The Impact of the Multicolor Asian Lady Beetle (*Harmonia axyridis*) on Wine Quality

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Abstract

The Impact of the Multicolor Asian Lady Beetle (*Harmonia axyridis*) on Niagara Wine Quality

The possible influence of *Harmonia axyridis* (the Multicolored Asian Lady Beetle) on the sensory properties of wine was investigated. *H. axyridis* beetles were added to white and red grape musts at a rate of 0, 1 or 10 per L, and a trained panel evaluated the finished wines using flavor-profiling techniques. Significant modification of both wine aroma and flavor characteristics were observed in the 10 beetle/L treatments, with smaller effects noted at the 1 beetle/L rate. Vinification in the presence of *H. axyridis* gave higher intensity scores for peanut, bell pepper and asparagus aromas and flavors in the white wines, and peanut, asparagus/bell pepper, and earthy/herbaceous aromas and flavors in the red wines. In addition, sweet, acid and bitter tastes were affected in red wines, and a general trend of decreasing fruit and floral intensities with increasing beetle rate was observed in both white and red wines. 15 ng/L Isopropylmethoxypyrazine was added to control wines and sensory profiles similar to high beetle treatments were obtained, supporting the hypothesis that methoxypyrazines from beetles are implicated in the taint.

A trained panel evaluated the treated wines after 10 months of aging using the same sensory methods described above. Sensory profiles were very similar.

Fermenting in the presence of *Harmonia Axyridis* (*HA*) had little influence on the chemical composition of the finished wine. The notable exception is

Isopropylmethoxypyrazine content, which was assessed using GC-MS analysis and showed increased concentration with increasing beetle number for both white and red wines.

The influence of potential remedial treatments on the sensory properties of white and red wines tainted by Harmonia axyridis were also investigated. Bentonite, activated charcoal, oak chips, de-odorized oak chips, and UV or light irradiation were applied to tainted wine, and these wines evaluated chemically and sensorially. Both white and red wines treated with oak chips had strong *oak* characteristics, which masked the *Harmonia axyridis*-associated aroma and flavour attributes. In red wine, *asparagus/bell pepper* characteristics were decreased by bentonite and charcoal treatments. Only activated charcoal significantly decreased methoxypyrazine levels and only in white wine.

Hypothesis

Hypothesis 1: *Harmonia* in grape juice adversely affects wine quality

Hypothesis 2: Methoxypyrazines are the compound class responsible for the off-taint observed in *Harmonia*-affected wines

Hypothesis 3: Selected wine or juice treatments will affect the sensory profile of *Harmonia*-treated wines

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Chapter 1. Research Review of *Harmonia axyridis* and tainted wine

1.1. Introduction:

Since 2001, *Harmonia axyridis* (Pallas), a new species that formally resided outside of North America, has been a threat to the local wine industry, especially in the Niagara region, the most important wine region in Canada. It has been claimed that the beetle impacts a "peanut butter" or "musty" aroma to wine and adversely affects wine quality. (Martinson, 2002). It is essential to obtain an accurate sensory description of these tainted wines to finally characterise the problem, and, it is urgent that suitable methods are found to improve these tainted wines, not only for the present wines, but also for the future. Obviously, it is also necessary to understand the biological characteristics of *H. axyridis*, and the chemical substance responsible for the taint. These issues are reviewed below.

1.2. *Harmonia axyridis:* an introduced species

1.2.1. Biological characteristics

Harmonia axyridis (Pallas) (common name: Multicolored Asian Lady Beetle), belongs to Coccinellids (Coleoptera: Coccinellidae). This is a famous group consisting of various species that have the ability to predate aphids, therefore they are considered to be beneficial insects to the agriculture industry and to be a welcome part in many ecosystems (Koch, 2003, Brown & Miller, 1998).

Similar to other related species of Coccinellids, *H. axyridis* are oval, from yellow-straw color to brick red, with spots on their back. The colour of the ventral surface is yellow-orange to black. The body can be 4.8-7.5 mm in length and 3.9-5.9 mm wide. They can not be simply differentiated from other species by the number of spots or the body colour. They have a distinguishable M-shaped mark on the pronotum, which consists of two lines of spots jointed with the lateral spots (Chapin and Brou, 1991). The biological life cycle (approx. 20 days) is from egg to larvae, to pupa, and finally to adult. It has been observed under lab conditions that the female can lay 25 eggs per day, up to a total of near 4,000 eggs (Koch, 2003). According to early research quoted by Nalepa, the beetle can live up to 3 years (Nalepa *et al.,* 1996). As observed in Japan, the beetle has two or three generations each year: first mating and laying eggs in April and May with the second generation appearing in autumn (Osawa, 2000). *H. axyridis* can be found on various trees where the aphids exist, such as maple, walnut, oak, apple orchards, etc. (Chapin & Brou, 1991, Brown & Miller, 1998).

There are some natural enemies of *H. axyridis,* such as some phorids and birds. It is possible that other coccinellids also prey on *H. axyridis* (Koch 2003). Joseph *et al.* (1999) even discussed the cannibalism of *H. axyridis*, which can have both negative and positive consequences for the species (Joseph *et al.* 1999). There is an obvious concern as to whether pesticides will be harmful to these beneficial insects such as *H. axyridis.* Researchers in Korea examined 12 agents, which were chosen from various insecticides, acaricides and fungicides with recommended dosages, on different stages of *H. axyridis.*

They found that some of these agents were quite harmful to some life stages of *H. axyridis* (Youn *et al.* 2003).

1.2.2. An introduced biological control agent: positive or negative?

As an insect that can predate some pests, which are harmful to agriculture, such as aphids, *H. axyridis* is considered to be a beneficial species and a good candidate for biological control. They are not indigenous to North America, but originated in Asia. It was in the early part of last century that the lady beetle was first introduced to North America. Although the first attempt to establish the species in the New World seems to have failed, during the late 1970s and early 1980s, the United States frequently released *Harmonia axyridis* (both adult beetles and larvae) in several states. *H. axyridis* became established as the third species of the genus in the United States, and with a high ability for movement, they were quickly found in those states where they had not been introduced (e.g. Oregon) (Koch 2003, LaMana and Miller, 1995, Chapin & Brou1991). They were also found in Canada through the migrant way from the United States to south Canada (Damsker, 2001).

It is hard to predict what will happen when a new species is introduced to a different ecosystem. Some researchers in the USA began to focus on *H. axyridis* since they are widely found in all parts of North America. In 1988, Brou used several light traps to collect beetles in his local area, and found the combination of UV light sources (e.g. fluorescent blacklight and mercury vapour light) could attract beetles from dusk to dawn in different seasons (Chapin & Brou, 1991). Nalepa *et al* gathered beetles in the autumn

of 1993 and 1994 in both urban and agriculture areas of eastern United States. After comparing the samples from the two years, female beetles in 1994 were present at significantly larger numbers than males, which was different than the 1:1 ratio in 1993 (Nalepa *et al.*, 1996). LaMana and Miller compared the distribution of beetles in 1993 and 1994 in western United States and found an increase in the range of *H. axyridis*. They found that *H. axyridis* were predominant in samples from trees and shrubs, compared with a lower ratio among other species of Coccinellidae in herbaceous crops (LaMana & Miller, 1995). After several years of field studies on both *H. axyridis* and their prey (aphids), Osawa in 2000 suggested that the prey searching ability of beetles was high over a large area. He also found the beetles could distinguish the quality and quantity of their foods during their long-distance movement. Interestingly, females had a higher ability of prey searching than males, and were also stronger than male beetles. This may be the explanation for the sex ratio difference in Napela's study. (Osawa, 2000) Another explanation for this sex related character is the presence of male-killing bacteria in coccinellids, of which *H. axyridis* is a typical example. PCR and 16s rDNA gene analysis now offer improved tools for studying the biodiversity and impact of these bacteria (Majerus *et al.*, 1999).

The high ability for immigration meant the *H. axyridis* could quickly become the dominant species in those non-designated areas. However, some researchers believed if *H. axyridis* did not have such a high rate of movement they would remain longer in one location, and that would benefit the local area over a long period. The French introduced the *H. axyridis* in 1982, almost at the same time as the United States. French researchers

fed normal *H. axyridis* with a chemical mutant, and they selected the adults without the ability to fly over 17 generations. The differences in locomotory behavior, growth and reproductive rate, and foraging ability between mutated and normal beetles were not significant (Tourniaire *et al.*, 2000). This research may have positive implications for biological control in the future, however naturally existing *H. axiridis* are naturally good flyers.

Another important characteristic of *H. axiridis* is its phenotypic plasticity. Here, phenotypic plasticity means the degree to which they can express trait changes between environments, for a particular genotype. For *H. axiridis*, as a new introduced species, this is an important means for living in unpredictable, novel habitats. A high flexibility of phenotypic plasticity can offer better opportunity to overcome the difficulties in a new environment. Grill *et al.* (1996) used *H. axiridis* as test subjects by exposing them to different food sources. Their results showed the potential evolution of phenotypic plasticity. They suggested that in a much larger population, *H. axiridis* would have greater ability for evolution in different habitats and climates, and with different food sources and selection pressures (Grill *et al.*, 1996). Genetic research was also carried out on *H. axyridis* to attempt to answer the question that the roles of selection and genetic drift play on *H. axyridis* in North America. Due to its potential adaptable ability, Researchers predicted possible "boom and bust" population dynamics in *H. axyridis* (Krafsur *et al.* 1997).

All the observations show that *H. axiridis* is a successful species living and moving in North America. There are already some positive indications that the introduced species do control pests on pecans, strawberry, and roses (Koch, 2003). However, they are not always welcome as will be discussed in the next session.

1.2.3. The 2001 North American vintage

When the introduced H. axyridis brought its benefits to local agriculture, new unpredicted problems also appeared. One is Winter Aggregation, which means that beetles aggregate in mass in buildings before the onset of winter. Although aggregation enables researchers to catch beetles more conveniently, it may frequently cause the hosts to be annoyed. Fortunately, there is no evidence that these beetles bring any hazardous disease, and it seems they do not destroy the buildings or the furniture (Nalepa, 1996, LaMana and Miller, 1995). In the wine industry, problems were beginning to emerge.

1.3. *Harmonia axyridis*, methoxypyrazine and wine

1.3.1. What has happened to the wine in 2001: "Peanut butter"

In northern areas of the United States and some areas in Canada (including the Niagara region), some grapes harvested in 2001 produced wine with an unusual odor, often referred to as "Peanut butter" (Martinson, 2002). This appeared to correlate with the high number of *H. axyridis* beetles in the vineyards this year. Did the beetles cause the problem? If so, how could they?

1.3.2. Methoxypyrazine: from Harmonia axyridis to grape

H. axyridis also have their own predators. Some mechanisms have been advanced in Coccinellids to enable them to escape from potential predators. The bright color on their backs can be a warning signal to predators. Another important defense system of Coccinellids is they can emit substances in haemolymph from the tibio-femoral joints of their legs ("reflex bleeding"), when they are attacked. (Koch 2003, Laurent *et al.*, 2001, Abassi *et al.*, 1998) The main effective compounds of these volatile repellants are alkaloids. According to Laurent's summary, there have been more than 50 alkaloids characterized from ladybirds. Many of the biosynthetic pathways still need more research (Laurent *et al.*, 2001). Among these alkaloids, alkyl-methoxypyrazines, such as isopropyl- and isobutyl-methoxypyrazine (IPMP and IBMP), are considered as important olfactory alerting signals. Abassi *et al* (1998) argued that the emission isopropyl-methoxypyrazine could be not only a warning signal to possible attackers, but also might be used as a signal to attract adults of near kin and as a sex pheromone. If this is true, these high odor compounds will play an important role in beetle's behavior such as in aggregation and migration (Abassi *et al.*, 1998).

Pyrazines are flavour substances that can be found in foods. By heating potatoes and coffee they can be formed through a reaction called "Maillard reaction" (Buchbauer, *et al.*, 2000). Methoxypyrazines, such as isopropyl- and isobutyl-methoxypyrazine, are found in fresh vegetables such as green bell pepper, beans, asparagus, and even grapes (Boubee *et al.*, 2000, Buchbauer *et al.*, 2000, Allen & Lacey, 1998). Some bacteria, such as *Lysobacter enzymogenes*, can produce IPMP and may cause water taint of musty-

earthy odors in spring water (Emde *et al.*, 1992). Some amino acids are a possible source of methoxypyrazine biosynthesis. Allen suggested that leucine, isoleucine, and valine might play important roles in this biosynthesis (Fig1, Allen & Lacey, 1998).

leucine → leucunamide + C-C → pyrazinone → isobutylmethoxypyrazine

Figure 1. Supposed biosynthetic pathway to isobutylmethoxypyrazine (Allen & Lacey, 1998)

This proposed pathway has been shown in certain bacteria, but it is still not clear in plants and insects (Allen & Lacey, 1998, Hashizume *et al.*, 2001).

IBMP is also used as a fragrance ingredient although no more than 1 ton (!) is produced annually worldwide. Human skin-irritation test and genotoxicity of bacterial studies showed no signs of hazards. FEMA limits the permissive concentration in foods to 10 ppm (Letizia *et al.*, 2000).

1.3.3. Sensory characteristics of methoxypyrazines

Methoxypyrazines can elicit a broad spectrum of sensory properties in humans. Green, earthy, herbaceous, vegetative, and bell pepper, are common descriptions. (Sala *et al.*, 2002, Buchbauer *et al.*, 2000, Buchbauer *et al.*, 2000, Allen *et al.*, 1994 & 1998) The thresholds of these compounds are quite low: 1-2 ng/L in water; thus, even a very trace concentration presented in wine can have a remarkable impact on wine (Sala *et al.*, 2002, Allen & Lacey, 1998). However, some studies showed that thresholds in wines were higher than in water. Particularly in red wine, it could reach 10-16 ng/L. (Boubee *et al.*,

2000, Sala *et al.*, 2002). In white wine, if they reached about 4-8 ng/L, the recognizable characteristics would be caught (Allen & lacey, 1998). Allen concluded:

> It is possible that the greater flavour complexity and intensity of some red wines may mask methoxypyrazine aroma to some degree, permitting the presence of higher levels of methoxypyrazines in those wines than in wines with less intense and less complex flavour. Curiously, a study of the perception of added methoxypyrazines to a red wine found a higher aroma threshold for IBMP than for IPMP. This suggests that low levels of IPMP may be more important to the perception of red wines than is indicated by the sensory detection threshold in water (Allen & Lacey, 1998, pg 36).

Because of the low threshold of methoxypyrazines, they have been used in studies to reveal the interaction between the flavour molecules and the specific receptors in the olfactory mucosa. (Buchbauer *et al.*, 2000) Isobutyl-methoxypyrazine was the first material used for identifying the odorant binding protein (OBPS) in 1979, and it stimulated other research on those olfactory receptors. (Burova *et al.*, 1999, Leffingwell, 2002).

1.3.4. Methoxypyrazine in wine

Methoxypyrazines are presented in some grape varieties, such as Sauvignon blanc and Cabernet Sauvignon. In some instances, their distinctive aroma could be a style of special regions. Within a suitable range, they offer an acceptable and desirable flavour to Sauvignon blanc and Cabernet Sauvignon. However, even in these wines, a high concentration of methoxypyrazines can be unpleasant (Allen & Lacey, 1998).

There are two main factors influencing the level of methoxypyrazines in grapes: the grape variety and the growing condition. While Cabernet Sauvignon and Sauvignon blanc can produce appreciable levels of methoxypyrazines, they are difficult to detect in other grape varieties. During the berry maturity, IBMP decreases, from a surprisingly high level (100ng/L) to a very low level. Climate is also an important factor in the methoxypyrazine levels in grape. Allen and Lacey assumed that compared with warm regions where the IBMP concentration in fruit could be below the sensory threshold, the concentration in the same variety of grape in cool areas could reach 20-30 ng/L. In vineyard practice, canopy management also could affect the level of methoxypyrazine: the more exposure to sunlight, the lower the level will be (Allen & Lacey, 1998). Hashizume and Samuta (1999) also found that picked fruit that had been exposed to artificial light had decreased levels of methoxypyrazines after the light exposure.

Boubee *et al.* (2002) isolated the different parts of the grape from grape bunches, and used 12% ethanol solution to extract the IBMP. They found there was no IBMP in the flesh, but it was present in stems, skins, and seeds. With maturity, the level in stems and seeds decreased, whereas in the skins an increase was observed. However, all extractions into wine were quite fast and quickly reached a stable level. (Boubee *et al.*, 2002) Their result partially matched Allen's observation that skin contact could increase the concentration of IBMP, but Allen found the rate of increase was slow. (Allen & Lacey, 1998) Other research has also shown that stems are the main source of methoxypyrazines. (Hashizume & Samuta, 1997)

1.3.5. Measurement of ultra-trace levels of methoxypyrazine

Usually there are only trace amounts of methoxypyrazine found in wine, whether they exist naturally or exogenously, thus, establishing a reliable method of measurement is important for research. As they are highly volatile compounds, a gas chromatograph (GC) is a suitable instrument for analyzing. However, standard gas chromatography is not sensitive enough to give both qualitative and quantitative measurements. The first attempt at detecting methoxypyrazine in 1980 used 170L of Cabernet Sauvignon, yet still failed. (Allen *et al.*, 1994)

For the analysis of ultra-trace compounds such as methoxypyrazines, sample preparation is the first crucial step. Wilkes *et al.*, offered a detailed review on sample preparation for flavours in various foods (Wilkes *et al.*, 2000). Allen *et al.* added an internal standard IPMP ($^2H_3$), and treated it with several steps: distillation, ion-exchange, extraction, and concentration. This method is called stable isotope dilution, and has been used for quantitative measurement of methoxypyrzines in wine for over 15 years. It can detect to levels of 0.1 ng/L (Allen *et al.*, 1994). Hashizume & Samuta (1999), who found that the detection limit is less than 0.2 ng/kg in grapes, used a similar method. The greatest disadvantage of this method is that it needs considerable preparation time and labor cost. A simpler method is liquid-liquid extraction, which is easier to prepare and does not require special instruments; however, the sensitivity is limited to near threshold concentration of methoxypyrazines. Sala *et al.* in 2002 applied headspace solid-phase microextraction (HS-SPME) to source IBMP from wines without any solvent. This method has been used for analyzing the aroma compounds in foods and wines, and has

some advantages: short time, saving solvent, and keeping sensitivity near to 0.1 ng/L (Sala *et al.*, 2002). A similar SPME method was also used by Haetman *et al.* in 2002 on a model wine. They reported some factors, such as pH, ethanol, and oak, could affect the extraction of methoxypyrazine level from synthetic wine matrices. They assumed that pH<2 depressed the volatility of methoxypyrazine; however, wine pH is normally above this level (Hartman *et al.*, 2002). Measurement of IPMP and IBMP in water regions has also been researched using a granular adsorbent, Ambersorb572, and isotope dilution with an internal standard. The sensitivity of both analytes was less than 1 ng/L (Palmentier & Taguchi, 2001).

A variety of columns have been used in the research literature involving methoxypyrazines, but almost all have used Mass Spectrometry for detection. GC-MS is broadly applied in the detection and quantification of trace flavour compounds in foods, such as pepper, bell pepper, and oil of lavender, because of its ability to identify the targeted substance (Korany & Amtmann, 1997, Pallado *et al.*, 1997, Luning *et al.*, 1994). Allen *et al.* (1994) used stable isotope dilution and GC-MS to measure 18 commercial wines, and obtained successful results (sensitivity levels reached 0.1 ng/L). Boubee *et al.* (2000) used a similar method to check the IBMP levels in red Bordeaux and Loire wines. They confirmed there were higher levels of methoxypyrazines in Cabernet Sauvignon and Cabernet franc, than in Merlot. They also found a correlation between malic acid content and IBMP during grape ripening (Boubee *et al.*, 2000).

As previously mentioned, Sala *et al.* (2002) used HS-SPME to analyze methoxypyrazine levels and obtained data with high sensitivity, similar to that obtained by Allen. Other differences included the use of a nitrogen-phosphorus detection system and two different fused-silica capillary columns: one for analyzing and another for peak confirming (Sala *et al.* 2002).

1.4. Improvement of beetle tainted wines

In this section we will review traditional practices and agents used in treating and processing wine, and consider their possible application in improving the quality of wine affected by *Harmonia axyridis*. Non-traditional applications such as light and UV will also be considered.

1.4.1. Fining

As defined in Jackson's "Wine Science", "fining is commonly used to accelerate the spontaneous precipitation of suspended material in wine"; however, "fining may … (also) eliminate certain off-odors" (Jackson, 2000, p369).

Normally, in practice, fining means the addition of various materials into wine. These materials could be natural biological substances such as proteins: gelatin, isinglass, casein, and egg white, or natural substances such as bentonite and silica gel (Kieselsol), or synthetic materials such as PVPP (polyvinylpolypyrolidone). The fining agents can be used individually or combined together. Although there is a long history of practicing

fining in wine making, the mechanisms of fining are quite different and complex. The main mechanism involves the interaction of the charges between the fining agents and the targeted substances in the wine. However, fining may also cause undesired sensory changes in the wine, either through the introduction of off-odors or the removal of desirable flavour compounds. As Rankine states, "Thus, fining should be used only when necessary and at the minimum effective rates" (Rankine 1998, p154). In this research, we considered a number of fining agents that might decrease the aroma taint of *H. axyridis*, and these are reviewed below.

1.4.2. Bentonite

Bentonite, an unpurified aluminum silicate, is a montmorillonite clay that has been commonly employed for wine stabilization for more than 70 years since it was invented in California (Fig. 2). After soaking in hot water, the surface area of the bentonite can increase to about 750 m^2 per gram (Rankine 1998, p165-167). At the wine pH, the negative charge on the surface of the bentonite can effectively attract positively charged substances, such as amino acids and positively charged proteins. Other mechanisms such as hydrogen-bonding or van der Waals interactions also help bentonite absorb those neutral or negatively charged compounds. These interactions make bentonite become an effective fining agent for stabilizing and removing some aromas in wine making. There are two types of bentonite used for wine fining: calcium and sodium bentonites. In practice, for more efficiency, natural calcium-rich bentonites are treated with solid Sodium carbonate. It is believed that sodium-bentonite absorbs nearly twice the amounts

of protein than calcium-bentonite. (Gougeon *et al.*, 2003) The normal doses are from 0.2 to 0.4 g/L, but can be as high as 0.8-1.0 g/L (Ferreira *et al.*, 2002).

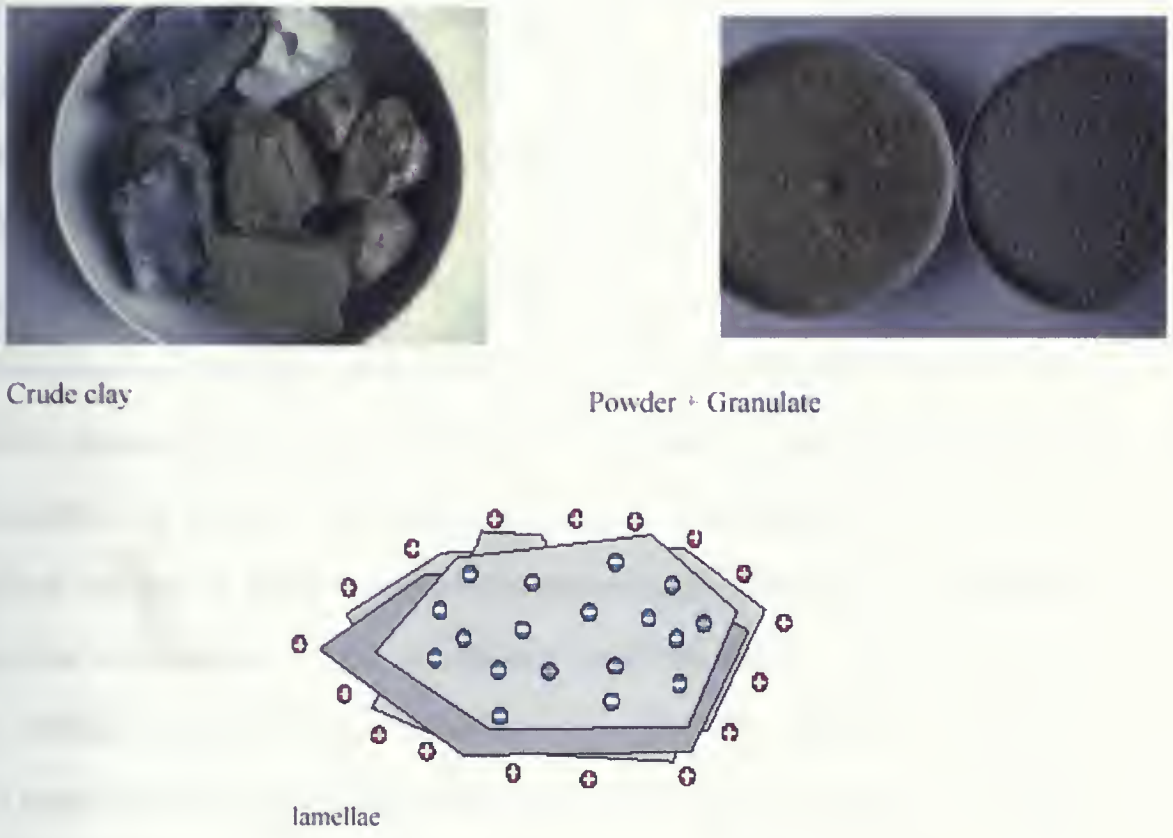

Figure 2. Bentonite in its original and refined appearances, and its crystal structure
From: http://www.swiftco.com.au/swift/pdf/Bentonite.pdf

Some researchers have used bentonite and other fining agents such as charcoal to remove exogenous substances such as fungicides, pesticides, and herbicides. Cabras *et al.* (1997) evaluated the influence of several clarifying agents, including bentonite (100g/hL, 2 days), on some fungicides (cyprodinil, fludioxonil, pyrimethanil, and tebuconazole).

They found bentonite caused Cyprodinil to decrease by 42% but there was no significant effect on the others (Cabras *et al.* 1997).

As a regular fining agent, bentonite has been widely used in wine treatment; however, some negative effects may be introduced. As a cation exchanger, bentonite can reduce some important charged components in wine, although compared with other fining agents such as silica gel/gelatin, bentonite has less effect on wine quality (Ferreira *et al.*, 2002). One of the most recent studies involves applying bentonite to sparkling wines. Researchers added bentonite to the *tirage solution* before the second fermentation. They tried to determine the effects of bentonite in this treatment by measuring the nitrogen composition and by sensory evaluation. They observed a significant decrease of protein nitrogen and peptide nitrogen after adding bentonite; thus, bentonite also affected the important characteristics of foam in sparkling wine. The sensory evaluation confirmed the chemical analysis (Martinez-Rodriguez & Polo, 2003). An alternative method is to mix bentonite with potassium caseinate and micro-crystalline into grape juice. This kind of treatment can produce wines with minor residual sugar and lower polyphenol content, compared with the addition of bentonite only. The sparkling wine treated by this mixture did not change in its sensory profile (Puig-Deu *et al.*, 1999). Other research also indicated that bentonite and other fining agents could influence the content of resveratrol, an important beneficial compound to humans (Castellari, 1998).

In our research, we used bentonite as one of the treatments on beetle tainted wines, and tried to determine its effect on those wines. Bentonite was selected based on some

positive anecdotal comments from industry, although generally methoxypyrazines are not positively charged compounds in wine at wine pH. (methoxypyrazine: pK = 0.75, "*Dictionary of Organic Compounds*", Vol. 4, p3716, Chapman & Hall, NY, 1982)

1.4.3. Charcoal (Activated carbon)

The charcoal used in wine fining is usually activated charcoal. It has a large surface area and has significant ability to absorb many small compounds. It can significantly decrease some odor components in wine (Jackson, 2000, p369). However, its electrical charge also enables it to absorb phenols and their derivatives, and cause loss of color and aroma compounds in treated wine. Charcoal may also introduce some chemically –adsorbed oxygen, which is an important negative factor in wine making and storage. Lopez *et al.* (2001) studied the effects of charcoal and other combined fining agents on sherry wine. They confirmed that charcoal has the most significant effect on polyphenolic content among the various fining agents. They used GC-MS identified aroma compounds as the aromatic profile, and this profile was used and verified by an expert panel. The panel did not find a significant difference based on this aroma profile. This could have been due to the low dosage of charcoal used (180mg/L) (Lopez *et al.* 2001).

As previously mentioned, research on fungicides has shown that charcoal reduced levels of all four of the examined fungicides (cyprodinil, fludioxonil, pyrimethanil, and tebuconazole) at a dosage of 20g/hL, and sometimes almost completely. (Cabras *et al.* 1997) Other similar research found charcoal effectively reduced herbicide residue in the treated wine (Ying & Williams, 1999). They found the most effective treatment was the

use of a filter with 5% charcoal filter pads that can remove more than 96% of the norflurazon in the wine. In a review, it is pointed out that charcoal can effectively eliminate most pesticides when the residues are low and less soluble (Cabras & Angioni, 2000).

1.4.4. Oak

Oak is widely used with wine since its long history as a storage container for wines (mainly red wines). Its sensorial affect on wine has been widely studied. Traditionally, oak barrels are used for aging wine and spirits and offer improved color stability and a more complex aroma. The aroma of oak is due to the complex compounds in the wood and the reaction with wine components during aging (Perez-Prieto *et al.,* 2002). For example, wine can absorb up to 30% of its total tannin content from the oak and this changes the color and bouquet of the wine. Yeast and bacteria also may modify the extracted oak-components. (Jackson, 2000, p383-399) Although it was believed such an influence of oak might change taste and mouth-feel, Pocock *et al.* (1994) reported that panelists were more influenced by the aroma of oak, rather than the taste of oak tannin. However, aging in oak barrels is not suitable for all wines, especially those wines with high fruity aromas.

In the wine industry, there are two main kinds of oak used, French oak and American oak. French oak (as well as oak from other European areas) has more extractable materials than American oak. There are factors other than oak species that can have an affect on wine flavour and aroma. These include seasoning of the oak (toasting) time and

methods, temperature and humidity of the storage environment, cooperage size (surface area), and the number of times used (Jackson, 2000, p383-399, Chatonnet, 1999, Hale *et al.* 1999, Rankine 1998, p207-212).

There are many reports that have discussed the effects of oak on wine composition sensory attributes. Compared with fermentation in stainless steel and oak wood on an industrial scale, Ibern-Gomez *et al.* found the presence of oak derived phenolic compounds and some other volatile compounds only in the wine fermented in the oak barrel (Ibern-Gomez *et al.*, 2001). Perez-Prieto *et al.* observed the dynamics of oak-related volatile components during aging and bottle storage. They also validated that the largest barrel (1000L) had the lowest level of volatile compound accumulation. They found a large difference between used and new barrels, but they did not find a large difference between French oak and American oak (Perez-Prieto *et al.*, 2003). Revilla and Gonzalez-SanJose (2001) obtained similar results. They considered the new anthocyanin pigments and even vitamin B and other anthocyanin derivatives, and concluded that French Nevers casks give the wine the best chromatic quality among 15 different oak woods (Revilla and Gonzalez-SanJose, 2001).

The complex compounds in wine may interact with those extracted components from oak. It is even possible that some substances are absorbed by the wood. Ramirez *et al.* (2001) investigated the sorption of aroma compounds by oak in a model wine. They put eight aroma compounds, such as terpene alcohols, and ethyl esters, into a model wine

with 12.6% ethanol. They found that most of those added aroma compounds could be absorbed by the oak. They set up a coefficient (K) between the solution and the sorbent:

$$K = C_{wood} / C_{solution}$$

Here, C is the concentration in unit mg/kg at equilibrium. They observed that linalool could reach K = 111.21 after the treatment. The ratio of surface area, again, was the most significant factor of the efficiency of sorption (Ramirez *et al.*, 2001). Another research focused on the interactions between 12 components from oak and pre-existing compounds in red wine. Researchers observed some changes in the aging with oak, and suggested that volatile and phenolic compounds interacted during the maturation (Escalona *et al.*, 2002).

Although oak maturation offers some benefits to certain wines, it has limitations. There are high costs associated with the barrel fermenting and aging. An alternative method is to put oak chips (or powder) or oak extracts into wine. The large surface area may help decrease the time for the interaction to occur between the wood chips and the wine. Obviously, there is concern regarding the chemical and sensory differences between traditional barrel aging and oak chip treatment. Afonso (2002) set up a panel of 15 panelists to evaluate the sensory attributes of barrel and oak chip (4-8g/L) fermented wines using both aroma and flavour-by-mouth. He found an obvious difference between these two treatments. Oak chips trended to give the wine more of a wood aroma: coconut and vanilla, and a greater impact in taste (bitterness and astringency), but different sources of oak chips did not show such differences (Afonso 2002). In another research, both French and American oak chips (4 and 7 g/L) were put into the juice, and the juice

was then fermented. After chemical analysis and sensory evaluation, Perez-Coello *et al.* found that wine with oak chips had increased yields of alcohols, acetates and some esters. They believed this increase was due to the chips offering a microenvironment for immobilized yeast cells. Wood components, such as furfural and vanillin were extracted and found in the wine although it was observed that yeast could metabolize them. Fresh and fruity characteristics were reduced by oak chips, leaving mainly oak characteristics. The fact that higher levels of esters and acetates were found in oak treated wines hinted that the lower fruity characteristics in the control wines could be masked by oak aromas. Panelists preferred low doses of oak and rejected extremely high doses (15g/L). Differences between French and American oak chips were also found (Perez-Coello *et al.*, 2000).

Importantly, Aiken and Nobel (1984) compared the sensory attributes of aromas from oak- and glass-aged Cabernet Sauvignon, and found both American and French oak could significantly decrease the intensity of "green bean" attribute, as well as increase the "vanilla" and "oak blend" attributes. This result hinted that oak attributes could mask the vegetative characters in the wine, or absorb the substances that cause the high vegetative characters (Aiken & Nobel, 1984). However, recent research of Hartmann *et al.* (2002) did not show evidence that oak treatment has a strong affinity for alky-methoxypyrazines, (Hartmann *et al.*, 2002). This result suggested that masking by oak-derived aromas plays a more important role in high methoxypyrazine wine. In our research, oak chips were used as a treatment for methoxypyrazine tainted wine, and both the sensorial and chemical effect on *H. axyridis* tainted wines were assessed.

1.4.5. UV and light: novel treatments

There are many reports about the photolysis of light and ultraviolet (UV). Targeted items can be polayacrylonitrile, polycarbonate, phenolic acids (Ramani & Ranganathaiah, 2000, Aggour & Aziz, 2000, Benitez *et al.*, 1997). Photolysis may not only break the molecular bonds, but may cause reactions between other compounds. For example, after phenols and di-*t*-butylketone were exposed for 5 h, with a 125 W medium pressure mercury lamp, some phenols were totally converted, and some complex reactions occurred. 66% 4-Methoxyphenol were substituted by *t*-butyl radicals and yielded 4-*t*-butylphenol, and finally produced *t*-butyl-4-*t*-butylphenyl ether (Galindo *et al.*, 1998).

UV is broadly used in wine and food science for the detection of compounds by various instruments. Some research has been conducted on photodegradation. One of most recent studies involved researchers trying to establish an online UV detector monitor system for monitoring sugar changes during fermentation. The mechanism was based on the detection of byproducts produced by the photodegradation of sugars (Roig & Thomas, 2003). In the food area, similar applications based on UV and light photolysis have also been investigated. Volmer (1998) investigated the photochemical behavior of pesticides in food samples. In order to help detect potential toxic substances in food samples, Solis *et al.* (2002) used non-thermal pulsed UV photolysis to help digest food samples. In wine, a similar method was used for digesting the wine sample in an oxidative UV photolysis system (Buldini *et al.*, 1999). However, in this research the temperature reached 85 ^{0}C

and pH changed to near 9 due to the decomposition of acids under a 500 W high-pressure mercury lamp. Obviously, it is impossible to treat wine under these conditions.

Applying UV/light to wine may have limited practical applications in wine making. Firstly, the penetration of UV is limited. It is estimated that 50% of radiation energy will be lost at a depth of 0.04 cm in white wine, and 0.004 cm in red wine (Rankine, 1998, p237). For this reason, UV is not an effective agent for sterilization of wine. Secondly, UV/light may cause the serious problem of oxidation by producing ozone in the irradiated air (Rankine, 1998, p237). Finally, UV and light may cause unpredictable changes on wine flavour compounds. Benitez *et al.* (2003) observed the changes in Fino sherry wines when exposed to UV and visible radiation. Significant changes in volatile and polyphenolic content were observed even before a higher visual browning happened in these wines.

With regards to the risks, moderate UV or light exposures on wine have been investigated for some applications. Stavropoulos *et al.* (2001) exposed bottled wines to diffuse daylight (light above 370 nm could pass through these bottles). The proposal was to observe the effect of light on two pesticides, pyrazophos and methidathion in wine. Compared with the wine stored in the dark, they did not find the light could significantly decompose these pesticides.

Some studies have investigated grapes treated with UV light. Resveratrol, one of the phenolic compounds, is a beneficial component in wine, possessing some cardio-

protective activity. Researchers are interested in how to obtain a high level in wine. There are many factors affecting the level of resveratrol in wine, one being light exposure. Threlfall *et al.* (1999) investigated whether UV light could affect the resveratrol level in cut grapes. They used a 254 nm UV light, irradiated clusters of grapes for five minutes and stored them at 21^0C for 20 hours before making the wine. After making the wines, they compared resveratrol levels in UV treated and non-treated wine. They obtained some significant results showing that UV exposure increased the resveratrol level in some wines, but this was not observed in all wines. Even the same variety of grape from a different year gave different results (Threlfall *et al.*, 1999). More recently, similar methods were used in some studies on the level of stilbene in red wines (Cantos *et al.* 2003).

We are concerned about whether methoxypyrazines will be affected by UV or light exposure. One study conducted by Hashizume and Samuta (1999) showed that light exposure could decrease the IPMP and IBMP levels in grape. They put unripe Cabernet Sauvignon grapes under fluorescent light and kept them there for several days. The rate at which the level of IPMP and IBMP decreased was higher in the light treated fruit than in fruit kept under dark conditions. The researchers suggested there was a balance between the synthesis and photodegradation of methoxypyrazines in grapes (Hashizume & Samuta, 1999).

In our research, we have hypothesized that UV/light may affect the methoxypyrazines in wines.

1.5. Sensory evaluation

Although modern chemical analytical instruments, such as GCO (gas chromatography olfactory), have been widely used for detecting and analyzing wine aroma and flavour components (Rapp, 1998), tasting is still the only universal way to evaluate wine sensory properties. Today, sensory evaluation plays an important role in food (wine) science and industry. Sensory perception is not just a personal feeling about the magical wine world. As defined by Nobel, "Sensory evaluation is a scientific discipline used to quantify, analyse and interpret reactions to the sensory properties of wine or any product." (Nobel, 1999, p98) It is important to select a suitable evaluation protocol and have suitable panelists and a statistical analysis method.

There are two types of sensory tests: analytical sensory test and affective test. The later one is related to consumer preference. Analytical tests can be divided into two groups: discrimination methods, which is used for judging whether difference between samples; and descriptive methods, which is used not only judge the difference, also what kind of human sensations could vary. Second, the descriptions of the attributes must be well understood in the same way by all panelists. Panelists must also know how to use the scales to match the felling of sensations. In practice, the testing protocol must be carefully prepared, such as the tasting environment, the randomization of samples, the serving sequences and codes. Then, the data must be accurately and reasonably explained, and conclusions reached (Lawless & Heymann, 1998).

Most descriptive analysis methods need panelists who have sufficient knowledge of the items, usually food or wine. They will be involved in intensive discussion and judgment of wines. Sensations need to be recorded precisely and accurately. Proficiency testing is often planned for measuring panel performance, especially for descriptive profile panels. McEwan *et al* (2002) pointed out that although training will help panelists become familiar with the samples, an expert panel is more reliable than a panel composed of novices. Research has shown that experts could perform more successfully in a triangle test that novices. To verify the hypothesis that experts could take advantage of their experience and extend it to an incidental task, and novices tend to offer ineffective descriptions due to their perceptual inexperience, Hughson & Boakes (2002) investigated wine experts and novices when describing wine. When the panelists were given varietal descriptions, experts had higher performance levels than novices. But, if given a shuffled description list, the situation was reversed. Another finding was if novices were given a short list of descriptions, their descriptive results were higher than chance, but if the list was long, although performance was higher than control, the Chi-square test did not show difference between samples (Lawless & Heymann, 1998, p13). In our study, we need to determine how *Harmonia axyridis* affect the sensory characteristics of wine; thus, descriptive sensory analysis will be employed.

1.5.1. Descriptive analysis and sensory profile

Murray *et al.* (2001) had a detailed review on descriptive sensory analysis. They believe that descriptive analysis is still the most useful sensory method available because of its

complexity, flexibility, and detailed information about all sensory properties. (Murray *et al.*, 2001)

Table 1. Characteristics of some main descriptive sensory methods

	Flavour Profile	**Texture Profile Method**	**Quantitative Descriptive Analysis**	**Sensory Spectrum**	**Free-choice Profiling**
Goal	Consensus; vocabulary development; rating	Description of texture; accounts for the temporal aspect of attributes	Describe the sensation; quantitative data of attributes	High accurate data of sensory attributes	Know the demands of marketing and consumer's perception
Size of panel	4~6	About 10	About 10	About 10	Large
Panel training	Very high	Medium to high	Medium to high based on understanding of persons	Very high	Avoid
Scale	Number or symbol	Number or category from standard scale	Line scale, panelist may use own way	Number of scale, all panelists use same way	Descriptions
Reference	Yes	Yes	Yes	Very important	No
Language	From panel	Standard terminology	From panel	From a standard lexicon	Panelist's own descriptors
Role of the leader	Active	Trainer	Not active	Trainer	Have to guess the meaning of words from panelists

There are different protocols for descriptive analysis: Flavour Profile (FP, or Profile Attribute Analysis), Texture Profile Method, Quantitative Descriptive Analysis, Sensory Spectrum, Generic Descriptive Analysis, Free-choice Profiling, Quantitative Flavour

Profiling, etc. These protocols are based on similar methods, although they have different philosophies and concerns. When a facilitator establishes a descriptive protocol, he/she may also use a combination of approaches for specific objectives (Murray *et al.*, 2001, Lawless & Heymann, 1998). Table1 list the main characters of the different descriptive methods.

All these methods have several similar steps, except Free-choice Profiling. Firstly, they need a small group of panelists. After the recruitment and screening process, the panelists need to be trained to a precise understanding of the objects, wines or foods. Secondly, the panel will produce suitable descriptive attributes. At this stage, references are normally presented to panelists, for calibrating the individual's sensory "frame" of references. Finally, quantitative data is obtained from the panelists. In these three steps, there are four points we must carefully consider. Here, a reliable panel is the foundation. All future descriptions and quantitative data are produced from the panelists; however, we know a significant difference between the control and the long-list test. An explanation is that the short-list provided some knowledge about the wine and allowed novices to match the task better (Hughson & Boakes, 2002). However, the panel leader would prefer panelists who have more knowledge about the target, and could use more precise language. Although a panel is selected from a professional environment, training will still play an important role in a descriptive sensory project (Brochet and Dubourdieu, 2001).

Statistical analysis is the key to interpreting sensory data. Analysis of variance (ANOVA) is usually used and both fixed and mixed models can be applied. A fixed model can be

used for assessing the performance of the panel. However, for repeating the protocol with another panel, panelists must be considered as random effects; thus a mixed model should be used for validating product differences (Carlucci & Monteleone, 2001). Multivariate analysis of variance (MANOVA) is provided for dependent variables simultaneously, and is a suitable method for descriptive analysis. Statistical methods used for reducing the complexity of data sets are Principle component analysis (PCA) and Procrustes analysis (Lawless & Heymann, 1998, p587-598).

After the statistical analysis, descriptive data can be displayed on radar plots, summarizing attributes and their intensities (Lawless & Heymann, 1998, p355). At this stage, a specific sensory profile is obtained.

1.5.2. Application of sensory evaluation and related topics

In our research, we need to establish the sensory profile of wine tainted by *Harmonia axyridis*. We also need to know the relationship between the sensations and certain compounds, specifically, methoxypyrazines. There has been a great deal of research based on the relationship between instrument analysis and sensory analysis. To understand the relationship between sensory analysis data and chemical analysis data, Nobel and Ebeler (2002) introduced multivariate statistics in analyzing wine flavour. Generalized procrustes analysis and Partial least square regressions were used not only for sensory data, but for data obtained from instruments (Nobel & Ebeler, 2002). Kotseridis *et al.* (2000) set up a panel with 17 enology students selected from 30 persons. The panel was trained and undertook a descriptive analysis of aroma from certain Merlot

and Carbernet Sauvignon wines. A two-way ANOVA and a LSD-test were used for statistical analysis. The panel also analyzed the chemical level of two odorants (HDMF and HEMF) and combined the chemical data with the sensory data of caramel. The results showed that chemical data and sensory data were well matched (Kotseridis *et al.*, 2000).

Sensory profiling is broadly used as a technique to obtain certain objective characterizations, and the quantitative data can also be used for product discrimination. Many studies used this technique for different proposals, such as differences among different Champagne wines, BC local wines, glucose oxidase treatments, and oak chip treatments (Afonso, 2002, Pickering *et al.*, 1999, Vannier *et al.*, 1999, Cliff & Dever, 1996).

1.6. Summary

The possibility that *Harmonia axyridis* may taint wine provides a new challenge to the wine industry and academia. Understanding this problem will be of considerable benefit to the wine industry, particularly in North America. The nature and extent of the problem needs to be quantified using sensory and chemical analysis. It is also necessary to explore potential methods for resolving this problem through appropriate treatment of affected juice/wine.

Chapter 2. Sensory Influence of *Harmonia axyridis* on White and Red Wine

2.1 Introduction

As reviewed in Chapter 1., *Harmonia axyridis* (Pallas) can produce high volatile repellents, mainly alkaloids. Alkyl-methoxypyrazines, such as isopropyl- and isobutyl-methoxypyrazine (IPMP and IBMP) which can be found in fresh vegetables such as green bell pepper, beans, asparagus, and even grapes, contribute a broad spectrum of sensory properties to human beings. *Green, earthy, herbaceous, vegetative, bell pepper*, etc. are usual descriptions of the aroma. (Sala *et al.*, 2002, Buchbauer *et al.*, 2000, Allen *et al.*, 1999 & 1994) The thresholds of these compounds are quite low: 1-2 ng/L in water; thus, even a very trace concentration presented in wine can cause a remarkable impact on wine (Sala *et al.*, 2002, Allen & Lacey, 1998). However, some studies showed that thresholds in wines were higher than in water. Especially in red wine, thresholds could be as high as 10-16 ng/L (Sala *et al.*, 2002, Boubee *et al.*, 2000).

It would, therefore, seem plausible that *H. axyridis* are capable of influencing wine quality via transfer of haemolymph onto grapes, or directly into juice when beetles become incorporated into the harvested fruit or associated material. A number of winemakers reported seeing significant numbers of beetles in grape musts after the harvest and processing of grapes in 2001. What influence, if any, the incorporation of *H. axyridis* beetles into grape juice might have on the quality of finished wines has not been

established, although much anecdotal comment and news media speculation has been noted. The objective of this study was to characterize the sensory properties of white and red wine fermented in the presence or absence of *H. axyridis*. Elucidation of the effects of *H. axyridis* on the chemical composition of wine will be reported in Chapter 4.

2.2 Materials and Methods

2.2.1 Wine preparation.

Two commercial juice concentrates from South American grapes were used: White Bourgeron™ and Red Bergamais™ (both Vinco International, St Catharines, Ontario). The concentrates were re-hydrated according to manufacturer's directions.

The basic composition of the re-hydrated juices were:

White Bourgeron™: °Brix, 21.9; pH, 3.14, and titratable acidity (TA), 5.9 g/L.

Red Bergamais™: °Brix, 22.7; pH, 3.32, and TA, 6.0 g/L.

Lady beetles were sourced from the local area and screened for identity. Identification of *Harmonia axyridis* was based on the morphological criteria detailed by Chapin and Brou (1991), and in particular on the presence of the characteristic dark M-shaped mark on the pronotum, extending to the anterior margin (Chapin & Brou, 1991; F.M. Oi & W. Foshee, http://www.aces.edu/department/ipm/ladybugs.htm).

Live *HA* beetles were then added to re-hydrated juice in 20 L glass carboys at rates of 0, 1, or 10 beetles per L of juice. Three 20 L replicates of each of the beetle treatments and

four 20 L replicates of the control juice (no beetle) were prepared and processed separately. Juices were then inoculated with a re-hydrated freeze-dried preparation of *Saccharomyces bayanus* (EC-1118®, Lallemand Inc.) at 5 to 6 x 10^6cells/mL, according to manufacturer's directions.

Fermentations were conducted at 18°C, and fermented to dryness and wines were moved to 4°C room. Wines were then racked (including removal of beetles), sulfited, and cold stabilized following standard microvinification protocol (Pickering et al. 1999). After four weeks, the individual replicates from each treatment were assessed by a small expert tasting panel (four faculty and senior students from Brock University's Cool Climate Oenology and Viticulture Institute) and considered not to differ sensorially. The replicate wines within each treatment were then pooled and bottled, without filtration. Wines were stored in a cellar at 14°C until required.

Basic composition of the bottled wines are shown in Appendix1, Table 15. (Methods were based on Zoecklein, *et al* 1995)

2.2.2 Sensory Methodology

2.2.2.1. Panel recruitment and training. The panel was recruited from Brock University staff and students. A questionnaire was used to screen prospective panelists for anosmias or other conditions which might limit their suitability. Further selection was based on their interest and availability. The final panel consisted of six females aged between 21 and 63 years. The gender distribution reflected availability of suitable personnel rather

than any target composition. All participants signed an Informed Consent Form, and the project was approved by the Brock University Research Ethics Board (file 01-290).

Nine training sessions of one-hour duration each were held over five weeks. A minimum of information on the nature of the study was provided in order to reduce potential bias.

During Sessions 1 and 2 the panel was presented with samples of wine from all of the six treatments (three white and three red). Samples were always presented blind, in coded ISO wine glasses, and were expectorated. The panel was instructed to generate appropriate descriptors for the appearance, aroma, and flavour of each wine. The panel leader facilitated the process of discussing terms as a group and looking for overlap and redundancy amongst descriptors. Terms that were used by only one person were removed from the developing lexicon. By panel consensus, appearance attributes (hue, density, and clarity) were removed from further consideration as wines within each style (white or red) did not appear to differ.

In subsequent training sessions, reference standards were developed and refined for each of the terms and evaluated for suitability by reference to specific wine samples from the study. 15cm line scales were developed for each descriptor, with the scale ends indented 1 cm to avoid endpoint effects (Lawless and Heymann, 1998). The left end of each scale was anchored with the phrase "absent" at the 1 cm indent mark, and the right end with "very high" at the corresponding 1 cm indent mark. The panel gained experiences by rating the intensities of both beetle-treated and control wines for each of the descriptors.

By panel consensus, the intensity of each of the reference standards was deemed to correspond to the "very high" anchor of respective line scales.

The final training session consisted of an orientation to the computer program and sensory laboratory that would be used for collecting data, and as a 'practice run' under experimental conditions. Tables 2 and 3 give the final lexicon of descriptive terms along with reference standard composition for the white and red wines, respectively.

Table 2 – White wine aroma and flavour descriptors with corresponding reference standards

Descriptor	***Reference composition[a]***
Melon	2 tsp fresh honeydew melon juice
Citrus	1 tsp fresh grapefruit juice + ½ tsp fresh lime juice
Floral	5 drops of mixture of: 10 mL 'Green/herbaceous' [#8947] + 10 mL 'Geranium leaf' [#9077] (both Wine Awakenings Inc®) + 10 mL linalool (Sigma Aldrich) in 20 mL distilled water
Asparagus	1 tsp canned asparagus juice (Equality™)
Bell pepper	10 mm square of fresh bell pepper heated with naked flame for 20 sec soaked in base wine for 20 min
Peanut	8 whole raw white peanuts crushed and soaked in 60 ml base wine for 30 min
Humus	50 g dried plant material (primarily bark) sourced from 2 cm below soil surface. Presented in plastic container without base wine
SO_2	700 mg/L aqueous solution of potassium metabisulfite (Fisher Scientific) without base wine
Sweet	12.5 g/L sucrose in aqueous solution
Acid	1.5g/L tartaric acid in aqueous solution
Bitter	12 mg/L quinine sulfate in aqueous solution

[a]All standards made up 1-2 hours before tasting in 60 ml of un-oaked neutral Chardonnay base wine (Cool Climate Oenology and Viticulture Institute Pilot Winery, Brock University) unless otherwise indicated. All standards presented as 30 ml samples in ISO wine glasses unless otherwise indicated. Standards represent the "very high" anchor term at the far right end of the respective line scales (15 cm)

Table 3 – Red wine aroma and flavour descriptors with corresponding reference standards

Descriptor	*Reference Composition*[a]
Red berry	2-3 fresh whole blackberries heated in microwave oven for 20 sec + 1/3 tsp strawberry jam
Cherry	10 ml cherry cocktail (DelMonte Quality™) + 1/2 tsp canned cherry juice (E.D. Smith™)
Plum	2 tsp plum jam (S&F™)
Asparagus/bell pepper	½ tsp of canned asparagus juice (Equality™) + one 5 x 10 mm strip of fresh bell pepper heated with naked flame for 20 sec
Cheesy	1g ripe Château Versailles™ brie cheese
Peanut	8 whole raw white peanuts crushed and soaked in 60 ml base wine for 20 min
Earthy/ Herbaceous	50 g dried plant material (primarily bark) sourced from 2 cm below soil surface. Presented in plastic container without base wine
SO_2	700 mg/L aqueous solution of potassium metabisulfite (Fisher Scientific) without base wine
Sweet	12.5 g/L sucrose in aqueous solution
Acid	1.5g/L tartaric acid in aqueous solution
Bitter	12 mg/L quinine sulfate in aqueous solution

[a]All standards made up 1-2 hours before tasting in 60 ml of un-oaked neutral Chardonnay base wine (Cool Climate Oenology and Viticulture Institute Pilot Winery, Brock University) unless otherwise indicated. All standards presented as 30 ml samples in ISO wine glasses unless otherwise indicated. Standards represent the 'very high' anchor term at the far right end of the respective line scales (15cm).

2.2.2.2. Data collection and analysis. Formal assessment of the wines took place over three sessions. The evaluations were conducted in individual white booths with red lighting (130 volt, 100 W Haskellite® red bulb covered with red cellophane) in the ventilated sensory lab at the Cool Climate Oenology and Viticulture Institute, Brock University. The following six samples were evaluated in triplicate for the aroma and

flavour intensity of predefined attributes (Tables 2 and 3) using a randomized complete block design, with order of presentation of samples randomized within each flight:

(i) White Bourgeron™ wine fermented without *Harmonia axyridis* (*HA*)

(ii) White Bourgeron™ wine fermented with 1 *HA* beetle per L

(iii) White Bourgeron™ wine fermented with 10 *HA* beetles per L

(iv) Red Bergamais™ wine fermented without *HA*

(v) Red Bergamais™ wine fermented with 1 *HA* beetle per L

(vi) Red Bergamais™ wine fermented with 10 *HA* beetles per L

In order to investigate the hypothesis that methoxypyrazines were associated with the taint characteristics, we added 15 ppt IPMP into control wines, and these were evaluated with the other six wines.

The wines were assessed ten weeks after bottling, and white and red wines were evaluated in separate sessions. In addition, panelists were asked to list any additional descriptive terms they felt applicable. All data was collected using Compusense™ software (C5V4, Guelph, Ontario, Canada). Before each flight, panelists were instructed to re-familiarize themselves with each reference standard. The standards were also available during data collection for reference if required. All wines were presented as 30-mL samples in covered ISO tasting glasses coded with 3-digit random numbers at ambient temperature (21°C +/- 1°C).

The aroma and flavour of each sample was assessed separately in order to reduce halo effects (Lawless and Heymann, 1998). Two flights of three samples were evaluated for aroma first, with a minimum 30-sec break between samples and a 5-min break between flights. Following a 15-min break, the same two flights were re-presented to the panel (with changed 3-digit codes), and assessed for flavour under the same assessment protocol.

Data for white and red wines were examined separately. Wine sensory attribute by treatment scores were assessed using ANOVA, with judge and session fitted as random effects, and all 2-way interactions included in the model. If the p of the treatment F-value was <0.05, the Bonferroni test was applied to separate means using the GLM procedure of SPSS (Version 11.0, SPSS Inc., Chicago, Illinois).

2.3. Results and Discussion

2.3.1. Treatment differences.

Table 4 gives the mean intensity scores and the results from Bonferroni means separation tests for the eight aromas and 11 flavour terms used to profile the white wines. Figure 3 displays the mean intensity scores of white wine in a cobweb diagram format. At 1 beetle/L, bell pepper aroma and peanut flavour showed an increase in intensity with respect to the control wine (0 beetles/L), with no significant differences noted for the other attributes. At a rate of 10 beetles/L, however, a number of attributes were impacted compared with the control. As well as the increase in bell pepper aroma and peanut

flavour noted above, peanut and asparagus aromas were increased, as were bell pepper and asparagus flavours.

Table 4. Mean sensory attribute scores for white wine as a function of level of *Harmonia axyridis* beetle addition to juice [a,b]

	Treatment (beetles/L)					
Attribute	**0**		**1**		**10**	
AROMA						
Melon	3.51	a	1.66	a	1.79	a
Citrus	3.14	a	1.88	a	1.96	a
Floral	2.22	a	2.04	a	1.15	a
Peanut	1.55	a	2.96	ab	4.59	b
Bell pepper	1.54	a	3.98	b	6.43	c
Asparagus	3.17	a	3.87	a	6.79	b
Humus	2.53	a	4.33	a	4.39	a
SO_2	2.66	a	2.93	a	2.84	a
FLAVOUR						
Melon	2.31	a	1.91	a	2.31	a
Citrus	3.11	a	3.48	a	3.12	a
Floral	1.28	a	2.02	a	1.14	a
Peanut	1.63	a	2.85	b	4.34	b
Bell pepper	1.51	a	1.97	a	3.77	b
Asparagus	1.17	a	2.35	a	3.89	b
Humus	2.40	a	3.26	a	3.23	a
SO_2	1.94	a	2.36	a	1.47	a
Sweet	3.83	a	3.60	a	3.57	a
Acid	6.02	a	7.31	a	5.74	a
Bitter	4.70	a	4.10	a	4.85	a

[a] Values shown are the mean intensity scores of six judges and triplicate assessments

[b] Treatment means identified with different letters are significantly different at the 5% level (p of F-value from ANOVA <0.05, followed by Bonferroni means separation test)

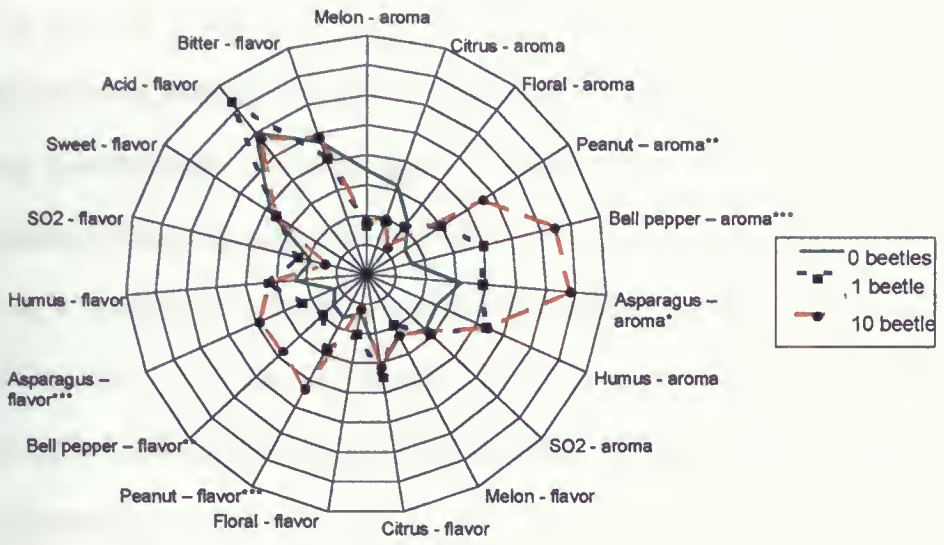

Figure 3 - Cobweb diagram showing mean sensory attribute scores for white wine for three levels of addition of *Harmonia axyridis* beetles to juice. (*=Significance level = <0.05, **= Significance level =<0.01, and ***= Significance level =<0.001, for Bonferroni test following significant F-value from ANOVA, α=0.05)

Bell pepper and asparagus aroma and flavour intensities were also significantly higher in the 10 beetle/L treatments compared with the 1 beetle/L wines. The 'beetle-induced' attributes (peanut, bell pepper and asparagus) appear to be more intense when assessed ortho-nasally than retro-nasally, suggesting that smelling may be more reliable than tasting when assessing a white wine for potential *H. axyridis* influence. While not statistically significant, a trend of decreasing fruit and floral aroma intensities is suggested when beetle treatments as a whole are compared with the control wines. This would be consistent with a masking effect from the relatively strong aromatic components apparently introduced in the beetle-treated wines.

Table 5 gives the mean intensity scores and the results from Bonferroni means separation tests for the eight aroma and 11 flavour terms used to profile the red wines. Figure 4 displays the mean intensity scores in a cobweb diagram format. At 1 beetle/L, only bitter intensity was affected (increased) when compared to the control wine (0 beetles/L), with no significant differences noted for any of the other attributes. At a rate of 10 beetles/L, however, a number of attributes were impacted compared with the control. Plum and cherry aroma and sweet flavour intensities were decreased, while peanut, asparagus/bell pepper and earthy/herbaceous aromas and flavours, along with acid and bitter intensities, were all increased.

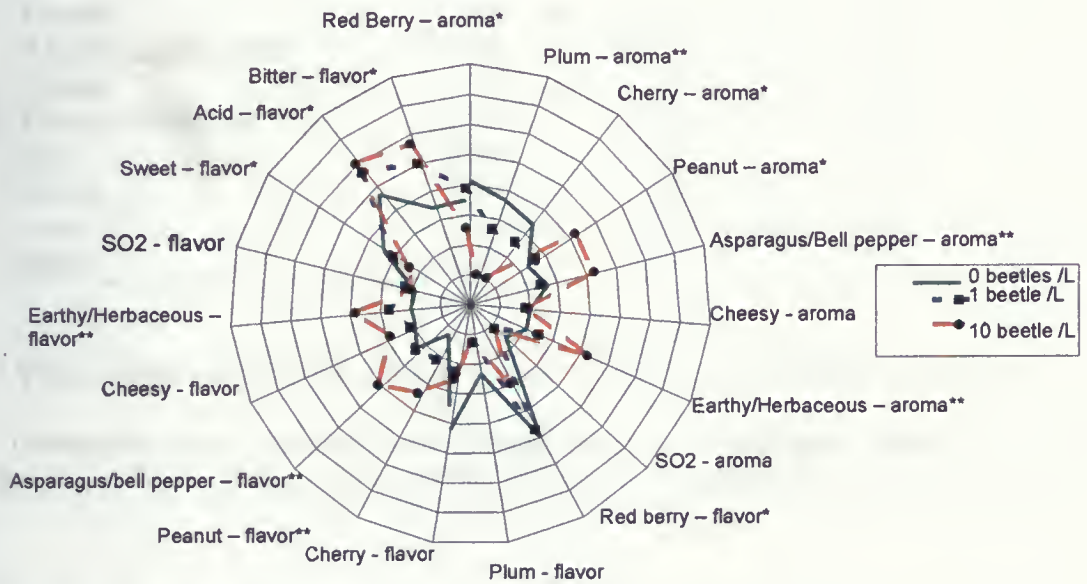

Figure 4 - Cobweb diagram showing mean sensory attribute scores for red wine for three levels of addition of *Harmonia axyridis* beetles to juice. (*=Significance level = <0.05, **= Significance level =<0.01, and ***= Significance level =<0.001, for Bonferroni test following significant F-value from ANOVA, α=0.05)

Table 5. Mean sensory attribute scores for red wine as a function of level of *Harmonia axyridis* beetle addition to juice [a,b]

	Treatment (beetles/L)					
Attribute	**0**		**1**		**10**	
AROMA						
Red Berry	4.12	ab	4.59	a	3.01	b
Plum	3.67	a	3.14	a	1.22	b
Cherry	3.39	a	3.11	a	1.27	b
Peanut	2.31	a	3.23	ab	5.10	b
Asparagus/Bell pepper	2.67	a	3.02	a	5.16	b
Cheesy	2.01	a	2.33	a	2.43	a
Earthy/Herbaceous	2.04	a	3.12	a	5.21	b
SO_2	1.61	a	1.49	a	1.51	a
FLAVOUR						
Red berry	4.98	ab	5.74	a	3.66	b
Plum	2.33	a	1.60	a	1.57	a
Cherry	4.19	a	2.77	a	3.08	a
Peanut	1.42	a	2.56	a	4.07	b
Asparagus/bell pepper	2.61	a	2.73	a	4.76	b
Cheesy	2.19	a	2.41	a	3.28	a
Earthy/Herbaceous	2.16	a	3.06	a	4.38	b
SO_2	2.04	a	2.47	a	2.22	a
Sweet	3.83	b	3.39	ab	2.67	a
Acid	5.52	a	6.64	ab	7.08	b
Bitter	4.03	a	5.89	b	6.74	b

[a] Values shown are the mean intensity scores of six judges and triplicate assessments

[b] Treatment means identified with different letters are significantly different at the 5% level (p of F-value from ANOVA <0.05, followed by Bonferroni means separation test)

Red berry, plum and cherry aroma and red berry flavour intensities were significantly lower in the 10 beetle/L treatments compared with the 1 beetle/L wine and control wine, while asparagus/bell pepper and earthy/herbaceous aroma scores were higher. The result of no significant difference in red berry aroma and flavour at 10 beetle/L compared with

control wines may be a consequence of the relatively low statistical power associated with a small panel. Interestingly, all three taste attributes were affected by the presence of beetles in the red wines assessed, while none were influenced in the white wines. The chemical origin of the increased bitterness observed in red wine fermented with beetles is less clear. To the author's knowledge, bitterness is not an attribute that has been ascribed to methoxypyrazines. Alkaloid compound(s) are a possibility, as they are typically bitter and over 50 have been reported in Coccinellidae haemolymph (King & Meinwald, 1996).

The wines with 15ppt IPMP added showed similar profiles to the 10 beetle/L wines. Figures 5 and 6 display their mean intensity scores in a cobweb diagram format. There is no significant difference between these wines and 10 beetle/L wines for any sensory attribute. This result partly verifies the hypothesis that IPMP is a key component contributing to the characteristic taint in *H. axyridis* affected wines.

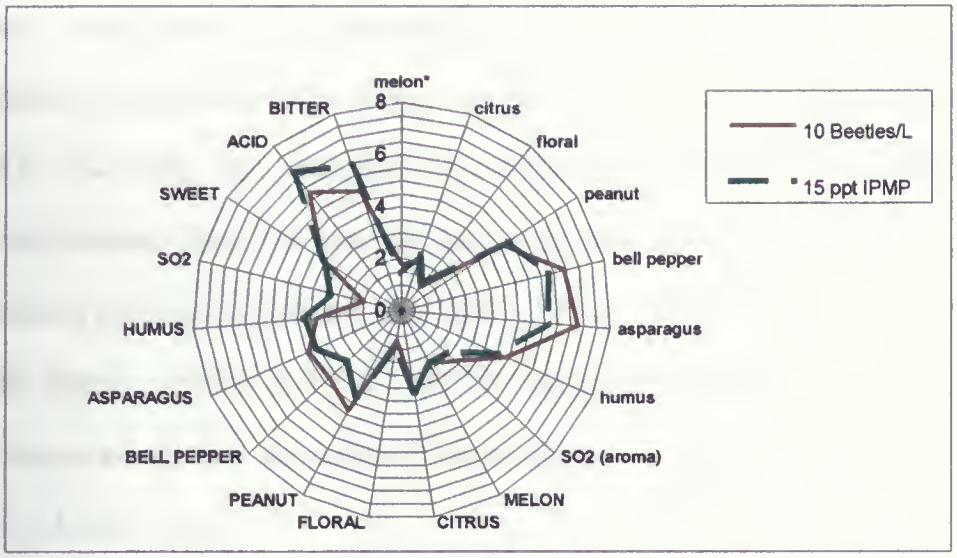

Figure 5. Cobweb diagram showing mean sensory attribute scores of white wines for 10 beetle/L level (in juice) and control wine with addition of 15ppt Isopropylmethoxypyrazine

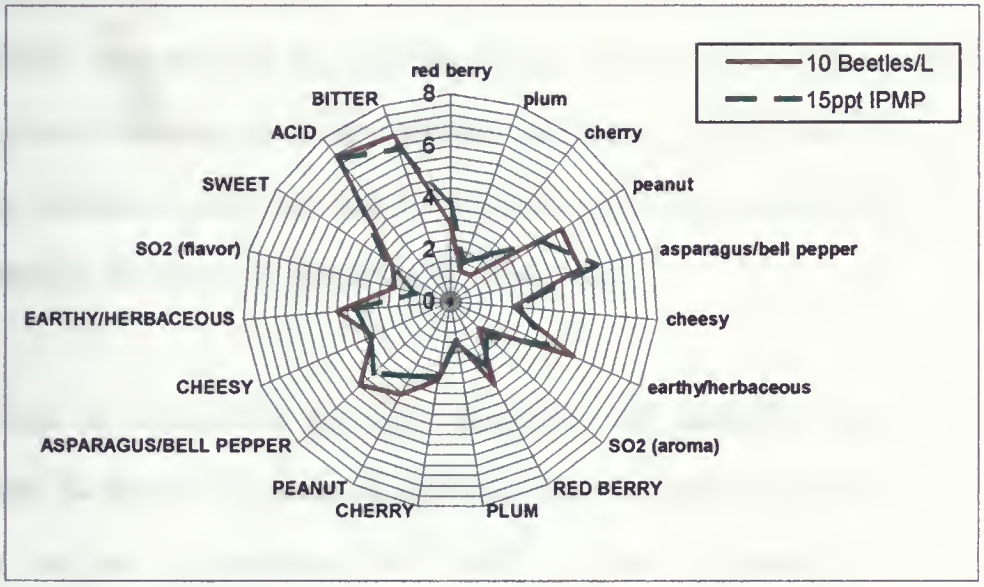

Figure 6. Cobweb diagram showing mean sensory attribute scores of red wines for 10 beetle/L level (in juice) and control wine with addition of 15ppt Isopropylmethoxypyrazine.

Further considerations. As previously noted, 2-isopropyl-3-methoxypyrazine has been identified in the effluent of the related species *Coccinella septempunctata* (Abassi et al. 1998). The ortho- and retro-nasal aroma descriptors shown here to characterize *H. axyridis*-affected wines are generally consistent with the known sensory properties of substituted pyrazines (Buchbauer et al. 2000) and how methoxyprazines are perceived in a wine medium (Allen et al. 1994, 1998). We therefore speculate that *H. axyridis*-derived methoxypyrazine(s) are the dominant aroma-active component(s) underlying the unique profile of these wines.

While the panel size (n=6) was at the lower end of the typical range used for flavour profiling (Lawless and Heymann, 1998), a number of significant main effects have been observed. All members of the panel were female; it would be of interest to determine if any gender differences exist with respect to the relative flavour profiles derived for these wines, particularly given our result for bitterness and the increased sensitivity of females to bitterants reported in the literature (Bartoshuk, 2000).

It should be stressed that the altered sensory profiles shown here do not predict or indicate the direction or extent of consumer preference and acceptability of commercial wine that may be influenced by *H. axyridis.* Indeed, determination of consumer perception and purchase behavior toward these wines would be a logical extension of this study.

2.4. Conclusion

Significant modification of wine aroma and flavour characteristics was observed in both white and red musts fermented in the presence of 10 *H. axyridis* beetles/L. Smaller effects were observed at a dosage rate of 1 beetle/L. A number of sensory attributes were enhanced in the beetle treatments; *peanut, bell pepper* and *asparagus* aromas and flavours in white wine, and *peanut*, *asparagus/bell pepper*, and *earthy/herbaceous* aromas and flavours in red wine. In addition, sweet, acid and bitter tastes were affected in red wine, and a general trend of decreasing fruit and floral intensities was observed with increasing beetle rate in both wines.

Taken overall, these results do indicate the potential for *H. axyridis* to influence wine quality. The effects were dose-dependent, and the 'external validity' of the beetle addition rates employed here; thus, the concentration of *H. axyridis* or effluent that might be incorporated into grape juice during commercial winemaking requires further investigation. The relative impact of the beetles is also likely to be variety and wine-style dependent. For instance, in these trials the typicality of the red wine appeared to be more adversely affected than that of the white wine.

Research has been undertaken to fully characterize the influence of *H. axyridis* on the chemical composition of wine (in Chapter 4). Associated with this, we will further test the hypothesis that methoxypyrazines are the principal odor-active compounds in *H. axyridis*-affected wine. Sensory work in this part has partly verified this hypothesis. The

effect of bottle aging on the sensory properties shown here will also be examined in Chapter 3. It may now also be appropriate to investigate the effectiveness of various remedial juice and wine treatments aimed at removing or reducing undesirable aroma and flavour contributions from *H. axyridis*. The sensory profiles developed here should serve as a useful base line for this work.

Chapter 3. Chemical composition and aging

3.1. Introduction

Previous research (Chapter 2) has shown significant sensory influences from *Harmonia axyridis* (Pallas). It is also important to further test the hypothesis that methoxypyrazines are the principal odor-active compounds in *H. axyridis*-affected wine. Methoxypyrazines, mainly isopropyl- and isobutyl-methoxypyrazine, are flavour compounds found in many foods. They are present in some grape varieties, such as Sauvignon blanc and Cabernet Sauvignon (Boubee *et al.*, 2000, Buchbauer *et al.*, 2000, Allen & Lacey, 1998). Although they offer acceptable and desirable flavours (e.g. vegetative aroma) in these certain wines within a particular range (e.g. from 5-15 ng/L), a high concentration of methoxypyrazines could be unwelcome (Allen & Lacey, 1998). GC-MS is usually used for measuring methoxypyrazine concentration, and some reports showed sensitivity levels as low as 0.1 ng/L using the most sensitive method of stable isotope dilution (Sala *et al.*, 2002, Boubee *et al.*, 2000, Allen *et al.*, 1994).

It is also important to evaluate how *Harmonia axyridis* tainted wines age, as wine is often consumed some months or years after bottling. Since a sensory profile has been obtained, it offers a sensory tool to investigate these wines again after 10-month aging.

3.2. Materials and Methods

3.2.1. Wine and basic chemical analysis

White (Bourgeron™) and Red (Bergamais™) wines were made in 2002 from concentrated juice (both Vinco International, St Catharines, Ontario), as described in Chapter 2. Bottled wines were stored in a cellar at 14°C until required. Some bulk wines from these trials were kept in carboys with tight rubber bungs in a 4°C room.

Methods of basic chemical analysis were from Zoecklein *et al.* (1995) as follows:

Titratable acidity (TA) is a measure of the organic acid content in a wine sample. 10 ml samples of wine were degassed by vacuum and titrated with standardized sodium hydroxide (0.1N). The end point was determined at pH 8.2 using an Accumet® AB15 (Fisher Scientfic) pH meter.

The major component of volatile acid (VA) in wine is acetic acid. It is an important indicator of wine quality, and was determined as follows. Steam distillation was conducted using a cash volatile acid still assembly, and 100ml distilled wine sample was gathered and titrated with standardized sodium hydroxide (0.1N) with 1% phenolphthalein indicator added. Immediately after, 1 ml 25% sulfuric acid and starch indicator were added, and the sample titrated with iodine (0.02N). The latter titration accounts for the contribution of SO_2 to the VA results.

SO_2 (sulfur dioxide) is normally added to wine as an antioxidant and inhibitor of microbial activity. The Ripper titrametric method (using iodine) is widely used for

measuring SO_2. Free SO_2 was titrated using a similar method as that introduced above for VA: 25ml samples were mixed with 5ml of 1% starch indicator and 5ml of 25% sulfuric acid, and titrated with standard iodine solution to a blue end point. To determine total sulfur dioxide, samples need be treated with 25 ml of 1*N* sodium hydroxide. After 10 min, bound SO_2 is released, and the sample is titrated with iodine. However, in red wine the pigments make the end point difficult to determine using this method. Therefore, in 2003, the aeration oxidation distillation method (Zoecklein *et al.*, 1995) was used to determine the SO_2 levels. Freshly prepared hydrogen peroxide (0.3%) is used to oxidize the sulfur dioxide into sulfuric acid, and then the sample is titrated with sodium hydroxide. (Full details are given on pg 497-501 in Zoecklein *et al.*, 1995).

The Lane-Eynon procedure was used for determining reducing sugars (R.S.) in wine, which is based on a known volume of alkaline copper sulfate at boiling temperature (pg 474-477, Zoecklein *et al.*, 1995).

Terpene measurement of aged wines was based on Dimitriadis and Williams (1984) as modified by Reynolds and Wardle (1989).

3.2.2. Measurement of methoxypyrazines

All measurements of methoxypyrazines were done in the LCBO Quality Assurance Laboratory in Toronto. Wine samples were concentrated in a C-18 SPE cartridge and eluted by ethyl acetate. The ethyl acetate extract was analyzed by GC-MS in an id DB5-MS column. Pyrazine was determined by selective ion monitoring of the target ion of m/z

152 and qualifying ions of m/z 137 and 124. Quantitative concentrations of both IPMP and IBMP were determined by comparison to a standard solution of 2-methoxy-3-isopropylpyrazine spiked into ethyl acetate. The limit of quantitation was 5 ppt.

3.2.3. Sensory Methodology

Panel recruitment and training. The panel was recruited from Brock University staff and students. A questionnaire was used to screen prospective panelists for anosmias or other conditions that might limit their suitability. Further selection was based on their interest and availability. The final panel consisted of seven females and one male aged between 21 and 53 years. All participants signed an Informed Consent Form, and the project was approved by the Brock University Research Ethics Board (file #01-290).

Seven training sessions of one-hour duration each were held over four weeks. A minimum of information on the nature of the study was provided in order to reduce potential bias. In all sessions, references were presented to panelists, using the same recipes from the previous profiling panel (Chapter 2). Samples were always presented blind, in coded ISO wine glasses, and were expectorated. In the first and second sessions, the panel was introduced to the white and red wines prepared as outlined in Chapter 2, and aged 10 months. The panel was also introduced to the tainted wines (blend) subjected to the different remedial treatments (in Chapter 4). The panel leader facilitated the process of discussing the terms as a group and looking for overlap and redundancy among the descriptors. Panelists were encouraged to generate new appropriate descriptors for the aroma and flavour of each wine. New terms were added to the previous reference

list if most panelists agreed with them. The same 15cm line scales used in Chapter 2 were introduced to the panel, with the scale ends indented 1 cm to avoid endpoint effects (Lawless and Heymann, 1998). The left end of each scale was anchored with the phrase "absent" at the 1 cm indent mark, and the right end with "very high" at the corresponding 1 cm indent mark. The panel was trained to rate the intensities of each of the attributes in the different wines. It was decided by panel consensus that the intensity of each of the reference standards would correspond to the "very high" anchor of the respective line scales. The final training session consisted of an orientation to the computer program and sensory laboratory that would be used for collecting data, and as a practice procedure under experimental conditions. Tables 6 and 7 give the lexicon of descriptive terms along with reference standard composition for the white and red wines, respectively.

Data collection and analysis: Formal assessment of the wines took place over six sessions. The evaluations were conducted in individual white booths with red lighting (130 volt, 100 W Haskellite® red bulb covered with red cellophane) in the ventilated sensory lab at the Cool Climate Oenology and Viticulture Institute (CCOVI), Brock University. All wines were evaluated in duplicate for the aroma and flavour intensities of the predefined attributes using a randomized complete block design, with order of presentation of samples randomized within each flight. Table 8 lists the wines evaluated after 10 month-aging.

Table 6. White wine aroma and flavour descriptors with corresponding reference standards

Descriptor	*Reference composition*[a]
Melon	2 tsp fresh honeydew melon juice
Citrus	1 tsp fresh grapefruit juice + ½ tsp fresh lime juice
Floral	5 drops of mixture of: 10 mL 'Green/herbaceous' [#8947] + 10 mL 'Geranium leaf' [#9077] (both Wine Awakenings Inc®) + 10 mL linalool (Sigma Aldrich) in 20 mL distilled water
Asparagus	1 tsp canned asparagus juice (Equality™)
Bell pepper	10 mm square of fresh bell pepper heated with naked flame for 20 sec soaked in base wine for 20 min
Peanut	8 whole raw white peanuts crushed and soaked in 60 ml base wine for 30 min
Humus	50 g dried plant material (primarily bark) sourced from 2 cm below soil surface. Presented in plastic container without base wine
SO_2	700 mg/L aqueous solution of potassium metabisulfite (Fisher Scientific) without base wine
Diesel	120mg (148ul) Isoamyl & 300 mg (375ul) isobutyl alcohol in 300 ml base wine
Oak	0.3/L French oak chips (Winemaster™, Vin Bon-Brew, St. Catharines, Ontario) in base wine
Sweet	12.5 g/L sucrose in aqueous solution
Acid	1.5g/L tartaric acid in aqueous solution
Bitter	12 mg/L quinine sulfate in aqueous solution

[a]All standards made up 1-2 hours before tasting in control white wine, unless otherwise indicated. All standards presented as 30 ml samples in ISO wine glasses unless otherwise indicated. Standards represent the "very high" anchor term at the far right end of the respective line scales (15 cm)

Table 7. Red wine aroma and flavour descriptors with corresponding reference standards

Descriptor	*Reference Composition*[a]
Red berry	2-3 fresh whole blackberries heated in microwave oven for 20 sec + 1/3 tsp strawberry jam
Cherry	10 ml cherry cocktail (DelMonte Quality™) + 1/2 tsp canned cherry juice (E.D. Smith™)
Plum	2 tsp plum jam (S&F™)
Asparagus/ bell pepper	½ tsp of canned asparagus juice (Equality™) + one 5 x 10 mm strip of fresh bell pepper heated with naked flame for 20 sec
Cheesy	1g ripe Château Versailles™ brie cheese
Peanut	8 whole raw white peanuts crushed and soaked in 60 ml base wine for 20 min
Earthy/ Herbaceous	50 g dried plant material (primarily bark) sourced from 2 cm below soil surface. Presented in plastic container without base wine
Vanilla	one drop vanilla extract (wine standard of "Wine Awakening Inc.™") in 60ml base wine
Diacetyl	0.1g/L Diacetyl (Sigma®) in base wine
Oak	0.3/L French oak chips (Winemaster™, Vin Bon-Brew, St. Catharines, Ontario) in ml base wine per 60ml + 0.5 ul "Art tost(smok) flavour, #8038-9138, Wine Awakering Inc.™
SO_2	700 mg/L aqueous solution of potassium metabisulfite (Fisher Scientific) without base wine
Sweet	12.5 g/L sucrose in aqueous solution
Acid	1.5g/L tartaric acid in aqueous solution
Bitter	12 mg/L quinine sulfate in aqueous solution

[a] All standards made up 1-2 hours before tasting in control white base wine, unless otherwise indicated. All standards presented as 30 ml samples in ISO wine glasses unless otherwise indicated. Standards represent the 'very high' anchor term at the far right end of the respective line scales (15cm)

Table 8. Wine evaluated

White wine (Bourgeron™, 10 month)	**Red wine (Bergamais™, 10 month)**
Aged white wine fermented without *Harmonia axyridis* (*HA*)	Aged red wine fermented without *Harmonia axyridis* (*HA*)
Aged white wine fermented with 1 *HA* beetle per L	Aged red wine fermented with 1 *HA* beetle per L
Aged white wine fermented with 10 *HA* beetles per L	Aged red wine fermented with 10 *HA* beetles per L

White and red wines were evaluated in separate sessions. In addition, panelists were asked to list any additional descriptive terms they felt applicable. All data was collected using Compusense™ software (C5V4, Guelph, Ontario, Canada). Before each flight, panelists were instructed to re-familiarize themselves with each reference standard. The standards were also available during data collection for reference if required. All wines were presented as 30-mL samples in covered ISO tasting glasses coded with 3-digit random numbers at ambient temperature (21°C +/- 1°C).

The aroma and flavour of each sample were assessed separately in order to reduce halo effects (Lawless and Heymann, 1998). Two flights of three samples were evaluated for aroma first, with a minimum 30-sec break between samples and a 5-min break between flights. Following a 15-min break, the same two flights were represented to the panel (with changed 3-digit codes), and assessed for flavour under the same assessment protocol.

3.3. Results and Discussion

3.3.1. Chemical analysis

Table 9. Basic chemical compositions of wines after 10 months aging (Mean ± STD)

Wine	pH	Titratable Acidity (g/L, as tartaric acid)	Volatile Acidity (g/L, as Acetic acid)	Ethanol (v/v%, GC)	Free SO_2 (mg/L)	Total SO_2 (mg/L)
White wine, no beetle	3.30	6.70±0.00	0.472±0.009	12.9±0.1	8.3±1.2	127.3±4.1
White wine, 1 Beetle/L	3.28	6.68±0.02	0.444±0.010	13.0±0.1	13.3±2.4	141.4±1.8
White wine, blended*	3.29	6.46±0.02	0.402±0.001	N/A	17.4±1.2	149.7±0.6
White wine, 10 beetle/L	3.30	6.73±0.00	0.377±0.006	13.1±0.0	17.8±1.8	153.5±3.5
Red wine, no beetle	3.39	6.77±0.02	0.562±0.013	13.8±0.1	12.0±0.6	105.8±1.8
Red wine, 1 Beetle/L	3.39	6.83±0.01	0.596±0.004	13.8±0.1	13.7±4.1	111.6±0.6
Red wine, blended*	3.39	6.86±0.04	0.576±0.004	N/A	12.0±4.1	108.7±4.7
Red wine, 10 beetle/L	3.39	6.71±0.02	0.484±0.005	13.4±0.1	8.3±1.2	100.0±0.6

* Tainted wine (blend) was a blend of 1 beetle/L and 10 Beetle/L wines from carboys at a ratio of 5:4. See Chapter 4.

Table 9 shows the basic chemical composition. After 10 month aging, the chemical composition is similar to that at bottling (Chapter2). The suggested increase in ethanol was not statistically significant (t-test, $p< 0.05$). However, significant increases of VA were observed, and some under the significant level: 1 beetle/L in white wines ($p< 0.01$),

no beetle/L ($p<0.05$) and 10 beetle/L ($p<0.05$) in red wines. This may be due to experimental error, although the same analytical protocols were used.

Table 10. Free volatile terpenes (FVT) and Potentially volatile terpenes (PVT) in wines after 10 month-aging (Mean ± STD)

Wine	FVT (ppm, as linalool)	PVT (ppm, as linalool)
White wine, no beetle	1.27±0.31	1.05±0.23
White wine, 1 Beetle/L	0.92±0.18	1.23±0.27
White wine, 10 beetle/L	1.11±0.18	1.06±0.03
Red wine, no beetle	1.64±0.57	1.92±0.31
Red wine, 1 Beetle/L	1.39±0.19	1.46±0.22
Red wine, 10 beetle/L	1.27±0.27	1.94±0.42

Terpenes are considered to be important aromatic components in many wines and can be influenced by different factors, such as wine variety and yeast strain (Schlosser, 2003). *Harmonia axyridis* have also been reported to release mono- and secqui-terpenes (Aldrich and Riddick, 2002). However, the introduction of *H. axyridis* did not influence the level of terpene in these wines (Table 10).

3.3.2. Methoxypyrazine

Samples were sent to the lab at the LCBO, Ontario in July 2002 and September 2003. Table 11 gives the mean and standard deviation of the data. Isopropylmethoxypyrazine (IPMP) was detected and quantified. The concentration of Isobutylmethoxypyrazine

(IBMP) was around or below 5ppt, which is the limitation of the method, and these data are not reported here. The results showed a trend of increasing levels of methoxypyrazines with increasing numbers of beetles.

Table 11. Concentration of methoxypyrazine (ppt) in *Harmonia axyridis* related wines [a]

Compound		*White wine*			*Red wine*		
		Treatment (beetles/L)			**Treatment (beetles/L)**		
		0	**1**	**10**	**0**	**1**	**10**
isopropyl-methoxy-pyrazine (ng/L)	*Bottling*	8.0 a ± 2.6	12.3 a ± 1.2	37.7 b ± 12.7	7.5 a ± 1.7	9.7 a ± 2.1	30.0 b ± 1.0
	11 months aging	< 5	< 5	20.7 ± 0.1	< 5	< 5	20.9 ± 0.7
isobutyl-methoxy-pyrazine (ng/L)	*Bottling*	< 5	< 5	6.0 ± 1.4	< 5	6.5 ± 0.7	4.5 ± 0.7
	11 months aging	< 5	< 5	< 5	< 5	< 5	< 5

[a] Bottling data are mean values of single measurements of triplicate fermentations (4 fermentations for red 0 beetles/L treatment) ± standard deviations; 11 months aging data are mean values of duplicate measurements ± standard deviations; for IPMP concentration at bottling, treatment means of each of the two wine styles with different letters are significantly different (LSD after significant F-value from ANOVA, α=0.05).

3.3.3 Aging effect on sensory characteristics

Table 12 gives the mean intensity scores and the results from Bonferroni means separation tests for the eleven aroma and fourteen flavour terms used to profile the white wines with different levels of beetles in wine after ten months aging. "*Diesel*", "*oak*" and "*overall intensity*" were new attributes, according to the descriptions produced from the panel. Figure 7 displays the mean intensity scores of white wine in a cobweb diagram format. There was no significant difference between the control wine (0 beetles/L) and 1 beetle/L wine. This contrasts with the data from the newly bottled wine where "*peanut*" character could be identified by the panel between white control wine and 1 beetle/L

white wine for both aroma and flavor. Although non significant, there is a general trend of higher intensities of the beetle-derived characters, *bell pepper* aroma, *humus* and *peanut* flavor in the 1 beetle/L wine, and decreased fruity attributes.

At a rate of 10 beetles/L, however, a number of attributes were impacted compared with the control. Intensity of *melon*, *citrus*, *floral* and *diesel* aromas were decreased, and *peanut*, *bell pepper*, and *asparagus* aromas increased. *Peanut, bell pepper, and asparagus* flavours were increased in 10 beetles/L level wine, and *melon* flavour was decreased. The difference between the two treatments with respect to SO_2 and overall intensity was low. There was no significant difference between these wines with respect to the new attributes, except *diesel* and *oak* in aroma part, both of which are lower in the 10 beetle/L wine. Oak was not added to either of these wines during production. It is possible that panelists require more training on this attribute. Similar results are observed when compared to the sensory data from last year's the newly bottled wine (Chapter 2). As with the data from last year, the fruit attributes appeared to be more intense when assessed ortho-nasally than retro-nasally, suggesting that smelling may be more sensitive than tasting when assessing a white wine for potential *H. axyridis* influence.

Table 12. Mean sensory attribute scores for aged white wine as a function of level of *Harmonia axyridis* beetle addition to juice [a,b]

	Treatment (beetles/L)					
Attribute	**0**		**1**		**10**	
AROMA						
Melon	3.81	a	3.64	a	2.11	a
Citrus	4.03	b	2.94	ab	2.01	a
Floral	4.23	b	2.68	ab	1.84	a
Diesel	2.36	a	2.84	a	1.37	b
Peanut	1.52	a	2.59	a	7.03	b
Bell pepper	2.06	a	3.06	ab	5.33	b
Asparagus	2.15	a	4.34	ab	6.68	b
Oak	2.73	b	2.28	a	1.51	a
Humus	1.50	a	2.51	a	2.74	a
SO_2	3.86	a	4.24	a	3.94	a
Overall aroma intensity	6.68	a	6.49	a	7.32	a
FLAVOUR						
Melon	4.06	b	3.03	ab	1.46	a
Citrus	4.84	a	4.56	a	2.74	a
Floral	3.45	a	1.81	a	1.48	a
Diesel	1.51	a	1.74	a	1.44	a
Peanut	1.63	a	2.15	a	6.31	b
Bell pepper	1.74	a	1.93	ab	4.39	b
Asparagus	2.16	a	2.22	a	5.90	b
Oak	1.91	a	2.06	a	2.05	a
Humus	1.91	a	1.74	a	2.85	a
SO_2	2.44	b	2.43	b	2.46	b
Sweet	2.46	a	2.29	a	2.49	a
Acid	6.41	a	6.21	a	6.55	a
Bitter	4.21	a	4.64	a	6.38	a
Overall flavour intensity	6.36	a	6.14	a	7.13	a

[a] Values shown are the mean intensity scores of eight judges and duplicate assessments

[b] Treatment means identified with different letters are significantly different at the 5% level (p of F-value from ANOVA <0.05, followed by Bonferroni means separation test)

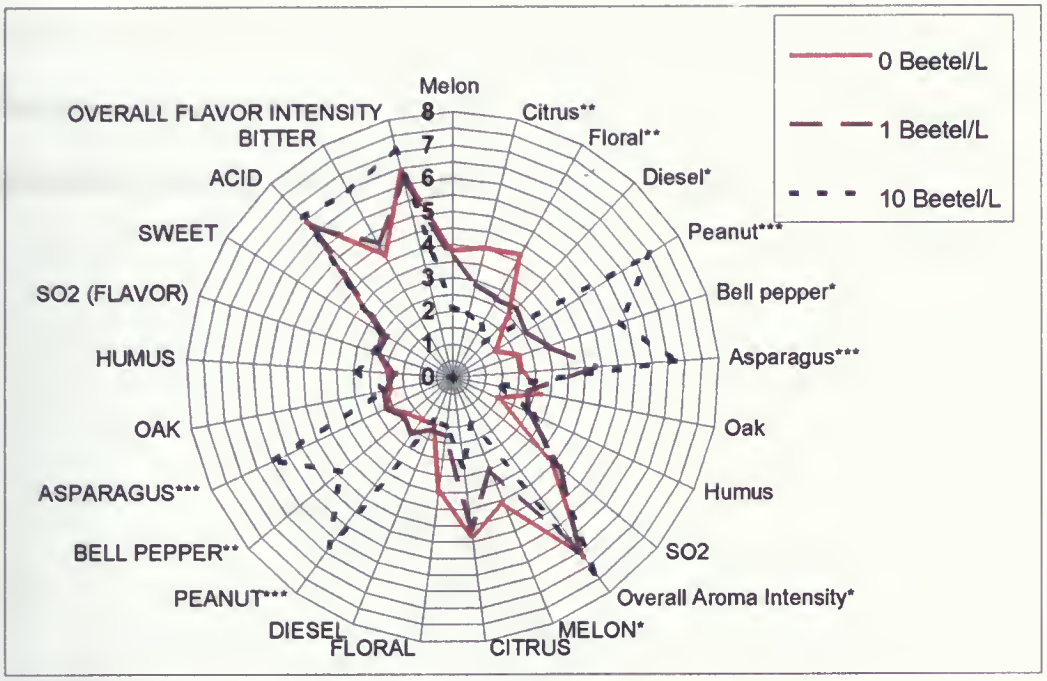

Figure 7 - Cobweb diagram showing mean sensory attribute scores for white wine after 10 month-aging for different levels of addition of *Harmonia axyridis* beetles to juice. Non capitalized attributes are aromas, and capitalized attributes are flavours. (*=Significance level =<0.05, **= Significance level =<0.01, and ***= Significance level =<0.001, for Bonferroni test following significant F-value from ANOVA, α=0.05)

Table 13 gives the mean intensity scores and the results from Bonferroni means separation tests for the twelve aroma and fifteen flavour terms used to profile the red wines. Figure 8 displays the mean intensity scores in a cobweb diagram format. At 1 beetle/L, only the intensity of *plum* flavor was affected (decreased) when compared to the control wine (0 beetles/L) (p=0.019), with no significant differences noted for any of the other attributes. Unlike the data from newly bottled wines, there were no differences in *bitterness* detected. At a rate of 10 beetles/L, however, a number of attributes were significantly impacted as compared with the control. *Red berry, plum* and *cherry* aroma

intensities decreased, while intensities of *peanut, asparagus/bell pepper* and *diacetyl* aromas increased. In contrast, with flavor, significant differences were observed in only *plum* and *peanut* intensities. As in white wine, this strongly suggests that smelling rather than tasting could play an important role in identifying *H. axyridis* tainted wines.

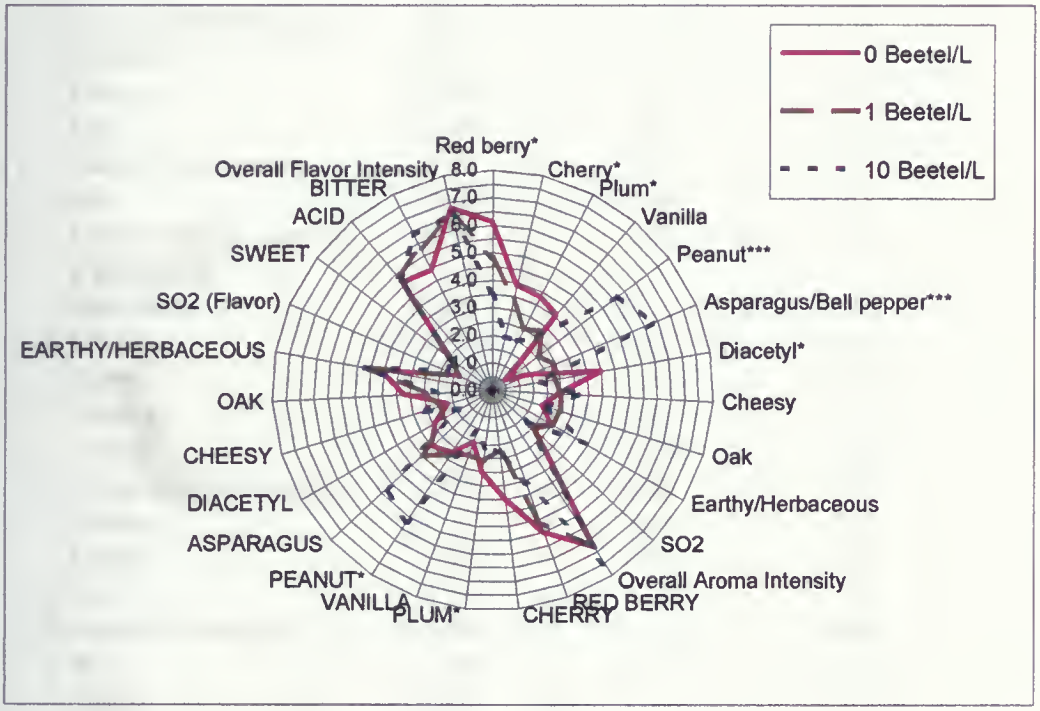

Figure 8 - Cobweb diagram showing mean sensory attribute scores for red wine after 10 month-aging for different levels of addition of *Harmonia axyridis* beetles to juice. Non-capitalized attributes are aromas, and capitalized attributes are flavours. (*=Significance level =<0.05, **= Significance level =<0.01, and ***= Significance level =<0.001, for Bonferroni test following significant F-value from ANOVA, α=0.05)

Table 13 Mean sensory attribute scores for red wine as a function of level of *Harmonia axyridis* beetle addition to juice [a,b]

	Treatment (beetles/L)					
Attribute	**0**		**1**		**10**	
AROMA						
Red Berry	6.09	b	4.77	ab	3.49	a
Cherry	3.39	b	3.36	ab	1.97	a
Plum	3.78	b	2.48	ab	2.00	a
Vanilla	3.56	a	2.83	a	2.50	a
Peanut	0.60	a	2.06	a	5.71	b
Asparagus/Bell pepper	1.41	a	2.44	a	6.31	b
Diacetyl	3.97	b	2.29	ab	1.82	a
Cheesy	2.41	a	2.45	a	3.05	a
Oak	1.93	a	2.56	a	2.02	a
Earthy/Herbaceous	2.49	a	2.54	a	3.86	a
SO_2	2.16	a	1.90	a	1.55	a
Overall aroma intensity	6.79	a	6.83	a	7.52	a
FLAVOUR						
Red berry	5.50	a	5.14	a	3.58	a
Cherry	2.97	a	2.61	a	2.12	a
Plum	4.00	b	2.23	a	2.27	a
Vanilla	2.00	a	2.47	a	1.57	a
Peanut	2.71	a	2.79	a	5.66	a
Asparagus/bell pepper	2.95	a	3.48	a	5.25	a
Diacetyl	2.41	a	2.01	a	1.42	a
Cheesy	1.67	a	2.03	a	2.55	a
Oak	3.16	a	2.40	a	1.96	a
Earthy/Herbaceous	4.08	a	4.63	a	4.66	a
SO_2	1.38	a	1.30	a	1.76	a
Sweet	1.86	a	1.94	a	1.81	a
Acid	5.16	a	5.41	a	5.23	a
Bitter	4.91	a	5.84	a	6.31	a
Overall flavour intensity	6.76	a	6.61	a	6.61	a

[a] Values shown are the mean intensity scores of eight judges and duplicate assessments

[b] Treatment means identified with different letters are significantly different at the 5% level (p of F-value from ANOVA <0.05, followed by Bonferroni means separation test)

A two-tailed t-Test of sensory data from the wines of bottling ans after 10 months aging showed few significant differences. Only the *floral* aroma (p=0.015) in the white control wine and *melon* aroma (p=0.040) in the wine of 1 Beetle/L had significant differences after aging. After aging, these two attributes had higher intensities. There was no significant difference in high level beetle tainted wines after aging in bottles. These two profiles matched well, for both studies, adding some "validity" to the profiles of *Harmonia axyridis* affected wines, although the panelists were not same.

In *H. axyridis* tainted wines, white wine showed more differences with age than red wine. It is reported that in white wine, methoxypyrazines have a lower threshold than in red wine. (Allen & Lacey, 1998, Boubee *et al.*, 2000, Sala *et al.*, 2002) Our sensory results support this. This may be due to the fact that red wine has more complex flavours and aromas.

Statistical results showed that the interaction between replications and panelists was significant for *humus* (p=0.035) and *floral* (p=0.034) aroma and *diesel* (p=0.031) and *bitter* (p=0.012) flavors. This might be due to some panelists using the scales differently between replications. Familiarity with the reference standards by more training may reduce the difference.

3.4. Conclusion

Few changes occurred during 10 months aged wines as a function of different levels of *H. axyridis* fermented with juice. Although the data suggests that methoxypyrazine levels may decrease after aging, there is no sensory evidence showing this trend. However, aging data verified the influence of the *H. axyridis* on wine quality.

The GC-MS analysis has shown that the number of *H. axyridis* beetles directly influences the level of IPMP in the finished wine. The hypothesis that IPMP is the principal odor-active compound in *H. axyridis*-affected wine has been further supported. Further research is required into the role of methoxypyrazines in this taint, and on determining the critical stages of grape and wine processing for the introduction of *H. axyridis* and formation of the taint

Chapter 4. Influence of potential remedial treatments

4.1 Introduction

Finding a method to remove the characteristics taint described in Chapter 2 and 3 will the focus in this part of our research Given that methoxypyrazines are implicated in the taint, any treatment that can remove them may decrease the intensity of taint characteristics. However, even if the levels of methoxypyrazines are not decreased, it is still possible that other effects such as "masking" may decrease the sensory intensities of beetle-derived attributes.

There are many factors that affect the level of methoxypyrazines in grapes. Researchers have found that sunlight and artificial light exposure can decrease the content of methoxypyrazines in fruit (Hashizume & Samuta, 1999 Allen & Lacey, 1998). This may be a clue that light or UV irradiation may have a photolysis effect on methoxypyrazines. Generally speaking, directly applying UV/light to wine is not a practical choice in wine making because of the risk of oxidation and light induced taints; however, Stavropoulos *et al.* exposed bottled wines to diffuse daylight, and these bottles allowed light above 370nm to pass through (Stavropoulos *et al.*, 2001).

Fining is used in the wine industry to stabilize fermented wine, and is also used to eliminate some off-odors. In practice, fining means the addition of various materials into wine. These materials can be natural biological substances such as proteins: gelatin,

isinglass, casein, and egg white, or natural substances such as bentonite and silica gel (Kieselsol), or synthetic materials such as PVPP.

At wine pH, the negative charge on the surface of bentonite can effectively attract positively charged substances, such as amino acids and positively charged proteins. Other mechanisms such as hydrogen-bonding or van der Waals interactions also help bentonite absorb neutral or negatively charged compounds. These interactions make bentonite an effective fining agent for stabilizing wine and removing some odors (Gougeon *et al.*, 2003). Although generally methoxypyrazines are not positively charged at wine pH (pK = 0.75, "*Dictionary of Organic Compounds*", Vol. 4, p3716, Chapman & Hall, NY, 1982), it may still be possible to remove some of them with bentonite.

Activated charcoal has a large surface area and has significant ability to absorb many odor components in wine. Many researchers have used charcoal as a fining agent to remove taint substances, such as fungicides, herbicides, and pesticides (Cabras & Angioni, 2000, Ying & Williams, 1999, Cabras *et al.* 1997). However, wine treated with activated charcoal may lose color and desirable aroma compounds, and there is a risk of oxygen being introduced into the wine by charcoal.

Traditionally, oak barrels are used for aging wine and spirits and offer improved color stability and a more complex aroma. The aroma of oak is due to the complex compounds in the wood and their reaction with wine components during aging (Perez-Prieto *et al.*, 2002). Components from the oak change the color, taste, mouth-feel, and bouquet of the

wine. Wood also absorbs some components of the wine, such as terpene alcohols and ethyl esters. The ratio of surface area is the most significant factor in the efficiency of sorption (Ramirez *et al.*, 2001). Aiken and Nobel compared the aroma sensory attributes from oak- and glass-aged Cabernet Sauvignon, and found both American and French oaks could significantly decrease the intensity of "green bean" aroma attribute, as well as increasing the "vanilla" and "oak blend" attributes. This result showed the possibility that oak attributes could mask the vegetative characteristics in the wine, or absorb the compounds that cause the high vegetative characteristics in the wine (Aiken & Nobel, 1984). However, more recent research by Hartmann *et al.* indicated evidence that oak did not have a strong affinity for alky-methoxypyrazines, (Hartmann *et al.*, 2002). An alternative method is to put oak chips (or powder) into wine. The extra large surface area may help decrease the time for the interaction between the wood chips and the wine to occur (Perez-Coello *et al.* 2000).

4.2. Materials and Methods

4.2.1. Wine preparation

White (Bourgeron™) and Red (Bergamais™) wines were made in 2002 from concentrated juice (both Vinco International, St Catharines, Ontario), as described in Chapter 2. Bottled wines were stored in a cellar at 14°C until required. Some bulk wines from these trails were kept in carboys with tight rubber bungs in a 4°C room.

1 beetle/L and 10 Beetle/L wines from the carboys were blended at a ratio of 5:4. Both blended white and red wines were used for the remedial treatments. Here, the tainted wines (blend) were considered to have approximately 15ppt level of IPMP (based on measured IPMP content of 1 and 10 beetle/L wines-Chapter 3), and this concentration may be close to the IPMP content of *HA*-affected commercial wines.

4.2.2. Remedial treatments

Small scale bench tests investigating both dosage levels and treatment duration times were conducted on the following: Bentonite, isinglass, combination of bentonite and isinglass, activated charcoal, French medium toast oak chips, UV light and normal light. After informal sensory assessment, five treatments were selected for both the white and red wines.

4.2.2.1. Bentonite

10g of commercial bentonite (Pilot Winery, Brock University, Ontario) was put into boiling water and stirred overnight. 40 ml of this solution was added into 4 L of the tainted wine (blend) (both white and red) in a 4L small glass jar, to give a final

concentration of 1g/L. The wine was moved to 7°C room and racked on the third and seventh day.

4.2.2.2 Activated charcoal

0.8g activated charcoal (Sigma®) was directly added into 4 L of tainted wine (blend) (both white and red) in a 4L small glass jar (final concentration is 0.2g/L). The wine was moved to 7°C room and racked on the third day and seventh day.

4.2.2.3. Oak

There were two kinds of oak chip treatment:

Normal oak chips (French medium toast oak chips, Winemaster™ (Vin Bon-Brew, St. Catharines, Ontario))

(i) Normal oak chips

(ii) Deodorized oak chips: 60g of oak chips were put into 300ml 40% ethanol solution overnight. On the second day, they were washed three times, boiled in water for 10 minutes, and dried in 60°C oven. After these steps, little oak aroma remained in the oak chips.

16 g de-odorized and non de-odorized oak chips were put into each of 4 L tainted wine (blend) (both white and red) in a 4L small glass jar (final concentrations are 4g/L). The wine was moved to 7°C room and racked on the second day and seventh day.

4.2.2.4 UV and light treatment

UV and white light were also included as treatments under the following conditions:

(i) Reactor (Fig. 9): made of translucent plastic. Inner dimension 15 x 5 x 1.5 (cm), and wine pass through the reactor portion which is 12 cm long, and 0.5cm deep.

(ii) UV light source (for red wine): UVG-43, Wave length=254nm, 18.3W, directly over on the reactor (15 x 5 x 1.5 cm).

(iii) Light (for white wine): halogen bulb, 120W, 30cm distance above the reactor.

(iv) Wine flow for both UV and light: 100ml/min, and protected by N_2 gas.

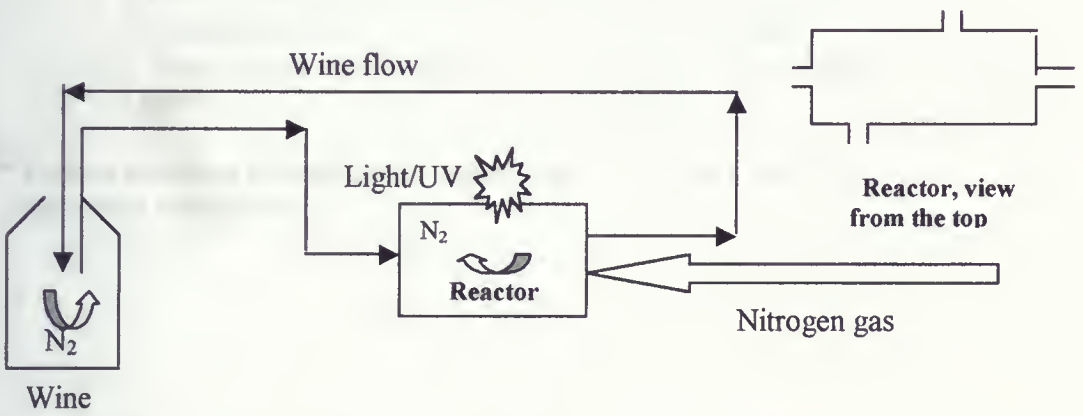

Figure. 9 Reactor used for light treatment of *Harmonia axyridis*-affected wines.

4.2.3. Sensory Methodology

Panel recruitment and training and data collection are described in Chapter 3. Table 14 lists the wines evaluated:

Table 14. Remediated wines evaluated

White wine (Bourgeron™, 10 month)	**Red wine (Bergamais™, 10 month)**
Tainted white wine * without any treatment	Tainted red wine * without any treatment
Tainted white wine treated with bentonite	Tainted red wine treated with bentonite
Tainted white wine treated with activated charcoal	Tainted red wine treated with activated charcoal
Tainted white wine treated with non-deodorized oak	Tainted red wine treated with non-deodorized oak
Tainted white wine treated with de-odorized oak	Tainted red wine treated with de-odorized oak
Tainted white wine treated with light	Tainted red wine treated with UV light

* Tainted wine was blended from 1 beetle/L and 10 Beetle/L wines from carboys at the ratio of 5:4.

4.3. Results and Discussion

4.3.1. Methoxypyrazine

GCMS results show none of the treatments, except activated charcoal in white wine, had significant affect on the level of IPMP in remedial wines. (Fig 10, Appendix 2. Table 16).

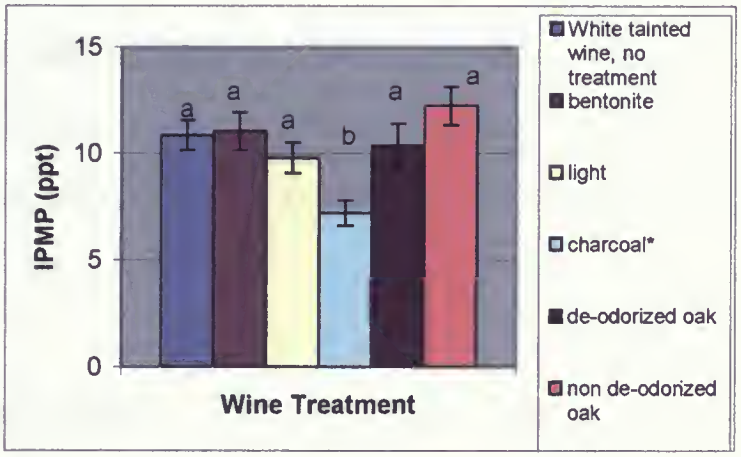

Figure 10. IPMP level in white wine after treatment. (Mean of duplicated measurement; *p of F-value from two-tail ANOVA <0.05)

4.3.2. Sensory Evaluation of remedial treatments

4.3.2.1. The blended wine for remedial treatments

The blended white wine did not show differences compared to the control (no beetle wine), except for *oak* aroma (p=0.013) and *bell pepper* flavour (p=0.041). It is interesting to note that although there was no oak treatment in them, the white control wine (no beetle) had the highest *oak* intensity (Appendix 3, Table 21). The blended white wine had the lowest intensity of SO_2 flavour (p=0.024; 0.026; 0.021), compared with control (0 beetle/L), 1 beetle/L, and 10 beetle/L wines. This hinted that there may be some

differences between wine stored in carboys and wine stored in bottles, although the chemical data for SO_2 did not show a large difference. The blended red wine only showed significant difference in *diacetyl* aroma, compared with the control wine ($p=0.007$), and it showed the lowest *diacetyl* intensity (Appendix3, Table 22). Here, the attribute of *diacetyl* is not related to *Harmonia axyridis* and its chemical content is unknown without further chemical analysis. However, the blended wines, both red and white wines, showed expected trends of higher intensities of beetle-derived attributes than beetle-free wines, with values generally between the 1 beetle/L and 10 beetle/L wines (Appendix 3, Table 21 and 22).

4.3.2.2. Remedial treatments

Table 17 to 20 in Appendix 2 shows means and standard errors of sensory data of the different remedial wines. Figure 11 and 12 display the mean intensity scores of remedial white wines and red wines respectively in a cobweb diagram format.

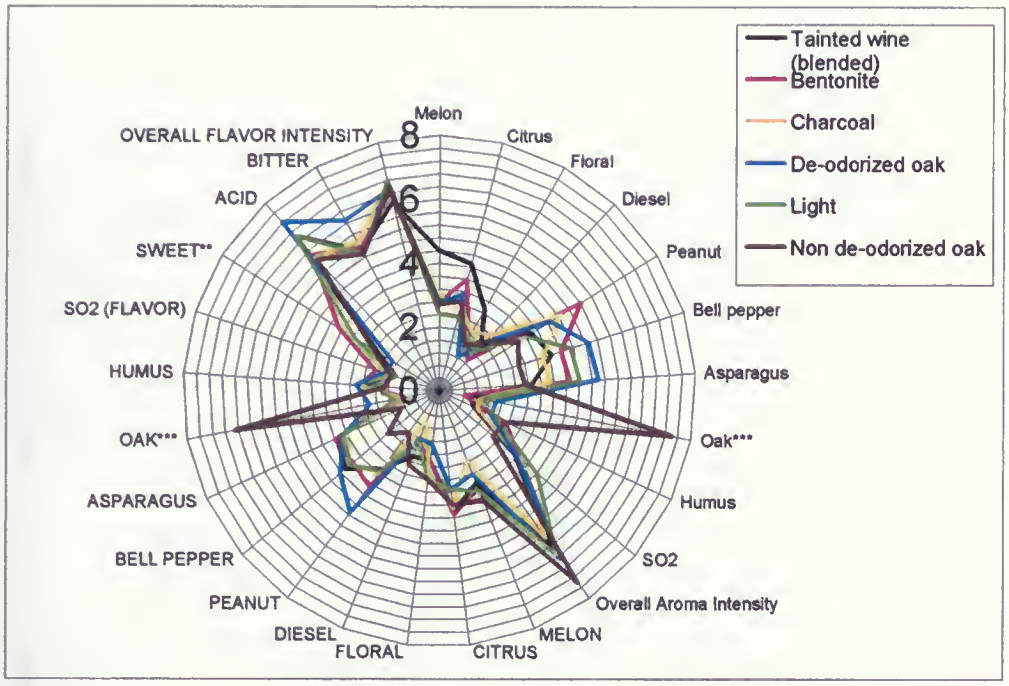

Figure 11. Cobweb diagram showing mean sensory attribute scores of white wines for different treatments of tainted white wine (blend). Aromas are in non-capitalized letters, and flavours in capitalized letters. (*=Significance level =<0.05, **= Significance level =<0.01, and ***= Significance level =<0.001, for Bonferroni test following significant F-value from ANOVA, α=0.05)

In treatments of white wine, non de-odorized oak chips contributed strong oak characteristics. Interestingly, compared with the de-odorized oak chip treatment, bentonite had a higher sweet intensity. All treatments showed lower intensities of *melon* aroma, and non de-odorized oak showed a decrease in the intensity of *peanut* flavour. When using LSD, non de-odorized oak treatment had significantly lower *peanut* flavor intensity (p=0.032), compared with the non-treated control wine (blend).

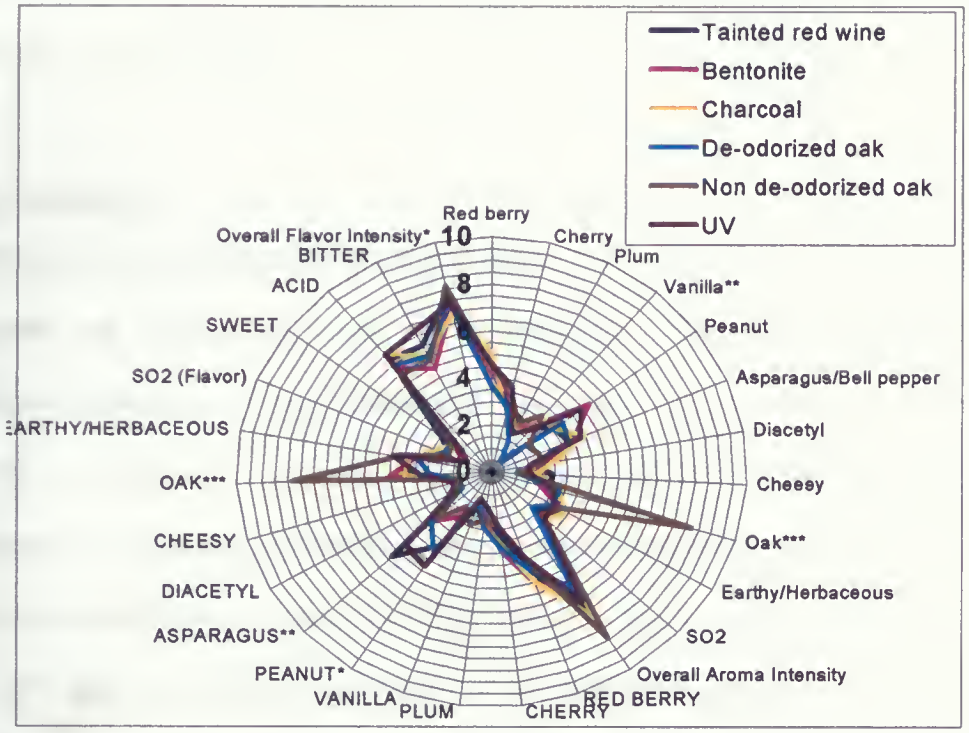

Figure 12. Cobweb diagram showing mean sensory attribute scores of red wines for different treatments of tainted red wine (blend). Aromas are in non-capitalized letters, and flavours in capitalized letters. (*=Significance level =<0.05, **= Significance level =<0.01, and ***= Significance level =<0.001, for Bonferroni test following significant F-value from ANOVA, α=0.05)

In red wine, non de-odorized oak chips also gives strong oak characteristics, and they also increased the overall aroma intensity compared with bentonite and de-odorized oak chips. Vanilla aroma, an attribute related to oak, was significantly higher in charcoal and non de-odorized oak treatments, as compared with de-odorized oak, The removal of oak odor in the treatment of the de –odorized oak chips was successful. With regards to flavour, non de-odorized oak, bentonite, and charcoal significantly decreased the *asparagus/bell pepper* attribute in red wine. Charcoal also decreased the *overall* flavour

intensity. This hinted that modified fining methods might have an affect on tainted wine. It still requires further research.

4.4. Conclusion

Remedial treatments slightly affect the intensities of some attributes in wines, except non de-odorized oak chips. The strong aroma from oak chips offered a strong oak characteristic. However, in the treatment of oak chips, only one of the *H. axyridis* attributes, *asparagus/bell pepper*, was decreased in red wine flavour. The methoxypyrazine level did not change in this treatment suggesting that only a masking affect occurs in red wine, as reported in Hartmann *et al.* (2002). Again, only in red wine flavour, was there a decrease in the intensity *of asparagus/bell pepper* from bentonite and charcoal treatments. The lower complexity in white wine appears make to the tainted attributes more prominent than in red wines.

Although the treatments did not offer a cure-all for the wine industry, we have some clues for future research. If fining does not greatly improve the tainted wine, preventing beetles in the vineyard may be more important. Future research will investigate at what stage during wine processing the beetles contribute their taint, and how methoxypyrazines change the wine attributes. Determining the threshold level of beetles per weight of grapes is also important. As mentioned before, there are different thresholds for methoxypyrazines in white wine and red wine. It is expected that white wine will have a lower threshold for the taint.

Chapter 5. Summary

The possible influence of *Harmonia axyridis* (the Multicolored Asian Lady Beetle) on the sensory properties of wine was investigated. *H. axyridis* beetles were added to white and red grape musts at a rate of 0, 1 or 10 per L, and a trained panel evaluated the finished wines using flavour-profiling techniques. Significant modification of both wine aroma and flavour characteristics were observed in the 10 beetle/L treatments, with smaller effects noted at the 1 beetle/L rate. Vinification in the presence of *H. axyridis* gave higher intensity scores for peanut, bell pepper and asparagus aromas and flavours in the white wines, and peanut, asparagus/bell pepper, and earthy/herbaceous aromas and flavours in the red wines. In addition, sweet, acid and bitter tastes were affected in red wines, and a general trend of decreasing fruit and floral intensities with increasing beetle rate was observed in both white and red wines. Similar sensory effects were observed in wines with 15 ppt isopropyl-methoxypyrazine added, which partly supported the hypothesis that methoxypyrazines from beetles are implicated in the taint characteristics. Sensory profiles of control and beetle-tainted wines were largely unchanged after 10 month aging.

The influences of different remedial treatments on the sensory properties of white and red wines tainted by *Harmonia axyridis* were investigated after 10 months of aging. Bentonite, activated charcoal, French oak chips, and UV/light were applied to modify the tainted wines. Methoxypyrazine levels were measured using a GC-MS. Using descriptive analysis techniques, a trained panel evaluated the treated wines. Wines treated with oak chips had strong *oak* characteristics and masked the *peanut* aroma in white wine. In the

red wine, only the characteristics of *asparagus/bell pepper* were decreased by bentonite, charcoal and oak chip treatments. The concentration of IPMP is related to the level of *H. axyridis* introduced into juice. However, in the remedial treatments, only activated charcoal significantly decreased the methoxypyrazine levels in white wine although sensory data did not show such a significant decrease in methoxypyrazine-related attributes.

This research developed a useful sensory profile for describing *H. axyridis* tainted wines. This should help in better understanding the beetle problem in North America, including the Niagara region. The hypothesis that *H. axyridis* and methoxypyrazines are responsible for the taint characteristics is supported by this research; however, further research into the role of methoxypyrazines in the taint still needs to be conducted with more sensitive measurement. Furthermore, present remedial methods do not offer a cure-all for tainted wines in the industry. Other methods may be worth investigating in the future. Without effective ways to significantly improve tainted wines, control of the pests in the vineyard and winery is critical. Finally, further research could investigate sensory thresholds for the taint, obtain consumer hedonic responses, and look at biotechnical way to improve the quality of tainted wine.

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Appendix 1.

Table 15. Basic chemical composition of wines (2002)

Wine	pH	Residual Sugar (Lane-Eynon Method)	Titratable Acidity (g/L, as tartaric acid)	Volatile Acidity (g/L, as Acetic acid)	Ethanol (v/v%, GC)
White wine, no beetle	3.28±0	4.9±1.1	6.79±0.02	0.338±0.017	12.5±0.0
White wine, 1 Beetle/L	3.27±0.01	4.7±0.5	6.71±0.01	0.240±0.010	12.7±0.6
White wine, 10 beetle/L	3.27±0.02	5.9±0.4	6.65±0.01	0.328±0.048	12.4±0.1
Red wine, no beetle	3.35±0.01	5.3±0.4	6.80±0.02	0.275±0.027	12.7±0.0
Red wine, 1 Beetle/L	3.34±0.01	6.2±0.9	6.78±0.07	0.290±0.064	12.3±0.2
Red wine, 10 beetle/L	3.36±0.01	4.2±1.0	6.83±0.03	0.271±0.025	12.6±0.5

*Data represent: mean value ± standard deviation

Appendix 2

Table 16 Concentration of isopropylmethoxypyrazine (ppt) in remedial wines

Wine	*Isopropylmethoxypyrazine (ppt)*
Tainted white wine [a]	10.9±0.7
Tainted white wine (bentonite)	11.0±0.9
Tainted white wine (light)	9.8±0.7
Tainted white wine (charcoal)	7.2±0.6[b]
Tainted white wine (de-odorized oak)	10.4±1.0
Tainted white wine (non-de-odorized oak)	12.2±0.9
Tainted red wine*	10.8±0.2
Tainted red wine (bentonite)	11.8±0.9
Tainted red wine (UV)	10.4±0.5
Tainted red wine (charcoal)	9.6±0.6
Tainted red wine (de-odorized oak)	10.1±0.1
Tainted red wine (non-de-odorized oak)	10.8±0.2

a. Tainted wine was blended from 1 beetle/L and 10 Beetle/L wines from the carboys at the ratio of 5:4.

b. Mean of duplicated measurement, *$p < 0.05$, two-tail ANOVA t-test.

Table 17. Mean of sensory aroma attribute scores for remediated white wines [a, b]

Wine & SE	Melon	Citrus	Floral	Diesel	Peanut	Bell pepper
A	4.37	4.14	2.91	1.91	3.38	3.68
SE	0.86	0.69	0.80	0.60	0.92	0.77
B	2.83	3.59	1.93	1.33	5.23	3.96
SE	0.72	0.73	0.68	0.37	1.22	0.76
C	3.02	2.73	2.06	2.39	4.51	3.41
SE	0.74	0.60	0.58	0.47	1.14	0.71
D	2.79	3.12	1.17	2.12	4.06	4.88
SE	0.68	0.75	0.36	0.67	0.91	0.91
E	2.70	2.94	1.56	2.08	2.93	2.61
SE	0.68	0.75	0.36	0.67	0.91	0.91
F	2.44	2.41	1.92	1.68	3.20	4.31
SE	0.72	0.69	0.54	0.56	0.82	0.94

Wine & SE	Asparagus	Oak	Humus	SO_2	Overall Intensity
A	2.93	0.97	1.23	3.13	5.92
SE	0.72	0.45	0.51	0.84	0.47
B	4.10	0.80	2.34	3.29	6.43
SE	0.84	0.25	0.62	0.83	0.53
C	3.51	1.31	1.33	3.34	5.68
SE	0.71	0.56	0.40	0.94	0.64
D	4.98	1.77	1.76	3.21	6.46
SE	0.82	0.58	0.53	0.92	0.62
E	2.65	7.38	2.27	2.16	7.35
SE	0.82	0.58	0.53	0.92	0.62
F	4.43	1.39	1.97	4.04	6.27
SE	0.80	0.43	0.56	0.91	0.38

[a] Wine:

A: Tainted wine (blend); B: Bentonite C: Charcoal D: Deodorized oak
E: Untreated oak F: Light
SE: Standard Error (= Standard deviation/ Square root of replications (16))

[b] Values shown are the mean intensity scores of eight judges and duplicate assessments

Table 18. Mean of sensory flavor attribute scores for remediated white wines [a, b]

	Melon	Citrus	Floral	Diesel	Peanut	Bell pepper	Asparagus
A	3.09	3.81	2.21	2.16	3.03	3.79	3.49
SE	0.68	0.85	0.79	0.66	0.87	0.89	0.68
B	3.33	3.90	2.24	1.70	3.69	3.46	3.59
SE	0.80	0.78	0.86	0.51	0.74	0.65	0.84
C	2.25	3.70	2.59	1.00	3.17	3.55	3.18
SE	0.70	0.84	0.67	0.34	0.76	0.84	0.68
D	2.84	3.10	1.69	1.60	4.74	4.06	3.26
SE	0.70	0.78	0.50	0.53	0.93	0.72	0.64
E	3.68	3.63	2.79	2.50	1.63	2.11	1.31
SE	0.61	0.77	0.83	0.76	0.71	0.61	0.41
F	3.38	3.07	2.83	1.69	3.11	3.56	3.27
SE	0.76	0.76	0.70	0.61	0.87	0.62	0.60

	Oak	Humus	SO_2	Sweet	Acid	Bitter	Overall Intensity
A	1.29	2.41	1.44	2.23	6.64	4.96	6.14
SE	0.38	0.57	0.44	0.41	0.82	1.07	0.54
B	1.15	2.48	2.19	3.74	5.78	5.14	6.39
SE	0.47	0.75	0.60	0.84	0.71	1.02	0.70
C	1.16	2.56	1.64	3.26	5.66	5.28	6.65
SE	0.40	0.69	0.52	0.68	0.63	0.90	0.50
D	2.20	2.63	1.84	1.71	7.23	6.11	6.53
SE	0.71	0.68	0.53	0.40	0.73	0.72	0.47
E	6.50	1.66	1.60	2.37	5.64	4.89	6.56
SE	1.11	0.55	0.47	0.62	0.79	0.86	0.53
F	1.37	2.06	1.44	3.07	6.56	4.87	6.77
SE	0.56	0.61	0.49	0.76	0.92	0.92	0.45

[a] Wine:

A: Tainted wine (blend); B: Bentonite C: Charcoal D: Deodorized oak
E: Untreated oak F: Light

[b] Values shown are the mean intensity scores of eight judges and duplicate assessments

Table 19. Mean of sensory aroma attribute scores for remediated red wines [a, b]

Wine & SE	Red berry	Cherry	Plum	Vanilla	Peanut	Asparagus/ Bell pepper
A	4.54	2.94	2.51	2.42	3.14	3.33
SE	0.77	0.75	0.72	0.69	0.88	0.80
B	4.32	3.31	2.24	2.53	4.81	3.54
SE	0.70	0.93	0.66	0.73	1.01	0.77
C	5.09	2.61	2.24	3.11	3.01	3.99
SE	0.68	0.84	0.75	0.85	0.80	0.85
D	3.67	2.76	1.49	0.63	3.54	3.31
SE	0.65	0.83	0.41	0.22	1.05	0.57
E	4.53	3.53	2.33	3.07	1.79	2.01
SE	0.87	0.86	0.58	0.74	0.63	0.64
F	4.62	3.73	1.76	2.30	4.44	3.84
SE	0.78	0.74	0.47	0.66	0.90	0.96

Wine & SE	Diacetyl	Cheesy	Oak	Earthy/Herbaceous	SO2	Overall Intensity
A	1.67	2.36	2.39	3.11	2.41	7.34
SE	0.55	0.62	0.78	0.71	0.65	0.52
B	2.24	1.43	1.95	3.22	2.69	6.18
SE	0.55	0.43	0.57	0.76	0.77	0.49
C	2.45	1.78	2.79	3.36	2.99	7.26
SE	0.59	0.62	0.94	0.88	0.56	0.55
D	1.62	0.99	2.79	2.78	2.24	5.88
SE	0.54	0.37	0.94	0.76	0.44	0.60
E	2.10	1.06	8.24	3.06	2.76	8.44
SE	0.64	0.37	0.82	0.62	0.57	0.55
F	1.51	1.45	2.62	2.83	2.91	6.63
SE	0.45	0.47	0.72	0.61	1.06	0.48

[a] Wine:

A: Tainted wine (blend); B: Bentonite C: Charcoal D: Deodorized oak
E: Untreated oak F: UV

[b] Values shown are the mean intensity scores of eight judges and duplicate assessments

Table 20. Mean of sensory flavor attribute scores for remediated red wines [a, b]

Wine	Red Berry	Plum	Cherry	Vanilla	Peanut	Asparagus/Bell pepper	Diacetyl	Cheesy
A	4.43	2.21	2.74	1.56	3.81	5.31	1.39	1.36
SE	0.83	0.64	0.76	0.68	1.08	0.95	0.63	0.51
B	5.03	1.92	3.38	1.48	2.28	3.09	1.92	1.37
SE	0.72	0.51	0.73	0.45	0.66	0.68	0.65	0.50
C	4.91	2.31	3.07	1.33	4.18	3.11	1.43	1.46
SE	0.79	0.72	0.74	0.51	1.05	0.58	0.54	0.60
D	4.41	2.42	3.25	1.18	4.15	3.23	1.34	1.28
SE	0.73	0.66	0.74	0.46	1.01	0.67	0.50	0.51
E	3.91	2.08	2.44	2.36	1.84	2.94	1.48	1.23
SE	0.74	0.67	0.64	0.71	0.44	0.71	0.50	0.49
F	3.86	1.46	2.40	1.23	4.76	4.58	1.74	1.61
SE	0.92	0.47	0.74	0.62	0.86	0.58	0.59	0.52

Wine	Oak	Earthy/Herbaceous	SO_2	Sweet	Acid	Bitter	Overall Intensity
A	3.00	3.84	1.76	2.39	6.23	5.97	7.75
SE	0.94	0.89	0.49	0.61	0.71	1.09	0.32
B	3.99	3.14	1.24	1.77	5.71	4.89	7.20
SE	0.91	0.65	0.42	0.54	0.71	1.02	0.39
C	3.35	3.28	2.33	2.01	6.46	5.51	6.89
SE	0.81	0.73	0.63	0.50	0.78	0.97	0.55
D	2.35	2.89	2.00	1.91	5.93	5.46	7.38
SE	0.79	0.73	0.53	0.60	0.68	0.88	0.41
E	7.84	3.68	1.76	1.91	5.38	5.34	8.05
SE	0.82	0.68	0.45	0.57	0.70	0.99	0.35
F	2.76	3.94	0.99	1.37	6.48	6.67	7.37
SE	0.89	0.67	0.31	0.38	0.67	0.92	0.38

[a] Wine:

A: Tainted wine (blend); B: Bentonite C: Charcoal D: Deodorized oak
E: Untreated oak F: Light

[b] Values shown are the mean intensity scores of eight judges and duplicate assessments

Appendix 3.

Table 21. Mean sensory attribute scores for aged white wine as a function of level of *Harmonia axyridis* beetle addition to juice [a,b]

Attribute	*Treatment (beetles/L)* 0		1		5		10	
AROMA								
Melon	3.81	ab	3.64	ab	4.37	b	2.11	a
Citrus	4.03	b	2.94	ab	4.14	b	2.01	a
Floral	4.23	b	2.68	ab	2.91	ab	1.84	a
Diesel	2.36	a	2.84	a	1.91	a	1.37	b
Peanut	1.52	a	2.59	a	3.38	a	7.03	b
Bell pepper	2.06	a	3.06	ab	3.68	ab	5.33	b
Asparagus	2.15	a	4.34	ab	2.94	a	6.68	b
Oak	2.73	b	2.28	ab	0.97	a	1.51	ab
Humus	1.50	a	2.51	a	1.23	a	2.74	a
SO_2	3.86	a	4.24	a	3.12	a	3.94	a
Overall aroma intensity	6.68	a	6.49	a	5.92	a	7.32	a
FLAVOR								
Melon	4.06	b	3.03	ab	3.09	ab	1.46	a
Citrus	4.84	a	4.56	a	3.81	a	2.74	a
Floral	3.45	a	1.81	a	2.21	a	1.48	a
Diesel	1.51	a	1.74	a	2.16	a	1.44	a
Peanut	1.63	a	2.15	a	3.03	a	6.31	b
Bell pepper	1.74	a	1.93	ab	3.79	bc	4.39	c
Asparagus	2.16	a	2.22	a	3.49	a	5.90	b
Oak	1.91	a	2.06	a	1.29	a	2.05	a
Humus	1.91	a	1.74	a	2.41	a	2.85	a
SO_2	2.44	b	2.43	b	1.44	a	2.46	b
Sweet	2.46	a	2.29	a	2.23	a	2.49	a
Acid	6.41	a	6.21	a	6.64	a	6.55	a
Bitter	4.21	a	4.64	a	4.96	a	6.38	a
Overall flavor intensity	6.36	a	6.14	a	6.14	a	7.13	a

[a] Values shown are the mean intensity scores of eight judges and duplicate assessments

[b] Treatment means identified with different letters are significantly different at the 5% level (p of F-value from ANOVA <0.05, followed by Bonferroni means separation test)

Table 22. Mean sensory attribute scores for red wine as a function of level of *Harmonia axyridis* beetle addition to juice [a,b]

	Treatment (beetles/L)							
Attribute	**0**		**1**		**5**		**10**	
AROMA								
Red Berry	6.09	b	4.77	ab	4.54	ab	3.49	a
Cherry	3.39	b	3.36	ab	2.94	ab	1.97	a
Plum	3.78	b	2.48	ab	2.51	ab	2.00	a
Vanilla	3.56	a	2.83	a	2.42	a	2.50	a
Peanut	0.60	a	2.06	a	3.14	ab	5.71	b
Asparagus/Bell pepper	1.41	a	2.44	a	3.33	a	6.31	b
Diacetyl	3.97	b	2.29	ab	1.67	a	1.82	a
Cheesy	2.41	a	2.45	a	2.36	a	3.05	a
Oak	1.93	a	2.56	a	2.39	a	2.02	a
Earthy/Herbaceous	2.49	a	2.54	a	3.11	a	3.86	a
SO_2	2.16	a	1.90	a	2.41	a	1.55	a
Overall aroma intensity	6.79	a	6.83	a	7.34	a	7.52	a
FLAVOR								
Red berry	5.50	a	5.14	a	4.43	a	3.58	a
Cherry	2.97	a	2.61	a	2.21	a	2.12	a
Plum	4.00	b	2.23	a	2.74	ab	2.27	a
Vanilla	2.00	a	2.47	a	1.56	a	1.57	a
Peanut	2.71	a	2.79	a	3.81	ab	5.66	ab
Asparagus/bell pepper	2.95	a	3.48	a	5.31	a	5.25	a
Diacetyl	2.41	a	2.01	a	1.39	a	1.42	a
Cheesy	1.67	a	2.03	a	1.36	a	2.55	a
Oak	3.16	a	2.40	a	3.01	a	1.96	a
Earthy/Herbaceous	4.08	a	4.63	a	3.84	a	4.66	a
SO_2	1.38	a	1.30	a	1.76	a	1.76	a
Sweet	1.86	a	1.94	a	2.39	a	1.81	a
Acid	5.16	a	5.41	a	6.23	a	5.23	a
Bitter	4.91	a	5.84	a	5.97	a	6.31	a
Overall flavor intensity	6.76	a	6.61	a	7.75	a	6.61	a

[a] Values shown are the mean intensity scores of eight judges and duplicate assessments

[b] Treatment means identified with different letters are significantly different at the 5% level (p of F-value from ANOVA <0.05, followed by Bonferroni means separation test)