinylguaiacol and ethyl cinnamate--had the lowest concentrations in H3.

Both Vidal and Riesling H1 icewines had the highest concentrations of ethyl butyrate, ethyl hexanoate, linalool, 4-vinylguaiacol and ethyl octanoate, and the lowest concentrations of 1-octen-3-ol, *cis*-rose oxide and nerol oxide. The later harvest dates

[H4 (Vidal) and H3 (Riesling)] had the highest concentrations of ethyl isobutyrate, ethyl 3-methylbutyrate, 1-hexanol, 1-octen-3-ol, 1-octanol, *cis*-rose oxide, nerol oxide, ethyl benzoate, ethyl phenylacetate, γ-nonalactone, and β-damascenone.

All concentration values were converted to odour activity values (OAV) to determine which the aroma compounds were the most odour-potent in Vidal and Riesling icewines picked at different harvest dates (Table 3.6). In Vidal icewines; H4 had the most odour-potent compounds (OAV > 1) with 16 aroma compounds found above sensory threshold concentrations, followed by H1 and H2, with 14 compounds each above threshold, and H3 with 13 compounds above threshold. The most odour-potent compounds across all Vidal icewines were β-damascenone, *cis*-rose oxide, 1-octen-3-ol, ethyl octanoate, ethyl hexanoate, ethyl 3-methylbutyrate, isoamyl acetate and 4-vinylguaiacol. β-damascenone was the most potent compound in all four harvest dates.

In Riesling icewine; 12 aroma compounds were found above the sensory threshold (OAV>1) in all three harvest dates. Similar to Vidal icewines, β-damascenone was the most potent aroma compound in Riesling. Other highly odour-potent compounds in Riesling harvest date wines were ethyl octanoate, *cis*-rose oxide, ethyl hexanoate, ethyl isobutyrate and 4-vinylguaiacol.

Vidal icewines, in general, had more odour-potent compounds (OAV > 1) and the OAVs were higher than Riesling icewines, especially for the most odour-potent compounds. The highest OAVs in Vidal and Riesling, respectively, were: β-damascenone [1175 (H4) and 222 (H3)]; *cis*-rose oxide [247 (H4) and 48 (H3)]; 1-octen-3-ol [229 (H4) and 43 (H3)]; and ethyl octanoate [246 (H1) and 221 (H1)].

Principal components analysis. PCA was performed first on the all aroma compounds since they were all different according to harvest date ($p<0.05$), and second on only those aroma compounds found above their sensory threshold (OAV>1). The PCA of all the aroma compounds for Vidal icewines showed that the first two factors (F1 and F2) explained 85.7% of the variation in the data and were retained; all but two compounds were heavily loaded on F1 and F2 (Figure 3.1). The exceptions were acetophenone, which was heavily loaded on F3, and decanal, which was heavily loaded on F4. Fifteen compounds were associated with F1, which explained 60.9% of the variation; with the exception of geranyl acetone, all compounds were positively loaded. Four compounds, isoamyl acetate, ethyl hexanoate, *cis*-rose oxide, and 4-vinylguaiacol, were associated with F2, which explained 24.8% of the variation. Three compounds were equally loaded on F1 and F2, ethyl butyrate, linalool and ethyl octanoate. F1 separated H1 from H4 with the other two harvest dates. 4-Vinylguaiacol, which was heavily loaded on F2, was associated with H1 and negatively related to H4. In general most attributes were associated with H4 and not with H2 and H3.

The PCA of the OAV for Vidal icewines explained 87.5 % of the variation on F1 and F2 and they were retained (Figure 3.2). In general, a similar loading pattern was observed with all attributes positively loaded on F1 and associated with H4. *Cis*-rose oxide, ethyl valerate, 1-octen-3-ol, decanal, 1-heptanol, ethyl isobutyrate, β-damascenone, and ethyl 2-methylbutyrate were all found positively loaded on F1 and F2 and associated with H4. *Cis*-rose oxide, ethyl valerate, 1-octen-3-ol, decanal, 1-heptanol were also inversely associated with H1. Phenethyl alcohol, ethyl 3-methylbutyrate, ethyl butyrate, ethyl octanoate, ethyl hexanoate and isoamyl acetate were all positively loaded

on F1 and negatively loaded with F2 and inversely associated with H2 and H3. 4-Vinylguaiacol was again associated with H1, the first harvest date.

The first two factors were retained, which described 89.3% of the variation in the Riesling harvest date PCA for all aroma compounds (Figure 3.3). Seventeen of the 23 compounds were heavily loaded on F1, only ethyl butyrate and geranyl acetone were negatively loaded all other were positively loaded. Ethyl hexanoate, ethyl octanoate, 4-vinylguaiacol, and ethyl cinnamate were heavily loaded on F2. These four compounds were inversely associated with H3. Ethyl isobutyrate and β-ionone were equally loaded on F1 and F2. β-Damascenone and 1-octen-3-ol were associated with H3 and inversely related to H1 and H2. In general, the positively loaded aroma compounds on F1 were not to be inversely associated with H1 and H2, such as β-damascenone, 1-octen-3-ol, *cis*-rose oxide, γ-nonalactone, β-ionone, ethyl 3-methylbutyrate, ethyl benzoate, ethyl phenylacetate, nerol oxide, and acetophenone.

The PCA showing the variation in the data based on OAV explains 90.8% of the variability on F1 and F2 (Figure 3.4). Factor 1 explained 54.1% of the variation and was described by ethyl butyrate, ethyl 3-methylbutyrate, 1-octen-3-ol, linalool, *cis*-rose oxide, phenethyl alcohol and β-damascenone. Most of these compounds were inversely correlated with H1 and H2. Factor 2 explained 36.7% of the variation and was described by ethyl hexanoate and ethyl octanoate, 4-vinylguaiacol, and ethyl cinnamate and was associated with H1 and inversely associated with H3.

Canonical variant analysis (CVA) was the performed on the Vidal and Riesling harvest date icewines to determine if the variation in the aroma compounds seen in PCA could be attributed to the harvest dates being different in the aroma composition. Harvest

date was found to differ in both cultivars ($p<0.05$). CVA on Vidal icewine showed H1 and H2 to be different from each other and H3 and H4; however, H3 and H4 were not different from one another (Figure 3.5). In Riesling icewine, all harvest dates differed from one another, with clear separation between H1, H2 and H3 (Figure 3.6). The same attributes found associated with the various harvest dates by PCA were used to discriminate the wine in CVA.

Discussion

Must and wine chemical analysis. During the typical grape maturation periods TA is found to decrease with an increase in sugar concentration during ripening (Winkler et al. 1974). The higher initial TA of icewines is likely the result of concentration from pressing frozen grapes, which also concentrate sugars, aroma and flavour compounds (Pickering 2006). The decrease in TA from early to later harvest dates in must is likely due to the acids precipitating out of the juice through freeze and thaw cycles while hanging on the vine. Since icewine grapes and must are not warmed up prior to pressing and fermentation the acids are not able to go back into solution and the results in reduced acids in the later harvest dates grapes.

Glycerol concentration in the finished icewine was found to increase with harvest date, with its highest concentration in the latest harvest date for Vidal and Riesling icewines. This is not a surprising results as many factors including grape ripeness and the microbial floral on the berry have been shown to increase glycerol concentrations (Scanes et al. 1998). Glycerol in wine is produced during yeast fermentation and also by infection from the fungus *Botrytis cinerea.* In dry table wines typical glycerol concentrations have

been reported from 4-10g/L and as high as 20g/L in late harvest botrytis affected wine styles (Ribéreau-Gayon et al. 2000). Increased glycerol concentrations are thought to contribute to the viscosity or mouthfeel of a wine, however it has been shown that a glycerol concentration of 26 g/L is required in order to detect an increase in perceived viscosity (Noble and Bursick 1984). This concentration is double that found in the experimental and commercial icewines. Therefore it cannot be said that the glycerol concentration is contributing to its mouthfeel or weight, this is in agreement with other studies and the effect of glycerol on wine mouthfeel (Nurgel and Pickering 2005). The increased glycerol concentrations in icewine is due to a stress response related to the hyperosmostic stress put on the yeast during icewine fermentation. During icewine fermentations, glycerol-3-phosphate encoded by *GPD1* that is upregulated to produce higher glycerol concentrations to balance the external osmotic pressure of the cell (Pigeau and Inglis 2007). Increased glycerol concentration with later harvest date is likely due to higher infection rates by *B. cinerea* and concentration effect due to desiccation of the berries.

Volatile analysis.

Effect of harvest date. In general, ethyl esters had the highest concentrations in the earliest harvest date (H1) in Vidal and Riesling icewines (Tables 3.4 and 3.5). An explanation for the decrease in ethyl ester concentrations with later harvest dates is probably related to the decrease in concentration of acids in the icewine must with later harvest dates (Table 3.3). If the TA is decreasing with harvest date, it would be expected that all other acids would also be decreasing resulting in less acid present to form the ethyl esters.

Another explanation for the lower concentration of esters with later harvest date could be related to free amino acid concentrations, which through the Ehrlich pathway make fusel alcohols to produce esters such as isoamyl acetate. Overripe grapes have been shown to have higher concentration of free amino acids, thereby reducing the requirement of yeast to produce higher alcohols (Kliewer 1968). A study investigating the sensory properties of sweet Fiano wines found base wines harvested at normal maturity (22 °Brix) had higher concentrations of fusel alcohols, esters, acetates and fatty acids than sweet wines harvested at late maturity (26 °Brix) and dried to 32 °Brix; the difference in concentration was related to yeast metabolic activity in mature and overripe grapes (Genovese et al. 2007). This is consistent with the results found by Bowen and Reynolds (2010), whereby table wine had higher concentrations of esters than icewines. Freeze and thaw cycles of the icewine grapes may be simulating a more extreme over-ripeness in the last harvest date, as cellular components have further broken down compared to H1.

Other compounds found to have the highest concentration in the first harvest were linalool and 4-vinylguaicol and decanal (Riesling only). Linalool is a monoterpene with a distinct floral aroma, it has been shown to increase in concentration until optimal maturity and then drop off (Marais and van Wyk 1986, Marais 1987). This would explain the higher concentration in the earlier harvest date if we think of the later harvest dates as being more over-ripe. Chemical reactions in the berries during freeze and thaw cycles on the vine may also contribute to this decrease in linalool with later harvest date, as it is converted to linalool oxides and alcohols.

4-Vinylguaiacol has the dual role of being considered both a important aroma contributor to wine and beer and an off-flavour depending on concentration and style of

beverage (Vanbeneden et al. 2008). It is a volatile phenol produced from the decarboxylation of ferulic acid during fermentation (Shinohara et al. 2000) characterized by an aroma of clove and a low odour threshold of 10ug/L (Ferreira et al. 2000). Ferulic acid is a hydrocinnamic acid, the main polyphenol found in white wines (Singleton et al. 1985a), these are important for determining the browning potential of a wine. It has been shown that many commercial *S. cerevisiae* wine yeast, wild yeast and non-*S. cerevisiae* yeast possess the ability to produced volatile phenols, known as phenolic off flavour (POFs), of which 4-vinylguaicol is included due expression of the *PAD1+* gene (Shinohara et al. 2000).

The total hydrocinnamates in Riesling and Vidal icewines were shown to decrease with later harvest dates, likely due to the freeze and thaw cycles causing a decline in concentration in the grape (Kilmartin et al. 2007). This decline in total hydrocinnamates with later harvest date explains why the highest concentration of 4-vinylguaiacol is found in H1 for both Vidal and Riesling icewines (101 and 131 ug/L, respectively), with a subsequent decrease in concentration with each subsequent harvest date (73 ug/L Vidal H4, and 90 ug/L Riesling H3). The absorbance at 420 nm in the chemical analysis of the wines was not lower for the H1 compared to the later harvest dates for browning (Table 3.2). The best explanation for this is likely that measuring the spectral absorbance at 420 nm accounts for all brown pigments in the wine not just the hydrocinnamates (Iland et al. 2004).

The majority of compounds were found to have the highest concentration in the last harvest date, which may be due to chemical changes occurring due to freeze and thaw cycles and / or extended hang time or may be due to concentration effects from

dehydration and desiccation of the fruit (Table 3.3). The 'mushroom alcohol' 1-octen-3-ol, several terpenes, *cis*-rose oxide and nerol oxide, and norisoprenoids, β-damascenone and β-ionone, all had the highest concentrations in the last harvest dates and the lowest concentrations in H1.

1-Octen-3-ol is characterized by a mushroom aroma and has been shown to have elevated levels in wines produced from overripe grapes (Genovese et al. 2007), Botrytis affected wine styles such as Sauternes and Tokaji Aszú (Miklosy et al. 2004), and in wines made from rotten grapes (Darriet et al. 2002, La Guerche et al. 2006). It is a metabolic by-product of fungal infection of the grapes by *Botrytis cinerea* producing either noble or grey rot and by other fungal infections such as powdery mildew (La Guerche et al. 2006). The concentration of 1-octen-3-ol increased with later harvest dates in both Vidal and Riesling wines (Table 3.4 and 3.5), likely due to increased levels of fungal infection of the grapes as they hung on the vine from mid – December (H1) to the end of January (H3) and into the beginning of February (H4, Vidal only). The concentration of 1-octen-3-ol was higher in sweet Fiano wine made in a passito style compared to dry table wines (Genovese et al. 2007), which is in agreement with differences in concentration of this compound in table wines compared to icewines (Bowen and Reynolds 2010c). While the grapes for icewine production in general are made from clean fruit, there is the possibility of infection from grey rot and/or noble rot and powdery mildew during the growing season.

Nerol oxide is a monoterpene ether similar to *cis*-rose oxide, and is produced from linalool. Therefore, it seems intuitive that as the concentration of linalool decreased with harvest date the concentration of one of it degradation compounds would increase. Nerol

oxide is the result of hydrolysis of diendiol I during juice storage and processing and is not naturally present in Muscat of Alexandria grapes during berry development (Williams et al. 1980). Nerol oxide was not found to have a high OAV value or concentration in experimental icewines and therefore is not an odour-potent compound of interest.

Cis-rose oxide has a floral aroma with a low sensory threshold value (0.2 μg/L), it is an impact odorant important to the varietal aroma of Gewurztraminer wines (Guth 1997, Ong and Acree 1999). *Cis*-rose oxide was found to increase in concentration with the later harvest dates in both Vidal and Riesling icewines and had one of the highest OAVs in both cultivars (Table 3.6). It has recently been shown that *cis*-rose oxide can be produced from the stereoselective reduction of geraniol followed by allylic hydroxylation and acid catalyzed cyclization (Luan et al. 2006). It is the result of geraniol derived diols I and II which act as the precursors for *cis*- and *trans*-rose oxide formation after yeast fermentation (Koslitz et al. 2008).

Luan et al. (2005) showed the stereoselective reduction of labeled geraniol to citronellol to produce cis –rose oxide in *in vivo* feeding experiments with Scheurebe grape berry mesocarp. They found an increase of geraniol reductase activity toward the end of ripening, which can produce high levels of citronellol, a precursor to *cis*- rose oxide production. These findings are in agreement with previous conclusions that high concentrations of *cis*-rose oxide can be achieved by leaving fruit on the vine for extended periods (Wilson et al. 1984). The extended hang time of grape berries destined for icewine production are in line with these findings and explain a possible mechanism as to why the highest concentration of this potent odorant is found in the latest harvest dates for both Vidal and Riesling icewines.

Norisoprenoids are carotene breakdown products formed from enzymatic and non-enzymatic reactions either through direct degradation or glycosylated intermediates. There are three main steps the formation of norisoprenoids; initial cleavage, enzymatic transformation to the non-aromatic intermediate metabolite, and finally acid hydrolysis non-aromatic precursor to aroma compounds (Winterhalter et al. 1990). The formation of norisoprenoids has been extensively reviewed in the literature (Mendes-Pinto 2009).

The C_{13} norisoprenoid, β-damascenone, is characterized by a stewed apple, floral aroma and a low sensory thresholds (2 ng/L in water). It had the highest OAV values in all experimental icewines and was one of the most odour-potent compounds in commercial icewines analyzed through GCO (Bowen and Reynolds 2010). This is in agreement with previous studies that also found β-damascenone to be an important odorant in Riesling (Chisholm et al. 1994, Komes et al. 2006) and Vidal (Chisholm et al. 1995) table wines. The concentration of β-damascenone increased with later harvest dates in Vidal and Riesling icewines and Vidal icewines were found to have higher concentrations of β-damascenone compared to Riesling at all harvest dates (Tables 3.4 and 3.5).

High concentrations of β–damascenone have been reported in the literature in wines made from sun dried grapes (Campo et al. 2008) and over ripe grapes (Pons et al. 2008), which are conditions that may be somewhat analogous to the extended hang time of icewine grapes. Generally β-damascenone concentrations in wines range from 1 to 4 μg/L, while the compound has high OAV due to it low sensory threshold it is generally not considered an impact odorant but rather acts as an aroma enhancer to fruity esters in

the wine and has been shown to contribute to the aroma buffer of a wine (Escudero et al. 2007).

Campo et al. (2008) found a key odour compound in Pedro Ximenez wines to be β-damascenone, with average concentrations of 10 μg/L and reaching up to 21.7 μg/L in the wines (five times greater than average table wines). They suggested β-damascenone may be the compound responsible for the characteristic raisin like aroma found in these wines, but would need to be confirmed with reconstitution and omission tests. This is of particular interest to this research since icewines are often described as having a raisin like aroma and experimental wines were found to have very high concentrations of β-damascenone, ranging from 45 to 56 ug/L for Vidal (Table 3.4) and 7.8 to 11 ug/L in Riesling (Table 3.5). The concentration of β–damascenone in Vidal icewines was over double that found in Pedro Ximenez wines and Riesling icewine concentrations were similar to Pedro Ximenez wines suggested that β-damascenone is an important odorant in this wine style.

Conclusions

Chemical analysis showed differences due to harvest date on aroma compounds, with the majority of the aroma attributes having the highest concentration in later harvest dates for both Vidal and Riesling icewines. The highest concentration for most aroma compounds was found in the latest harvest date, 16 of 24 for Vidal (H4) and 17 of 23 for Riesling (H3). The latest harvest date had the highest concentrations of ethyl isobutyrate, ethyl 3-methylbutyrate, 1-hexanol, 1-octen-3-ol, 1-octanol, *cis*-rose oxide, nerol oxide, ethyl benzoate, ethyl phenylacetate, γ-nonalactone and β-damascenone in both Vidal and

Riesling. H1 for both cultivars had the highest concentrations for ethyl butyrate, ethyl hexanoate, linalool, 4-vinylguaiacol, and ethyl octanoate. Odour activity values were calculated; the most odour-potent compounds were β-damascenone, *cis*-rose oxide, 1-octen-3-ol, ethyl octanoate, ethyl hexanoate, and 4-vinylguaiacol in both cultivars across harvest dates. PCA found most attributes were associated with the last harvest date, with the exception of 4-vinylguaicol, which was associated with H1. Harvest date was identified as a discriminating dimension using canonical variant analysis in Vidal and Riesling for both sensory and volatile compounds. The high concentrations of β-damascenone in icewines required further investigation into its potential role as an impact odorant in this wine style, as does the elevated levels of glycerol in icewines with increased hang time. The concentration of both β-damascenone and glycerol could be used a marker compounds of icewine made from grapes naturally frozen on the vine, however further investigation would be required.

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Table 3.1 Chemical standards, quantitative and qualitative ions, and calibrated intervals for Vidal and Riesling icewines and table wines.

Analyte	Supplier	CAS no.	Quantitative ion (m/z)	Qualitative ions (m/z)	Calibrated interval ([µg/L]) Vidal	r^2 value	Riesling	r^2 value
ethyl isobutyrate	Aldrich	97-62-1	43	71, 88, 116	50-150	0.977	150-600	0.923
ethyl butyrate	Aldrich	105-54-4	71	43, 88, 116	20-180	0.962	125-500	0.915
ethyl 2-methylbutyrate	Aldrich	7452-79-1	57	102, 74, 130	5-125	0.997	-	-
ethyl 3-methylbutyrate	Aldrich	108-64-5	88	41, 70, 130	15-135	0.993	15-135	0.973
1-hexanol	Sigma-Aldrich	111-27-3	56	43, 69, 84	1000-6250	0.964	300-4800	0.979
isoamyl acetate	Aldrich	123-92-2	43	70, 55, 87	150-600	0.935	-	-
ethyl valerate	Sigma-Aldrich	539-82-2	88	57, 101, 130	1.0-36	0.995	-	-
1-heptanol	Acros Organics	111-70-6	70	56, 83, 98	2-200	0.996	-	-
1-octen-3-ol	Aldrich	3391-86-4	57	72, 85, 99	1-400	0.999	1.0-400	0.904
ethyl hexanoate	Aldrich	123-66-0	88	99, 60, 144	300-1200	0.999	300-1200	0.993
acetophenone	Aldrich	98-86-2	105	77, 120, 51	1.0-25	0.985	1.0-25	0.979
1-octanol	Sigma-Aldrich	111-87-5	56	41, 69, 84	10.0-20	0.918	2.0-32	0.979
linalool	Sigma-Aldrich	78-70-6	71	93, 121, 154	5-125	0.972	20-500	0.989
cis rose oxide	Fluka	16409-43-1	139	69, 83, 154	3-108	0.998	0.5-24.5	0.998
phenethyl alcohol	Acros Organic	60-12-8	91	122, 65, 51	15000-135000	0.977	15000-60000	0.942
nerol oxide	Bedoukian	1786-08-9	68	83, 41, 152	5.0-80	0.997	20-80	0.993
ethyl benzoate	Aldrich	93-89-0	105	122, 77, 150	1.0-9	0.993	1.0-9	0.995
ethyl octanoate	Aldrich	106-32-1	88	101, 127, 172	400-1600	0.992	300-12000	0.975
decanal	Sigma-Aldrich	112-31-2	57	70, 82, 112	0.25-16	1.000	0.5-4.5	0.984
ethyl phenylacetate	Aldrich	101-97-3	91	164, 65, 136	2.0-50	0.984	2.0-50	0.987
2-phenethyl acetate	Aldrich	103-45-7	104	43, 91, 78	-	-	3.0-75	0.991
4-vinylguaiacol	Alfa Aesar	7786-61-0	150	135, 107, 77	20-320	0.944	25-1225	0.987
γ-nonalactone	Aldrich	104-61-0	85	41, 114, 156	0.5-200	0.981	10-160	0.976
β-damascenone	Gift	23726-93-4	69	105, 121, 190	2.0-8	0.994	2.0-32	0.996
geranyl acetone	Alfa Aesar	3796-70-1	43	69, 151, 194	0.15-0.60	0.991	0.15-0.60	0.990
ethyl cinnamate	Aldrich	103-36-6	131	103, 176, 77	-	-	3.0-27	0.975
β-ionone	Aldrich	79-77-6	177	43, 135, 192	-	-	0.01-0.25	0.992

Table 3.2. Impact of harvest date (H1 to H4) on Vidal and Riesling icewine must chemical variables, Garphil Farms, St. Catharines, ON, 2004-05.

		H1	H2	H3	H4	Significance
Vidal	Brix	37.9 ± 0.1a	37.0 ± 0.1a	37.6 ± 0.1a	37.2 ± 0.1a	ns
	pH	3.5 ± 0.0b	3.4 ± 0.0c	3.6 ± 0.0a	3.5 ± 0.0b	*
	Titratable acidity (g/L)	11.2 ± 0.1a	10.3 ± 0.1b	9.6 ± 0.1c	9.5 ± 0.0c	*
Riesling	Brix	38.0 ± 0.2a	38.1 ± 0a	37.9 ± 0.2a	---	ns
	pH	3.7 ± 0.0a	3.8 ± 0a	3.7 ± 0.04a	---	ns
	Titratable acidity (g/L)	8.5 ± 0a	8.0 ± 0.0b	7.2 ± 0.16c	---	*

ns, *: not significant or significant at $p < 0.05$, respectively. Treatments with the same letter are not significantly different, least significant difference, $p<0.05$.

Table 3.3 Impact of harvest date (H1 to H4) on Vidal and Riesling icewine wine chemical variables, Garphil Farms, St. Catharines, ON, 2004-05.

		H1	H2	H3	H4	Significance
Vidal	pH	3.64 ± 0.0b	3.61 ± 0.0c	3.73 ± 0.0a	3.64 ± 0.0b	***
	A420	0.51 ± 0.1c	0.61 ± 0.0a	0.47 ± 0.1 d	0.53 ± 0.0b	***
	TA (g/L)	11.1 ± 0.2a	11.2 ± 0.1a	10.6 ± 0.1b	10.7 ± 0.1b	***
	Acetic acid(g/L)	0.85 ± 0.0c	0.83 ± 0.0d	0.87 ± 0.01b	1.00 ± 0.0a	***
	Glycerol (g/L)	10.7 ± 0.3a	10.9 ± 0.2a	13.6 ± 0.5b	15.2 ± 0.3c	***
	Ethanol (%v/v)	10.9 ± 0.6a	10.9 ± 0.3a	11.7 ± 0.1c	11.4 ± 0.0b	***
	Residual Sugar (g/L)	182 ± 4.6a	166 ± 10.6d	172 ± 1.4b	170 ± 3.4c	***
Riesling	pH	3.85 ± 0.0a	3.90 ± 0.0c	3.88 ± 0.0b		***
	A420	0.55 ± 0.1b	0.65 ± 0.0c	0.47 ± 0.0a		***
	TA (g/L)	9.40 ± 0.1a	9.62 ± 0.1b	9.45 ± 0.1a		**
	Acetic acid(g/L)	0.87 ± 0.0a	0.89 ± 0.0a	0.94 ± 0.0b		***
	Glycerol (g/L)	9.99 ± 0.4a	10.3 ± 0.2b	12.5 ± 0.3c		***
	Ethanol (%v/v)	12.2 ± 0.1b	12.1 ± 0.2a	12.5 ± 0.2c		***
	Residual Sugar (g/L)	178 ± 3.2c	176 ± 1.2b	160 ± 2.2a		***

, *: significant at $p < 0.01$ and 0.001, respectively. Treatments with the same letter are not significantly different, least significant difference, $p<0.05$.

Table 3.4 Impact of harvest date on Vidal icewine aroma compound concentrations (μg/L), Garphil Farms, St. Catharines, ON, 2004-05.

NO	Compound	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Significance	F-value	p-value
1	Ethyl isobutyrate	77.62 b	74.08 c	73.09 d	89.46 a	***	772.6	<0.0001
2	Ethyl butyrate	98.74 a	96.06 a	70.78 c	88.46 b	***	140.8	<0.0001
3	Ethyl 2-methylbutyrate	22.79 b	20.44 c	17.67 d	25.67 a	***	64.7	<0.0001
4	Ethyl 3-methylbutyrate	30.69 a	26.34 b	24.94 c	29.98 a	***	128.2	<0.0001
5	1-hexanol	1394 b	1281 d	1334 c	1607 a	***	94.9	<0.0001
6	Isoamyl acetate	311.9 a	234.5 c	230.9 c	248.9 b	***	128.1	<0.0001
7	Ethyl valerate	4.24 d	5.11 c	7.34 d	12.33 a	***	2224.2	<0.0001
8	1-heptanol	11.57 c	11.09 c	13.84 b	30.37 a	***	3138.5	<0.0001
9	1-octen-3-ol	74.65 d	95.54 c	133.7 b	229.3 a	***	646.4	<0.0001
10	Ethyl hexanoate	720.9 a	555.9 c	481.1 d	590.1 b	***	113.0	<0.0001
11	Acetophenone	1.57 b	1.61 a	1.52 c	1.58 b	**	23.0	<0.0001
12	1-octanol	10.42 b	10.02 c	10.13 c	11.85 a	***	116.2	<0.0001
13	Linalool	49.68 a	27.15 b	33.09 b	37.44 b	**	10.0	0.009
14	*Cis*-rose oxide	16.20 d	32.20 c	40.40 b	49.37 a	***	593.9	<0.0001
15	Phenethyl alcohol	31989 a	20936 b	22587 b	29767 a	***	48.5	0.00013
16	Nerol oxide	18.00 d	23.26 c	27.02 b	36.09 a	***	548.4	<0.0001
17	Ethyl benzoate	1.09 cb	1.02 c	1.17 b	2.07 a	***	461.7	<0.0001
18	Ethyl octanoate	1227 a	983 b	788 c	1022 b	***	125.8	<0.0001
19	Decanal	0.98 b	1.84 a	1.32 b	2.03 a	**	18.7	0.002
20	Ethyl phenylacetate	7.91 b	6.32 c	7.64 b	9.66 a	***	257.5	<0.0001
21	4-vinylguaiacol	101.6 a	83.80 b	78.17 c	72.66 d	***	124.0	<0.0001
22	γ- nonalactone	6.49 c	4.58 d	8.01 b	15.01 a	***	369.5	<0.0001
23	β-damascenone	45.90 b	46.06 b	34.72 c	58.77 a	***	188.9	<0.0001
24	Geranyl acetone	0.28 c	0.30 b	0.35 a	0.24 d	***	146.1	<0.0001

, *: significant at $p < 0.01$ or 0.001, respectively. Treatments with the same letter are not significantly different, least significant difference, $p<0.05$.

Table 3.5 Impact of harvest date on aroma compound concentrations (μg/L) in Riesling icewines, Garphil Farms, St. Catharines, ON, 2004-05.

NO	Compound	Harvest 1	Harvest 2	Harvest 3	Significance	F-value	p-value
1	ethyl isobutyrate	191 b	193 a	198 a	**	24.4	0.0060
2	ethyl butyrate	147 a	1467 a	141 b	**	135	0.0020
3	ethyl 3-methylbutytate	20.9 b	21.4 b	26.2 a	***	98.1	0.0004
4	1-hexanol	798 b	795 b	867 a	**	30.2	0.0040
5	1-octen-3-ol	15.3 c	31.5 b	43.8 a	***	775	<0.0001
6	ethyl hexanoate	635 a	590 b	425 c	**	143	0.0002
7	acetophenone	1.90 b	1.91 b	2.90 a	***	102	0.0004
8	1-octanol	4.31 b	4.60 b	6.60 a	***	349	<0.0001
9	linalool	74.5 a	64.6 b	75.9 a	*	15.0	0.0140
10	*cis*-rose oxide	2.20 c	5.80 b	9.60 a	***	473	<0.0001
11	phenethyl alcohol	16635 b	16097 c	17377 a	**	84.6	0.0010
12	nerol oxide	19.3 b	18.5 b	22.9 a	**	43.3	0.0020
13	ethyl benzoate	1.30 b	1.30 b	2.40 a	***	401	<0.0001
14	ethyl octanoate	1105 a	957 b	669 c	***	93.5	0.0004
15	decanal	1.40 a	0.50 b	1.30 a	**	65.9	0.0010
16	ethyl phenylacetate	5.30 b	5.21 b	6.90 a	***	176	0.0012
17	2-phenethyl acetate	7.02 b	6.41 c	7.71 a	***	107	0.0003
18	p-vinylguaicol	130 a	112 b	89.9 c	***	780	<0.0001
19	γ-nonalactone	16.3 c	17.5 b	21.2 a	***	4773	<0.0001
20	β-damascenone	7.81 c	8.10 b	11.1 a	***	1018	<0.0001
21	geranyl acetone	0.41 b	0.45 a	0.36 c	***	552	<0.0001
22	ethyl cinnamate	7.32 a	6.30 b	5.92 c	***	531	<0.0001
23	β-ionone	0.01 b	0.01 b	0.04 a	***	179	0.0001

*, **, ***: significant at $p < 0.05$, 0.01, or 0.001, respectively. Treatments with the same letter are not significantly different, least significant difference, $p<0.05$.

Table 3.6. Impact of harvest date on odour activity values (OAV) of aroma compounds in Ontario Vidal and Riesling icewines, based on published sensory threshold values.

		VIDAL				RIESLING			
NO	Compound	Harvest 1 OAV	Harvest 2 OAV	Harvest 3 OAV	Harvest 4 OAV	Harvest 1 OAV	Harvest 2 OAV	Harvest 3 OAV	Threshold µg/L
1	ethyl isobutyrate	5.18	4.94	4.87	5.96	12.8	12.9	13.2	15
2	ethyl butyrate	4.94	4.80	3.54	4.42	7.34	7.33	7.06	20
3	ethyl 2-methylbutyrate	1.27	1.14	0.99	1.43				18
4	ethyl 3-methylbutyrate	10.2	8.78	8.31	9.99	6.96	7.13	8.74	3
5	1-hexanol	0.17	0.16	0.17	0.20	0.10	0.10	0.11	8000
6	isoamyl acetate	10.4	7.82	7.70	8.30				30
7	ethyl valerate	2.83	3.41	4.89	8.22				1.5
8	1-heptanol	3.86	3.70	4.62	10.12				3
9	1-octen-3-ol	74.7	95.5	134	229	15.3	31.5	43.8	1
10	ethyl hexanoate	51.5	39.7	34.4	42.2	45.4	42.1	30.3	14
11	acetophenone	0.02	0.03	0.02	0.02	0.03	0.03	0.05	65
12	1-octanol	0.10	0.09	0.09	0.11	0.04	0.04	0.06	110
13	linalool	1.99	1.09	1.32	1.50	2.98	2.58	3.04	25
14	*cis*-rose oxide	81.0	161	202	247	10.9	29.0	48.0	0.2
15	phenethyl alcohol	2.29	1.50	1.61	2.13	1.19	1.15	1.24	14000
16	nerol oxide	0.01	0.01	0.01	0.01	0.01	0.01	0.01	3000
17	ethyl benzoate	0.002	0.002	0.002	0.004	0.002	0.002	0.004	575
18	ethyl octanoate	246	197	158	205	221	191	134	5
19	decanal	0.49	0.92	0.66	1.02	0.70	0.27	0.67	2
20	ethyl phenylacetate	0.11	0.09	0.11	0.13	0.07	0.07	0.09	73
21	2-phenethyl acetate					0.03	0.03	0.03	250
22	4-vinylguaiacol	10.2	8.38	7.82	7.27	13.1	11.2	8.99	10
23	γ-nonalactone	0.22	0.15	0.27	0.50	0.54	0.58	0.71	30
24	β-damascenone	918	921	694	1175	155	162	223	0.05
25	geranyl acetone	0.01	0.01	0.01	0.004	0.01	0.01	0.01	60
26	ethyl cinnamate					6.65	5.71	5.36	1.1
27	β-ionone					0.10	0.10	0.40	0.09

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Figure 3.6. Canonical variant analysis of Ontario Riesling icewines produced from different harvest dates (H1 to H3).

Figure 3.1. Chemical map of Ontario Vidal icewines produced from different harvest dates (H1 to H4) displaying variation in the products with principal component analysis.

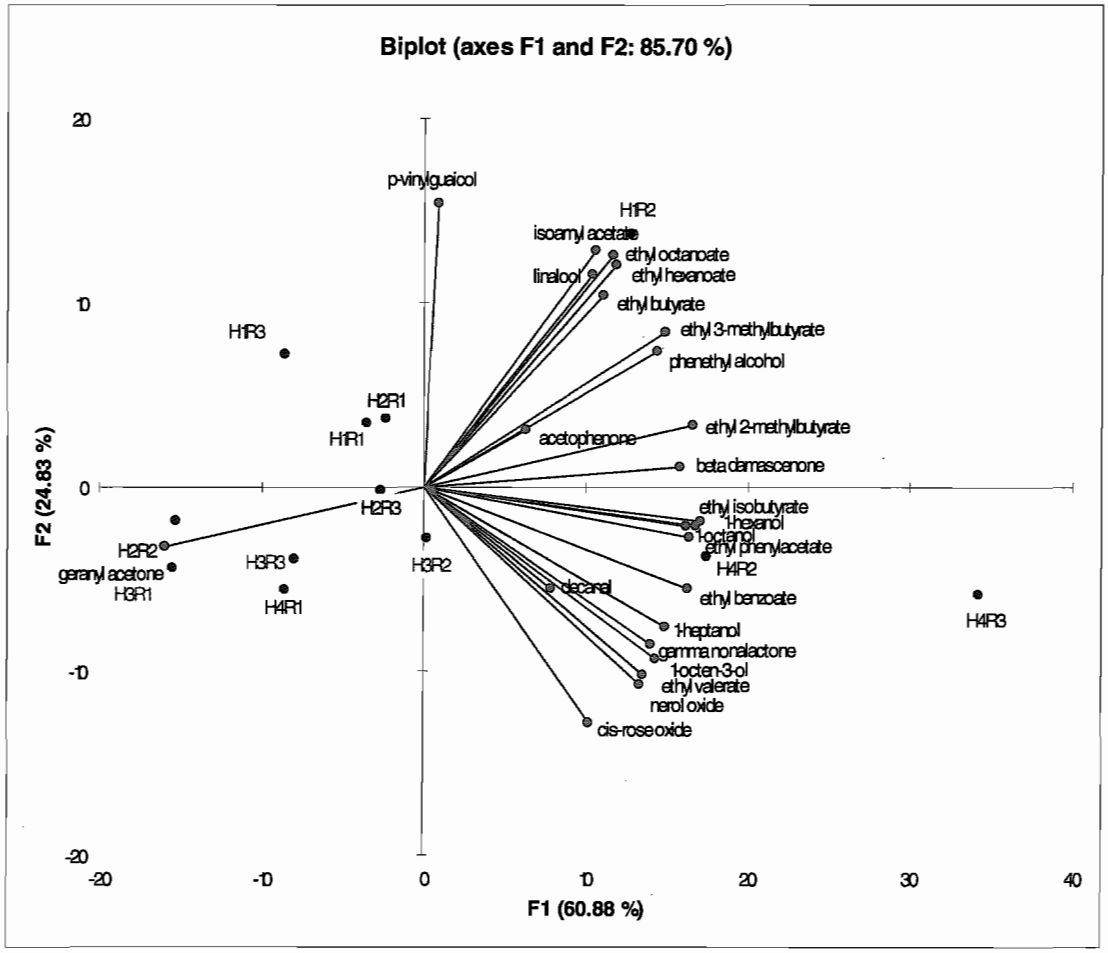


Figure 3.2. Chemical map of the variation in aroma compounds above their sensory threshold (OAV > 1) in Ontario Vidal icewines produced from different harvest dates (H1 to H4) through principal component analysis.

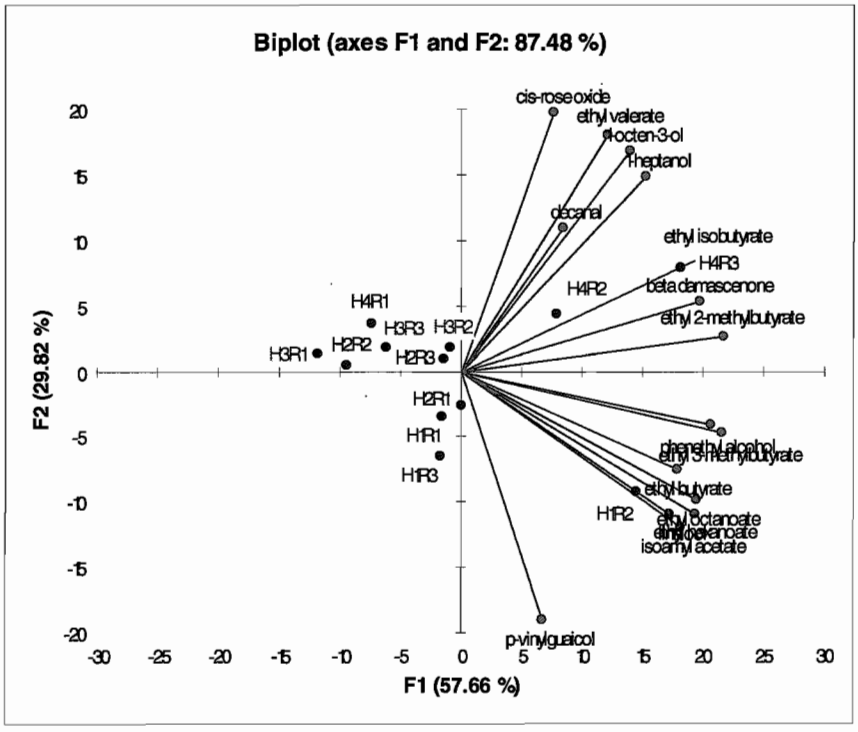


Figure 3.3. Chemical map of Ontario Riesling icewines produced from different harvest dates (H1 to H3) showing the variation in the products using principal component analysis.

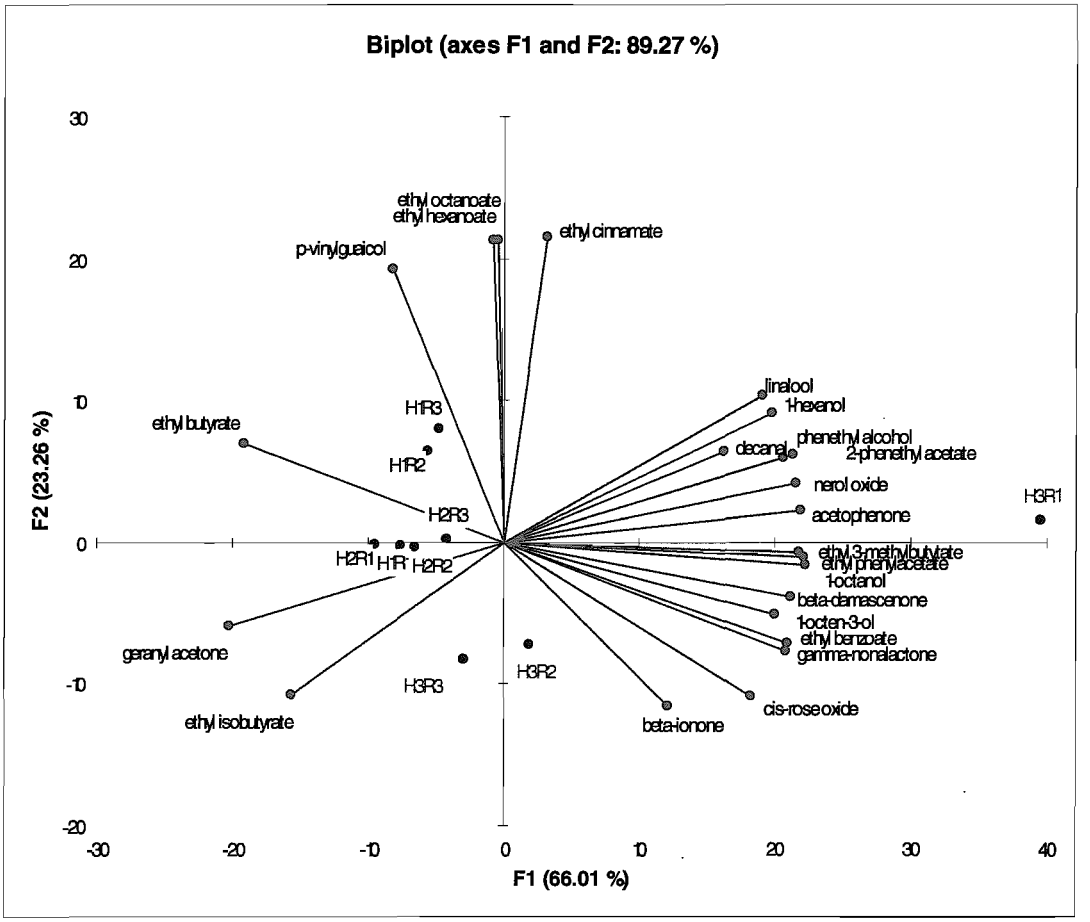


Figure 3.4. Chemical map of Ontario Riesling icewines produced from different harvest dates (H1 to H3) with aroma compounds found above their sensory threshold (OAV > 1) showing the variation in the attributes.

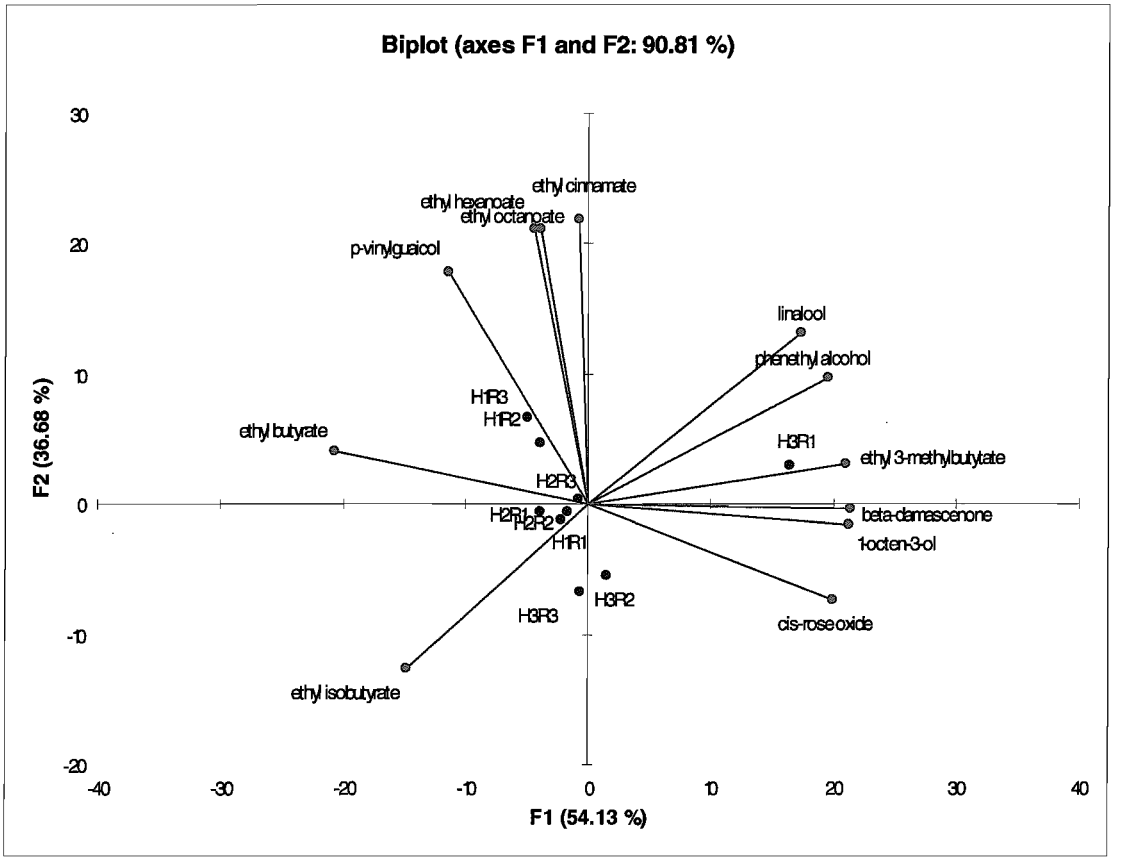
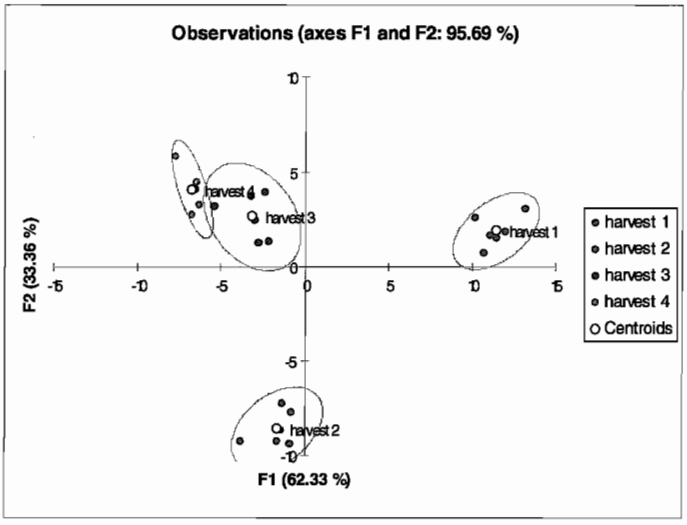


Figure 3.5. Canonical variant analysis of Ontario Vidal icewines produced from different harvest dates (H1 to H4).



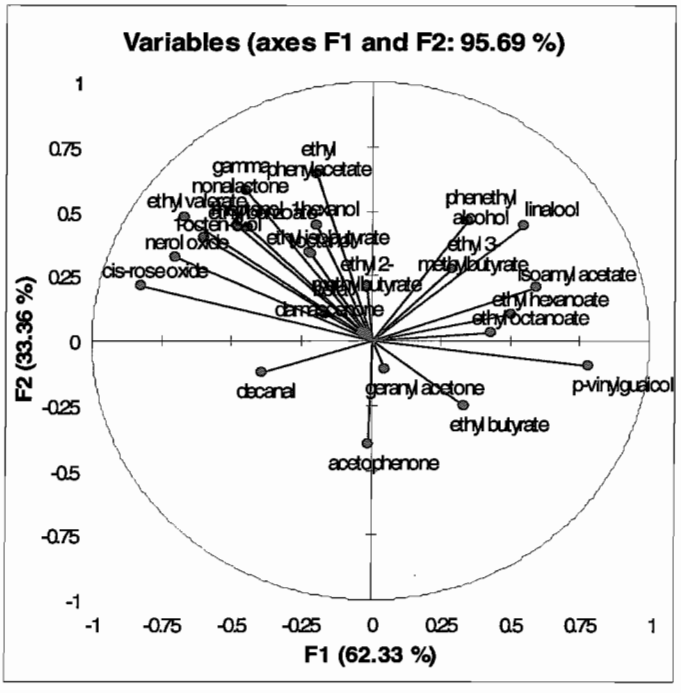
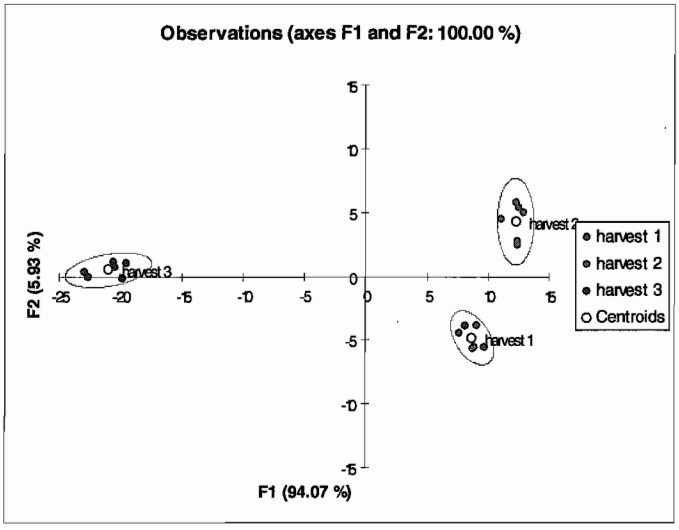
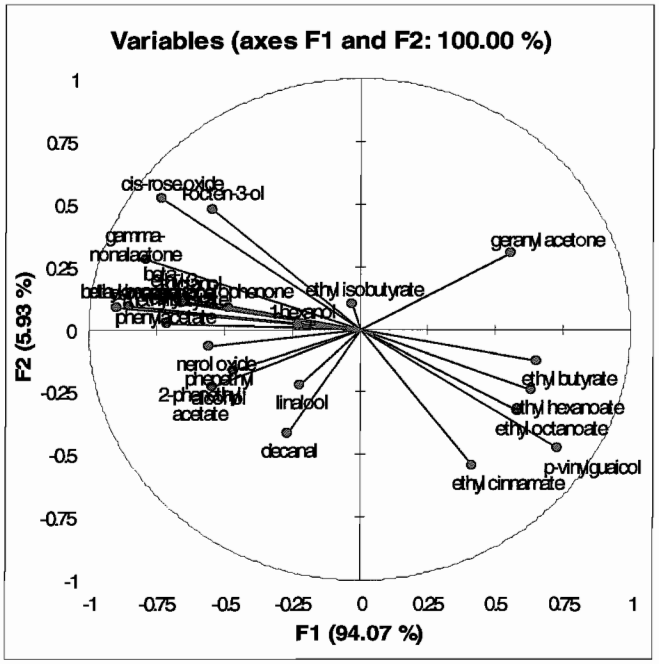


Figure 3.6. Canonical variant analysis of Ontario Riesling icewines produced from different harvest dates (H1 to H3).





Chapter 4
The Effect of Crop Level on Vidal blanc and Riesling Icewine from the Niagara Peninsula: I. Chemical Variables and Aroma Compounds

Amy J. Bowen and Andrew G. Reynolds

Abstract

Icewine is a sweet dessert wine made from pressing grapes naturally frozen on the vine. Currently, many grape growers crop their grapevines designated for icewine at levels often double those of table wines; therefore, it was of interest to ascertain whether reducing crop level might impact icewine chemical and aroma compound profiles. Three vineyard treatments [control (fully cropped), cluster thin at fruit set to one (basal) cluster per shoot (TFS), and cluster thin at veraison (TV)] were evaluated in a randomized block design for Riesling and Vidal cultivars over two seasons, 2003-04 and 2004-05. Musts differed between treatments in pH and titratable acidity in both years. Wines were different for most standard chemical variables, however; no clear trends existed between years or cultivars. Vidal icewines had the highest concentration of aroma compounds in the control and TV wines in 2003 and in TFS wines in 2004. Almost all compounds differed ($p < 0.05$) according to crop level treatment in Vidal: 17 of 24 in 2003 and 23 of 24 in 2004. The compounds with the highest odour activity values in Vidal were β-damascenone, ethyl octanoate, *cis*-rose oxide, 1-octen-3-ol, ethyl hexanoate and isoamyl acetate in 2003 and β-damascenone, 1-octen-3-ol, ethyl octanoate, *cis*-rose oxide, and ethyl hexanoate in 2004. Principal components analysis (PCA) found β-damascenone, ethyl 2- and 3- methylbutyrate, ethyl isobutyrate, ethyl butyrate and 1-heptanol to be correlated and associated with the control in 2003. In 2004, the PCA found most attributes positively loaded on F1 and associated with treatment replicate (block) and not crop level. Riesling icewines differed ($p < 0.05$) according to crop level treatment for all aroma compounds in 2003 and 22 of 23 in 2004. In both years, the majority of the aroma compounds were had the highest concentration in TV wines and the lowest concentration in TFS wines. Odour activity values in Riesling icewines were highest for β-damascenone, ethyl octanoate and ethyl hexanoate in both 2003 and 2004; *cis*-rose oxide was also highly odour potent in 2004. The PCA from the 2003 Riesling icewines showed most attributes loaded on F1 and associated with TV wines. We concluded that freeze and thaw events in November and December were likely more important in aroma compound development than crop level.

Key words: wine aroma, odour activity values, gas chromatography-mass spectrometry, cluster thinning

Introduction

Icewine is a sweet late harvest dessert wine produced from grapes that have frozen naturally on the vine. The frozen grapes are pressed, leaving water behind as ice

crystals, which results in a must concentrated with sugar, acids, aroma, and flavour compounds. Pressing grapes frozen for icewine production reduces the yield to 15 -20% that of table wine significantly increasing the amount of vine acreage required for production (Pickering 2006). To compensate for some of this loss in yield currently, many grape growers crop their grapevines designated for icewine at levels often double those of table wines. It was therefore of interest to ascertain whether reducing crop level might impact icewine chemical and aroma compound profiles.

Riesling and Vidal blanc are excellent cultivars for icewine production. Vidal blanc (syn. Vidal) is a white French-American hybrid consisting of 75% *V. vinifera* genetic background, from a cross between Ugni blanc and Rayon d'Or (Seibel 4986) (Galet 1998). A key viticultural feature is its winter hardiness. The cultivar has large cylindrical clusters, with medium-sized, thick-skinned berries that are disease resistant (Galet 1998). It is a high acid cultivar prone to overcropping, which enables it to produce large yields for icewine production. Cluster thinning is essential for table wine production. Vidal is a late maturing cultivar harvested usually in mid- to late October in eastern North America (Chisholm et al. 1994).

Riesling is considered by many to be the best choice of cultivar for producing icewines of the highest quality and ageability due to its high natural acidity. It is the noble grape of Germany, known for producing a wide range of wine styles from bone dry to ultra sweet, both clean and Botrytis-affected. Riesling has all the characteristics of the ideal icewine grape; it is late maturing, high acid, thick skinned providing some disease resistant and winter hardiness (for *V. vinifera*) (Galet 1998).

Yield calculations for icewine crops are based on October estimates for regular harvest (Ziraldo and Kaiser 2007) because it becomes more difficult to accurately estimate crop late in the season as the individual berries are at different stages of desiccation due to climatic conditions. Yield estimations of 150 L/tonne, or approximately 7 tonnes/acre for Vidal, and 125 L/tonne or 5 tonnes/acre for Riesling are used by one of Ontario's largest icewine producers as a guideline for maximum yield and quality (Ziraldo and Kaiser 2007). However, anecdotally it is not uncommon for growers to crop vines up to 10 tonnes/acre to increase the volume of icewine juice they can sell to the wineries, especially for Vidal.

Cluster thinning is a standard viticultural practice performed to keep the grapevine in balance, thus, preventing overcropping. The net effect of cluster thinning is to improve the quality of the grapes, which is achieved due to an increase in the ratio of leaf area to crop and maintain the health of the vine (Winkler et al. 1974). Reynolds (1989a, 1989b) found that cluster thinning changes the composition of the grapes with an increase in sugar content and pH and a decrease in titratable acidity. It can also increase cluster weight, berries per cluster, and berry weight and can advance maturation of the fruit (Bravdo et al. 1984).

The main goal of cluster thinning is to reduce the crop load of a vine to advance grape maturation and improve wine quality (Keller et al. 2005). Cluster thinning after bloom will reduce yield, but increase berry size, cluster weight and berries per cluster, and can advance harvest date (Bravdo et al. 1984). Cluster thinning early in the season allow the vine to compensate for the crop removed, shown as an increase in berry size, number of clusters formed and number of berries per cluster. These results are generally

not desired in wine grape production because low yields are perceived to increase aroma compound production and enhance the perceived wine quality (Jackson and Lombard 1993). It is therefore common for wine grape production to cluster thin just prior to veraison (Reynolds et al. 2007).

Cluster thinning was initially used to improve fruit composition in French-American hybrids such as De Chaunac and Seyval blanc, and later on in *V. vinifera* cultivars (Reynolds 1989a). As of 2009, no research has focused on the effect of crop level on the composition of dessert wines. However, in table wines, several studies have established a clear relationship between crop level and varietal characteristics. Reynolds et al. (1996) found that cluster thinning produced Pinot noir wines which were rated by panelist as having less grassy and vegetative characteristics and were rated higher for descriptors such as black pepper, cherry, and currant. PCA results showed correlations between typical Pinot noir descriptors and cluster thinning.

Studies with Riesling have shown similar results; where cluster thinned grapes have a higher concentration of monoterpenes (McCarthy et al. 1985). The effect of cluster thinned vines to three different crop levels; 1, 1.5, and 2 clusters per shoot were studied to determine the effect of vineyard treatments on Riesling composition and sensory response (Reynolds et al. 1994a). Monoterpene concentrations decreased with increasing number of clusters per shoot. Linalool was positively correlated with ripe fruit character and sweetness and negatively correlated with green-fruit flavor and cluster thinning was increased the perception of ripe fruit character in the wines. Monoterpenes such as linalool, linalool oxides, terpineol and citronellol were associated with lower crop levels and low to moderate shoot densities and increased in concentration with bottle age.

The objective of this study was to determine the effect of crop level; control (fully cropped), thin at fruit set and thin at veraison, on Vidal and Riesling chemical, and aroma compound profiles. Currently there is nothing in the literature pertaining to this topic so the results will be of interest to grape growers, winemakers and other wine professionals.

Materials and Methods

Chemicals. Analytical standards (Table 4.1) were purchased from Aldrich (Oakville, ON,), Sigma-Aldrich (Oakville, ON), Fluka (Oakville, ON), Bedoukian (Danbury, CT, USA), Acros organic (Geel, Belgium). β-Damascenone was a gift from Dr. T. Acree, Cornell University. Chemical standards were diluted in dichloromethane (Caledon; Georgetown, ON) and stored at -25°C.

Treatments. Two commercial vineyard plots were chosen for the crop level study. Both vineyard experiments were made up of randomized block designs containing six blocks each with three treatments. The Vidal block was located at Garphil Farms in west St. Catharines, in the Creek Shores sub appellation. It consisted of six rows of grape vines; each one was designated as a block. Each row (block) was then divided into three treatments; control (fully cropped), thin to one basal cluster per shoot at fruit set, and thin to one basal per shoot cluster at veraison. The first vine at each end of the row was untouched and left as a buffer. The Riesling block was located in Niagara-on-the-Lake at Lambert Farms, in the Four Mile Creek sub appellation. It consisted of two rows of grapevines; each row was divided into three blocks. Each block was then divided in three treatments; control (fully cropped), thin to one basal cluster per shoot at fruit set, and thin to one basal cluster per shoot at veraison. The first postlength at each end of the row was

untouched and left as a buffer. All vines were sprayed and maintained at the discretion of the grower. Grapes were harvested once proper icewine conditions were met (≤ -8 °C) on 7 January 2004 and 15 January 2005.

Harvest and pressing. Treatments were kept separate at harvest by placing them into labeled bins indicating their block and treatment. All grapes were brought back to the pilot winery at Brock University and pressed in the basket press with an inflatable rubber bladder by treatment at 2 bar. The resultant must was collected in 20-L food grade pails for each treatment until it reached 35 °Brix , it was then sulphited to 75 mg/L and stored at 4°C until fermentation. For each treatment, a 250-mL must sample was frozen at -25°C for future analysis.

Fermentation. The must was inoculated with Lalvin® K1-V1116 *Saccharomyces cerevisiae* (Lallemand) as per the yeast rehydration procedure of Kontkanen et al. (2004) into 20 L carboys. The fermenting must was left at room temperature overnight and was then placed in an 18 °C fermentation chamber. Fermentation was stopped by addition of 75 mg/L potassium metabisulphite (Sigma, Oakville, ON) when the ethanol, determined by GC, was 10 % v/v, after which the carboys were moved to the -2°C chamber for cold stabilization. The icewines were left to settle for up to 2 weeks then racked into clean carboys to remove the lees.

Bottling. At bottling, icewines were brought to room temperature, 100 mg/L of potassium metabisulphite (Sigma, Oakville, ON) and 100 mg/L potassium sorbate (Sigma, Oakville, ON) were added to the wines. A 250-mL wine sample was taken and frozen at -25°C for future wine analysis. The icewine was filtered through a 1 μ pad filter (Scott Laboratories, Pickering, ON) and 0.45 μ membrane filter (Millipore, Bedford,

MA). It was then bottled in 375-mL bottles, corked (Scott Laboratories, Pickering, ON) and put in the wine cellar for storage at 12 °C until analysis.

Must and wine analysis. For a detailed description of must and wine analysis refer to materials and methods from Chapter 3. Titratable acidity (TA) by autotitrator (Man-Tech Associates Ltd., Guelph, ON) to a pH endpoint with 0.1% NaOH, pH by pH meter (model 825MP, Fisher Scientific, Ottawa, ON) and soluble solids (°Brix) by refractometer (Abbé model 10450; American Optical Corp., Buffalo, NY) were determined for the icewine musts. Chemical analysis was conducted on the finished wines for TA, pH, absorbance at 420 nm, acetic acid, glycerol and glucose-fructose concentrations, and percent ethanol. TA and pH were determined as per must above. Absorbance at 420 nm (A420) using a Ultrospec 2100 Pro UV/Visible spectrophotometer (Biochrom Ltd., Cambridge, England) at 420nm. Megazyme enzyme kits (Megazyme, Bray, Ireland) were used to determine acetic acid (g/L), glycerol (g/L), glucose-fructose (g/L) following manufacturing instructions. Ethanol (% v/v) was determined with an Agilent 6890 GC-FID (Agilent Technologies, Mississauga, ON), according the method of Nurgel et al. (2004).

Volatile extraction. Wine volatiles were extracted by stir bar sorptive extraction (SBSE), commercially known as Twister, using 10-mm stir bar (Gerstel, Baltimore, MD) coated with polydimethylsiloxane (PDMS, 0.5 mm film thickness). A 10-ml sample of icewine was poured into a 10 mL extraction vial and spiked with an internal standard, 100 µg/L *n*-dodecanol (Sigma; Oakville, ON) in GC-grade dichloromethane. The stir bar was added to the wine and extracted for 60 minutes at 1000 rpm. The stir bar was

removed from the extraction vial, dried with a lint free tissue, rinsed with Milli-Q water (Millipore, Bedford, MA) and stored in a 4-mL amber vial at 4°C until analysis.

Gas chromatography-mass spectrometry (GC-MS). Instrument: Agilent 6890N/5975B gas chromatograph mass spectrometer equipped with a Gerstel thermal desorption unit (TDS2; Gerstel, Baltimore, MD) and cooled injection system (CIS4) programmable temperature vaporization (PTV) inlet and an olfactometry port (DATU, Geneva, NY). Analytical column: Agilent HP-5MS, 5% phenyl methyl siloxane, 30m length, 0.25 mm internal diameter and 0.25 μm film thickness. Carrier gas: 1.4 mL/min 5.0 purity helium (Praxair, Mississauga, ON). Oven program: initial temperature 35 °C held for 3 min, increased by 4 °C/min to 155 °C, increased by 30 °C/min to a final temperature 240 °C. Thermal desorption: initial temperature 30 °C, increased by 60 °C/sec to 250°C and held for 3 min. TDS transfer line temperature 275°C connected to CIS4 inlet cryo-cooled to -70°C with liquid nitrogen in solvent vent mode. After desorption, CIS4 inlet temperature was increased at 12 °C/sec to 280 °C and held for 5 min while analytes are released on the column. The MS was run in scan mode, 30 to 400 Da for compound identification and in select ion monitoring (SIM) mode selecting for one quantitative ion and three qualitative ions for each compounds as shown in Table 2.1 (Chapter 2) for quantification.

Identification and Quantification. The compounds were identified as the 'top 15' odour potent volatiles by GC-O CharmAnalysis (Bowen and Reynolds 2010c) and are listed in Table 5.1. The compounds were identified by comparison of retention time, odour perception and mass spectra (Wiley library) to pure standards. Three-point calibration curves were run for each analyte in model wine solution to ensure linearity (r^2

> 0.9) (Table 2.1). The icewine model wine solution contained 11.57 g/L tartaric acid (EMD Chemical Inc., Darmstadt, Germany), 153 g/L fructose (Caledon; Georgetown, ON), 11% (v/v) ethanol (Commercial Alcohols Inc.; Brampton, ON), with a pH 3.61. Standard curve concentrations and compounds were quantified based on the ratio of the peak area of the compound relative to the peak area of the internal standard to determine the concentration of the analytes. Analysis was run in duplicate with relative standard deviation calculated. Odour activity values (OAV) for the compounds in each wine were calculated by dividing the concentration by its sensory threshold, a value greater than one indicated the compound contributed to the aroma of the wine.

Statistical analysis. All statistical analysis was performed using XLSTAT (Addinsoft, Paris, France) statistical software. To determine if differences exist between crop level for must and wine chemical variables a two factor (treatment x rep) analysis of variance (ANOVA) was performed. Least significant difference (LSD) values were determined for significant attributes ($p < 0.05$). A three factor ANOVA (crop level x fermentation rep x GC rep) with two-way interactions was used to determine if differences existed between aroma compounds in crop level wines ($p < 0.05$). Mean scores and LSD were calculated for aroma compounds that differed by crop level treatments.

The mean concentration of the aroma compounds was analyzed by principal components analysis (PCA) for each fermentation replicate using the correlation matrices to determine the compounds which best describe the variation due to crop level. Next, canonical variant analysis (CVA) using a stepwise forward model ($p < 0.05$) was used to determine if icewines from different crop level treatments could be differentiated based

on difference in the aroma compounds. Only those attributes that differed in concentration by crop level were included in the PCA and CVA.

Results

Must chemical variables. For both cultivars, control treatments had lower pH and higher TA than TFS or TV wines over two vintages (Table 4.1). Vidal control icewines had pH of 3.4 vs. 3.55 for the thinned treatments, and TA values of 8.6 g/L and 8.3 g/L, respectively. TA values were highest in the control treatments for both cultivars (Vidal 8.60 g/L in 2003 and 8.74 g/L in 2004; Riesling 10.25 g/L and 9.02 g/L in 2003 and 2004, respectively) and lowest in the TV wines (Vidal 8.25 g/L in 2003 and 7.53 g/L in 2004; Riesling 9.23 g/L and 7.75 g/L in 2003 and 2004, respectively).

Wine chemical variables. Differences were found between treatments in both Vidal and Riesling wines (Table 4.2). Similar patterns for pH and TA were found in the finished wines as previously described in the musts, with control treatments having lower pH and higher TA than TFS or TV wines for both cultivars over two vintages. However there were some exception; neither pH nor TA were different for the 2004 Riesling icewines, likely due to the desiccation of the fruit. Another exception was the 2003 Vidal icewines, where the TV treatment had a TA of 9.13 g/L, which was higher than the control (TA = 8.93 g/L). Glycerol concentrations were also higher in control icewines from the 2003 vintage for both cultivars; however, this result was not found in the 2004 vintage. Control wines had glycerol concentration of 14.01 g/L for Vidal and 34.17 g/L for Riesling, while the concentration was 12.70 g/L and 32.28 g/L for the TFS wines, and 13.64 g/L and 30.99 g/L for the TV wines for Vidal and Riesling, respectively. The

much higher glycerol concentration found in the Riesling icewines is likely due to the condition of the fruit upon harvest, which was very desiccated.

Vidal aroma compounds. ***Analysis of variance.*** Vidal crop level icewines differed ($p<0.05$) for 17 of the 24 aroma compounds identified in the top 15 in 2003 (Table 4.3) and 23 of the 24 compounds in 2004 (Table 4.4). Compounds that did not differ by crop level in 2003 were ethyl valerate, ethyl hexanoate, 1-octanol, phenethyl alcohol, nerol oxide, ethyl octanoate, and γ-nonalactone. In 2004 only ethyl butyrate did not differ by cropping level.

Vidal icewines from 2003 had higher concentrations of aroma compounds in the control (fully cropped) and TV treatments and lowest concentration for TFS wines (Table 4.3). The opposite was found in 2004 when almost all the aroma compounds had the highest concentration in the TFS treatment compared to the control and TV. In 2004 only ethyl 2-methylbutyrate, decanal, β-damascenone and geranyl acetone did not have the highest concentration in the TFS treatment, all four compounds had concentrations between the control and TV treatments.

The OAV were calculated for all compounds in the three Vidal crop level treatments (Table 4.4). In 2003 the following compounds had the highest OAV in descending order; β-damascenone, ethyl octanoate, *cis*-rose oxide, 1-octen-3-ol, ethyl hexanoate and isoamyl acetate. Eight other compounds--ethyl isobutyrate, 4-vinylguaiacol, 1-heptanol, ethyl butyrate, ethyl valerate, ethyl 2-methylbutyrate, phenethyl alcohol and linalool--were also found above their sensory threshold (OAV>1) in 2003 but their odour potency values were not consistent across treatments. In 2004, the five compounds with the highest OAVs across all treatments were β-damascenone, 1-

octen-3-ol, ethyl octanoate, *cis*-rose oxide, and ethyl hexanoate. β-Damascenone had the highest OAV for all treatments, followed by *cis*-rose oxide in the two thinned treatments and 1-octen-3-ol in the fully cropped treatment. Other compounds found above their sensory threshold in all treatments were ethyl 3-methylbutyrate, 4-vinylguaiacol, isoamyl acetate, ethyl valerate, ethyl isobutyrate, 1-heptanol, ethyl butyrate, phenethyl alcohol, ethyl 2-methylbutyrate and linalool.

Principal components analysis. The PCA of the 2003 Vidal crop level aroma compounds explained 75.2 % variation on F1, 41.7%, and F2 with 33.5% (Figure 4.1). Geranyl acetone was inversely correlated with ethyl 2- and 3- methylbutyrate, decanal, and ethyl phenylacetate. The latter four compounds along with acetophenone and ethyl isobutyrate were associated with the fully cropped treatment. The TFS and TV replicates 1&2 were located together on the PCA and negatively loaded on F1 and F2 and were inversely related to 1-octen-3-ol, 1-hexanol, ethyl benzoate and 1-heptanol. Ethyl butyrate and β-damascenone were positively associated with the two other TV replicates. The other two TFS replicates were positively associated with geranyl acetone, 4-vinylguaiacol, linalool and *cis*-rose oxide and positively loaded on F2.

The PCA looking at the aroma compounds above sensory threshold (OAV>1) for the 2003 Vidal icewines explained 82.5% of the variation on F1 and F2. F1 explained 45.7 % of the variation and it separated the control treatments from TFS wines. Therefore, the control treatments were associated with attributes positively loaded on F1 and F2 such as β-damascenone, ethyl 2- and 3- methylbutyrate, ethyl isobutyrate, ethyl butyrate and 1-heptanol. The TFS treatments were associated with those compounds loaded positively on F1 and negatively on F2 such as 1-octen-3-ol, isoamyl acetate,

linalool, cis rose oxide and 4-vinylguaiacol. While ethyl hexanoate and ethyl octanoate were found much higher than the sensory threshold, their concentrations did not differ by crop level treatment and they were omitted from the PCA.

The PCA of the 2004 Vidal crop level aroma compounds explained 88.6% of the variation in the data (Figure 4.1). All attributes, with the exception of acetophenone, were loaded on F1 and F2 therefore only these two factors were retained. Factor 1 explained 78.8% of the variation and was positively loaded with 20 of the aroma compounds. Factor 2 explained 9.8% of the variation, and only geranyl acetone was heavily loaded on this factor. Decanal was not well described by any factor. Geranyl acetone was inversely correlated to decanal and acetophenone was inversely correlated to ethyl 2- and 3- methylbutyrate. Otherwise, all aroma compounds were positively loaded on F1. Most aroma compounds were either associated with TFS treatments 1&2 or 3&4 and were loaded positively on F1 and F2, or they were associated with control and TV replicates 3&4 and loaded positively on F1 and negatively on F2. Isoamyl acetate, *cis*-rose oxide, nerol oxide, 4-vinylguaiacol, ethyl isobutyrate, linalool and γ-nonalactone were inversely associated with control replicates 1&2 and 5&6. Ethyl valerate, 1-hexanol, 1-octanol and phenethyl alcohol were inversely related to the TFS and TV 1&2. It is worthy of note that treatments did not sort based on crop level but by replicate (i.e. block). Replicates 1&2 and 3&4 were found to group together regardless of crop level and were inversely related on F1.

A PCA of the 2004 Vidal aroma compounds found above sensory threshold (OAV>1) that also differed by ANOVA was conducted and explained 93.8% of the variation on two factors: F1 with 86.3% and F2 with 7.4% of the variation. Fourteen

compounds were included, and all were heavily loaded on F1 and showed a similar pattern the PCA of all aroma compounds. β-Damascenone, ethyl 2-and 3-methylbutyrate, phenethyl alcohol and ethyl valerate were positively associated with control and TV 3&4 and negatively correlated with TFS 1&2 and TV 1&2 and 3&4 and loaded positively on F1 and F2. Ethyl octanoate, ethyl hexanoate, ethyl octanoate and 1-heptanol were negatively associated with control replicates 1&2 and 5&6 and loaded positively on F1 and negatively on F2.

CVA was used to separate the crop level treatments, but unfortunately with only three treatments the maximum number of factors that could be used was two. The CVA could not be run on GC replicates for each crop level treatment due to co-linearity of the data set; too many of the variables were highly correlated resulting in redundancy and overlap. This is evident by looking at the PCA correlation matrix. However based on two factors, the crop level treatments differed from one another in 2003 and 2004. In 2003, fully cropped and TV wines differed from TFS but not from each other (Figure 4.2). The TFS wines were associated with linalool, *cis*-rose oxide, geranyl acetone and 4-vinylguaiacol. In 2004, all crop level treatments differed from one another, and most aroma attributes were associated with TFS wines (Figure 4.2).

Riesling aroma compounds. ***Analysis of variance.*** Riesling crop level icewines differed ($p < 0.05$) for all attributes in 2003 (Table 4.6) and for 22 or 23 compounds in 2004, (Table 4.7); only 2-phenethyl acetate was not different in 2004. 4-Vinylguaiacol was not detected in any of the wines in 2003 but was detected in 2004 and differed with crop level, having the highest concentration in TV wines and lowest concentration in the fully cropped treatment. In both 2003 and 2004, the majority of the aroma compounds

had the highest concentration in the TV treatments and the lowest concentration in TFS wines. Only geranyl acetone had the lowest concentration in TV wines in both 2003 and 2004.

The odour potency of the Riesling crop level icewines was determined by calculating their OAVs (Table 4.8). In 2003, the most odour potent compounds across all treatments, in descending order, were β-damascenone, ethyl octanoate and ethyl hexanoate. Eight other compounds were found above their sensory threshold (OAV>1) in all treatments ethyl isobutyrate, ethyl 3-methylbutyrate, 1-octen-3-ol, *cis*-rose oxide, ethyl butyrate, ethyl cinnamate, linalool, and phenethyl alcohol. In general, the profile of aroma compounds found above their sensory threshold (OAV>1) in 2004 was similar to 2003 (Table 4.7). The compounds with the highest OAV across all cropping levels in 2004 were β-damascenone, ethyl octanoate, ethyl hexanoate and *cis*-rose oxide. The only differences between 2003 and 2004 in terms of odour potent compounds were 4-vinylguaiacol, which was found above its sensory threshold across all treatments in 2004, and β-ionone, which was found above its sensory threshold (OAV>1) in 2004 in the fully cropped and TFS treatments, but below its sensory threshold in the 2004 TV wines and all crop levels in 2003.

Principal components analysis. The PCA of the 2003 Riesling crop level icewines explained 83.7% of the variation on two factors, with F1 and F2 accounting for 65.8% and 17.9% respectively (Figure 4.3). Most aroma compounds were positively loaded on F1, except of geranyl acetone which was negatively loaded. Four compounds were associated with F2, acetophenone, *cis*-rose oxide, ethyl cinnamate and β-ionone. Geranyl acetone was associated with TFS and inversely correlated to β-damascenone,

ethyl hexanoate, ethyl octanoate, ethyl phenylacetate and g-nonalactone. Most aroma compounds were heavily loaded on F1 and associated with TV. β-Damascenone was heavily loaded on F1 and was highly correlated with 1-octen-3-ol, ethyl hexanoate, ethyl benzoate, ethyl octanoate, ethyl phenylacetate, phenethyl alcohol and γ-nonalactone.

The PCA of the 2003 Riesling crop level aroma compounds found above their sensory threshold (OAV>1) explained 86.3% of the variations on two factors, with F1 and F2 accounting for 66.3% and 19.9% of the variation in the data, respectively. All but two of the aroma compounds found above their sensory threshold were highly correlated and heavily loaded on F1 in the positive direction. These compounds were separated by F2 with β-damascenone highly correlated with 1-octen-3-ol, ethyl octanoate and ethyl hexanoate and inversely associated with TFS. Linalool was highly correlated with ethyl butryrate, ethy-3-methylbutyrate, isobutyrate and phenethyl alcohol and most strongly associated with TV wines. Two compounds were loaded on F2, *cis*-rose oxide and ethyl cinnamate, and they were inversely correlated to each other.

Due to the limited volumes of Riesling icewines produced in 2004, only three non-replicated wines were ultimately available for aroma compound analysis. As a result, the maximum number of factors was two, which explained 100% of the variation. However, based on the PCA, all the compounds, with the exception of β-ionone, were positively loaded on F1, which explained 85.8% of the variability (Figure 4.3). Ethyl isobutyrate and β-ionone were equally loaded on F1 and F2, and acetophenone and 4-vinylguaiacol were heavily loaded on F2, which explained the remaining 14.2% of the variation. Most compounds were associated with TV wines on F1.

The PCA of the 2004 Riesling crop load aroma compounds found above their sensory threshold (OAV>1) explained 85.8% of the variability on F1 and 14.2% on F2. Two compounds, ethyl isobutyrate and 4-vinylguaiacol, were equally loaded on F1 and F2, otherwise all compounds were heavily loaded on F1. β-Ionone was associated with the fully cropped treatment and was negatively loaded on F1 and inversely correlated to 4-vinylguaiacol and ethyl isobutyrate. All other compounds were positively loaded on F1 and highly correlated to each other.

CVA could only be used to separate the 2003 treatments since there were not enough data points for the 2004 icewines (Figure 4.4). Crop level was a discriminating factor for all three treatments; most aroma compounds were associated with the TV treatment.

Discussion

Must and wine chemical variables: Control (fully cropped) treatments had lower pH and higher TA in must and wine samples then thinned treatments, TFS or TV, these finding are in agreement with several other studies (Kliewer and Weaver 1971, Reynolds et al. 1994b, Reynolds et al. 2007). This is likely the result of more clusters per vine which will delay ripening and result in berries with higher TA.

Cluster thinning normally increases the sugar concentration of the berries because thinned vines have higher °Brix at harvest (Reynolds et al. 2007). However, in the case of icewine the soluble solids concentration was standardized at around 35 °Brix because the grapes were pressed frozen until they reached the desired sugar concentration. The

soluble solids concentration at commercial harvest for table grapes has little effect on the concentration of the icewine must and resultant wine.

Effect of crop level on Vidal aroma compounds. Although concentration differences existed between treatments, no clear trends were seen with respect to cluster thinning in the 2003 Vidal icewines (Table 4.3). This suggests that crop level did not have an effect on the net aromatic profile of the wines, which is in agreement with studies by Keller et al. (2005) and Reynolds et al. (1994b) who both found that thinning had little effect on berry composition. In contrast, the 2004 vintage of Vidal icewines found TFS wines had the highest concentration for almost all aroma compounds, compared to TV and control (Table 4.4). This suggests that thinning can have an effect on the aroma profile of the wines because the TFS wines were likely more aromatic.

An explanation for the differences in aroma compound composition due to vintage is best explained through temperature variation in the growing season. Bravdo (1984) found a difference between crop load and harvest date in all thinning treatments in Carignane vineyards when the clusters were thinned just after bloom, the lower the crop load the earlier the harvest date. While the grapes were all harvested at the same date in this study, the onset of cold temperatures and the first freeze event should be considered as the time when maturation stops in icewine grapes.

The 2003 growing season in Niagara had an average number of growing degree days (GDD); June was cooler than normal but July and September were close the mean temperature for the region, and August was warmer than usual. The 2004 growing season was cooler than average in June, July and August with September being on slightly warmer than the mean. In the warmer 2003 vintage the effect of cluster thinning

in TFS vines was minimized because all treatments achieved optimal ripeness and the uniformity of the grapes at harvest negated the effects of cluster thinning. The 2004 growing season was cool through the summer, which slowed the ripening of the berries. The reduction of crop stress in TFS vines advanced maturation and resulted in riper fruit with higher concentrations of aroma compounds at harvest in this treatment compared to fully cropped or TV.

Since we were studying the effect of cluster thinning on icewine grapes it may be more important to consider temperature variations from maturation, commercial table wine harvest, in October to icewine harvest in December and January. The temperature variation during the hang time of icewine grapes will ultimately effect the composition of the wine. By looking at the temperature minimum and maximum from October to harvest (January), one can provide a plausible explanation of why cluster thinning only had an effect in 2004. The temperature data, specifically the daily highs and lows for October, November and December over the two vintages, suggests that the growing season was cooler in 2004 and it also had more freezing events (Figure 4.5). These freeze events would have frozen the constituents inside the grape, and therefore less desiccation likely occurred; hence their composition would not have changed much due to hang time. This prolonged freezing would have preserved any differences due to cluster thinning and the state of maturity in the grapes before the onset of cold temperature in October. The minimum temperatures in November and December 2004 were below zero 12 and 25 days respectively compared to only 8 and 23 days in 2003.

The 2003 vintage was warmer throughout the growing season and the hang time period. From October to December, the 2003 grapes experienced greater fluctuations in

temperature resulting in more freeze and thaw events than 2004. The freeze and thaw events and overall warmer temperatures in 2003 would have led to increased desiccation and therefore further concentration of aroma compounds in all treatments, negating the effects of cluster thinning past regular commercial harvest. There were 13 freeze and thaw events from October 7 to Dec. 31 in 2003 versus only eight in 2004. The greater number and larger temperature differences (between minimum and maximum) in freeze and thaw events in 2003 versus 2004 likely resulting in more desiccation of the fruit and concentration of aroma compounds (Figure 4.5).

From the differences in the growing season it could be suggested that the onset of cold and number and duration of freeze events has more of an effect on the volatile composition of icewine grapes than cluster thinning. It is well known that aroma compound volatiles increase up until maturation and then tend to drop off in concentration (Marais and Wyk 1986, Reynolds 1989b, Coelho et al. 2007). However, these studies were all on table wine grapes that do not experience the hang time or freeze and thaw events for several months past maturation like icewine grapes. Several more years of data will be required to determine the exact effect of cluster thinning on the aroma compound development Vidal icewine grapes and wine.

Effect of crop level on Riesling aroma compounds. Cluster thinning at veraison resulted in the highest aroma compound concentration in both the 2003 and 2004 vintages. The TV treatment had the highest concentration for most of the aroma compounds, with 18 of 22 compounds in 2003 (Table 4.6) (4-vinylguaiacol which was not detected in 2003) and 20 of the 23 compounds in 2004 (Table 4.7). This was not unexpected since cluster thinning later in the season , such as veraison, has been shown to

allow assimilates to be directed to the fruit, increasing soluble solids and aroma compounds, and not towards the roots and leaves increasing vigor (Keller et al. 2005). If vines are thinned too early in the season, they can compensate for the loss in fruit or may become too vigorous with negative effects on canopy microclimate and fruit quality (Jackson and Lombard 1993). The net effect in reducing the total number of cluster at the onset of ripening has been show to result in higher concentration of aroma compounds (Sinton et al. 1978, Balasubrahmanyam et al. 1979, McCarthy et al. 1985) and increased sugar accumulation in the fruit (Ferree et al. 2005, Reynolds et al. 2007). When cluster thinning was applied at different times throughout the growing season from bloom to veraison in Vidal and Chardonnay vines, fruit quality increased the most when cluster thinning was applied just prior to veraison (Ferree et al. 2005).

The TFS treatment had the lowest concentration for almost all aroma compounds, with the exception of geranyl acetone having its highest concentration in both 2003 and 2004 and ethyl cinnamate also having the highest concentration in 2003 TFS. These results are a contradiction from the results found in the Vidal wines, the most likely explanation being differences in cultivars and fruit quality.

Keller et al (2005) investigated the effect of cluster thinning on three deficit-irrigated cultivars and found response differences between cultivars with Riesling being less responsive to thinning than Chenin blanc or Cabernet Sauvignon. Since this study was looking at the effect of irrigation, perhaps Riesling was less responsive because it is a more drought tolerant cultivar (Gaudillere et al. 2002), and therefore limitation of water supply did not affect berry composition. While our study found Riesling icewine grapes to be responsive to cluster thinning, the vines were not irrigated and the conditions were

not dry. The study does highlight that response to cluster thinning could be related to differences in cultivar, which can affect the number of clusters per vine, cluster and berry size, maturation times and aroma compound development.

Differences in the growing season and hang time period did not have the same effect on Riesling wines as seen in Vidal. This can be related to sub-standard fruit quality in the Riesling grapes in both vintages. At icewine harvest, the Riesling grapes were highly desiccated compared to the Vidal grapes. The higher glycerol concentration, of the wine was also an indication of desiccated fruit, which activated the glycerol stress response in the yeast during fermentation (Table 4.2). Because of the dehydration present in all treatments the effect of freeze and thaw cycles was less evident because further significant desiccation could not occur.

Odour activity values: Any compound found above its sensory threshold, was found above its sensory threshold in all treatments. This suggests that while concentration ranges varied, compounds were odour-potent in all treatments. β-Damascenone was the most odour-potent compound in all treatments; its concentration was highest in the control (fully cropped) treatment in both 2003 and 2004 for Vidal and Riesling.

Conclusions

Vidal icewines had the highest concentration of aroma compounds in the control and TV wines in 2003 and in TFS wines in 2004. Almost all compounds were found to differ in Vidal: 17 of 24 in 2003 and 23 of 24 in 2004. The compounds with the highest odour activity values in Vidal were β-damascenone, ethyl octanoate, *cis*-rose oxide, 1-octen-3-ol, ethyl hexanoate and isoamyl acetate in 2003 and β-damascenone, 1-octen-3-

ol, ethyl octanoate, *cis*-rose oxide, and ethyl hexanoate in 2004. PCA found β-damascenone, ethyl 2- and 3- methylbutyrate, ethyl isobutyrate, ethyl butyrate and 1-heptanol to be correlated and associated with the control in 2003. In 2004, PCA found most attributes positively loaded on F1 and associated with vineyard replicate (block) and not crop level treatment. Riesling icewines differed for all aroma compounds in 2003 and 22 of 23 in 2004. In both years, the majority of the aroma compounds had the highest concentration in TV wines and the lowest concentration in TFS wines. Odour activity values in Riesling icewines showed that β-damascenone, ethyl octanoate and ethyl hexanoate were the most odour potent compounds both 2003 and 2004, while *cis*-rose oxide was also highly odour potent in 2004. The PCA from the 2003 and 2004 Riesling icewine showed most attributes loaded on F1 and associated with TV wines. Freeze and thaw events in November and December are likely more important in aroma compound development than crop level. Further investigation into how the effect freeze and thaw events and berry desiccation impact aroma compound development in icewine should be undertaken to fully understand the impact of weather on flavor development.

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Table 4.3. Impact of crop level treatment on 2003 Vidal icewine aroma compounds determined by GC-MS, Garphil Farms, St. Catharines, ON, 2003-04.

Table 4.4. Impact of crop level treatment on 2004 Vidal icewine aroma compounds determined by GC-MS, Garphil Farms, St. Catharines, ON, 2003-04.

Table 4.5. Impact of crop level treatment on odour activity values (OAV) of aroma compounds in Vidal icewines and their published sensory threshold values, Garphil Farms, St. Catharines, ON, 2003-04.

Table 4.6. Impact of crop level treatment on 2003 Riesling icewine aroma compounds determined by GC-MS, Lambert Farms, Niagara-on-the-Lake, ON, 2003-04.

Table 4.7. Impact of crop level treatment on 2004 Riesling icewine aroma compounds determined by GC-MS, Lambert Farms, Niagara-on-the-Lake, ON, 2003-04.

Table 4.8. Impact of crop level treatment on odour activity values (OAV) of aroma compounds in Riesling icewines and their published sensory threshold values, Lambert Farms, Niagara-on-the-Lake, ON, 2003-04.

Table 4.1. Impact of crop level treatment on Vidal and Riesling icewine must chemical composition, Garphil Farms, St. Catharines, ON, and Lambert Farms, Niagara-on-the-Lake, ON, 2003-04.

Cultivar	Year	Treatment	Brix	pH	TA (g/L)
Vidal	2003	C	35.3 ± 1.2	3.46 ± 0.08a	8.60 ± 0.38a
		TS	35.3 ± 0.43	3.60 ± 0.13c	8.26 ± 0.32b
		TV	35.3 ± 0.17	3.51 ± 0.14b	8.25 ± 0.36b
	Significance		ns	***	***
	2004	C	35.4 ± 0.25a	3.40 ± 0.07a	8.74 ± 0.50c
		TS	35.8 ± 0.40b	3.56c ± 0.05	8.27 ± 0.37b
		TV	35.9c ± 0.43	3.55b ± 0.08	7.53 ± 0.36a
	Significance		***	***	***
Riesling	2003	C	35.8 ± 0.05a	3.33 ± 0.04a	10.2 ± 1.0a
		TS	35.5 ± 0.04c	3.35 ± 0.01b	9.65 ± 0.98b
		TV	35.6 ± 0.09b	3.37 ± 0.02c	9.29 ± 0.80c
	Significance		***	***	***
	2004	C	34.9 ± 0.83b	3.52 ± 0.04a	9.02 ± 0.39c
		TS	35.5 ± 0.25c	3.56 ± 0.02b	8.92 ± 0.22b
		TV	34.5 ± 0.50a	3.60 ± 0.01c	7.75 ± 0.16a
	Significance		**	***	***

ns, **, ***: not significant or significant at $p < 0.01$ and 0.001, respectively. Means with the same letter are not significantly different.

Table 4.2. Impact of crop level treatment on Vidal and Riesling icewine chemical composition, Garphil Farms, St. Catharines, ON, and Lambert Farms, Niagara-on-the-Lake, ON, 2003-04.

		Control	Thin at Set	Thin at Veraison	Significance
Vidal 2003	pH	3.69 ± 0.07a	3.83 ± 0.08c	3.72 ± 0.13b	***
	A420	0.56 ± 0.05 a	0.80 ± 0.08b	0.96 ± 0.05c	***
	TA (g/L)	8.93 ± 0.24a	8.84 ± 0.10a	9.13 ± 0.22b	**
	Acetic acid (g/L)	0.84 ± 0.06	0.83 ± 0.02	0.86 ± 0.03	ns
	Glycerol (g/L)	14.1 ± 1.1c	12.7 ± 0.27a	13.6 ± 0.28b	***
	Ethanol (% v/v)	15.7 ± 0.60a	15.8 ± 0.21a	15.5 ± 0.37b	***
	Residual Sugar (g/L)	90.7 ± 8.9a	97.4 ± 2.4b	102 ± 2.3c	***
Vidal 2004	pH	3.58 ± 0.01a	3.70 ± 0.06c	3.68 ± 0.05b	***
	A420	0.34 ± 0.01a	0.47 ± 0.02c	0.41 ± 0.03b	***
	TA (g/L)	10.0 ± 0.24c	9.64 ± 0.20b	9.10 ± 0.17a	***
	Acetic acid (g/L)	0.74 ± 0.02b	0.72 ± 0.02a	0.74 ± 0.01b	**
	Glycerol (g/L)	10.8 ± 0.38b	9.85 ± 0.50a	10.8 ± 0.58b	***
	Ethanol (% v/v)	11.1 ± 0.20a	11.1 ± 0.53b	11.2 ± 0.42b	*
	Residual Sugar (g/L)	163 ± 6.4a	167 ± 6.3b	167 ± 7.1b	**
Riesling 2003	pH	3.50 ± 0.01b	3.49 ± 0.01a	3.52 ± 0.01c	***
	A420	0.39 ± 0.02a	0.43 ± 0.04b	0.48 ± 0.06c	***
	TA (g/L)	11.5 ± 0.64a	10.9 ± 0.80b	10.8 ± 0.63b	***
	Acetic acid (g/L)	0.80 ± 0.06a	0.79 ± 0.03a	0.75 ± 0.08b	*
	Glycerol (g/L)	34.2 ± 0.72c	32.3 ± 1.8b	31.0 ± 0.33a	***
	Ethanol (% v/v)	10.6 ± 0.47c	10.4 ± 0.66b	9.65 ± 0.77a	***
	Residual Sugar (g/L)	132 ± 14a	149 ± 11c	139 ± 23b	***
Riesling 2004	pH	3.66 ± 0	3.68 ± 0	3.67 ± 0	ns
	A420	0.42 ± 0	0.39 ± 0	0.38 ± 0	ns
	TA (g/L)	10.7 ± 0.14	11.0 ± 0.05	10.3 ± 0.10	ns
	Acetic acid (g/L)	0.70 ± 0.01	0.80 ± 0	0.68 ± 0.01	ns
	Glycerol (g/L)	23.5 ± 0.15	24.2 ± 0.19	24.9 ± 0.48	ns
	Ethanol (% v/v)	11.2 ± 0.04a	11.7 ± 0.03b	12.1 ± 0.03b	*
	Residual Sugar (g/L)	134 ± 1.2a	132 ± 0.47a	115 ± 0.24b	*

ns, *, **, ***: not significant or significant at $p < 0.05$, 0.01 and 0.001, respectively. Means with the same letter are not significantly different.

Table 4.3. Impact of crop level treatment on 2003 Vidal icewine aroma compounds concentrations (µg/L) determined by GC-MS, Garphil Farms, St. Catharines, ON, 2003-04.

NO	Compounds	Control	Thin at Set	Thin at Veraison	Significance	F-value	p-value
1	ethyl isobutyrate	77.3 a	72.7 b	76.0 a	**	37.0	0.003
2	ethyl butyrate	74.2 a	70.5 b	68.4 b	**	18.1	0.01
3	ethyl 2-methylbutyrate	30.1 a	23.0 b	30.6 a	***	160	0.0002
4	ethyl 3-methylbutyrate	31.7 a	27.6 b	31.7 a	***	92.9	0.0004
5	1-hexanol	1202 c	1229 b	1242 a	***	110	0.0003
6	isoamyl acetate	189 b	202 a	181 c	***	64.1	0.0009
7	ethyl valerate	4.97 a	5.03 a	5.30 a	ns	6.86	0.051
8	1-heptanol	13.0 b	12.4 c	14.1 a	**	45.2	0.002
9	1-octen-3-ol	35.6 b	40.0 a	41.1 a	*	9.75	0.029
10	ethyl hexanoate	453 a	465 a	468 a	ns	1.30	0.368
11	acetophenone	4.15 a	1.51 b	1.57 b	*	17.6	0.01
12	1-octanol	9.66 a	9.68 a	9.65 a	ns	0.25	0.791
13	linalool	32.0 b	39.8 a	30.5 b	**	42.0	0.002
14	*cis*-rose oxide	8.37 b	10.3 a	8.60 b	***	275	<0.0001
15	phenethyl alcohol	19694 a	19108 a	19610 a	ns	3.15	0.15
16	nerol oxide	16.9 a	16.1 a	17.4 a	ns	6.53	0.055
17	ethyl benzoate	1.12 a	0.93 b	0.88 b	*	17.0	0.011
18	ethyl octanoate	861 a	947 a	904 a	ns	5.08	0.08
19	decanal	1.50 b	1.36 c	1.70 a	***	88.9	0.0005
20	ethyl phenylacetate	5.78 a	5.21 b	5.74 a	**	43.2	0.002
21	4-vinylguaicol	51.2 c	59.2 a	55.9 b	***	476	<0.0001
22	γ nonalactone	3.42 a	3.46 a	3.54 a	ns	5.63	0.069
23	β damascenone	46.7 a	39.3 b	44.7 a	*	13.7	0.016
24	geranyl acetone	0.34 b	0.42 a	0.34 b	***	214	<0.0001

ns, *, **, ***: not significant or significant at $p < 0.05$, 0.01 and 0.001, respectively. Means with the same letter are not significantly different.

Table 4.4. Impact of crop level treatment on 2004 Vidal icewine aroma compound concentrations (μg/L) determined by GC-MS, Garphil Farms, St. Catharines, ON, 2003-04.

NO	Compounds	Control	Thin at Set	Thin at Veraison	Significance	F-value	p-value
1	ethyl isobutyrate	80.4 c	86.8 a	82.6 b	**	37.6	0.003
2	ethyl butyrate	77.1 a	80.8 a	78.2 a	ns	2.25	0.221
3	ethyl 2-methylbutyrate	25.8 a	25.0 b	23.1 c	**	61.1	0.001
4	ethyl 3-methylbutyrate	29.5 a	29.6 a	27.5 b	**	52.0	0.001
5	1-hexanol	1386 c	1462 a	1309 b	***	79.1	0.0006
6	isoamyl acetate	208 c	266 a	241 b	***	219	<0.0001
7	ethyl valerate	8.90 b	10.0 a	8.40 b	**	35.6	0.003
8	1-heptanol	14.0 b	17.8 a	13.3 b	**	37.6	0.003
9	1-octen-3-ol	208 b	279 a	198 b	***	149	0.0002
10	ethyl hexanoate	564 b	659 a	569 b	**	19.6	0.009
11	acetophenone	1.60 b	2.40 a	1.50 b	**	25.3	0.005
12	1-octanol	10.5 a	11.0 a	9.91 b	*	14.5	0.015
13	linalool	32.3 b	43.1 a	30.8 b	***	199	<0.0001
14	*cis*-rose oxide	37.2 c	67.4 a	43.5 b	***	385	<0.0001
15	phenethyl alcohol	30253 b	32291 a	26416 c	**	39.1	0.002
16	nerol oxide	26.3 b	44.2 a	27.9 b	***	334	<0.0001
17	ethyl benzoate	1.10 b	1.40 a	0.9 c	***	103	0.0004
18	ethyl octanoate	997 b	1201 a	1021 b	*	8.06	0.04
19	decanal	1.70 a	1.3 b	0.62 c	***	64.5	0.0009
20	ethyl phenylacetate	9.10 a	8.9 a	7.70 b	**	58.0	0.0011
21	4-vinylguaiacol	82.5 b	95.8 a	80.0 b	**	71.4	0.0007
22	γ- nonalactone	8.40 c	10.6 a	9.24 b	**	53.3	0.0013
23	β- damascenone	32.0 a	29.3 b	25.7 c	**	25.0	0.005
24	geranyl acetone	0.30 c	0.35 b	0.38 a	***	108	0.0003

ns, *, **, ***: not significant or significant at $p < 0.05$, 0.01 and 0.001, respectively. Means with the same letter are not significantly different.

Table 4.5. Impact of crop level treatment on odour activity values (OAV) of aroma compounds in Vidal icewines and their published sensory threshold values, Garphil Farms, St. Catharines, ON, 2003-04.

		2003 OAV			2004 OAV			
NO	Compound	Control	Thin at Set	Thin at Veraison	Control	Thin at Set	Thin at Veraison	Threshold (µg/L)
1	ethyl isobutyrate	5.15	4.85	5.07	5.36	5.79	5.51	15
2	ethyl butyrate	3.71	3.52	3.42	3.85	4.04	3.91	20
3	ethyl 2-methylbutyrate	1.67	1.28	1.70	1.44	1.39	1.28	18
4	ethyl 3-methylbutyrate	10.6	9.20	10.6	9.83	9.86	9.18	3
5	1-hexanol	0.15	0.15	0.16	0.17	0.18	0.16	8000
6	isoamyl acetate	6.30	6.74	6.06	6.92	8.86	8.02	30
7	ethyl valerate	3.31	3.35	3.54	5.93	6.63	5.60	1.5
8	1-heptanol	4.34	4.13	4.69	4.68	5.92	4.42	3
9	1-octen-3-ol	35.6	40.0	41.1	208	279	198	1
10	ethyl hexanoate	32.4	33.2	33.5	40.3	47.1	40.7	14
11	acetophenone	0.06	0.02	0.02	0.02	0.04	0.02	65
12	1-octanol	0.09	0.09	0.09	0.10	0.10	0.09	110
13	linalool	1.28	1.59	1.22	1.29	1.72	1.23	25
14	*cis*-rose oxide	41.8	51.6	43.0	186	337	218	0.2
15	phenethyl alcohol	1.41	1.37	1.40	2.16	2.31	1.89	14000
16	nerol oxide	0.01	0.01	0.01	0.01	0.02	0.01	3000
17	ethyl benzoate	0.002	0.002	0.002	0.002	0.002	0.002	575
18	ethyl octanoate	172	189	180	199	240	204	5
19	decanal	0.75	0.68	0.85	0.87	0.63	0.29	2
20	ethyl phenylacetate	0.08	0.07	0.08	0.12	0.12	0.11	73
21	4-vinylguaicol	5.12	5.91	5.59	8.25	9.58	7.99	10
22	γ nonalactone	0.11	0.12	0.12	0.28	0.35	0.31	30
23	β damascenone	934	786	894	639	585	513	0.05
24	geranyl acetone	0.01	0.01	0.01	0.01	0.01	0.01	60

Table 4.6. Impact of crop level treatment on 2003 Riesling icewine aroma compound concentrations (μg/L) determined by GC-MS, Lambert Farms, Niagara-on-the-Lake, ON, 2003-04.

NO	Compound	Control	Thin at Set	Thin at Veraison	Significance	F-value	p-value
1	ethyl isobutyrate	204 b	205 b	214 a	***	281	<0.0001
2	ethyl butyrate	137 b	136 c	141 a	***	80.7	0.0005
3	ethyl 3-methylbutytate	32.2 b	31.2 b	43.3 a	***	527	<0.0001
4	1-hexanol	490 b	475 c	502 a	***	157	0.0002
5	1-octen-3-ol	7.70 a	6.41 b	7.61 a	***	186	0.0001
6	ethyl hexanoate	295 b	246 c	342 a	***	114	0.0003
7	acetophenone	1.89 c	1.95 b	1.96 a	***	356	<0.0001
8	1-octanol	5.00 b	4.50 c	5.71 a	***	322	<0.0001
9	linalool	41.5 c	42.6 b	49.0 a	***	478	<0.0001
10	*cis*-rose oxide	1.50 a	1.01 c	1.20 b	***	2866	<0.0001
11	phenethyl alcohol	16733 b	16584 b	17671 a	***	104	0.0004
12	nerol oxide	27.8 c	31.2 b	38.5 a	***	1156	<0.0001
13	ethyl benzoate	1.4 b	1.21 c	1.51 a	***	886	<0.0001
14	ethyl octanoate	575 b	425 c	670 a	***	61.8	0.0009
15	decanal	1.30 b	0.60 c	1.90 a	***	835	<0.0001
16	ethyl phenylacetate	5.00 b	4.61 c	5.40 a	***	1088	<0.0001
17	2-phenethyl acetate	7.31 b	7.00 c	8.10 a	***	1138	<0.0001
18	4-vinylguaiacol	ND	ND	ND	-	-	-
19	γ-nonalactone	15.9 b	15.7 c	16.2 a	***	190	0.0001
20	β-damascenone	10.9 b	8.91 c	11.8 a	***	630	<0.0001
21	geranyl acetone	0.31 b	0.36 a	0.27 c	***	260	<0.0001
22	ethyl cinnamate	4.90 c	5.70 a	5.50 b	***	195	0.0001
23	β-ionone	0.02 a	0.003 c	0.01 b	***	1010	<0.0001

***: significant at $p < 0.001$. Means with the same letter are not significantly different.

Table 4.7. Impact of crop level treatment on 2004 Riesling icewine aroma compound concentrations (μg/L) determined by GC-MS, Lambert Farms, Niagara-on-the-Lake, ON, 2003-04.

NO	Compound	Control	Thin at Set	Thin at Veraison	Significance	F-value	p-value
1	ethyl isobutyrate	199b	200 a	202 a	*	16.6	0.024
2	ethyl butyrate	141 b	139 b	151 a	**	34.8	0.008
3	ethyl 3-methylbutytate	27.5 b	26.9 b	37.1 a	**	46.6	0.006
4	1-hexanol	535 b	517 b	599 a	**	39.1	0.007
5	1-octen-3-ol	10.8 b	9.80 b	13.9 a	**	54.1	0.004
6	ethyl hexanoate	461 b	399.3 c	572 a	**	55.9	0.004
7	acetophenone	1.91 a	1.88 b	1.88 a	**	42.7	0.006
8	1-octanol	4.50 b	4.10 c	5.81 a	***	254	0.000
9	linalool	43.7 b	42.9 b	57.6 a	***	247	0.000
10	*cis*-rose oxide	3.10 b	2.80 c	3.50 a	*	29.3	0.011
11	phenethyl alcohol	17593 b	17169 b	18800 a	**	32.2	0.009
12	nerol oxide	17.3 b	16.7 b	23.8 a	**	159	0.001
13	ethyl benzoate	1.60 b	1.41 c	1.90 a	**	129	0.001
14	ethyl octanoate	882 b	803 b	1100 a	**	51.7	0.005
15	decanal	0.55 b	0.22 c	0.67 a	***	563	0.000
16	ethyl phenylacetate	6.01 a	5.40 b	6.30 a	*	24.9	0.013
17	2-phenethyl acetate	7.70	7.80	7.92	ns	0.35	0.728
18	4-vinylguaiacol	71.6 c	74.6 b	75.8 a	**	181	0.001
19	γ-nonalactone	17.2 b	16.5 c	17.5 a	**	47.2	0.005
20	β-damascenone	10.1 a	8.90 b	10.6 a	*	18.1	0.021
21	geranyl acetone	0.36 b	0.41 a	0.32 c	***	245	0.000
22	ethyl cinnamate	4.71 b	4.62 b	5.00 a	**	37.9	0.007
23	β-ionone	0.22 a	0.14 b	0.03 c	***	468	0.000

ns, *, **, ***: not significant or significant at $p < 0.05$, 0.01 and 0.001, respectively. Means with the same letter are not significantly different.

Table 4.8. Impact of crop level treatment on odour activity values (OAV) of aroma compounds in Riesling icewines and their published sensory threshold values, Lambert Farms, Niagara-on-the-Lake, ON, 2003-04.

		2003 OAV			2004 OAV			
NO	Compound	Control	Thin at Set	Thin at Veraison	Control	Thin at Set	Thin at Veraison	Threshold (µg/L)
1	ethyl isobutyrate	13.6	13.6	14.2	13.2	13.4	13.4	15
2	ethyl butyrate	6.84	6.78	7.05	7.05	6.95	7.55	20
3	ethyl 3-methylbutytate	10.74	10.4	14.4	9.17	8.96	12.4	3
4	1-hexanol	0.06	0.06	0.06	0.07	0.07	0.07	8000
5	1-octen-3-ol	7.66	6.36	7.56	10.8	9.83	13.9	1
6	ethyl hexanoate	21.1	17.6	24.5	32.9	28.5	40.8	14
7	acetophenone	0.03	0.03	0.03	0.03	0.03	0.03	65
8	1-octanol	0.05	0.04	0.05	0.04	0.04	0.03	110
9	linalool	1.66	1.71	1.96	1.75	1.72	2.30	25
10	*cis*-rose oxide	7.48	5.08	5.83	15.3	13.9	17.5	0.2
11	phenethyl alcohol	1.20	1.19	1.26	1.26	1.22	1.34	14000
12	nerol oxide	0.01	0.01	0.01	0.01	0.01	0.01	3000
13	ethyl benzoate	0.002	0.002	0.003	0.003	0.002	0.003	575
14	ethyl octanoate	115	85.0	134	176	161	220	5
15	decanal	0.63	0.32	0.95	0.28	0.11	0.34	2
16	ethyl phenylacetate	0.07	0.06	0.07	0.08	0.08	0.09	73
17	2-phenethyl acetate	0.03	0.03	0.03	0.03	0.03	0.03	250
18	4-vinylguaicol	-	-	-	7.17	7.46	7.58	10
19	γ-nonalactone	0.53	0.52	0.54	0.57	0.55	0.58	30
20	β-damascenone	218	179	237	202	178	212	0.05
21	geranyl acetone	0.01	0.01	0.004	0.01	0.01	0.01	60
22	ethyl cinnamate	4.48	5.18	4.99	4.27	4.14	4.56	1.1
23	β-ionone	0.18	0.03	0.13	2.49	1.58	0.34	0.09

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Figure 4.1. Chemical maps of 2003 and 2004 Ontario Vidal icewines produced from different crop levels, displaying variation in the products with principal component analysis. A: 2003; B: 2004.

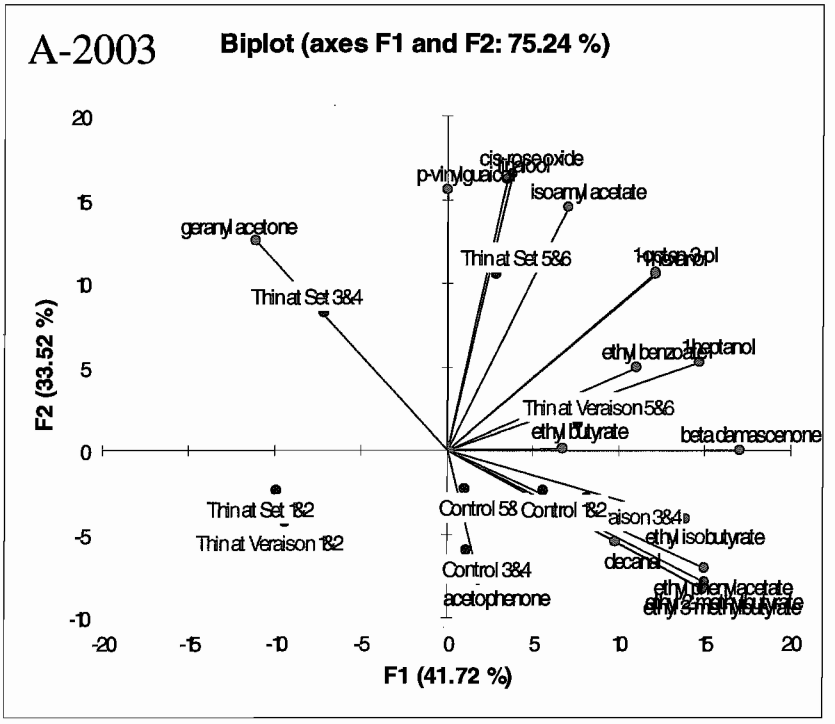
Figure 4.2. Canonical variant analysis of 2003 and 2004 Ontario Vidal icewines produced from different crop levels, showing the separation between treatments and associated aroma compounds. A: 2003; B: 2004.

Figure 4.3. Chemical maps of 2003 and 2004 Ontario Riesling icewines produced from different crop levels, displaying variation in the products with principal component analysis. A: 2003; B: 2004.

Figure 4.4. Canonical variant analysis of 2003 Ontario Riesling icewines produced from different crop levels, showing the separation between treatments and associated aroma compounds.

Figure 4.5. Comparison of daily maximum, minimum and mean temperature data from October to December in 2003 (A-C) and 2004 (D-F), St. Catharines, ON. Highlights difference in temperature patterns during hang time of icewine grapes between the two years.

Figure 4.1. Chemical maps of 2003 and 2004 Ontario Vidal icewines produced from different crop levels, displaying variation in the products with principal component analysis. A: 2003; B: 2004.



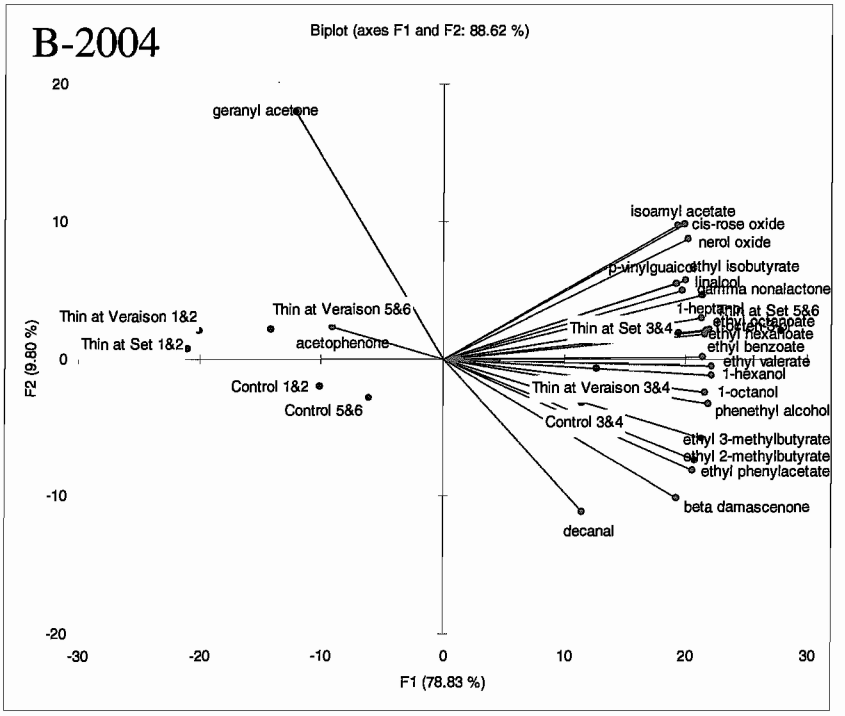
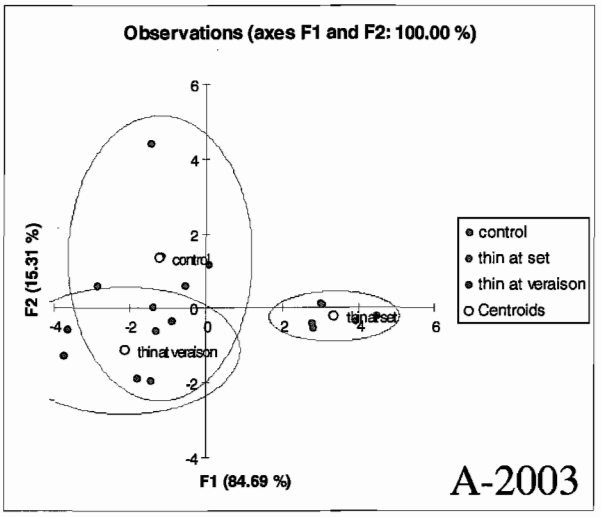
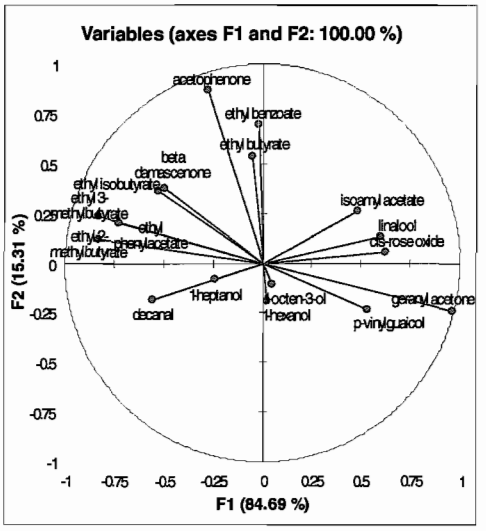
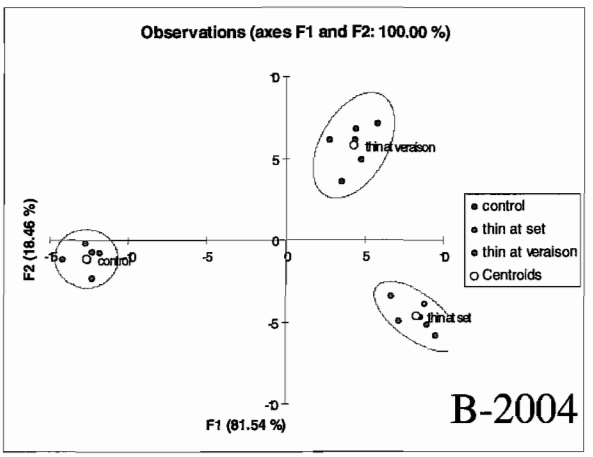


Figure 4.2. Canonical variant analysis of 2003 and 2004 Ontario Vidal icewines produced from different crop levels, showing the separation between treatments and associated aroma compounds. A: 2003; B: 2004.







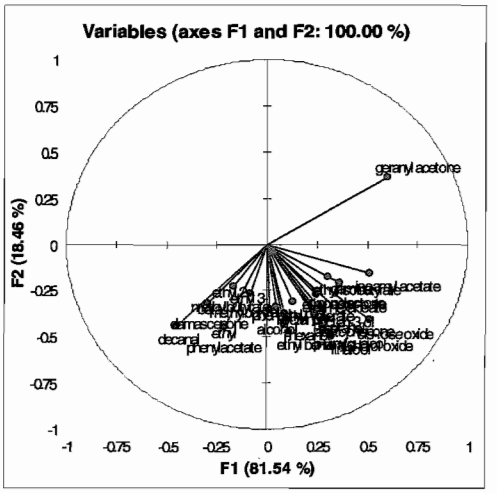
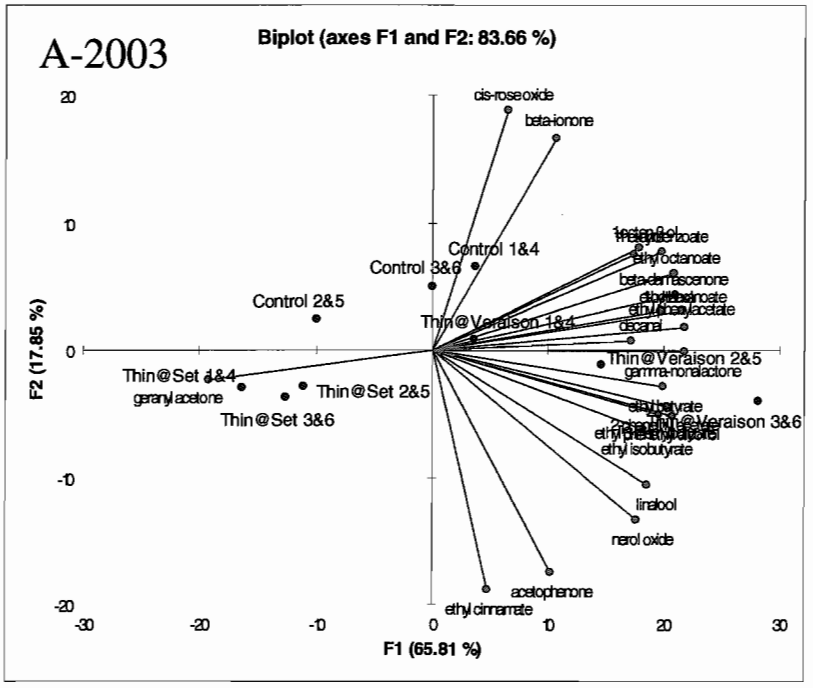


Figure 4.3. Chemical maps of 2003 and 2004 Ontario Riesling icewines produced from different crop levels, displaying variation in the products with principal component analysis. A: 2003; B: 2004.



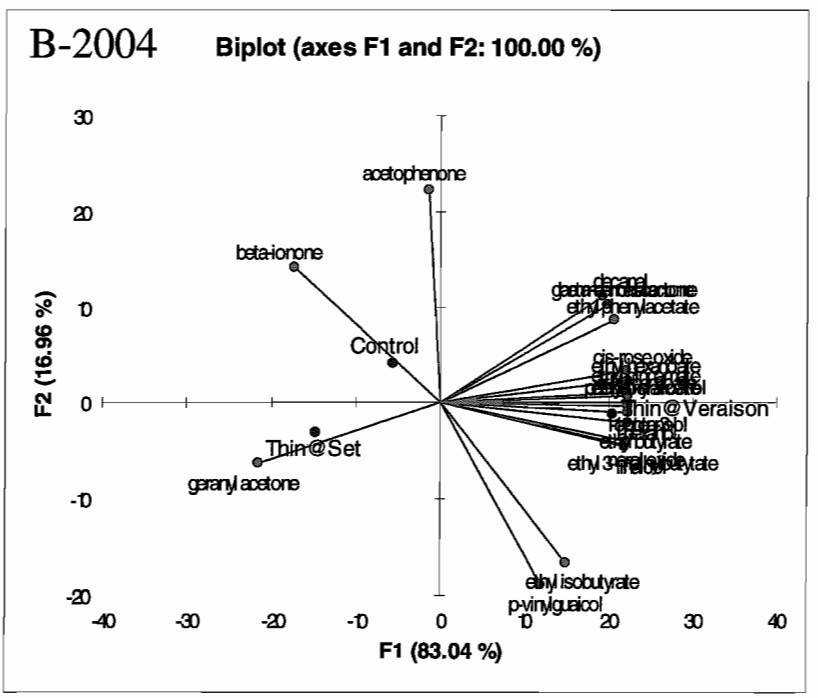
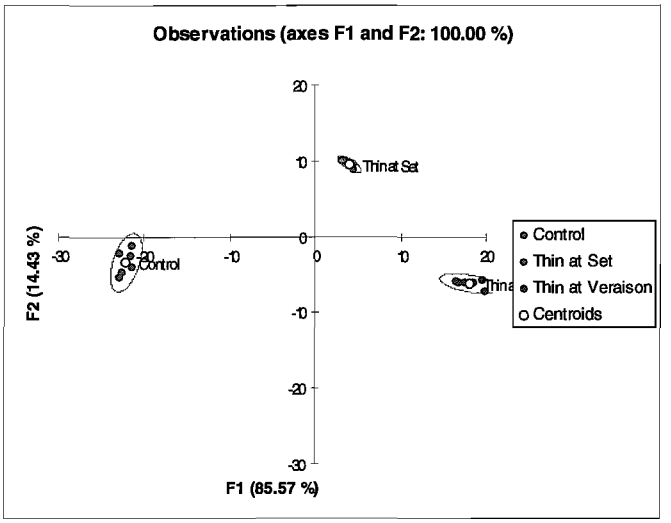


Figure 4.4. Canonical variant analysis of 2003 Ontario Riesling icewines produced from different crop levels, showing the separation between treatments and associated aroma compounds.



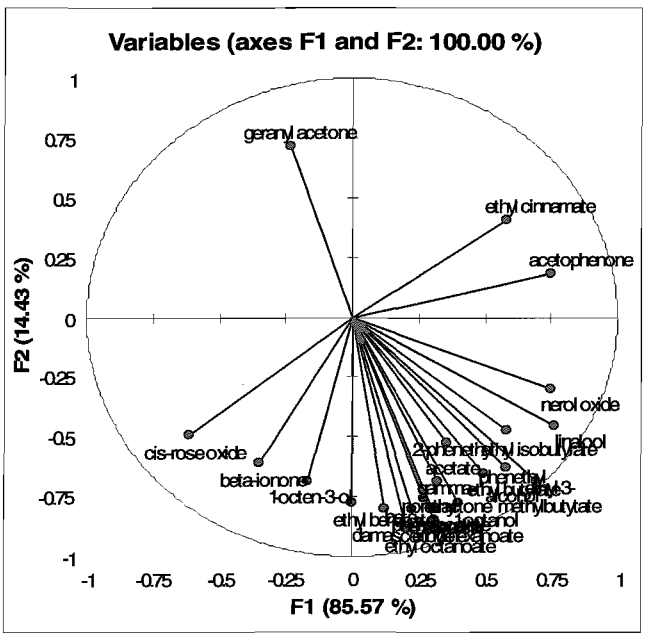
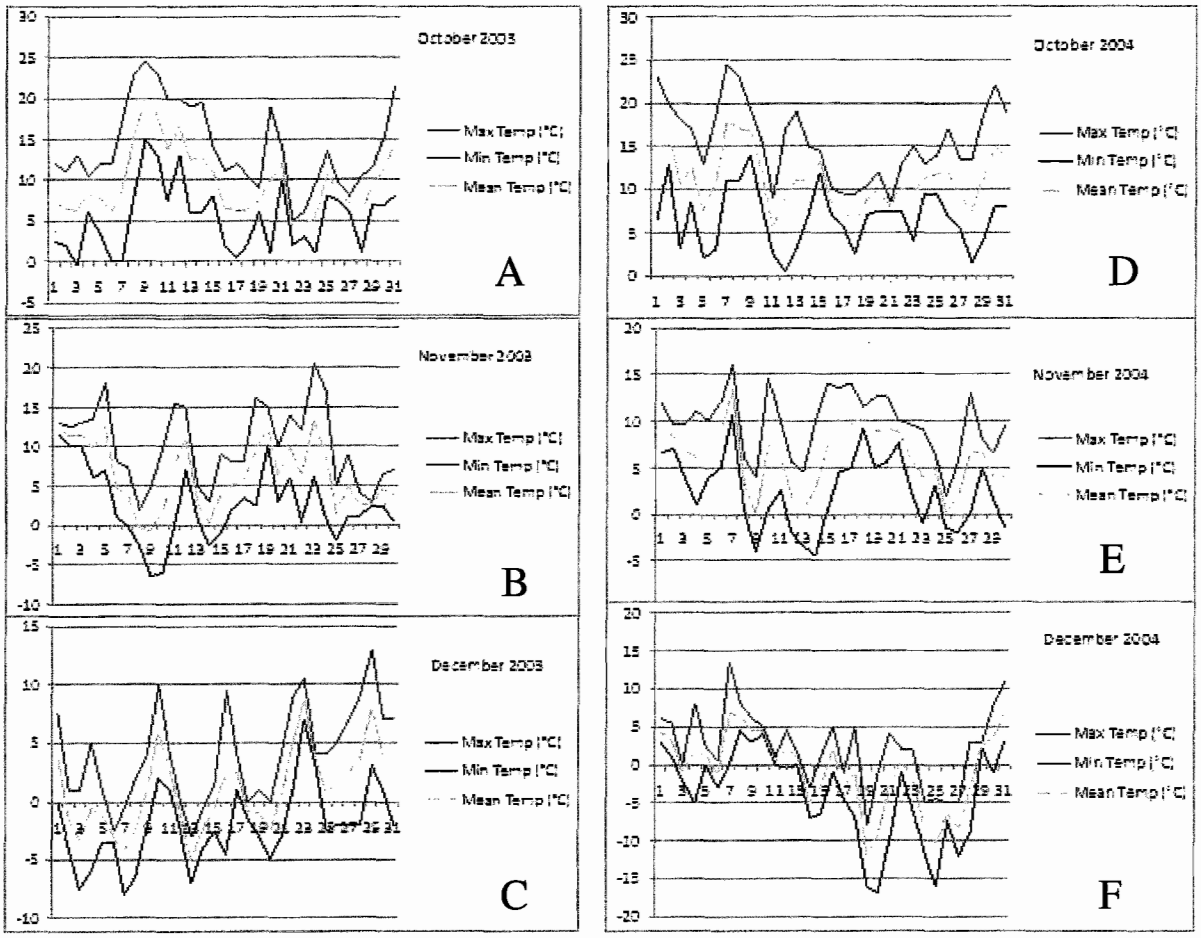


Figure 4.5. Comparison of daily maximum, minimum and mean temperature data from October to December in 2003 (A-C) and 2004 (D-F), St. Catharines, ON. Highlights difference in temperature patterns during hang time of icewine grapes between the two years.



Source : Environment Canada (Canada)

Chapter 5
The Effect of Harvest Date and Crop Level on Vidal blanc and Riesling Icewines from the Niagara Peninsula: II. Relating Sensory and Instrumental Analysis

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Abstract

We hypothesize that the freeze and thaw cycles endured by icewine grapes change their chemical and sensory profiles due to climatic events. The objective of this study was to determine the influence of harvest date and crop level on icewine sensory profiles and their interaction with chemical parameters. Harvest date (HD): Riesling and Vidal icewines were made from four HD; Harvest 1: 19 December; Harvest 2: 29 December; Harvest 3: 18 January; Harvest 4: 11 February (Vidal only). Crop level (CL): Riesling and Vidal icewines from three vineyard treatments [control (fully cropped), cluster thin at fruit set to one (basal) cluster per shoot (TFS), and cluster thin at veraison (TV)] were evaluated in a randomized block design for Riesling and Vidal cultivars over two seasons, 2003-04 and 2004-05. Triangle tests showed significant differences between HD and CL for both cultivars ($p<0.05$). Wines were then evaluated by descriptive analysis using 14 trained judges. Ten and eleven aroma and flavor attributes were significantly different ($p<0.05$) in Vidal and Riesling HD icewines, respectively. For Vidal, later HD had significantly higher intensity scores for aroma and flavor descriptors than H1. For Riesling, H1 wines had higher intensity ratings for fresh fruit descriptors whereas H 3 wines were higher for dried fruit and nutty descriptors. Partial least squares regression (PLS) found Vidal icewines to be described by dried fruit/raisin and honey flavors and viscosity, these sensory attributes were correlated several aroma compounds and were associated with later HD . Riesling icewines had more complex interactions between sensory descriptors and aroma compounds. Sensory differences were also found in the CL icewines, thinned treatments were found to have higher intensity ratings of fruity, honey, sherry and nut aroma and flavors in both cultivars. PLS showed sherry flavor the most important explanatory variable in 2003 correlated with 4-vinylguaiacol and banana flavor in 2004. No clear relationship was determined by PLS for Riesling. Harvest date and crop level do affect the sensory profile and chemical composition of icewine from the Niagara Peninsula.

Key words: sensory analysis, partial least squares analysis, harvest decisions, cluster thinning

Introduction

Icewine is described as a wine style with intense aroma and flavors. However, very little is known regarding what effects the sensory properties of this wine style. The sensory profiles of icewines have previously been studied to determine the effect of yeast

strain (Erasmus et al. 2004, Kontkanen et al. 2005) and region of origin (Cliff et al. 2002, Nurgel et al. 2004) on sensory profiles and chemical variables (Setkova et al. 2007b, Soleas and Pickering 2007).

Erasmus et al. (2004) studied the effect of yeast strain on the production of acetic acid, glycerol and sensory attributes of icewine. They found icewines produced with the yeast strains N96 and EC1118 were associated with high-quality icewine, had a light yellow colour and low sulfur-like aromas and were recommended as the most suitable strains for icewine production. Kontkanen et al. (2005) found Vidal icewines produced by direct inoculation with K1-V1116 without micronutrients were described with raisin, butter and spicy aromas whereas those with micronutrient were described as sweet in taste with honey and orange flavors. Icewines produced by the stepwise acclimatization without micronutrient were described with peach and terpene aromas and with micronutrient were described with pineapple and alcohol aromas and alcohol and honey flavors.

The sensory properties of icewines have been shown to vary due to geographic location and vintage. Cliff et al. (2002) compared the sensory profiles of icewines from Ontario, British Columbia and Germany . Ontario icewines were associated with the highest fruit and floral aromas and golden copper colour. British Columbia icewines were rated high for sweetness, body/viscosity and intensity of aftertaste. German icewines were associated with a nutty/oily character and had the highest acidity. Principal component analysis showed separation for the Canadian and German wines but not between Ontario and British Columbia wines. Nurgel et al. (2004) found Ontario icewines had higher intensity ratings for apricot, raisin, honey and oak aromas compared

to British Columbia icewines and that British Columbia wines had higher intensities for pineapple and oxidized aromas than Ontario icewines. This study found that icewines from Ontario and British Columbia while different in their sensory profiles differed more based on chemical composition.

These studies indicate that differences in yeast strain, region of origin and vintage changed the sensory profile of the resultant wine. However the effect of viticultural treatment, such as harvest date and crop level, on icewine sensory properties has not been studied. The effect of harvest date on other wines styles has been shown to affect the sensory profile of the wines. A study investigating the effect of grape maturity on the composition and quality of Vidal wines from Ohio rated wines on a seven-point hedonic scale from three different harvest dates; early –1 Oct, mid – 5 Oct and late – 31 Oct. Wines from the mid harvest date treatment were rated the highest, therefore the most preferred wines by the panelist for aroma and taste over two vintages and described as more fruity than the early or late harvest date (Gallander 1983). Reynolds and Wardle (1997) found that wines from six and three different cultivars for aroma and flavor, respectively could be differentiated through triangle tests when produced from "early" and "late" harvested fruit. The sensory properties of sweet Fiano wines found base wines harvested at normal maturity (22 ^{o}Brix) to be described by fruity (banana, apple, pear, and pineapple), floral (lime, rose and acacia) and vegetal notes (mint, grass and wild fennel) whereas sweet wines harvested at late maturity (26 ^{o}Brix) and dried to 32 ^{o}Brix were described using terms of citrus jam, dried apricot, dried figs, prune, honey and coconut (Genovese et al. 2007).

The effect of crop level on other wines styles has been shown to affect the sensory profile of the wines. Reynolds et al (1996) found that cluster thinning produced Pinot noir wines which were rated by panelist as having less grassy and vegetative characteristics and were rated higher for descriptors such as black pepper, cherry, and currant. PCA results showed correlations between typical Pinot noir descriptors and cluster thinning. In another study by Reynolds et al (1994a) the effect of vineyard treatments on Riesling composition and sensory response in cluster thinned vines to three different crop levels; 1, 1.5, and 2 clusters per shoot was examined. They found that monoterpene concentrations decreased with increasing number of clusters per shoot. Linalool was positively correlated with ripe fruit character and sweetness and negatively correlated with green-fruit flavor and cluster thinning was found to increase the perception of ripe fruit character in the wines. Monoterpenes such as linalool, linalool oxides, terpineol and citronellol were found to be associated with lower crop levels and low to moderate shoot densities and were found to increase in concentration with age (Reynolds et al. 1994a). Cluster thinning, yeast strain and enzyme treatment were found to affect the sensory properties for Chardonnay Musqué wines (Reynolds et al. 2007). Wines from the cluster thinned treatment generally had increased colour depth, higher sweetness and herbaceous / grassy aromas and lower tropical fruit aroma than fully cropped treatments.

Wine flavor is a complex interaction of aroma, taste and tactile sensation elicited by hundreds of chemical compounds which are affected by viticultural and enological practices. There is much interest in the fields of oenology and sensory science to understand wine flavor, which involves not only a description of the sensory profile of a wine but how it is affected by the wines chemical composition and odor-active profile

(Ebeler 2001, Fischer 2007, Ferreira and Cacho 2009). Multivariate statistical analysis such as partial least square regression (PLS), provide the tools that enables the relationships between these chemical variables and sensory attributes to be visualized and interpreted (Noble and Ebeler 2002). PLS is considered a 'soft modeling' technique that uses linear combinations of one set of variables (such as aroma compounds) to predict the variations in another set of variables (such as sensory attributes), essentially it indicates how well one set of variables a can predict the variation in the other set of variables (Noble and Ebeler 2002). Numerous studies on table wines have used PLS to elucidate the relationship between chemical and sensory parameters; profiling Zinfandel (Noble and Shannon 1987) and California Chardonnay wines (Lee and Noble 2006), flavor characteristics of New Zealand Sauvignon blanc wine (Lund et al. 2009), prediction of wine sensory properties (Aznar et al. 2003, Campo et al. 2005) and aroma differentiation between Bordeaux varieties (Kotseridis et al. 2000).

In the Vintners Quality Alliance Act of Ontario, the term icewine is trademark protected and can only be used for wines made from grapes naturally frozen in the vineyard in specified viticultural areas, at temperatures $\leq$ -8 °C after the 15 November of the vintage year (Government of Ontario 1999). Higher yields of icewine juice are usually obtained in December since there is less desiccation of the fruit, and the air temperature does not have to be as cold to achieve the required 35 °Brix value. It is believed anecdotally that the freeze and thaw cycles are critical to developing the sensory profile for which icewines are known. As a result, some producers in Ontario will have several icewine harvests from mid- December to late January in order to achieve a balance between flavor profile and yield.

Pressing grapes frozen for icewine production reduces the yield to 15 -20% that of table wine significantly increasing the amount of vine acreage required for production (Pickering 2006). To compensate for some of this loss in yield currently, many grape growers crop their grapevines designated for icewine at levels often double those of table wines. It was therefore of interest to ascertain whether reducing crop level might impact icewine chemical and aroma compound profiles. It is not know how crop level will affect the sensory profiles of icewines.

In two previous studies (Bowen and Reynolds 2010a, Bowen and Reynolds 2010b), the chemical variables and aroma compounds of Vidal and Riesling icewines from four distinct harvest dates; December to February, and three different crop levels; fully cropped, thin at fruit set and thin at veraison were reported. Harvest date and crop level were found to affect the concentration of the aroma compounds in both cultivars. The objective of this study was to determine the effect of harvest date and crop level on: 1) the sensory profiles of the icewines through descriptive analysis and; 2) to correlate those sensory profiles with the aroma compounds previously identified (Bowen and Reynolds 2010a, Bowen and Reynolds 2010b) using PLS of the Vidal and Riesling icewines described in the previous studies.

Materials and Methods

Harvest date wines. The Riesling and Vidal icewines were the same as those described in Bowen and Reynolds (2010b). Riesling and Vidal icewines were made from grapes harvested from Garphil Farms in West St. Catharines, over the course of the icewine season. The grapes were picked as follows: H1 on Dec.19, 2004; H2 Dec.29,

2004; H3 on Jan.18, 2005; and H4 on Feb.11, 2005. There were only three harvest dates for Riesling; the grapes from the fourth harvest were lost to bird predation.

Grapes were pressed by cultivar and harvest date in the large membrane press at two bars until the must measured approximately 37 °Brix. The exact starting °Brix was measured on each pressing (harvest date), the must was then divided into three 20-L carboys for triplicate fermentations.

Crop level wines. The Riesling and Vidal icewines were the same as those described in Bowen and Reynolds (2010a). Two commercial vineyard plots were chosen for the crop level study. Both vineyard experiments were made up of randomized block designs containing six blocks each with three treatments. The Vidal block was located at Garphil Farms in west St. Catharines. It consisted of six rows of grape vines; each one was designated as a block. Each row (block) was then divided into three treatments; control (fully cropped), thin to one basal cluster per shoot at fruit set, and thin to one basal per shoot cluster at veraison. The Riesling block was located in Niagara-on-the-Lake at Lambert Farms. It consisted of two rows of grapevines; each row was divided into three blocks. Each block was then divided in three treatments; control (fully cropped), thin to one basal cluster per shoot at fruit set, and thin to one basal cluster per shoot at veraison. All vines were sprayed and maintained at the discretion of the grower. Grapes were harvested once proper icewine conditions were met ($\leq$ -8 °C) in on 7 January 2004 and 15 January 2005.

Treatments were kept separate at harvest by placing them into labeled bins indicating their block and treatment. Grapes were pressed in the basket press with an inflatable rubber bladder by treatment at 2 bar. The resultant must was collected in 20-L

food grade pails for each treatment until it reached 35 °Brix; it was then sulfited to 75 mg/L and stored at 4°C until fermentation.

Fermentation. The must was inoculated with Lalvin® K1-V1116 *Saccharomyces cerevisiae* according to Kontkanen et al. (2004). Fermentations were stopped with the addition of 75 ppm SO_2 when the alcohol reached 10%. Wines were place at -2°C for cold stabilization. Potassium sorbate was added prior to filtration and bottling to prevent further fermentation in bottle. Refer to Bowen and Reynolds (2010a) and Bowen and Reynolds (2010b) for a more detailed explanation of the winemaking protocol and the must and wine parameters.

Difference testing. Triangle tests were performed to determine if differences could be detected between replicate fermentations of the same treatment and between treatments for each cultivar. Twenty five mL samples of icewines were presented in clear ISO wine glasses, labeled with three digit codes, and randomized. Twenty four judges were given three wines and asked to pick out the different wine based on aroma and taste. Testing was conducted under red light to prevent colour bias in the sensory testing facility (Inniskillin Hall, Brock University) equipped with Compusense software (version 4.6, Guelph, ON).

Descriptive analysis. The DA panel was made up of 14 judges, nine women and five men, all staff or students at CCOVI. Training consisted of six, one hour, sessions during which time panelist tasted through all wines and developed a lexicon of terms to describe the icewines. The lexicon contained 14 attributes assessed for aroma and flavor; apricot, banana, caramelized, citrus, dried fruit/raisin, floral, honey, lychee, nut, peach, pear/apple, sherry, tangerine and tropical fruit, and three tastes; sweet, acid and bitter, and

one mouthfeel sensation; viscosity. Reference standards were generated for each lexicon term and were anchored the 15-cm line scales through panel consensus (Table 5.1). Panelists assessed each sample in duplicate on a 15-cm line scale. Each sample contained 20 mL of icewine in a clear ISO wine glass identified with a randomized three-digit code to assess aroma, then flavor and taste using a complete randomized block design. Data collection was completed in individual booths under red light using Compusense Software in the sensory testing facility using a Williams Latin Square design.

Statistical analysis. Difference testing, triangle tests, were analyzed using Compusense software (Guelph, ON). Three-way analysis of variance (ANOVA) [F= MS(wine)/MS(judge x wine)] was performed using SENPAQ version 3.7 statistical software (Qi Statistics 2007, England) at 95% confidence ($p < 0.05$) interval for the harvest data treatments and a 90% significance ($p < 0.10$) for the crop level treatment. Attributes that differed were analyzed by least significant difference (LSD) post-hoc tests to determine which wines differ for that attribute ($p<0.05$). Principal Components Analysis (PCA), Canonical Variant Analysis (CVA), and PLS were performed with XLSTAT statistical software (Addinsoft, Paris, France).

Only different attributes found through ANOVA at 95% confidence level were used in the PCA and CVA, as they are the attributes shown to contribute to the variability due to harvest date or crop level.

PLS was run on the sensory attributes described in this paper and the chemical compounds quantified and explained in Bowen and Reynolds (2010a) and Bowen and Reynolds (2010b). The sensory attributes were X variable and the chemical compounds

the Y variable. The regression was performed at 95% significance. All variables were well described by the model; therefore, reduction of the variables was not necessary.

Results

Difference testing. ***Harvest date.*** Triangle tests were performed to determine if differences existed between triplicate fermentation of each harvest date and between harvest dates. No differences were found between triplicate fermentations of each harvest date ($n=24$, $p \leq 0.05$) for either Vidal or Riesling icewines. Differences were found between harvest date treatments ($n=24$, $p \leq 0.05$) for Vidal. In Vidal Harvest Date wines, triangle tests showed that H1 and H2 were different from H3 and H4. Neither H1 and H2 nor H3 and H4 differed from each other.

In Riesling Harvest Date wines, triangle tests showed no differences between harvest dates at 95% confidence level. However, differences existed between treatments at 90% confidence level ($n=24$, $p \leq 0.10$) H1 was different from H2 and H3. H2 and H3 did not differ from each other.

Crop level. Triangle tests showed no differences ($n=24$, $p<0.05$) between replicate fermentations (with one exception: Vidal fully-cropped 2003), but differences between crop level treatment were found for both Vidal and Riesling icewines for the 2003 and 2004 vintages. In Vidal; fully cropped block 1 was different from fully cropped block 2, TFS, and TV in 2003 and the fully cropped treatment was different from TFS in 2004. For Riesling; TFS was different from TV in 2003 and fully cropped and TFS were different from TV in 2004.

Descriptive analysis. ***Vidal harvest date.*** Honey, peach, sherry, and tropical fruit aromas, caramelized, dried fruit/raisin, honey, and nut flavors, bitter taste and viscosity differed due to harvest date. For all significant attributes, the judge x sample interaction was not significant. This is important because it indicates that even though the judges may not have used the scale in the same way, they were consistent in how the rated the attributes among products.

From the mean scores (Table 5.2), some differences can be found between the various harvest dates. Harvest 1 had the lowest mean score for a majority of the attributes; these were honey, peach, sherry and tropical fruit aromas, and caramelized, dried fruit/raisin flavors as well as the lowest bitterness score. Harvest 2 generally had intermediate scores somewhere between Harvest 1 and Harvests 3 and 4. Harvest 3 and 4 had the highest scores for all the aroma and flavor attributes. Of the significant attributes (Table 5.2), Harvest 1 was found to be different from Harvest 3 for five of the significant attributes; honey, peach, sherry, tropical fruit aromas and bitterness and different from Harvest 4 for seven of the attributes; honey, peach aromas and caramelized, dried fruit/raisin, honey and nut flavors and bitterness.

The PCA for the 2004 Vidal icewines was three dimensional based on the results of the scree plot. The first three principal components explain 85.57% of the total variance in the wines and were retained (Figure 5.1). Honey, peach, sherry aromas and caramelized, dried fruit/raisin, honey, and nut flavors were highly positively loaded on Factor 1 and associated with wines from Harvests 3 and 4. Viscosity was heavily loaded on the Factor 2 and associated with wine from Harvest 1. With the exception of

viscosity, the wines from Harvest 3 and 4 were associated with all the sensory attributes located positively loaded on F1.

Riesling harvest date. Citrus, dried fruit/raisin, lychee, and nut aromas, and citrus, floral, lychee, nut, sherry, tangerine, and tropical fruit flavors differed between harvest dates. For most attributes which differed, the judge x sample interaction was not significant. Only dried fruit/raisin aroma and lychee flavor had significant judge x sample interactions. Harvest 1 had higher mean intensity scores for citrus and lychee aroma and flavor, as well as floral, tangerine and tropical fruit flavors. Harvest 2 had intermediate intensity scores between Harvest 1 and 3 for most attributes. Harvest 3 had the highest intensity ratings for nut aroma and flavor, dried fruit/raisin aroma and sherry flavor (Table 5.3).

PCA explained 90.91% of the variation in the attributes on two factors which were retained; all attributes were heavily loaded on F1 which explained 81.36% of the variability (Figure 5.2). Citrus and lychee aroma and flavor, and floral, tangerine and tropical fruit flavors were all negatively loaded on F1 and associated with Harvest 1. Dried fruit/raisin aroma, nut aroma and flavor, and sherry flavor were positively loaded on F1 and associated with Harvest 3. Harvest 2 was found in the centre of the PCA and not well described by any of the sensory attributes.

Vidal crop level. **2003.** Few sensory differences in the icewines were found in 2003. Only caramelized aroma and sherry flavor differed between treatments at a 95% confidence interval. The ANOVA was re-run at a 90% confidence interval and nine sensory attributes differed: caramelized aroma, dried fruit/raisin aroma, peach aromas; pear/apple aroma and flavor; sherry, tangerine, and tropical fruit flavors, and bitter taste.

For all significant attributes, the judge x sample interaction was not significant. Mean intensity scores for all attributes are reported (Table 5.4). Sensory attributes with high intensity scores in all treatments but not different according to crop level were honey aroma and flavor, sherry aroma, caramelized, dried fruit/raisin and nut flavors (Table 5.4). With the exception of bitter taste, no taste (sweet, acid) or mouthfeel (viscosity) attributes differed. Thinned treatments (TFS and TV) had higher intensity ratings for caramelized and dried fruit/raisin aromas, and pear/apple, sherry, and tangerine flavors compared to control (fully cropped) wines (Table 5.4 and Figure 5.3a). In general, fully cropped wines were rated lowest for all attributes.

The sensory map of the 2003 Vidal crop level icewines explained 63.83 % of the variation on two factors, F1 and F2, which were retained (Figure 5.3a). Factor 1 explained 43.7% of the variability in the data was positively correlated with the sensory attributes caramelized, dried fruit/raisin, sherry, and tangerine aromas, and pear/apple aroma and flavor. Factor 2 explained 20.1% of the variation and was positively correlated with peach aroma and negatively correlated with bitter taste. Fully-cropped treatments were separated by F2 from the thinned treatments that were positively loaded on F1 and associated with most sensory attributes (Figure 5.3a). Control block 1 was inversely associated with bitterness and Control block 2 was inversely associated with peach and pear/apple aromas, and tropical fruit flavor. Both thinned treatments were most associated with caramelized, tangerine, and sherry aromas, and apple/pear flavor. Control block 1 was different from Control block 2, TFS and TV wines through CVA.

2004. All judges were retained and six sensory attributes differed between treatments at a 90% confidence interval. These were: citrus, floral, and lychee aromas;

banana, honey, and nut flavors (Table 5.5). None of the attributes that differed had judge x sample interactions. Sensory attributes which had high intensity ratings in the 2004 icewines but did not differ due to crop level were honey and tropical fruit aromas, peach and pear/apple aromas and flavors, and dried fruit/raisin flavor (Table 5.5). Wines from the thinned treatments had the highest intensity ratings for all attributes that differed (Table 5.5). TV wines were highest in floral and lychee aromas and honey flavors and TFS wines were highest in citrus aroma and banana flavor. Similar to 2003 wines, the fully cropped treatment had the lowest intensity ratings.

The sensory map of the Vidal crop level icewines in 2004 showed a similar pattern to 2003; it explained 79.8% of the variation by two factors--56.5% on F1 and 23.3% on F2, and both were retained (Figure 5.3b). Four attributes: citrus, floral, and lychee aromas, and honey flavor, were positively loaded on F1, and were positively associated with TV wines. F1 was driven by an increasing perception of floral and lychee aromas and honey flavor which were highly correlated. Banana and nut flavors were loaded on both the F1 and F2, but more heavily on F2 in a positive direction. Fully cropped wines were negatively associated with lychee, citrus and floral aromas (Figure 5.3b).

***Riesling crop level.* 2003.** Panel performance was assessed for all judges, two judges were removed from the data set due to their low ability to discriminate attributes, non-reproducibility and contributing to the interaction (error term). The ANOVA was re-run at a 90% confidence interval ($p<0.1$, n=12) and six sensory attributes differed due to crop level: apricot and tangerine aromas, honey, nut, and tropical fruit flavors, and bitter taste (Table 5.6). The judge x wine interaction term was not significant for the first four

attributes but was significant at $p < 0.10$ for tropical fruit flavor and bitter taste. Attributes that did not differ by cropping level but had high intensity ratings in all wines were dried fruit/raisin, pear/apple, and sherry aromas and flavors, as well as apricot flavor (Table 5.6). The fully cropped Riesling icewines were rated higher for tangerine aroma, nut flavor, and bitter taste. TFS wines had higher intensities of apricot aroma, honey flavor, tropical fruit flavor than the control, and TV wines had intensities intermediate between TFS and control wines.

The sensory map, PCA, explained 87.1% of the variation in the wines on the first two factors that were retained; 53.9 % and 33.2% were explained by F1 and F2, respectively (Figure 5.4a). Bitter taste and honey flavor were inversely correlated and loaded on F1. Bitter taste was associated with the fully cropped wines, whereas honey was associated with TFS wine on the positively loaded on F1. The rest of the attributes that differed were equally loaded on F1 and F2 and negatively associated with the TV wines, indicating that they were lower in tangerine, apricot, nut and tropical fruit attributes.

2004. Panel performance was assessed and all judges were retained. Only four attributes differed due to cropping level: dried fruit/raisin and floral aromas, citrus flavor, and viscosity (Table 5.7). Acidity did not differ; therefore the panel was not confusing acid taste and citrus flavor. None of the attributes showed significant judge x sample interactions. Only apricot and dried fruit/raisin flavors had high intensity scores for attributes that did not differ due to cropping level

The sensory map of the 2004 icewines explained 85.3% of the variation in the data on the first two factors, with 60% explained by F1 and 25.3% explained by F2

(Figure 5.4b). Floral aroma was negatively loaded on F1 and associated with TV wines, whereas, citrus flavor and viscosity were all loaded positively on F1 and associated with control wines. Dried fruit/raisin aroma was positively loaded on F2 and associated with TFS wines (Figure 5.4b).

Correlation between sensory and analytical results. ***Vidal harvest date.*** The PLS of the 10 sensory attributes and 24 chemical compounds found the global goodness-of-fit and predictive quality of the model to be fairly good with a Q^2 cumulated index of 0.553 on two components which increased to 0.659 on four components. PLS explained 83.9% of the variability of the dependent; chemical compounds, and 69.8% of the variability of the explanatory; sensory attributes, on the first two components (Figure 5.5). The first dimension contrasted the wines from the later harvest dates (Harvest 3 and Harvest 4), that had higher intensities for all aroma and flavor attributes vs. the wines from Harvest 1, which had more viscosity. Most of the esters, ethyl octanoate, ethyl hexanoate, ethyl butyrate, isoamyl acetate and ethyl 3-methylbutyrate were associated with Harvest 1 and inversely correlated to tropical fruit, sherry, peach and honey aromas. 4-Vinylguaiacol was also associated Harvest 1 and negatively correlated to honey aroma, caramelized flavor, and bitter taste, which were most associated with Harvest 3. Harvest 4 was associated with most of the odor-active compounds, such as the terpenes and norisoprenoids, which were positively correlated to dried fruit/raisin, honey and nut flavors. Harvest 2 was not well represented by the model since it was located near the centre of the map (Figure 5.5); it was negatively associated with Harvest 4 and therefore most odor-active compounds and the dried fruit/raisin, nut and honey flavors.

The individual models for each of the dependent variables were assessed to determine what sensory attribute (explanatory variable) were most important to the model (Table 5.8). Dried fruit/raisin flavor was an explanatory variable for 12 of the 24 odor-active compounds (ethyl isobutryrate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, 1-hexanol, ethyl valerate, 1-heptanol, 1-octanol, ethyl benzoate, ethyl phenylacetate, g-nonalactone, β-damascenone and geranyl acetone). Only geranyl acetone had an inverse relationship, for all other compounds it had a positive relationship; therefore, as the concentration of these compounds increases in icewine, so to should the dried/fruit raisin flavor. Viscosity was an important positive explanatory variable for 10 of the compounds (ethyl isobutyrate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, 1-hexanol, 1-heptanol, 1-octanol, phenethylalcohol). Honey was a significant positive explanatory variable for seven of the compounds (ethyl valerate, 1-heptanol, 1-octen-3-ol, nerol oxide, ethyl benzoate, ethyl phenylacetate, γ-nonalactone). Other noteworthy sensory attributes included sherry aroma, which was found to be inversely related to ethyl octanoate, and caramelized flavor, which was positively associated with ethyl valerate but negatively related to 4-vinylguaiacol along with bitter taste.

Riesling harvest date. The PLS of the 11 sensory attributes and 23 chemical compounds found the global goodness-of-fit and predictive quality of the model to be good with a Q^2 cumulated index of 0.702 on two components. PLS explained 96.9% of the variability of the dependent; chemical compounds, and 88.5% of the variability of the explanatory; sensory attributes, on the first two components (Figure 5.6). The first dimension contrasted the wines from Harvest 1, which were associated with lychee and citrus aromas and tangerine, tropical fruit, citrus and floral flavors with wines from

Harvest 3 which were associated with dried fruit/raisin and nut aromas and nut and sherry flavor. The esters; ethyl butyrate, ethyl hexanoate, ethyl octanoate and ethyl cinnamate were associated with Harvest 1 and correlated to fresh and tropical fruit aromas and flavors. 4-Vinylguaiacol was also associated with Harvest 1 and positively correlated with citrus aroma and flavor, floral, lychee, tangerine and tropical fruit flavors, and negatively correlated with nut aroma and flavor and sherry flavor. Nut aroma and flavor, sherry flavor and dried fruit/raisin aroma were associated with Harvest 3 and correlated with most of the aroma compounds (β-damascenone, β-ionone, *cis*-rose oxide, nerol oxide, γ-nonalactone, 1-octanol, 1-octen-3-ol, ethyl-3-methylbutyrate, ethyl phenylacetate). Harvest 2 was associated with the second dimension and negatively correlated with decanal and linalool. None of the sensory attributes were associated with Harvest 2 or the aroma compounds decanal and linalool (Figure 5.6).

The individual models for each of the dependent variables were assessed to determine which sensory attributes (explanatory variables) were most important to the model (Table 5.9). Nut and sherry flavor had the highest number of explanatory variables for 15 and eight of 23 odor-active compounds, respectively. Nut flavor was found to have a positive relationship with 11 of the compounds (ethyl 3- methylbutyrate, 1-hexanol, 1-octen-3-ol, acetophenone, 1-octanol, *cis*-rose oxide, ethyl benzoate, ethyl phenylacetate, γ-nonalactone, β-damascenone, β-ionone) and a negative relationship with four compounds (ethyl butyrate, ethyl hexanoate, ethyl octanoate, 4-vinlyguaiacol). Sherry flavor was positively related to five compounds (1-octen-3-ol, acetophenone, *cis*-rose oxide, γ-nonalactone, β-ionone) and negatively correlated with three compounds (ethyl hexanoate, ethyl octanoate, 4-vinylguaiacol). 4-Vinylguaiacol explained nine of

the 11 sensory descriptors, the exceptions were lychee and dried fruit/raisin aroma, and was associated with Harvest 1. *Cis*-rose oxide, 1-octen-3-ol, ethyl octanoate and γ-nonalactone were all associated with at least five sensory attributes (Table 5.9). *Cis*-rose oxide was negatively correlated with citrus aroma and flavor, floral, lychee, tangerine and tropical fruit flavors and positively correlated with nut and sherry flavor.

***Vidal crop level.* 2003.** The PLS of the nine sensory attributes and 17 chemical compounds found the global goodness-of-fit and predictive quality of the model to be fairly good with a Q^2 cumulated index of 0.577 on three components. The first two components explained 71.2 % of the variability of the dependent; chemical compounds, and 64.5 % of the variability of the explanatory; sensory attributes (Figure 5.7a). The first dimension contrasts the TFS and TV wines that had higher intensities for all the aroma and flavor attributes with the control wines that were not associated with any sensory attributes. Sherry flavor was the most important explanatory variable, it was positively correlated with 4-vinylguaiacol, linalool, *cis*-rose oxide and geranyl acetone and tangerine and tropical fruit flavors and associated with TFS wines and negatively correlated with β-damascenone, ethyl 2- and 3- methylbutyrate and ethyl phenylacetate and Control block 1 wines.

2004. The PLS of the six sensory attributes and 23 chemical compounds found the global goodness-of-fit and predictive quality of the model to be fairly good with a Q^2 cumulated index of 0.659 on two components. The first two components explained 92.4 % of the variability of the dependent; chemical compounds, and 80.4 % of the variability of the explanatory; sensory attributes (Figure 5.7b). The first dimension was driven by banana flavor which was positively loaded on component 1 and correlated with nut flavor

and 18 of the 23 aroma compounds and associated with TFS wines (Figure 5.7b). Banana flavor was found to be the most important explanatory variable; it was significant for 20 of the 23 aroma compounds, the exceptions were β-damascenone, geranyl acetone, ethyl butyrate, and decanal. β-damascenone was explained negatively by floral aroma and positively by nut flavor.

***Riesling crop level.* 2003.** The PLS of the six sensory attributes and 22 chemical compounds found the global goodness of fit and predictive quality of the model to be fairly good with a Q^2 cumulated index of 0.848 on two components. The first two components explained 93.4 % of the variability of the dependent; chemical compounds, and 90.9 % of the variability of the explanatory; sensory attributes (Figure 5.8). The first dimension was driven by apricot aroma, honey flavor and tropical fruit flavor, which were negatively loaded on t1 and associated with TFS wines. Tangerine aroma and nut flavor were driving the second dimension and negatively loaded on t2 and associated with the control wines, while bitter taste was equally explained by t1 and t2 (Figure 5.8). Most of the aroma compound variables were inversely correlated with the sensory attributes. The aroma compounds were loaded in the positive direction on both dimensions and associated with TV wines. Tropical fruit flavor was positively correlated with geranyl acetone and ethyl cinnamate and inversely correlated with all other aroma compounds. Honey flavor was inversely correlated with bitter taste, 1-octen-3-ol, *cis*-rose oxide, β-damascenone, and β-ionone and was the most important explanatory variable with a positive association on acetophenone and ethyl cinnamate but a negative association on 1-octen-3-ol, *cis*-rose oxide, and decanal concentrations. Nut flavor was a negative

explanatory variable for acetophenone and ethyl cinnamate. Ethyl cinnamate was also explained positively by the sensory attribute apricot aroma and negatively by bitter taste.

Discussion

Sensory profiles. *Harvest date.* Both difference testing and descriptive analysis found Vidal and Riesling icewines differed in their sensory profiles when wines were made from grapes picked at different harvest dates, specifically the first HD, Harvest 1 picked 19 Dec. had a different sensory profile than Harvest 3 (Riesling), picked 18 Jan. and Harvest 4 (Vidal), picked 11 Feb. These results indicated for the first time to the author's knowledge that viticultural practices such as harvest date changed the sensory profile of icewines. The changes in sensory profiles of icewine due to harvest date are in agreement with Bowen and Reynolds (2010b) who found odor-active volatile compounds concentration differed in icewine from different harvest dates. In general, icewine from later harvest dates had higher concentration of most odor-active volatiles in both Vidal and Riesling experimental wines.

Previous studies on icewine have only studied enological effects such as yeast strain (Erasmus et al. 2004, Kontkanen et al. 2005), and region of origin (Cliff et al. 2002, Nurgel et al. 2004, Setkova et al. 2007b) on the wines sensory profiles. Vidal icewine had higher intensity ratings of all sensory attributes in the later harvest date treatment, Harvest 3 and Harvest 4 compared to the early harvest date, Harvest 1. These results indicated that it is the hang time, which allowed the icewines to be differentiated by their sensory properties. Therefore it appears that the longer hang time of Harvest 3 and 4 results in an icewine with a perceived greater intensity of aroma and flavor attributes than

wines picked at the earlier harvest dates. This is supported by the chemical composition of the wines that were also found to have the highest concentration in later harvest dates (Bowen and Reynolds 2010b) and through PLS analysis [refer to next section (Figure 5.5 and 5.6)].

Vidal and Riesling icewine showed similar sensory profiles (Figures 5.1 and 2) to sweet Fiano wines, where the early HD were associated with fresh/tropical fruit characteristics and the later HD were associated with dried fruit notes (Genovese et al. 2007). However, in Vidal icewines all sensory attributes are associated with the later harvest dates. Marais and van Wyck (1986) also found harvest date to affect the sensory properties of Riesling and Bukettraube grapes, it was found that grape maturity increased the terpene concentration in the wines which resulted in higher intensity scores for terpene-like character and overall wine quality when evaluated by sensory analysis. Reynolds et al (1993) also showed that harvest date affects the sensory profiles of wines from different *V. vinifera* cultivars; Gewürztraminer, Kerner, Müller-Thurgau and Muscat Ottonel, Optima, Pearl of Csaba and Siegerrebe. Aroma differences of the wines made from two distinct harvest dates, early and late, were found through triangle tests for all cultivars except Pearl of Csaba. Wines had higher intensity of Muscat-like aroma in the later harvest dates; these differences were attributes to higher concentration of free and bound terpenes in the wines. The effect of grape maturity on the quality of Vidal wines from Ohio was assessed, wines from three distinct harvest dates (early, mid, late maturity) were assessed on a seven-point hedonic scale over two vintages (Gallander 1983). Wines from the mid-maturity treatment were the most preferred wines for aroma and flavor in both years; they were preferred for their fruitier and cleaner taste compared

to late maturity wines which were described as more mature and complex. Sensory evaluation alone using a panel of trained judges were able to provide consistent descriptions and differentiation of fruit based on their stage of maturity in Cabernet Franc grapes from different harvest dates (Le Moigne et al. 2008). Findings from all of these studies support the results of this research that harvest date impacts and changes the sensory profile of icewines.

Crop level. The sensory profiles of icewines from different crop levels differed with the thinned treatments, TFS and TV, having higher intensity ratings for aroma and flavor descriptors than control wines in both vintages of Vidal icewines (Figure 5.4). These findings are in agreements with other studies that found cluster thinning increased the aroma and flavor intensity and overall quality of the table wines such as Pinot noir (Reynolds et al. 1996), Chardonnay Musqué (Reynolds et al. 2007), Carignane (Bravdo et al. 1984), and Riesling (Reynolds et al. 1994a). Cluster thinning Vidal grapevines postbloom is recommend to improve juice soluble solid concentration and therefore wine quality (Ferree et al. 2005). Anecdotally, it was believed that crop level affects the sensory profiles of icewine, however this if the first time that crop level has been shown to do so.

In Riesling icewines, the intensity ratings of the sensory descriptors differ between vintages and treatments. In 2003 the TFS icewines had the highest intensity ratings for honey and tropical flavors and apricot aroma, all of which are commonly used descriptors of high quality Riesling icewines. This is in agreement with Reynolds et al. (1994a) that found an increase in ripe-fruit character with a decrease in number of clusters per shoot. The control icewines were high in tangerine aroma, nut flavor and

bitter taste. The higher rating of bitter taste could be related to a lower perceived sweetness, which while not different, due to the higher rating of honey and tropical fruit flavor in the TFS icewines, this was in agreement with Reynolds et al. (1994a), and McCarthy et al. (1985) who found a decrease in sweetness in control (fully cropped) treatments in Riesling table wines. While the 2004 Riesling icewines also show that thinned treatments had higher intensity ratings for sensory attributes of floral and dried fruit, very few sensory differences were found in these wines most likely due to the poor condition of the grapes at harvest.

Correlation between sensory and chemical descriptors. Cliff et al (2002)) used PCA to map the volatile concentrations and mean aroma scores of icewines in an attempt to determine relationships between the sensory and chemical variables of the wines. They concluded that icewines were differentiate by geographic location, i.e. Germany versus Canada, and that no single impact compound was found, instead that icewine aroma was complex with many volatile compounds and sensory attributes contributing to the end result. This current study builds on what was found by Cliff et al (2002) in that we can begin to identify how our regional differences in terms of cultural practices for icewine may contribute to the changes in the chemical and sensory profiles of the wines. This would help to understand what the key compounds are that characterized icewine. The current study has shown that harvest date and crop level change the sensory properties and found the chemical parameters were also affected by these viticultural treatments. If the majority of German icewines are harvested before Canadian icewines and/or contain differing crop levels this could explain why regional

differences were found in previous studies (Cliff et al. 2002, Nurgel et al. 2004, Setkova et al. 2007b)

Harvest date. The PLS results showed similar trends to both the sensory and chemical results. Harvest 1 was differentiated from the Harvest 3 and 4 (Figures 5.5 and 5.6). For Vidal icewines, dried fruit/raisin flavor, viscosity, and honey flavor were the best explanatory variables to predict changes in the chemical variables (Table 5.8). Compounds that were highly correlated with these sensory descriptors include β-damascenone, which was previously found to be an important aroma compound in Pedro Ximenez wines and may be responsible by for the dried fruit character (Campo et al. 2008). It was also present in high concentrations in Vidal and Riesling icewines (Bowen and Reynolds 2010b). Sensory descriptive terms used to describe β-damascenone are diverse and included honey, fruity, apple, canned peach, and pear. Since dried fruit flavor was also correlated with other aroma compounds such as 1-octen-3-ol (mushroom aroma), 1-heptanol (nutty aroma), ethyl butyrate (fruity aroma), *cis*-rose oxide (rose like aroma), ethyl phenylacetate (honey aroma) and γ-nonalactone (coconut/ tropical aroma), further research is required to elucidate which compounds are really contributing to the icewine sensory profile. Ethyl phenylacetate is an important odorant contributing the honey, sweet character to Aglianico del Vulture wines (Tat et al. 2007); this compound correlated with honey flavor in Vidal icewines, but almost all the same compounds highly correlated with dried fruit flavor were also highly correlated to honey flavor. This highlights the complexity of the wine matrix and the difficulty in determining what compounds are most important to the icewine aroma. However, they do provide direction of further research and insight into how harvest date changes the sensory profiles.

Another interesting result of note is that as dried fruit/raisin and honey flavor increased so did the perceived viscosity. While glycerol concentration did increase with later harvest (Bowen and Reynolds 2010b), the concentration was not found to be above the sensory threshold (Noble and Bursick 1984). The difference in perceived viscosity is therefore linked to increasing concentration of aroma compounds that contribute to the dried fruit /raisin and honey flavors and give the illusion of increased viscosity in the wines. Previous research on model icewines has also shown that glycerol levels are generally not high enough to elicit a detectable sensory response (Nurgel and Pickering 2005).

The results of the Riesling harvest date icewine were more complicated to interpret because more compounds and sensory attributes were highly correlated and there were many more explanatory variables to predict changes in the chemical variables (Table 5.9). This may have to do with those attributes associated with Riesling which permit it to produce icewines with more complexity (Nurgel et al. 2004) and table wines from bone dry to very sweet. These results support Cliff et al (2002), that icewine is a complex interaction of aroma compounds that result in its sensory profile. It is difficult to narrow down which aroma compounds and sensory attributes are the most important for Riesling icewines from these results, further sensory studies will be required to determine which if any of these compounds contribute most to the sensory profile of Riesling icewines. In general, nut and sherry flavor were the most important sensory variable that positively correlated to 1-octen-3-ol (mushroom aroma), acetophenone (almond, floral aroma), *cis*-rose oxide (rose floral aroma), γ-nonalactone (coconut aroma), β-damascenone (apple/pear, dried fruit aroma), β-ionone (violet floral aroma)

and Harvest 3 icewines and negatively correlated with ethyl hexanoate (fruity aroma), ethyl octanoate (fruity aroma), 4-vinylguaiacol (spicy, phenolic aroma) and Harvest 1 icewines. Other important sensory descriptors were citrus aroma and flavor, and floral, lychee and tropical fruit flavors, which were associated with Harvest 1, 4-vinylguaiacol and several fermentation esters. These results make sense since most of the fermentation esters are characterized by "fruity" odor descriptors (Ferreira and Cacho 2009). An increase in the concentration of aroma compounds with non-fruity aromas, such as 1-octen-3-ol (mushroom aroma) or *cis*-rose oxide (floral rose aromas), would decrease the overall fruity characteristic and contribute more complex aroma likely due to synergistic effects between the various aroma compounds. To date, the only way to determine these effects are through omission and reconstitution studies, though while tedious can provide true information about the individual role of an aroma compounds in the wine matrix (Campo et al. 2005, Nicolau et al. 2006)

Aroma compounds to investigate further in Riesling include 4-vinylguaiacol, *cis*-rose oxide, 1-octen-3-ol and γ-nonalactone and β-damascenone. 4-vinylguaiacol and β-damascenone have previously been found to be odor potent compounds in Riesling table wines contributing a spicy, smoked and fruity, honey character, respectively (Komes et al. 2006). The volatile phenol, 4-vinylguaiacol was highly correlated to the fermentation esters ethyl hexanoate and octanoate and the citrus, tropical sensory attributes and was found to be an odor potent compound in Riesling icewine (Bowen and Reynolds 2010b). It had the highest concentration in the early harvest date icewines likely due to precipitation of hydrocinnamates from freeze and thaw events (Bowen and Reynolds 2010a). Since no single impact odorant has been identified in previous characterization

of Riesling table wines (Chisholm et al. 1994, Komes et al. 2006), it is not surprising to find the same result with icewine which is in agreement with Cliff et al (2002).

Crop level. Vidal crop level icewines showed a similar trend to the sensory and chemical data (Figure 5.7), the sensory and chemical map obtained through PLS of the 2003 Vidal icewines found all the sensory attributes were positively correlated with the thinned treatments. In 2003, sherry flavor was the only significant explanatory variable and was positively predicted changes with the terpenes, linalool and *cis*-rose oxide, 4-vinylguaiacol and was associated with the thinned treatments (Figure 5.7a). The high sherry flavor in the wines may best be described by the presence of 4-vinylguaiacol, described by its clove, phenolic aroma was found about its sensory threshold in all crop level treatment in 2003 (Bowen and Reynolds 2010a). In 2004, the banana flavor was the most important explanatory variable and was positively predicted almost all aroma compounds and was associated with the TS treatments (Figure 5.7b). Vintage variation is best explained by differences in the climatic conditions between years during the hang time from October to harvest in January (Bowen and Reynolds 2010a). The 2003 vintage had more freeze and thaw events and larger temperature differences (between minimum and maximum) than the 2004 vintage, which in general was colder. These conditions likely resulted in more desiccation of the fruit and negated the effect of cluster thinning. The colder fall of 2004 resulted in almost all the aroma compounds being correlated with each other and associated with the TFS wines.

For the Riesling crop level icewines it is more difficult to understand the results of the PLS (Figure 5.8). They indicate that honey flavor is the most important explanatory variable to predict changes in the chemical parameters and that it is

associated with TFS wines. This intuitively makes sense when comparing to the sensory results (Figure 5.8) since honey was found to have the highest intensity ratings in TFS wines along with tropical fruit flavor and apricot aroma. However, this is in contrast to the results from the chemical analysis which found almost all of the aroma compound had the highest concentration in the TV icewines (Bowen and Reynolds 2010a). These results together indicate that the wines with the highest concentration of aroma compounds have the lowest sensory intensity which seems counter intuitive. The only plausible explanation for this finding is that the sub-standard fruit quality of the Riesling grapes might be masking the true results.

Conclusions

Harvest date was found to differentiate icewine based on sensory profile and chemical composition for both Vidal and Riesling. In Vidal, later harvest dates had significantly higher intensity scores for aroma and flavor descriptors than Harvest 1. PCA of sensory attributes that differed in Vidal found all aroma and flavor attributes in Vidal were associated with later harvest dates (H3 and H4) and factor 2 separated early harvest dates (H1 and H2) from late harvest dates (H3 and H4). Correlation between the sensory and chemical variables found PLS analysis found dried fruit/raisin and honey flavor to be important explanatory variable which were positively correlated with β-damascenone, ethyl butyrate (fruity aroma), *cis*-rose oxide (rose like aroma), ethyl phenylacetate (honey aroma) and γ-nonalactone (coconut/ tropical aroma). 4-Vinylguaiacol was always associated with the early harvest dates.

In Riesling, Harvest 1 wines had higher intensity ratings for fresh fruit descriptors whereas Harvest 3 wines were higher for dried fruit and nutty descriptors. PCA showed all attributes were heavily loaded on factor 1 with fresh fruit and tropical attributes associated with harvest 1 (H1) and dried fruit and nutty attributes associated with harvest 3 (H3). Correlation between the sensory and chemical parameters found Harvest 3 icewines associated with nut and sherry flavor and positively correlated to the aroma compounds β-damascenone, *cis*-rose oxide, 1-octen-3-ol and γ-nonalactone and negatively correlated to 4-vinylguaiacol, and ethyl hexanoate and ethyl octanoate and associated with Harvest 1 icewines.

Therefore, harvest date has been shown to affect the sensory profiles of Vidal blanc and Riesling icewines. The potential significance of these findings is that it provides information to winemakers about how time of harvest will affect the sensory profile of the resultant icewine. Therefore depending on the desired sensory profile of icewine, it could dictate whether grapes are picked in December for fresh and tropical fruit aroma and flavors or January for more nut and dried fruit characteristics. Perhaps both profiles are desired in an icewine, then grapes can be picked at different times throughout the icewine harvest season (December to February) fermented in separate tanks and blended before bottling to achieve the desired sensory profile.

Crop level, control (non-thinned), thinned at fruit set, and thinned at veraison also affected the sensory profiles of Vidal and Riesling icewines. In Vidal, all sensory attributes that differed were found to be associated with the thinned treatments in 2003 and 2004 experimental icewines. The thinned treatments were described as having higher intensity ratings for attributes such as peach, apple/pear, dried fruit and caramelized

aromas and tropical fruit, tangerine and apple/pear flavors. PCA found the all the sensory attributes were associated with the thinned treatments in both years, with the exception of nut flavor being associated with the control treatment in 2004. Correlation between the sensory and aroma compounds in 2003, found sherry flavor to be a most important explanatory variable which was correlated positively with to 4-vinylguaiacol, *cis*-rose oxide, and linalool and was associated with the thinned treatments. In 2004, banana flavor was the sensory variable that best predicted almost all of the aroma compounds. No clear relationships could be found using PLS on Riesling crop level wine, however sensory differences found the TFS to have the highest intensity rating of honey and tropical fruit flavor and apricot aroma while the control were had higher intensity ratings for tangerine aroma, nut flavor and bitter taste. Vintages differences were explained by number of freeze and thaw events and temperature fluctuations from October to January. The potential significance of these findings would be that cluster thinning icewines grapes intensifies the sensory profile of the resultant wines due to higher concentration of aroma compounds.

Changes in the sensory profiles of icewines due to harvest date and crop level have been demonstrated in this study. The next step would be: 1) to elucidate what are the key compounds in icewine through omission and reconstitution studies and by validating the PLS models and 2) to determine how consumers perceive these differences and what their preferences are. Understanding consumer preference would provide another tool to guide growers and winemaker on cultural practices such as how much crop to grow and when to harvest for optimal icewine quality.

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Table 5.1: Complete list of reference standards used for descriptive analysis of Ontario Riesling and Vidal icewines. All standards were made in a un-oaked neutral white base wine.

Reference Standard	Ingredients	Position on Line Scale (cm)
Apricot	6 dried apricots chopped / 100 mL base wine, let soak overnight	10
Banana	1 drop isoamyl acetate / 425 mL wine	15
Caramelized	15 g crunchy sponge toffee, add 60 mL wine and boil down, add 100 mL wine and let sit overnight	14
Citrus	15 mL each grapefruit and lime juice, 5 mL lemon juice / 100 mL wine	13
Dried fruit / Raisin	25 g dried fig, prune and raisin / 100 mL wine	8
Floral	fresh freesia flowers	11.5
Honey	15g honey / 100mL wine	11
Lychee	6 mL puree in 100mL wine	10
Nut	toasted chopped walnuts and hazelnuts	7.5
Peach	25 mL peach nectar / 100 mL wine	10
Pear / Apple	10 mL each pear and apple juice / 100 mL wine	8.5
Sherry	12.5 mL of Alvears Amontillado, Montilla Spain in 100mL wine	14
Tangerine	3 g tangerine peel / 250 mL wine	12
Tropical fruit	5 mL each mango, tropical fruit, passion fruit and guava / 100 mL wine	11
Sweet	150 g/L sucrose in distilled water	9.5
Acid	0.67 g/L tartaric acid in distilled water	11.5
Bitter	0.015 g/L quinine sulfate in distilled water	11.5
Viscosity	0.3 g/L carboxymethylcellulose in distilled water	5.5

Table 5.2. Impact of harvest date on mean sensory attribute scores from descriptive analysis by ANOVA (α>0.05) for Vidal icewines, Garphil Farms, St. Catharines, Ontario, 2004-05.

Sensory Attribute	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Significance	F-value	p-value
Apricot aroma	2.5	3.2	3.3	2.7	ns	1.38	0.2640
Banana aroma	1.8	1.2	1.1	1.2	ns	0.86	0.4714
Caramelized aroma	2.2	2.1	2.8	3.3	ns	1.75	0.1747
Citrus aroma	3.2	3.3	2.4	2.3	ns	2.73	0.0582
Dried fruit/Raisin aroma	2.5	2.8	2.6	3.2	ns	1.53	0.2234
Floral aroma	1.7	1.6	1.7	1.7	ns	0.00	0.9998
Honey aroma	**2.9b**	**3.2b**	**4.3a**	**4.3a**	**	**4.44**	**0.0094**
Lychee aroma	2.6	2.3	2.4	2.3	ns	0.23	0.8730
Nut aroma	0.9	0.9	1.0	1.2	ns	0.49	0.6925
Peach aroma	**2.5b**	**3.4a**	**3.5a**	**3.5a**	**	**5.09**	**0.0049**
Pear / Apple aroma	2.9	2.5	3.1	2.8	ns	0.55	0.6500
Sherry aroma	**2.6b**	**2.7b**	**3.7a**	**3.1ab**	*	**3.29**	**0.0314**
Tangerine aroma	1.7	1.9	1.5	1.4	ns	0.54	0.6593
Tropical fruit aroma	**2.8b**	**3.4ab**	**4.4a**	**3.4ab**	*	**3.72**	**0.0199**
Apricot flavor	3.9	3.8	3.9	4.1	ns	0.22	0.8848
Banana flavor	2.9	2.4	2.0	2.0	ns	1.68	0.1886
Caramelized flavor	**1.8b**	**2.4ab**	**2.6ab**	**3.1ab**	*	**3.27**	**0.0321**
Citrus flavor	3.7	3.6	3.4	3.5	ns	0.28	0.8419
Dried fruit/Raisin flavor	**3.2b**	**3.5b**	**3.4b**	**4.5a**	**	**5.65**	**0.0028**
Floral flavor	1.1	1.1	1.0	1.0	ns	0.10	0.9606
Honey flavor	**3.9b**	**3.7b**	**4.1ab**	**4.7a**	*	**2.96**	**0.0453**
Lychee flavor	2.2	2.2	2.3	2.0	ns	0.17	0.9162
Nut flavor	**0.9b**	**0.9b**	**0.9b**	**1.3a**	*	**3.99**	**0.0149**
Peach flavor	4.1	4.5	4.6	4.5	ns	0.72	0.5448
Pear / Apple flavor	3.8	3.6	4.0	3.7	ns	0.33	0.8055
Sherry flavor	1.8	2.0	2.4	2.5	ns	2.79	0.0546
Tangerine flavor	2.7	2.9	2.8	2.4	ns	0.75	0.5300
Tropical fruit flavor	4.6	4.1	4.8	4.2	ns	0.74	0.5359
Sweet	6.9	6.8	6.9	7.0	ns	0.30	0.8267
Acid	6.5	6.7	6.5	6.5	ns	0.17	0.9133
Bitter	**1.5b**	**2.3a**	**2.1a**	**2.5a**	*	**3.58**	**0.0231**
Viscosity	**6.5a**	**5.8b**	**6.2a**	**6.4a**	**	**4.51**	**0.0087**

ns, *, **, and *** represent not significant or significant at $p \leq 0.05$, 0.01, and 0.001 respectively. Means with the same letter were not significantly different by LSD.

Table 5.3. The impact of harvest date on mean sensory scores determined by ANOVA (α>0.05) from descriptive analysis of Riesling icewines, Lambert Farms, Niagara-on-the-Lake, Ontario, 2004-05.

Sensory Attribute	Harvest 1	Harvest 2	Harvest 3	Significance	F-value	p-value
Apricot aroma	3.1	3.0	3.2	ns	0.11	0.8966
Banana aroma	1.8	1.2	1.4	ns	2.03	0.1510
Caramelized aroma	1.6	1.7	2.1	ns	0.48	0.6261
Citrus aroma	**4.0a**	**3.4a**	**2.6b**	**	**6.33**	**0.0057**
Dried fruit / Raisin aroma	**2.0b**	**2.3b**	**3.6a**	**	**6.23**	**0.0062**
Floral aroma	3.5	2.8	2.5	ns	0.73	0.4892
Honey aroma	3.0	3.2	3.6	ns	1.55	0.2308
Lychee aroma	**3.4a**	**2.9a**	**1.8b**	*	**4.98**	**0.0147**
Nut aroma	**0.7b**	**0.7b**	**1.4a**	**	**7.05**	**0.0036**
Peach aroma	3.6	3.1	2.7	ns	1.80	0.1847
Pear / Apple aroma	3.0	2.7	2.8	ns	0.34	0.7124
Sherry aroma	1.5	1.5	2.3	ns	2.26	0.1244
Tangerine aroma	1.9	2.0	1.4	ns	1.26	0.3010
Tropical fruit aroma	3.4	2.8	2.7	ns	0.82	0.4504
Apricot flavor	3.6	3.6	3.9	ns	0.62	0.5463
Banana flavor	2.4	1.8	1.5	ns	2.25	0.1257
Caramelized flavor	2.7	2.3	2.8	ns	1.18	0.3235
Citrus flavor	**4.1a**	**3.6a**	**2.9b**	**	**6.57**	**0.0049**
Dried fruit / Raisin flavor	3.2	3.3	4.1	ns	2.83	0.0770
Floral flavor	**3.2a**	**1.9b**	**1.2b**	***	**12.52**	**0.0002**
Honey flavor	4.1	3.7	4.0	ns	0.64	0.5374
Lychee flavor	**3.2a**	**2.9a**	**1.8b**	*	**4.99**	**0.0147**
Nut flavor	**1.0b**	**1.1b**	**1.6a**	*	**4.50**	**0.0210**
Peach flavor	3.5	4.0	3.7	ns	0.61	0.5527
Pear / Apple flavor	3.6	2.8	2.9	ns	1.42	0.2589
Sherry flavor	**1.7b**	**1.7b**	**2.5a**	*	**5.00**	**0.0145**
Tangerine flavor	**2.6a**	**2.1ab**	**1.6b**	*	**5.07**	**0.0139**
Tropical fruit flavor	**3.8a**	**3.3ab**	**2.6b**	*	**4.28**	**0.0247**
Sweet	7.1	7.2	7.5	ns	1.02	0.3739
Acid	6.7	7.0	6.7	ns	0.81	0.4555
Bitter	1.0	1.3	1.4	ns	1.29	0.2920
Viscosity	6.4	6.4	6.4	ns	0.01	0.9907

ns, *, **, and *** represent not significant or significant at $p \leq 0.05$, 0.01, and 0.001 respectively. Means with the same letter were not significantly different by LSD.

Table 5.4. The impact of crop level on the mean sensory attributes found through descriptive analysis (ANOVA; α>0.10) in 2003 Vidal icewines, Garphil Farms, St. Catharines, Ontario.

Sensory Attribute	Control 1	Control 2	Thin at Set	Thin at Veraison	Significance	F-value	p-value
Apricot aroma	3.1	3.0	3.4	3.4	ns	0.54	0.6557
Banana aroma	1.6	1.5	1.5	1.5	ns	0.15	0.9282
Caramelized aroma	**4.8b**	**4.8b**	**5.1ab**	**5.7a**	**	**3.26**	**0.0325**
Citrus aroma	1.5	1.4	1.7	1.4	ns	1.15	0.3412
Dried fruit / Raisin aroma	**5.6b**	**6.2ab**	**6.1ab**	**6.6a**	*	**2.84**	**0.0513**
Floral aroma	0.9	0.9	1.1	1.1	ns	1.04	0.3887
Honey aroma	4.1	4.5	4.3	4.2	ns	0.86	0.4708
Lychee aroma	1.5	1.2	1.4	1.4	ns	0.72	0.5481
Nut aroma	3.2	3.2	3.4	3.4	ns	0.17	0.9155
Peach aroma	**2.1a**	**1.6b**	**1.7ab**	**2.1a**	*	**2.35**	**0.0882**
Pear / Apple aroma	**3.0ab**	**2.5b**	**3.0ab**	**3.3a**	*	**2.62**	**0.0658**
Sherry aroma	6.3	7.2	7.0	7.0	ns	1.16	0.3389
Tangerine aroma	1.2	1.3	1.3	1.7	ns	1.13	0.3511
Tropical fruit aroma	1.7	1.4	1.5	1.3	ns	1.47	0.2396
Apricot flavor	3.5	3.0	3.4	3.7	ns	1.21	0.3202
Banana flavor	1.2	1.0	1.2	1.2	ns	1.00	0.4056
Caramelized flavor	5.5	5.2	5.3	5.9	ns	1.69	0.1869
Citrus flavor	1.8	1.6	2.2	1.6	ns	1.84	0.1581
Dried Fruit/Raisin flavor	6.3	6.7	6.4	6.8	ns	1.07	0.3726
Floral flavor	0.9	0.8	0.9	0.9	ns	0.14	0.9341
Honey flavor	4.2	4.1	4.3	4.4	ns	0.23	0.8714
Lychee flavor	1.2	1.1	1.3	1.5	ns	1.59	0.2092
Nut flavor	3.9	3.7	4.0	4.0	ns	0.20	0.8959
Peach flavor	2.1	1.8	1.9	2.3	ns	1.06	0.3788
Pear / Apple flavor	**2.7b**	**2.7b**	**3.1ab**	**3.4a**	*	**2.64**	**0.0644**
Sherry flavor	**6.0b**	**6.1b**	**7.2a**	**7.1a**	**	**3.02**	**0.0421**
Tangerine flavor	**1.6b**	**1.5b**	**1.9ab**	**2.1a**	*	**2.60**	**0.0668**
Tropical fruit flavor	**1.7ab**	**1.3b**	**1.9ab**	**1.5ab**	*	**2.34**	**0.0898**
Sweet	7.2	6.6	6.9	7.0	ns	1.69	0.1870
Acid	6.2	5.8	6.1	5.9	ns	0.62	0.6065
Bitter	**2.6b**	**3.3a**	**3.1ab**	**3.4a**	*	**2.41**	**0.0830**
Viscosity	6.1	6.0	6.1	5.8	ns	1.12	0.3521

ns, *, **, ***and **** represent not significant or significant at $p \leq 0.01$, 0.05, 0.01, and 0.001 respectively. Means with the same letter were not significantly different by LSD.

Table 5.5. The impact of crop level on the mean sensory attributes found through descriptive analysis (ANOVA; α>0.10) in 2004 Vidal icewines, Garphil Farms, St. Catharines, Ontario.

Sensory Attribute	Control	Thin at Set	Thin at Veraison	Significance	F-value	p-value
Apricot aroma	3.2	3.2	3.4	ns	0.17	0.8433
Banana aroma	1.6	1.9	1.6	ns	0.79	0.4651
Caramelized aroma	3.1	2.6	2.2	ns	2.35	0.1151
Citrus aroma	**2.3b**	**3.1a**	**2.8ab**	**	**3.39**	**0.0491**
Dried fruit / Raisin aroma	3.5	3.5	3.3	ns	0.42	0.6634
Floral aroma	**1.7b**	**2.9a**	**3.3a**	***	**5.79**	**0.0083**
Honey aroma	3.6	4.0	3.7	ns	0.64	0.5342
Lychee aroma	**2.1b**	**3.0ab**	**3.2a**	**	**3.47**	**0.0460**
Nut aroma	1.1	1.1	0.8	ns	1.13	0.3363
Peach aroma	4.1	4.0	4.3	ns	0.24	0.7829
Pear / Apple aroma	3.9	4.2	3.6	ns	0.99	0.3856
Sherry aroma	1.9	2.1	2.1	ns	0.21	0.8122
Tangerine aroma	2.5	2.1	2.6	ns	1.57	0.2281
Tropical fruit aroma	3.6	4.3	4.0	ns	1.32	0.2851
Apricot flavor	3.8	4.3	3.9	ns	0.66	0.5248
Banana flavor	**1.3b**	**1.7a**	**1.4ab**	*	**2.61**	**0.0926**
Caramelized flavor	3.0	3.3	3.0	ns	0.48	0.6236
Citrus flavor	3.2	3.0	3.2	ns	0.16	0.8547
Dried Fruit / Raisin flavor	4.5	4.5	4.2	ns	0.85	0.4400
Floral flavor	1.3	1.3	1.5	ns	0.18	0.8369
Honey flavor	**3.8b**	**4.2ab**	**4.5a**	*	**2.97**	**0.0689**
Lychee flavor	2.4	2.8	2.5	ns	0.29	0.7899
Nut flavor	**1.2a**	**1.2a**	**0.9b**	**	**3.37**	**0.0498**
Peach flavor	4.7	4.4	5.2	ns	2.14	0.1379
Pear / Apple flavor	4.0	4.2	4.2	ns	0.25	0.7811
Sherry flavor	2.0	2.2	2.0	ns	0.59	0.5880
Tangerine flavor	2.8	3.0	2.6	ns	0.51	0.6080
Tropical fruit flavor	3.8	4.2	4.3	ns	1.06	0.3612
Sweet	7.1	7.0	7.4	ns	1.00	0.3801
Acid	7.3	6.5	6.7	ns	1.31	0.2867
Bitter	1.6	1.4	1.5	ns	0.19	0.8224
Viscosity	6.1	6.3	6.4	ns	0.39	0.6793

ns, *, **, ***and **** represent not significant or significant at $p \leq 0.01$, 0.05, 0.01, and 0.001 respectively. Means with the same letter were not significantly different by LSD.

Table 5.6. The impact of crop level on the mean sensory attributes found through descriptive analysis (ANOVA; α>0.10) in 2003 Riesling icewines, Lambert Farms, Niagara-on-the-Lake, Ontario.

Sensory Attribute	Control	Thin at Set	Thin at Veraison	Significance	F-value	p-value
Apricot aroma	**3.7ab**	**4.6a**	**3.2b**	*	**3.12**	**0.0639**
Banana aroma	0.9	0.8	0.7	ns	0.51	0.6063
Caramelized aroma	3.6	2.9	3.7	ns	1.18	0.3252
Citrus aroma	2.0	1.8	2.4	ns	0.79	0.4643
Dried fruit / Raisin aroma	5.8	5.8	5.5	ns	0.23	0.7935
Floral aroma	1.4	1.4	0.8	ns	0.89	0.4262
Honey aroma	3.2	3.4	3.5	ns	0.11	0.8977
Lychee aroma	1.5	1.2	1.2	ns	0.40	0.6757
Nut aroma	2.5	2.8	2.1	ns	1.01	0.3791
Peach aroma	2.8	2.8	2.8	ns	0.01	0.9880
Pear / Apple aroma	4.0	3.2	3.2	ns	1.46	0.2528
Sherry aroma	5.0	4.5	5.0	ns	0.36	0.7002
Tangerine aroma	**3.0a**	**2.4ab**	**1.9b**	*	**2.60**	**0.0969**
Tropical fruit aroma	2.3	2.2	2.0	ns	0.16	0.8558
Apricot flavor	4.7	4.8	4.6	ns	0.07	0.9331
Banana flavor	0.5	1.0	0.8	ns	0.71	0.5029
Caramelized flavor	3.8	3.9	3.5	ns	0.37	0.6952
Citrus flavor	2.3	2.8	2.4	ns	0.66	0.5258
Dried Fruit / Raisin flavor	6.6	7.1	6.8	ns	0.24	0.7867
Floral flavor	1.1	1.1	0.7	ns	0.61	0.5527
Honey flavor	**2.8b**	**3.6ab**	**3.2b**	*	**3.32**	**0.0550**
Lychee flavor	1.3	1.5	1.3	ns	0.48	0.6236
Nut flavor	**3.7a**	**2.9ab**	**2.5b**	*	**2.89**	**0.0771**
Peach flavor	2.9	2.9	3.3	ns	0.31	0.7384
Pear / Apple flavor	3.0	4.2	3.6	ns	1.74	0.1994
Sherry flavor	4.8	4.3	4.3	ns	0.84	0.4462
Tangerine flavor	2.6	2.5	2.3	ns	0.22	0.8041
Tropical fruit flavor	**2.3ab**	**3.4a**	**2.1b**	*	**2.67**	**0.0916**
Sweet	6.7	6.7	7.0	ns	0.60	0.5550
Acid	6.5	6.4	6.2	ns	0.25	0.7802
Bitter	**2.3ab**	**1.1b**	**1.4ab**	*	**2.99**	**0.0712**
Viscosity	5.9	5.9	6.1	ns	0.21	0.8098

ns, *, **, ***and **** represent not significant or significant at p≤0.01, 0.05, 0.01, and 0.001 respectively. Means with the same letter were not significantly different by LSD.

Table 5.7 The impact of crop level on the mean sensory attributes found through descriptive analysis (ANOVA; α>0.10) in 2004 Riesling icewines, Lambert Farms, Niagara-on-the-Lake, Ontario.

Sensory Attribute	Control	Thin at Set	Thin at Veraison	Significance	F-value	p-value
Apricot aroma	3.1	3.3	3.5	ns	0.26	0.77303
Banana aroma	1.0	1.1	0.9	ns	0.38	0.68565
Caramelized aroma	2.7	2.5	2.3	ns	0.81	0.45516
Citrus aroma	2.6	3.2	3.2	ns	1.78	0.18817
Dried fruit / Raisin aroma	**3.9ab**	**4.2a**	**3.1b**	*	**3.11**	**0.06141**
Floral aroma	**1.9b**	**1.9b**	**2.8a**	**	**3.54**	**0.04373**
Honey aroma	2.6	2.6	2.6	ns	0.01	0.99480
Lychee aroma	1.7	1.7	2.1	ns	1.42	0.26025
Nut aroma	1.6	1.5	1.0	ns	1.08	0.35581
Peach aroma	2.7	2.5	2.5	ns	0.26	0.76978
Pear / Apple aroma	2.3	2.6	2.4	ns	0.34	0.71595
Sherry aroma	3.5	2.9	2.8	ns	1.94	0.16448
Tangerine aroma	1.5	2.1	2.1	ns	1.41	0.26294
Tropical fruit aroma	2.5	2.4	2.9	ns	1.56	0.22859
Apricot flavor	4.1	3.8	3.8	ns	0.45	0.63944
Banana flavor	1.1	1.4	1.0	ns	1.65	0.21197
Caramelized flavor	2.9	2.5	2.9	ns	0.68	0.51637
Citrus flavor	**3.1ab**	**3.5a**	**3.0b**	*	**2.59**	**0.09441**
Dried fruit / Raisin flavor	4.8	4.5	4.6	ns	0.30	0.74394
Floral flavor	1.5	1.4	1.7	ns	0.35	0.71130
Honey flavor	3.5	3.2	3.4	ns	0.73	0.49334
Lychee flavor	1.4	1.3	1.6	ns	0.42	0.66200
Nut flavor	1.5	1.7	1.5	ns	0.41	0.66718
Peach flavor	3.5	3.3	3.6	ns	0.53	0.59549
Pear / Apple flavor	3.2	3.5	3.1	ns	1.27	0.29713
Sherry flavor	3.0	2.4	2.7	ns	0.87	0.43279
Tangerine flavor	2.0	1.9	2.3	ns	0.75	0.48088
Tropical fruit flavor	2.8	3.0	3.2	ns	0.36	0.70387
Sweet	6.8	6.9	6.8	ns	0.14	0.86577
Acid	6.8	6.8	6.4	ns	1.69	0.20498
Bitter	2.1	2.2	2.3	ns	0.31	0.73669
Viscosity	**5.9ab**	**6.2a**	**5.8b**	**	**3.41**	**0.04832**

ns, *, **, ***and **** represent not significant or significant at $p \leq 0.01$, 0.05, 0.01, and 0.001 respectively. Means with the same letter were not significantly different by LSD.

Table 5.8. Summary table of the Vidal PLS model results of the odor-active compounds and the sensory attributes found to be the most significant in the harvest date icewines. A positive association indicates as the concentration of the compound increase, so too will the intensity of the sensory attribute. The reverse would be found for a negative relationship.

	Significant sensory attributes from the standardized coefficients					
Compounds	Dried fruit/raisin flavor	Viscosity	Honey flavor	Caramelized flavor	Sherry aroma	Bitter
ethyl isobutyrate	+	+	ns	ns	ns	ns
ethyl 2-methylbutyrate	+	+	ns	ns	ns	ns
ethyl 3-methylbutyrate	+	+	ns	ns	ns	ns
1-hexanol	+	+	ns	ns	ns	ns
ethyl valerate	+	-	+	+	ns	ns
1-heptanol	+	+	+	ns	ns	ns
1-octen-3-ol	ns	ns	+	ns	ns	ns
1-octanol	+	+	ns	ns	ns	ns
phenylethyl alcohol	ns	+	ns	ns	ns	ns
nerol oxide	ns	ns	-	ns	ns	ns
ethyl benzoate	+	+	+	ns	ns	ns
ethyl octanoate	ns	ns	ns	ns	-	ns
ethyl phenylacetate	+	+	+	ns	ns	ns
4-vinylguaiacol	ns	ns	ns	-	ns	-
γ- nonalactone	+	+	+	ns	ns	ns
β- damascenone	+	ns	ns	ns	ns	ns
geranyl acetone	-	ns	ns	ns	ns	ns

* +, -, ns indicate whether the sensory attribute had positive association, negative association or was not significant respectively for each of the odor-active compounds listed.

Table 5.9. Summary table of the Riesling PLS model results of the odor-active compounds and the sensory attributes found to be the most significant in the harvest date icewines. A positive association indicates as the concentration of the compound increase, so too will the intensity of the sensory attribute. The reverse would be found for a negative relationship.

	Significant sensory attributes from the standardized coefficients									
Compounds	Nut Flavor	Sherry Flavor	Tropical Fruit Flavor	Citrus Flavor	Citrus Aroma	Floral Flavor	Lychee Flavor	Tangerine Flavor	Dried Fruit / Raisin Aroma	Nut Aroma
ethyl butyrate	-	ns	ns	ns	ns	ns	ns	ns	ns	ns
ethyl 3-methylbutytate	+	ns	ns	ns	ns	ns	ns	ns	ns	ns
1-hexanol	+	ns	ns	ns	ns	ns	ns	ns	ns	ns
1-octen-3-ol	+	+	-	-	ns	-	ns	-	+	ns
ethyl hexanoate	-	-	ns	ns	ns	ns	ns	ns	ns	ns
acetophenone	+	+	ns	ns	ns	ns	ns	ns	ns	ns
1-octanol	+	ns	ns	ns	ns	ns	ns	ns	ns	ns
cis-rose oxide	+	+	-	-	-	-	-	-	ns	ns
ethyl benzoate	+	ns	ns	ns	ns	ns	ns	ns	ns	ns
ethyl octanoate	-	-	+	+	+	ns	+	ns	ns	ns
ethyl phenylacetate	+	ns	ns	ns	ns	ns	ns	ns	ns	ns
4-vinylguaiacol	-	-	+	+	+	+	+	-	ns	-
γ-nonalactone	+	+	ns	-	-	ns	-	ns	ns	ns
β-damascenone	+	ns	ns	ns	ns	ns	ns	ns	ns	ns
ethyl cinnamate	ns	ns	+	ns	ns	+	ns	ns	ns	ns
β-ionone	+	+	ns	ns	ns	ns	ns	ns	ns	ns

* +, -, ns indicate whether the sensory attribute had positive association, negative association or was not significant respectively for each of the odor-active compounds listed.

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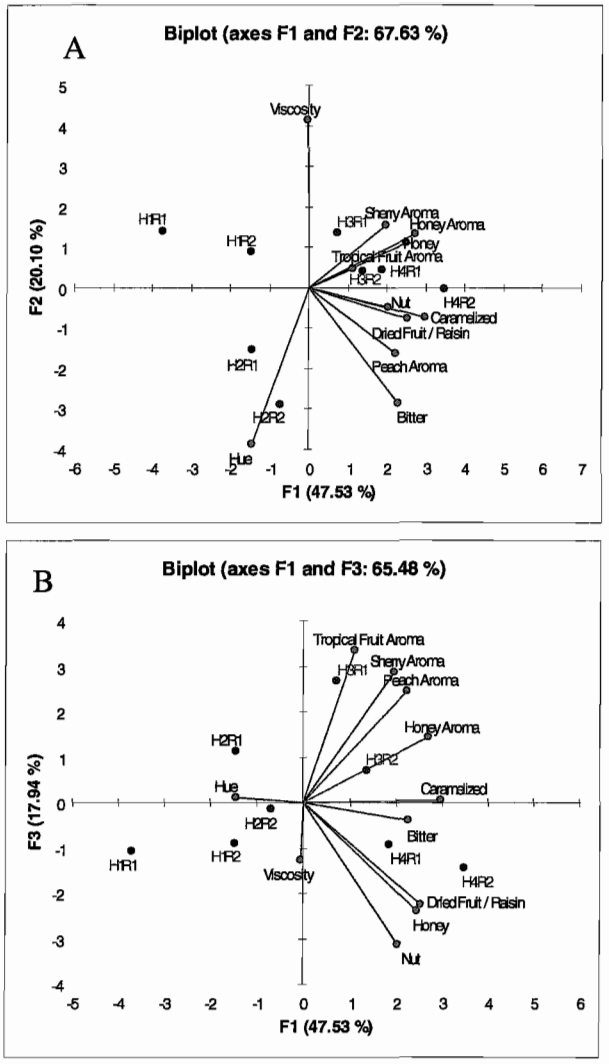
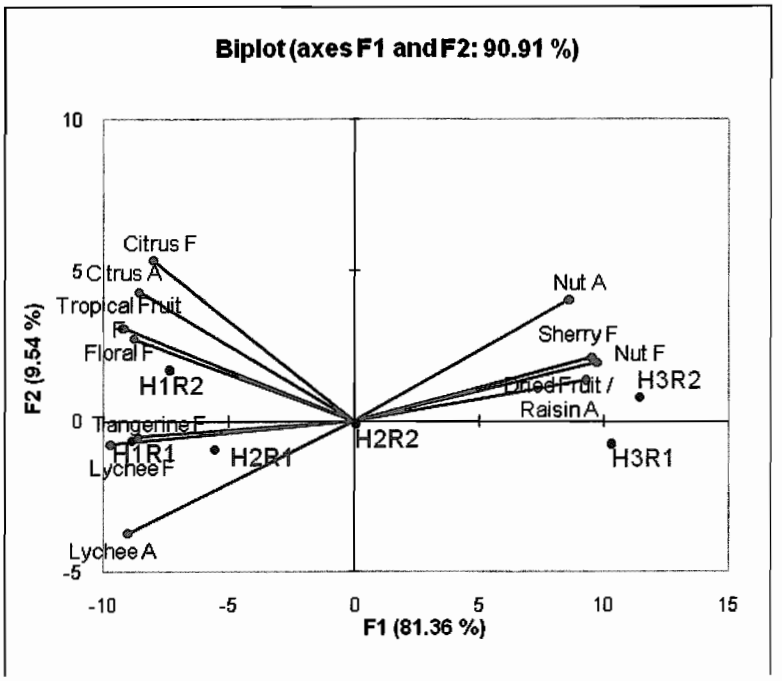


Figure 5.1: Sensory map of the Vidal icewines from four different harvest dates (H1 – H4) showing the variation in the products through PCA on Factors 1 and 2 (A) and Factors 1 and 3 (B), where R1 and R2 indication sensory rep 1 and 2, respectively.



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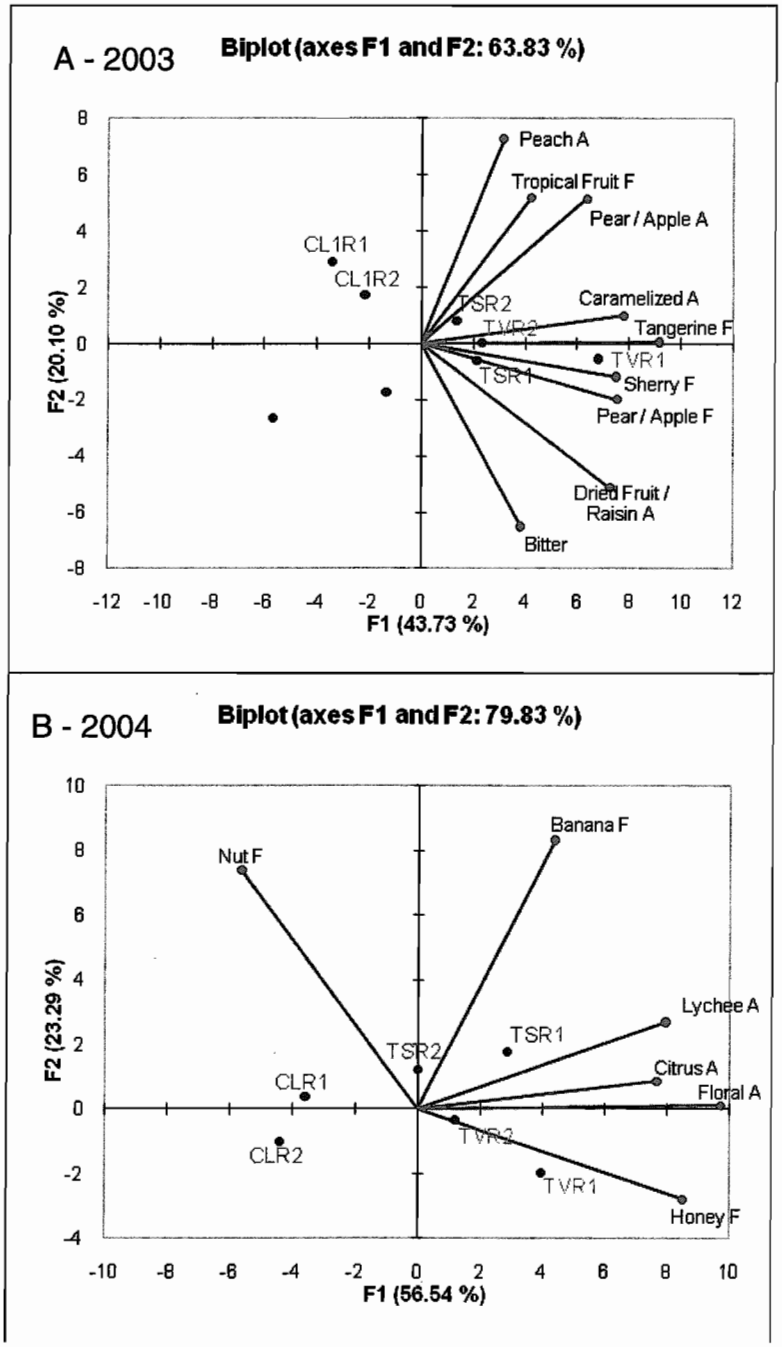

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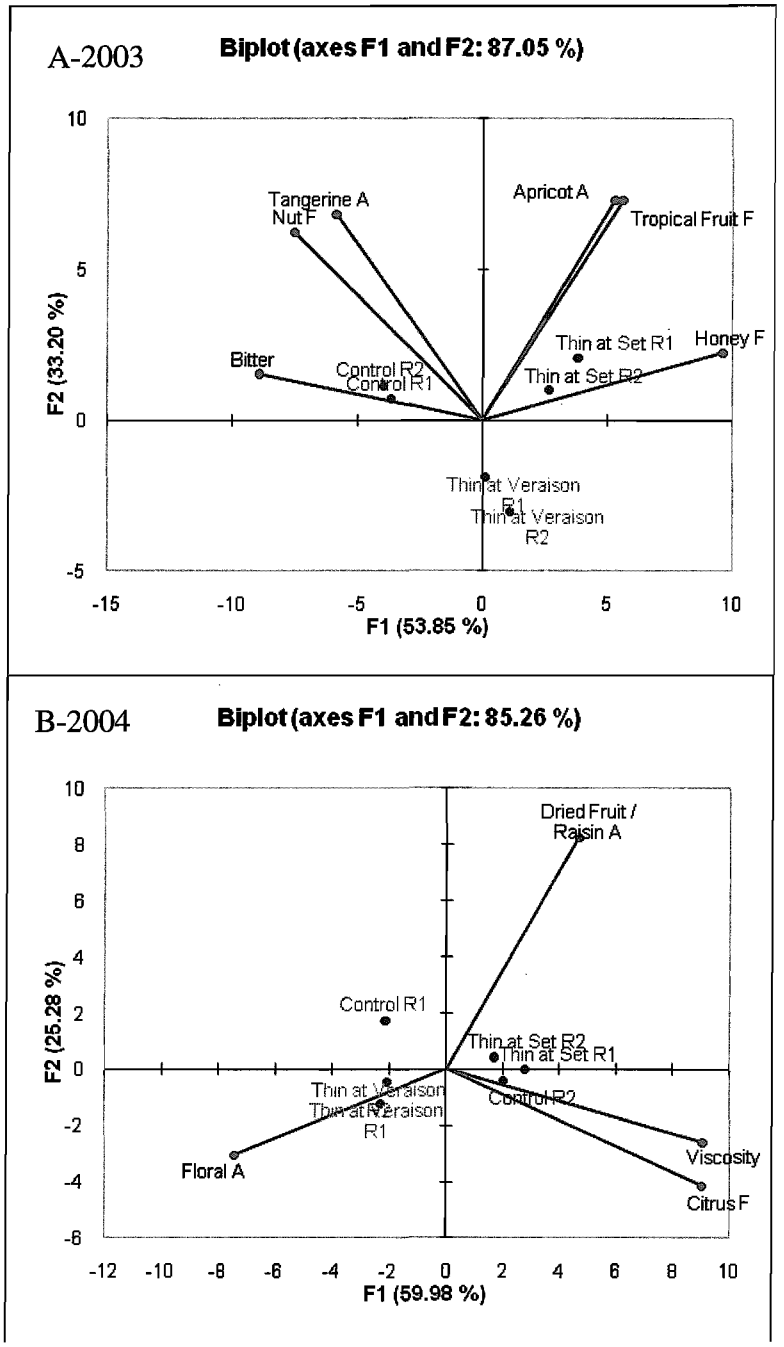

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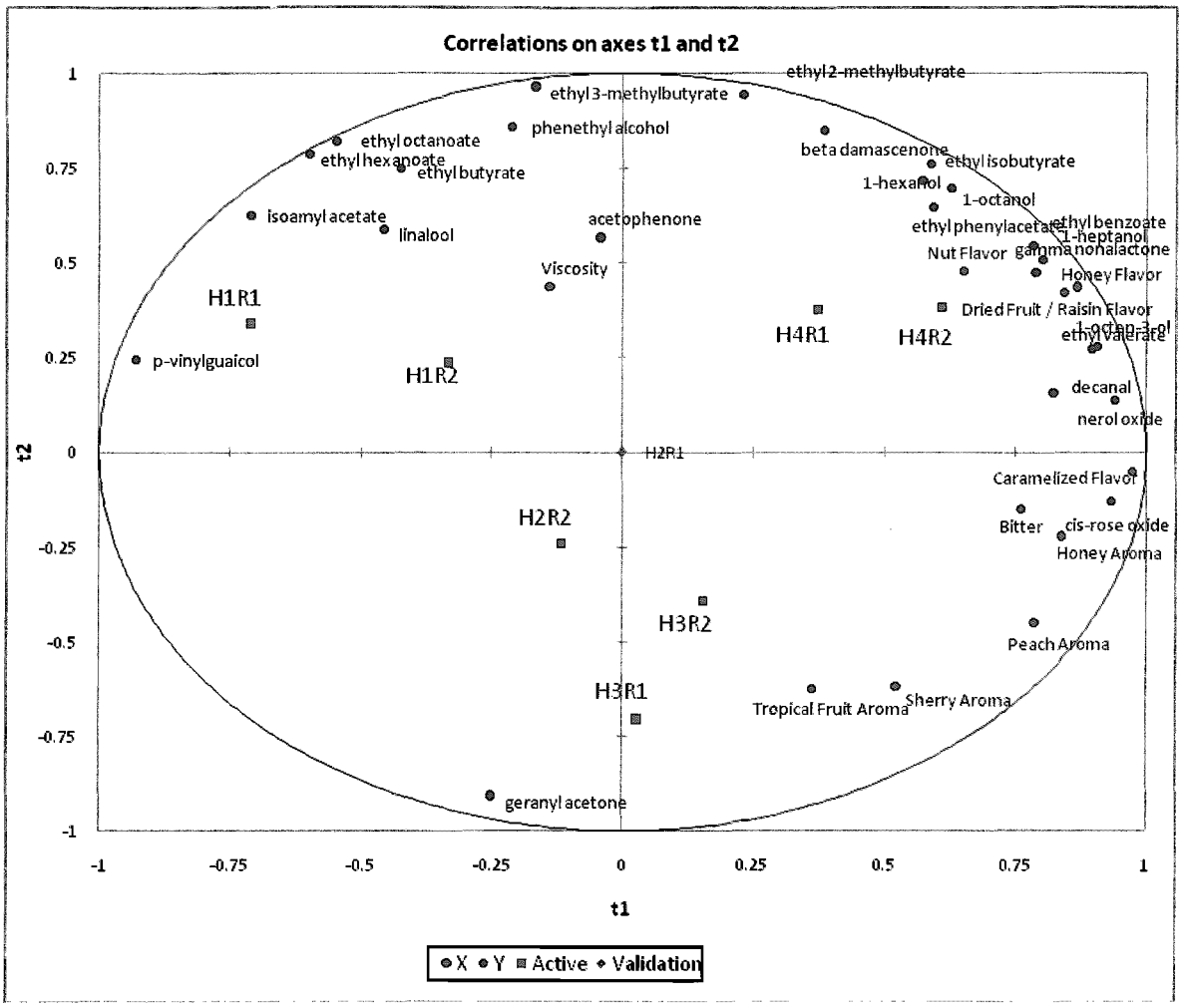
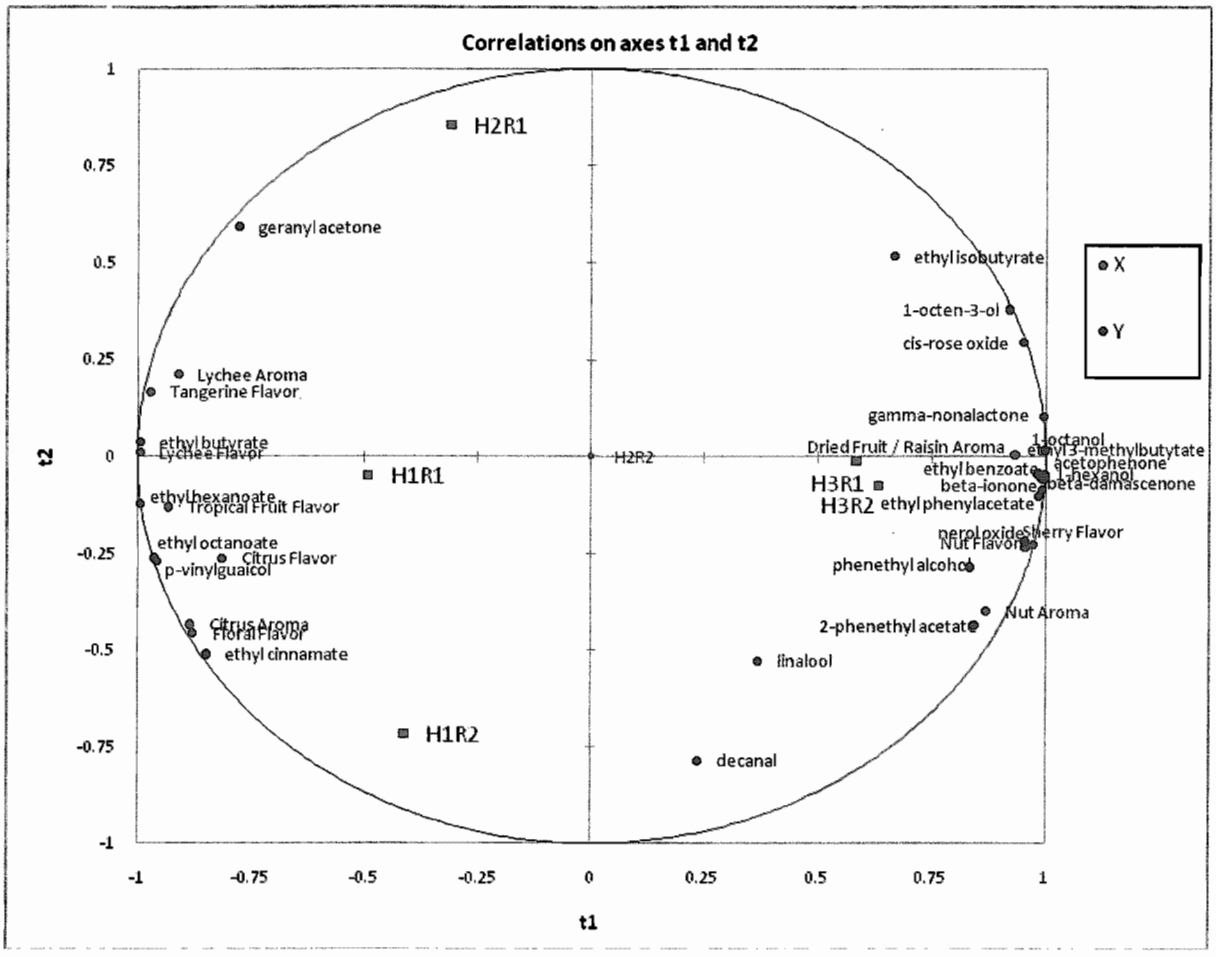


Figure 5.5 The impact of harvest date on the sensory and chemical profiles of Vidal icewines from four harvest dates (H1-H4) determined by PLS. R1 and R2 represent rep 1 and 2 for each harvest date, respectively.



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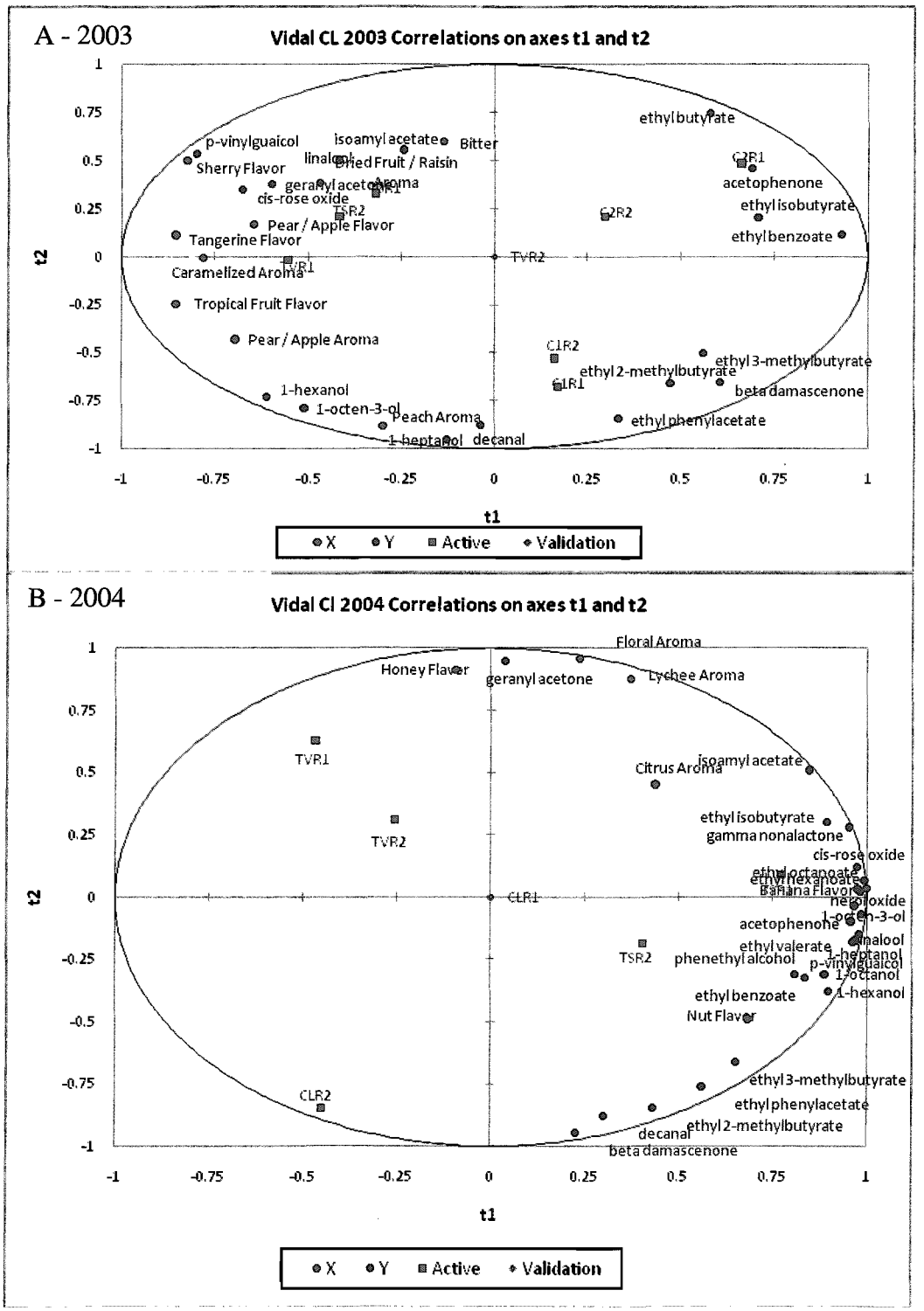

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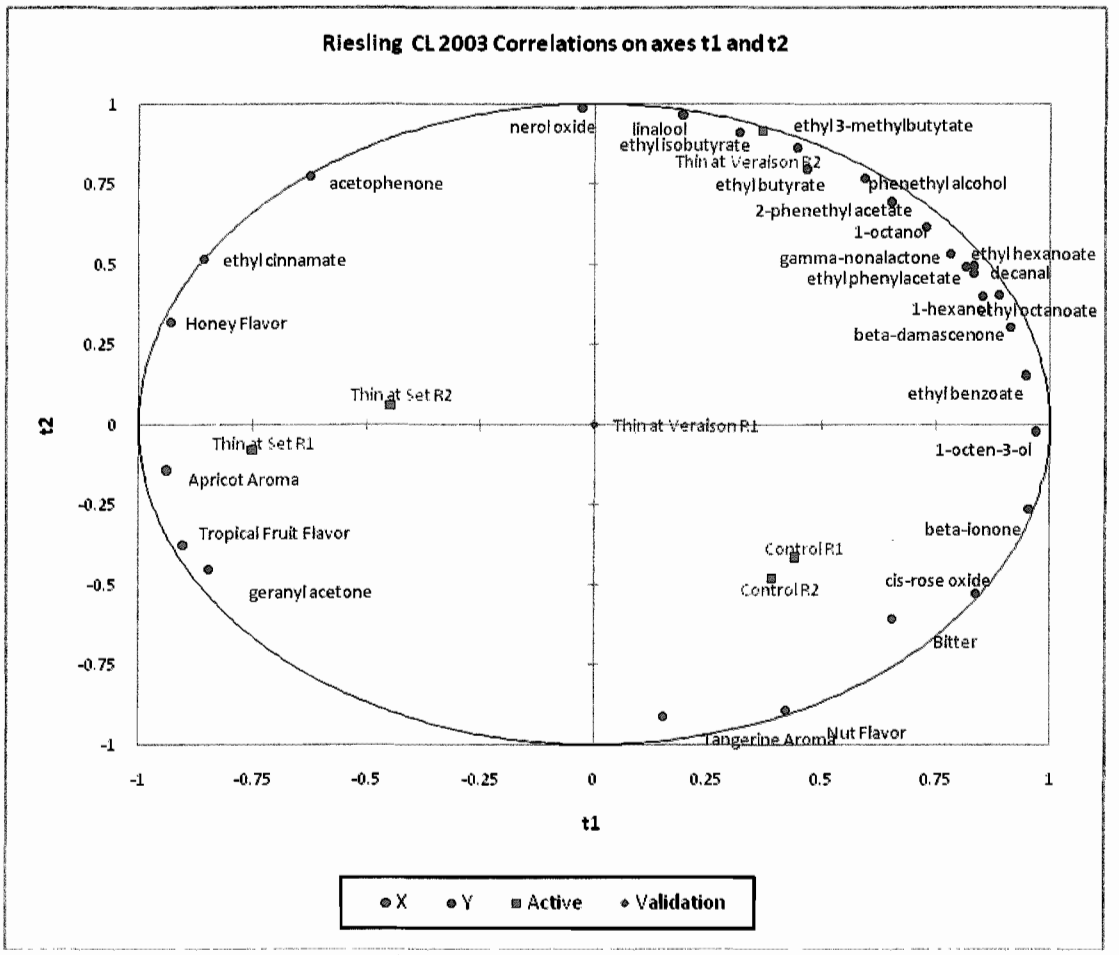

Figure 5.8 The impact of crop level on the sensory and chemical profiles of Riesling icewines from three different treaments; unthinned (CL), thin at fruit set (TS) and thin at veraision (TV) determined by PLS. R1 and R2 represent rep 1 and 2 for each crop level, respectively.

Chapter 6
General Discussion and Conclusions

6.1 Introduction

Wine aroma is the result of a complex interaction of hundreds of volatile compounds that together form a matrix to elicit a sensory response. This sensory response is what makes wine such an interesting topic of research and appreciation from the novice wine drinker to the wine connoisseur. Icewine is no exception; picking and pressing the grapes frozen long after commercial harvest results in a wine concentrated in sugar, acids, aroma and flavour compounds with descriptors of honey, peach, caramel with a sweet taste balanced by its high natural acidity. However, little is know about the aroma compounds responsible for icewines' characteristic sensory profile or how viticultural practices affect its aroma and flavour profile. The objectives of this thesis were: 1) to identify odour-active compounds which could be used to characterize Niagara icewine using sensory analysis and gas chromatography; 2) to determine the effect of crop level and harvest date on these odour-active compounds; 3) determine the sensory profiles of the icewine made from different harvest dates and crop levels; and 4) to correlate the analytical and sensory results for an overall profile of the icewines. For all these objectives it was hypothesized that the freeze and thaw cycles of icewine grapes would result in changes in their chemical composition and sensory properties of the finished icewines and that the chemical composition and sensory properties would be affected by harvest dates and crop levels.

6.2 Objective 1: Identify odour-active compounds that characterize Niagara icewine.

CharmAnalysis™, with two sniff judges, was used to determine the most odour potent compounds in Vidal and Riesling icewine and table wine. The top 15 odour potent compounds found through CharmAnalysis™ were quantified and odour activity values (OAV) calculated to understand difference in aroma profiles between icewine and table wine. The most odour potent compounds in commercial Vidal and Riesling icewine and table wines were found to be the same. However; in general the icewines had higher concentrations and OAV of most aroma compounds compared to table wines. This finding is in agreement with other research comparing the concentration of odour-potent compounds in table wine and later harvested dried grape Fiano wines from Southern Italy (Genovese et al. 2007) and sun-dried Pedro Ximenez wines of Spain (Campo et al. 2008).

The compound with the highest OAV in icewine was β-damascenone and in table wine was ethyl octanoate. In total, 23 and 24 odour-active compounds were quantified in Riesling and Vidal wines, respectively using stir bar sorptive extraction (SBSE) gas chromatography-olfactometry-mass spectrometry (GC-O-MS). The most odour-potent compounds in icewine were β-damascenone, 1-octen-3-ol, ethyl octanoate, *cis*-rose oxide, and ethyl hexanoate. In table wines, the most odour potent compounds were ethyl octanoate, β-damascenone, ethyl hexanoate, *cis*-rose oxide, ethyl 3-methylbutyrate and 4-vinylguaiacol. In general the most odour-potent compounds found through GC-O in this study were β-damascenone, ethyl octanoate, ethyl hexanoate, ethyl butyrate, ethyl 2-methylburtyrate, ethyl 3-methylbutyrate, phenylethyl alcohol, 4-vinylguaiacol, linalool and *cis*-rose oxide which have all previously been identified in Vidal and Riesling table

wines (Simpson and Miller 1983, Chisholm et al. 1994, Chisholm et al. 1995, Komes et al. 2006).

While no unique odour-potent compounds were found in the icewine, in agreement with Cliff et al (2002), the qualitative differences in concentration of the aroma compounds in the icewines and table wines provide useful information toward creating a chemical signature for icewine. While most compounds had higher concentration in icewines, the compounds that were always higher in table wines can also be used to as a marker to detect genuine icewine, such as; 4-vinylguaiacol, decanal, ethyl octanoate and ethyl hexanoate.

Through SBSE GC-O-MS of icewines using CharmAnalysis™, 23 and 24 odour-potent compounds were identified in Vidal and Riesling icewines from the Niagara Peninsula, respectively that can be used to assess the effect of freeze and thaw events, harvest date and crop level of aroma compounds and sensory profiles. Future research will determine if odour potent compounds identified in this study such as β-damascenone, 1-octen-3-ol, and/or 4-vinylguaiacol can be used as icewine marker compounds.

6.3 Objective 2: Effect of harvest date and crop level on odour-active compounds.

6.3.1. Harvest date.

Riesling and Vidal icewines were made from grapes picked between December 2004 and February 2005; Harvest 1:19 December; Harvest 2: 29 December; Harvest 3: 18 January; Harvest 4: 11 February (Vidal only). The role of harvest date on chemical variables and aroma compounds of icewine from different harvest dates was determined through SBSE-GC-MS on the aroma compounds identified in Objective 1. Chemical analysis of the icewine must found TA to decrease with later harvest date in both Vidal

and Riesling. In Vidal, pH was also found to decrease with later harvest date. Chemical analysis of the wine found difference between the harvest dates but no clear trend was observed with the exception of glycerol. Glycerol concentration increased with later harvest date, likely due to higher infection rates by *B. cinerea* and concentration effect due to desiccation of the berries through longer hang time.

The composition of aroma compounds was affected by harvest date, the latest harvest date had the highest concentration of 16 of 24 (H4) and 17 of 23 (H3) aroma compounds in Vidal and Riesling, respectively. The latest harvest date had the highest concentrations for ethyl isobutyrate, ethyl 3-methylbutyrate, 1-hexanol, 1-octanol, *cis*-rose oxide, nerol oxide, ethyl benzoate, ethyl phenylacetate, γ-nonalactone and β-damascenone in both Vidal and Riesling. The earliest harvest date had the highest concentration of ethyl butyrate, ethyl hexanoate, ethyl octanoate, 4-vinylguaiacol and linalool. Odour activity values were calculated and the most odour-potent compounds in both cultivars across harvest dates were β-damascenone, *cis*-rose oxide, 1-octen-3-ol, ethyl octanoate, ethyl hexanoate and 4-vinylguaicol. PCA found most attributes associated with the last harvest date with the exception of 4-vinylguaiacol which was always associated with H1 for both cultivars. β-Damascenone was the most odour potent compound with high concentrations in all treatments but increasing with later harvest date.

The higher concentration of the ethyl esters in the earliest harvest dates (H1) for both Vidal and Riesling icewines may be related to the decreasing TA from freeze events or differences in yeast metabolic activity in overripe grapes (Kliewer 1968). Ethyl esters were also found to have higher concentrations in table wines compared to icewines

(Bowen and Reynolds 2010b) and sweet Fiano base wines harvested at normal maturity had higher concentration of ethyl ester than overripe, late maturity wines (Genovese et al. 2007) which is agreement with the findings of this study.

Higher concentrations of 4-vinylguaicol in H1 is related to the total hydrocinnamate concentration in the grapes since it is formed from the decarboxylation of ferulic acid during fermentation (Shinohara et al. 2000). The total hydrocinnamate concentration decreases with later harvest dates in icewine likely due to freeze and thaw events that cause a decline in concentration in the grape (Kilmartin et al. 2007). This decline in hydrocinnamates is positively correlated with the decline in 4-vinylguaicol formation with later harvest date. This presence and concentration of 4-vinylguaicol has the potential to act as a marker compound for grapes subjected to freeze and thaw events or grapes frozen at normal maturity.

The high concentrations of β-damascenone in the harvest date wine are similar to those reported from sun-dried grapes (Campo et al. 2008) and over ripe grapes (Pons et al. 2008). With the high odour potency and concentration, further research will be required to determine the exact role and contribution of β-damascenone to icewine sensory profiles. However, it is know that its concentration increases with extended hang time (Bowen and Reynolds 2010a) and concentration are much higher in icewine than table wine (Bowen and Reynolds 2010b).

Harvest date was found to change the chemical variables and aroma compound composition of icewines. The six most odour-potent compounds found to differentiate by harvest date β-damascenone, 4-vinylguaiacol, 1-octen-3-ol, *cis*-rose oxide, ethyl hexanoate, and ethyl octanoate.

6.3.2 Crop level

Three vineyard treatments [control (fully cropped), cluster thin at fruit set to one basal cluster per shoot (TFS), and cluster thin at veraison to one basal cluster per shoot (TV)] were evaluated for Riesling and Vidal cultivars over two seasons, 2003-4 and 2004-5 to determine difference in chemical variables and aroma compounds of the resultant icewines. Must samples differed for both cultivars over two vintages, control treatments had lower pH and higher TA than TFS or TV. Similar to harvest date, differences were found in wine chemical variables but no clear trends were observed.

The compounds with the highest OAV values in Vidal were β-damascenone, ethyl octanoate, *cis*-rose oxide, 1-octen-3-ol, ethyl hexanoate and isoamyl acetate in 2003 and β-damascenone, 1-octen-3-ol, ethyl octanoate, *cis*-rose oxide and ethyl hexanoate in 2004. Almost all aroma compounds differed due to crop level in Vidal; 17 of 24 in 2003 and 23 of 24 in 2004. In 2003 Vidal icewine, the control and TV treatments had the highest concentration of aroma compounds and the lowest concentration of TFS, however no clear trends were seen with respect to cluster thinning. These findings are in agreement with Keller et al. (2005) and Reynolds et al. (1994) which both found that thinning had little effect on berry composition. However, the opposite was found in 2004; almost all aroma compounds had the highest concentration in the TFS treatment. These finding are supported by Bravdo (1984) who found difference in crop level and harvest date in Carignane vines, the lower the crop level the earlier the harvest date when cluster thinning was completed just after bloom. Since all grapes were harvested at the same time, the first freeze event could be considered the end of maturation. Therefore, the differences between vintages are related to differences in the number and severity of

freeze and thaw events in November and December. The warmer fall of 2003 had more freeze and thaw event and greater temperature fluctuations, which resulted in desiccation of the fruit and concentration of the aroma compounds negating the effect of cluster thinning.

Due to the difference in growing season, it is suggested that the onset of cold temperatures and the number and duration of the freeze and thaw events has more of an effect on the volatile composition of the Vidal icewine grapes than cluster thinning. Riesling icewines had the highest concentration of aroma compounds in TV treatment; 18 of 22 in 2003 and 20 of 23 in 2004. Odour activity values showed that β-damascenone, ethyl octanoate, ethyl hexanoate were the most odour-potent in 2003 and 2004, while *cis*-rose oxide was also highly odour-potent in 2004. The finding that most of the aroma compounds in Riesling were associated with TV is a contradiction to the results found in Vidal. The most likely explanations are related to difference in grape cultivar and fruit quality. Riesling has previously been found less responsive to cluster thinning than Chenin blanc or Cabernet Sauvignon (Keller et al. 2005), the same could be true for Vidal.

We conclude that freeze and thaw events in November and December were likely more important for aroma compound development than crop level.

6.4 Objective 3: Effect of harvest date and crop level on icewine sensory profiles.

6.4.1 Harvest date

Triangle tests found Vidal early harvest date wines (H1 and H2) to differ from the later harvest date wines (H3 and H4) and in Riesling H1 differed from H2 and H3. Descriptive analysis found 10 and 11 sensory attributes to differ in Vidal and Riesling

wines, respectively. Vidal icewine had higher intensity scores for all significantly different sensory attributes in the later harvest dates (H3 and H4); honey aroma and flavour, peach aroma, sherry aroma, tropical fruit aroma, caramelized flavour, dried fruit/raisin flavour, nut flavour, bitter taste and viscosity. For Riesling, fresh fruit and tropical attributes were associated with H1 and dried fruit, nut attributes with H3. These differences were also illustrated through PCA for both cultivars. These findings are in agreement with the aroma compound composition of the icewines (Bowen and Reynolds 2010a) and previous research which has shown the harvest date affects the sensory profiles of different *V. vinifera* cultivars (Reynolds et al. 1993) and Vidal wines (Gallander 1983).

6.4.2. Crop level

Vidal icewines from both thinned treatments, TFS and TV, had higher intensity rating for the significantly different sensory attributes than the control (fully cropped) treatment in both 2003 and 2004. The 2003 Vidal thinned icewines were described as having higher intensity rating for caramelize aroma, dried fruit/raisin aroma, peach aroma, pear/apple aroma and flavour, sherry flavour, tangerine flavour, tropical fruit flavour and bitter taste. In 2004, the thinned treatments were described by higher intensity ratings of citrus aroma, floral aroma, lychee aroma, banana flavour, honey flavour and nut flavour. PCA found all attributes that differed were associated with the thinned treatments.

In Riesling, control (fully cropped) wines had higher intensity ratings for tangerine aroma, nut flavour and bitter taste while TFS wines were rated highest for apricot aroma, honey flavour and tropical fruit flavour in 2003. In 2004 wines, only four

attributes differed due to crop level; dried fruit/raisin aroma, floral aroma, citrus flavour and viscosity.

The effect of harvest date and crop level on the sensory profiles of the icewines was determined through triangle test and descriptive analysis. Both Vidal and Riesling wines differed in their sensory profiles, however, more sensory differences were found due to harvest date than crop level in both cultivars.

6.5 Objective 4: Relating icewine sensory profiles to their odour-active compounds.

With the exception of Riesling crop level wines; there were strong correlations between sensory descriptors and odor-compounds using PLS analysis. Vidal and Riesling harvest date icewines were contrasted on the first dimension by wines from later harvest date with wine from early harvest dates. In Vidal, all aroma and flavour attributes were associated with the H3 and H4 and positively correlated with most of the aroma compounds such as β-damascenone and *cis*-rose oxide. The esters and 4-vinylguaiacol were associated with H1, the esters were inversely correlated to tropical fruit, sherry, peach and honey aromas and 4-vinylguaiacol was inversely correlated to honey, dried fruit/raisin and nut flavours. In Riesling, H1 was associated with lychee and citrus aromas and tangerine, tropical fruit, citrus and floral flavours which were positively correlated to many of the esters and 4-vinylguaiacol. Nut aroma and flavour, sherry flavour and dried fruit/raisin aroma were associated with H3 and positively correlated with most of the aroma compounds such as terpenes, norisoprenoids and alcohols.

In Vidal crop level icewines, TFS and TV were associated with all the sensory attributes and were contrasted by the control (fully cropped) wines by PLS, in 2003. Sherry flavour was positively correlated with the aroma compounds 4-vinylguaiacol,

linalool, *cis*-rose oxide and geranyl acetone and the sensory attributes tangerine and tropical fruit flavours. In 2004, most of the sensory and aroma compounds were positively loaded on the first dimension which was driven by banana flavour and associated with the TFS treatment by PLS. An exception was β-damascenone inversely correlated to floral aroma and positively correlated with nut flavour. In Riesling, most of the sensory attributes were inversely correlated with the aroma compounds. Honey was the most important explanatory variable and was inversely correlated with bitter taste, 1-octen-3-ol, *cis*-rose oxide, β-damascenone and β-ionone.

Several compounds were identified that require further investigation to determine their role in the icewine flavour, the most important for future research due to concentration difference between early and late harvest date and OAV are β-damascenone, *cis*-rose oxide and l-octen-3-ol in later harvest dates and 4-vinylguaiacol, ethyl hexanoate and ethyl octanoate in early harvest date icewines.

6.6 Overall relevance of the research and conclusions

The information put forth supports both the hypothesis and the objectives. Aroma compounds were identified in icewines from the Niagara Peninsula, their concentrations were quantified and the effect of harvest date and crop level on aroma compounds was found. The sensory profiles of the wines were found to differ and were correlated with odour-active compounds. It can be concluded that harvest date and crop level affect the chemical variables, aroma compounds and sensory properties of Vidal and Riesling icewines from the Niagara Peninsula and that freeze and thaw events change the sensory profile of the icewine.

The most odour-potent compounds were β-damascenone, *cis*-rose oxide, 1-octen-3-ol, 4-vinylguaiacol and ethyl octanoate and ethyl hexanoate. Early harvest date wines were characterized by higher esters, 4-vinylguaiacol and linalool whereas later harvest date wines were characterized by β-damascenone, 1-octen-3-ol and *cis*-rose oxide. The role of β-damascenone as a potential marker compound for icewine, contributing the dried fruit raisin, character requires further investigation.

Since this is the first in-depth study to assess the sensory and volatile composition of icewine due to cultural practices such as harvest date and crop level; there are many avenues for future research. These include, but are not limited to, identification of marked compounds in icewine from the Niagara Peninsula, sensory re-constitution and omission studies to determine impact odorants, understanding the exact role of β-damascenone and 4-vinylguaiacol to icewine sensory profiles, quantification of key compounds from the current study in commercial icewines and try to classify by harvest date, determination of the exact role of freeze and thaw events on icewine chemical composition, and consumer research studies to determine which sensory profile the consumer prefers. However, the research presented herein provides a strong foundation for understanding the odour active volatiles and sensory profiles important to icewine and is a step in the right direction toward finding marker compounds to identify genuine icewine.

6.7 Literature Cited

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