AN ABSTRACT OF THE DISSERTATION OF

Pallavi Mohekar for the degree of Doctor of Philosophy in Food Science and Technology presented on July 22, 2016.

Title: Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* Taint in Wine: Impact on Wine Sensory, Effect of Wine-processing and Management Techniques

Abstract approved:

_____

Dr. Elizabeth Tomasino

Brown marmorated stink bug (BMSB), (*Halyomorpha halys*, Hemiptera: Pentatomidae) is an invasive species that damages numerous agricultural crops including grapes. Related damage include lower berry weight with increased exposure and cracked berries as a result of BMSB feeding activity. The insect is currently detected in 43 US states including Oregon, Washington, California, and New York. The grape and wine industries in these areas hold significant economic value. BMSB damage has already resulted in major economic loss in the agricultural industry. Current data indicates that the infestation is spreading to new regions and its population density is increasing in regions where it has been detected.

Surveys show BMSB in vineyards of Oregon, Virginia and New York where BMSB can damage grapes, lowering their yield and quality. When harvested with grape clusters, BMSB can introduce volatile compounds, trans-2-decenal and tridecane, into wine. Prior work has shown that the presence of these compounds alters wine sensory. In this thesis, the focus is on analyzing BMSB's impact on wine quality and

consumer preference. Additionally, this work determines the action threshold (AT) for BMSB in the vineyard, which is likely to prove important to the grape and wine industry in designing control limits and maintaining wine quality.

The first contribution of this work establishes the sensory detection threshold (DT) and consumer rejection thresholds (CRT) for trans-2-decenal in red wine. Trans-2-decenal is one of the main aroma compounds in BMSB taint, having green, cilantro-like aroma characteristics that is undesirable in wine. Results conclusively show that trans-2-decenal in wine has a negative effect on its quality. In Pinot noir, consumers were able to perceive trans-2-decenal at 1.92 µg/L (DT). Consumer preference for Pinot noir and Merlot containing trans-2-decenal decreased significantly above the concentration of 4.8 µg/L (CRT). Pinot noir containing trans-2-decenal above CRT was described as green, herbal, musty and less fruity by wine professionals. Based on such findings, the use of CRT is recommended when establishing consumer tolerance levels of trans-2-decenal in wine.

The second contribution relates BMSB presence in vineyard with sensory threshold of trans-2-decenal in the finished wine. Pinot noir, Merlot and Pinot gris were produced using different densities of BMSB in grape clusters. The results of this study indicate BMSB density of three per cluster can result in Pinot noir containing trans-2-decenal at its DT and below its CRT. This density can be used as AT for BMSB in the vineyard since wines made from grapes contaminated at or greater than 3 BMSB per cluster are likely to experience low preference by wine consumers. The

same BMSB density can be used to devise control measures for Merlot since trans-2-decenal CRT was found to be similar for both Pinot noir and Merlot. Pinot gris was found to be free of trans-2-decenal even at BMSB density of 1 per cluster. Therefore, we believe that BMSB may not be a concern for white wines.

The third contribution provides methods to reduce BMSB taint in finished wine. This can be done by modifying the winemaking process or by applying corrective measures in the wine. During winemaking, destemming and pressing were identified to be the steps responsible for increasing BMSB taint levels in wine whereas alcoholic fermentation decreases taint levels. Consequently, finished white wine was found to be free of trans-2-decenal since fermentation occurs after pressing. However, trans-2-decenal was present in finished red wine since pressing occurs after fermentation. Taint levels in finished red wine are also affected by different pressing variants (free run versus press fraction, bladder press versus basket press), with press fraction and bladder press introducing more BMSB taint compared to free run and basket press. This information will allow winemakers to adjust processing steps in order to minimize BMSB taint levels in red wines. Further reduction in taint levels was shown to be possible through the use of reverse osmosis filtration. Alternatively, oak addition can be used to mask the sensory attributes of trans-2-decenal at the risk of introducing spicy notes into wine. None of the other common fining agents tested were found to be effective against trans-2-decenal.

Taken together, this thesis contributes a number of novel insights into the impact of BMSB on red and white wine. By relating consumer thresholds and descriptive analysis with chemical and wine processing, we are able to establish control densities for the pest in the vineyard, identify key processing steps and post-fermentation treatments to reduce BMSB taint. The information contained in this thesis is likely to prove valuable to the wine industry in its struggle against BMSB.

Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* Taint in Wine: Impact on Wine Sensory, Effect of Wine-processing and Management Techniques

by
Pallavi Mohekar

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented July 22, 2016
Commencement June 2017

Doctor of Philosophy dissertation of Pallavi Mohekar presented on July 22, 2016

APPROVED:

Major Professor, representing Food Science and Technology

Head of the Department of Food Science and Technology

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Pallavi Mohekar, Author

ACKNOWLEDGEMENTS

My deepest gratitude goes to my advisor, Dr. Elizabeth Tomasino. I'm extremely grateful for her mentorship during this challenging endeavor. She's taught me everything I know in this area. This work would not have been possible without her patience, guidance and support and I'm lost for words to describe how invaluable she has been to my graduate career.

I would also like to express my deepest gratitude to all the members of my committee. Dr. Sarah Emerson, my minor advisor, for her patience, advice and expertise with the statistical analyses. Dr. James Osborne who has been a constant mentor on all aspects of this project. His support has been critical with the winemaking. Dr. Vaughn Walton for providing much-needed support with the entomological aspects of the project.

I would also like to thank Dr. Nik Wiman who provided many (doomed) bugs, along with much needed guidance, which made much of this work possible. My graduate program would not have been possible without the tireless efforts of the FST department staff. Linda Dunn helped me navigate most of the procedural aspects of this endeavor and was always available to help with the paperwork as deadlines approached. Christina helped me on many occasions with order requests on short notice and Debby for all her assistance with travel arrangements.

I'm also incredibly grateful to my lab mates and friends at OSU who have made this experience one that I will forever cherish. Mei, in particular, has been a pillar of strength, making the lab an enjoyable place to work, always willing to help with every arbitrary request, particularly around GC-MS. Anthony, for his cheerful presence around the lab, his help with winemaking and lifting of various things around OSU. Alison, for making early morning sensory panels more fun. There are many others who made it all unforgettable but I would be remiss to mention Shiloh,

Amalia, Ying, Athena, Danielle and Todd for their assistance, friendship and general awesomeness.

I would also like to thank all my friends and well-wishers, old and new, from around the world. You make it all worthwhile.

This PhD is dedicated to my dear husband, Jervis. I would not be where I am today, personally and professionally, without his presence in my life. Finally, I am eternally grateful to our parents, siblings and the entire family for their unconditional love, support and encouragement through this journey. I feel lucky and blessed to have these wonderful people in my life.

CONTRIBUTION OF AUTHORS

Chapter 1: Pallavi Mohekar wrote Chapter 1 with input from Dr. Elizabeth Tomasino.

Chapter 2: The core study, the concept and research design was developed by Dr. Elizabeth Tomasino, Dr. Juyun Lim and Pallavi Mohekar. Data collection and its analysis was performed by Pallavi Mohekar. Dr. Elizabeth Tomasino and Pallavi Mohekar prepared the manuscript with inputs from Dr. Juyun Lim, Dr. Trina Lapis and Dr. Nik Wiman.

Chapter 3: The core study, the concept and research design was developed by Dr. Elizabeth Tomasino and Pallavi Mohekar. Data collection and its analysis was performed by Pallavi Mohekar. The manuscript was prepared by Dr. Elizabeth Tomasino and Pallavi Mohekar with inputs from Dr. Sarah Emerson.

Chapter 4: The core study, the concept and research design was developed by Dr. Elizabeth Tomasino, Dr. James Osborne and Pallavi Mohekar. Data collection and its analysis was performed and supervised by Pallavi Mohekar. The manuscript was prepared by Dr. Elizabeth Tomasino and Pallavi Mohekar with inputs from Dr. James Osborne, Dr. Vaugh Walton and Dr. Nik Wiman.

Chapter 5: The core study, the concept and research design was developed by Dr. Elizabeth Tomasino, Dr. James Osborne and Pallavi Mohekar. Data collection and its analysis was performed and supervised by Pallavi Mohekar. The manuscript was prepared by Dr. Elizabeth Tomasino and Pallavi Mohekar with inputs from Dr. James Osborne.

Chapter 6: Pallavi Mohekar wrote chapter 6 with input from Dr. Elizabeth Tomasino.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

Page

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

Figure Page

LIST OF TABLES

CHAPTER 1

INTRODUCTION

The US wine industry sold 38 billion dollars' worth of wine in 2015 (Gordon, 2016). In 2014, US was ranked the 6th largest country for wine export with a total value of $1.5 billion dollars (Wine America, The National Association of American wineries). The economic value of wine is mainly derived from its quality which, in turn, is derived from its sensory characteristics (Hopfer et al., 2015; Sáenz-Navajas et al., 2012; Villamor and Ross, 2013).

Wine sensory consists of color, aroma, taste and mouthfeel characteristics (Styger et al., 2011a) produced by a complex interaction between volatile and nonvolatile wine fractions (Sáenz-Navajas et al., 2012; Styger et al., 2011a; Villamor and Ross, 2013). The main component of wine sensory is its aroma which is extremely complicated due to the number of factors involved. For example, aroma is perceived both orthonasally (i.e., through the nostrils) and retronasally (i.e., through the back of the mouth) (Jones et al., 2008; Sáenz-Navajas et al., 2012, 2010; Villamor and Ross, 2013). Retronasal aroma involves disparate factors such as taste, mouthfeel, product and mouth temperature, saliva and enzymes (Bult et al., 2007; Genovese et al., 2009; Jones et al., 2008; Roberts and Acree, 1995; Sáenz-Navajas et al., 2012). There are more than 800 volatile compounds that contribute towards wine's aroma perception (Ebeler, 2001; Mendes et al., 2012). Their impact on wine aroma varies dramatically with concentration. For example, dimethyl sulfide contributes positively at very low

concentrations but is perceived as offensive at higher concentrations (Segurel et al., 2004; Spedding and Raut, 1982).

The large number of aroma compounds makes the study of wine sensory and its aroma extremely challenging. However, besides its obvious impact on wine quality, the study of wine sensory has additional significance and impact. For example, a key problem in the wine and food industry is being able to predict the effect of new compounds, product composition and processing steps on consumer preference. This can be achieved by leveraging sensory techniques, chemical analyses and statistics.

In this thesis, we draw from the above areas of research in order to quantify the impact of an invasive pest called the Brown Marmorated Stink Bug (BMSB) on consumer preference of wine.

Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys*

BMSB belongs to the Hemiptera, family Pentatomidae. It is a notorious pest for its damage to crops, resistance to control measures, rapid reproductive rates and significant hitchhiking ability (Basnet, 2014; Lee, 2015; Leskey et al., 2012a). Additionally, BMSB causes urban nuisance by invading human structures such as residential homes, and commercial businesses during its overwintering stage (Hamilton et al. 2008; Hamilton, 2009; Inkley 2012; Lara et al., 2016; Lee 2015; Leskey et al., 2012).

Figure 1.1: Life stages of BMSB (Adapted from Lara et al., 2016)

Its life cycle consists of an egg stage, five nymphal stages and finally, an adult stage (Figure 1.1) (Lara et al., 2016; Leskey et al., 2012a; Rice et al., 2014). Excluding the egg state, BMSB can harm agricultural crops at each stage of its life (Acebes, 2016; Pfeiffer et al., 2012; Smith et al., 2014) which has drawn the attention of agricultural communities around the world.

BMSB in the field:

Origin and current spread: BMSB was first detected in the United States in the mid-1990's in Allentown, PA (Hoebeke and Carter, 2003). The population in US is believed to have originated from China, most likely as a hitchhiker on shipping freight (Xu et al., 2014). Since its introduction, BMSB has spread rapidly across the US and is currently found in 43 states (Figure 1.2) (Northeastern IPM Center 2016, www.stopbmsb.org). BMSB has established itself in at least 26 states (Lara et al., 2016). Among these are included extremely important wine-producing states such as California, New York, Oregon, Washington. (Lara et al., 2016, Rice et al., 2014).

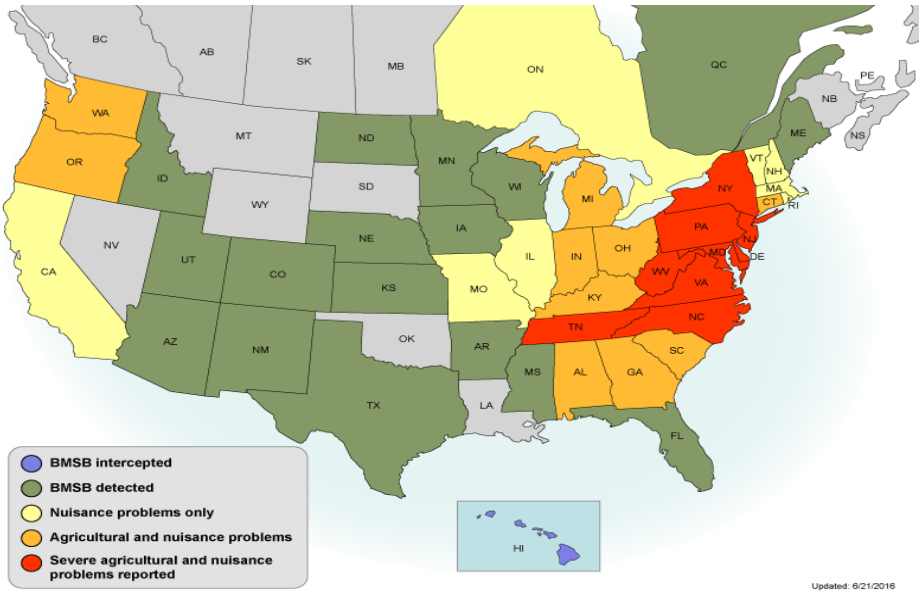

Figure 1.2. Current BMSB spread in USA (Adapted from Northeastern IPM Center 2016, www.stopBMSB.org)

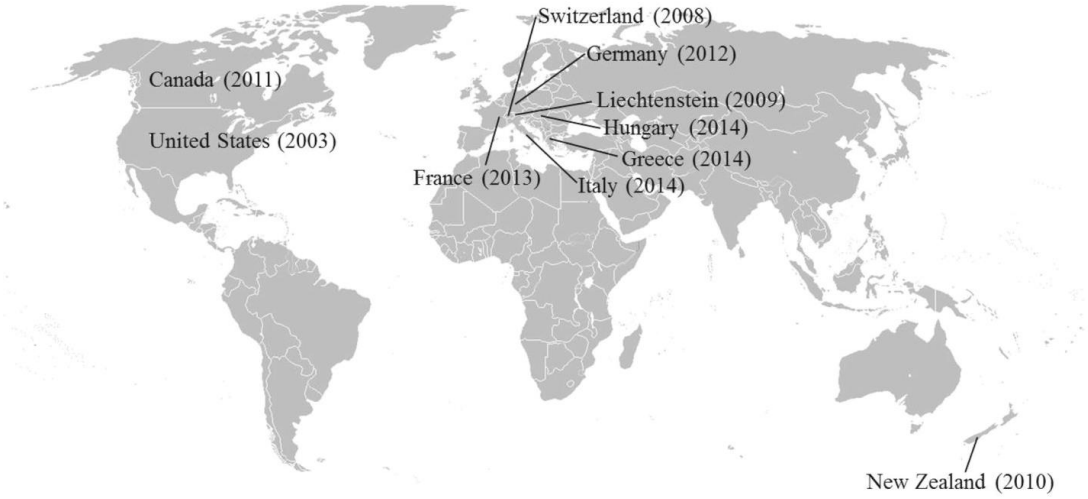

Figure 1.3. Countries where BMSB has been currently reported. The year of detection of establishment is also shown (Adapted from Lee, 2015)

Other affected countries include Canada (Fogain and Graff, 2011), Italy (Haye et al. 2014), Hungary (Vétek et al., 2014a), Switzerland (Wermelinger et al., 2008), Liechtenstein (Arnold, 2009), Greece (Milonas and Partsinevelos, 2014), France (Callot and Brua, 2013) and Germany (Heckmann, 2012) (Figure 1.3 Besides geographical spread, the BMSB problem is exacerbated by the fact that BMSB densities increase once they have been established in a location. From 2004 to 2011, the number of BMSB caught in trap networks have increased at a rate of 75% (Lee, 2015).

Agricultural impact: BMSB are known to feed on more than one hundred agricultural crops including grapes (Basnet, 2014; Leskey et al., 2012a; Pfeiffer et al., 2012; Rice et al., 2014). Feeding occurs through a piercing-sucking mouthpart which leaves visible brown or black marks and necrotic spots on fruit and leaf surfaces (Basnet, 2014; Haye et al., 2014; Leskey et al., 2012a; Nielsen and Hamilton, 2009a; Rice et al., 2014). BMSB can cause additional damage by injecting digestive enzymes that liquefy the crop tissue, which it then imbibes (McPherson and McPherson, 2000; Peiffer and Felton, 2014). Typically, the damaged crop becomes unsuitable as fresh produce. Worse, this damage increases the risk of secondary infections by other pests, lowering fruit quality and yield further (Basnet, 2014; Leskey et al., 2012a; Pfeiffer et al., 2012).

BMSB-related damage has caused major economic loss in the US. For example, in 2010, apple growers in Mid-Atlantic states lost 37 million dollars of their crop to BMSB (American/Western Fruit Grower 2011, Leskey et al., 2012a) and some Maryland growers experienced up to 90% loss of their peach crop that year (Leskey et al. 2012a). Finally, BMSB can also act as a vector of *phytoplasmas* which can lead to a type of plant disease known as "witches broom" in *P. tomentosa* (Basnet, 2014; Pfeiffer et al., 2012; Weintraub and Beanland, 2006). However, it is currently unknown if this disease can occur in the vineyard.

When dealing with pests and their impact, it is important to know when control measures are warranted. This can be done through the use of pest thresholds (Buntin, 1996; Isaacs et al., 2012). The key assumption underlying pest threshold is that a certain level of pest population is tolerable in the field (Higley and Pedigo, 1996). Thresholds are based on economic injury level (EIL), which is the minimal population density that will cause economic damage equivalent to the cost of control measures (Stern et al. 1959). Economic threshold (ET) is then defined as the pest density at which control measures need to be applied to prevent pest population from reaching the EIL (Stern et al. 1959). However, the establishment of ET requires extensive, long term research (Luna and House 1990). Therefore, ET is often replaced by action threshold (AT) which is based on previous local experiences, limited research and is often generalized over a large area (Isaacs et al., 2012; Luna and House 1990). The knowledge of these thresholds allow for an optimized pesticide application and time for biological control to take place (Isaacs et al., 2012; Luna and

House 1990). The economic threshold for BMSB in soybean has been reported to be five stink bugs per 15 sweeps (Bakken et al. 2015). However, currently there is no information available about BMSB threshold in the vineyard.

Industry response to infestation: Increasing BMSB populations and limited information about pest threshold in the field has led to a dramatic increase in insecticide application (Leskey et al., 2015, 2012a). These included broad-spectrum insecticides such as pyrethroids, organophosphates, carbamates, and neonicotinoids (Leskey et al., 2012a). Lacking any guidance on BMSB management, growers used insecticide application guidelines developed for the native stink bug (Leskey et al., 2012a). However, this did not sufficiently control BMSB, with insecticide effects wearing off three to seven days after application (Leskey et al., 2014). In some cases, populations were seen to recover even after insecticide application (Basnet, 2014). The applied insecticides proved to be toxic to beneficial arthropods which resulted in secondary outbreaks such as mites, aphids and mealybugs (Leskey et al., 2012a; Pfeiffer et al., 2012). Finally, the entire industry response conflicted with the EPA program for regulating insecticide application ("integrated pest management") (Leskey et al., 2012a). Consequently, better alternatives were sought that focused on developing more efficient monitoring and management tools for BMSB.

It is currently possible to detect nymph and adult BMSB using black pyramid traps baited with a pheromone-synergist combination (Leskey et al., 2015; Nielsen et al.,

2013). Work is ongoing on refining BMSB traps to be more practical and adoptable for growers (Morrison et al., 2015). Researchers are also exploring a long term solution using biological control for BMSB management. For example, *Trissolcus japonicus*, a primary natural enemy of BMSB in China, has shown parasitism rate of up to 70% (Yang et al., 2009). Current survey results suggest the presence of wild population of *Trissolcus japonicus* in Beltsville, MD, the District of Columbia, Virginia, and Washington (Acebes, 2016; Talamas et al., 2015). A risk assessment of biological control is underway to make sure that its release will not harm any beneficial species (Basnet, 2014). Another promising option under development is an 'attract-and-kill' system that uses semiochemicals to lure the pest to a specific area, after which insecticide is applied in small, well-contained areas (Lee, 2015). It is important to note that all of the above BMSB management techniques are still under development and not available for deployment in the field.

BMSB in the vineyard

In vineyards, BMSB has been detected in Virginia, New York and Oregon (Basnet 2014; Smith et al., 2014; Wiman et al., 2014). In Oregon, the average number of BMSB captured per trap increased from 34 BMSB in 2013 to 101 in 2015 (Walton et al., 2015). Even though BMSB has established itself in parts of California, its presence in California vineyards has not yet been reported. However, prior work shows that detection is often the precursor to a much larger BMSB population if early action is not taken (Lara et al., 2016; Pfeiffer et al., 2012).

All BMSB life stages have been observed in vineyards indicating grape to be a suitable crop for BMSB development (Basnet, 2014; Haye et al., 2015; Leskey et al., 2012a; Pfeiffer et al., 2012; Smith et al., 2014). Adult BMSB may fly back and forth between vineyards and across field borders. Nymphs, however, are restricted to vines and therefore may cause much more feeding damage to the host plant as they disperse from egg masses (Walton personal communication). It has also been estimated that presence of 5 BMSB per grape cluster may lead to 37% loss in grape yield as a result of BMSB damage (Smith et al., 2014).

BMSB populations appear to increase with higher temperatures, where as many as six generations have been seen in a warmer region compared to a single generation in a cooler climate (Leskey et al., 2012a). Additionally, temperature also affects the time BMSB exits its overwintering state. Current data suggests presence of BMSB in the field as early as April when the temperature exceeds 25^{o}C (Haye et al., 2014; Leskey et al., 2015). Data sampled from four commercial vineyards in Virginia show BMSB presence in the field as early as May and first egg masses in June (Basnet, 2014, Lee 2015). In agreement with this, the first peak in BMSB population has been observed in mid-June to early July in apple and pear orchards (Nielsen and Hamilton, 2009a). The second peak followed in late August to early September in soybean crop (Leskey et al., 2012c; Nielsen and Hamilton, 2009b), which incidentally occurs at the same time as grape harvest. Early harvest is not an option for wine grapes as it does not allow enough time for grape flavor development (Gonzalez-Barreiro et al. 2015).

Other problems occur as new BMSB generations have shown higher resistance to commonly used insecticides (Leskey et al., 2014).

BMSB in the winery

Given the lack of control measures and environmental conditions favoring BMSB population growth, it seems increasingly likely that BMSB can be harvested with grape clusters, thereby entering wine processing. Typically, this is not a significant concern in itself. For instance, fruit is washed and pre-processed before it is sold as fresh produce or processed further into juice and purees. However, these preprocessing steps are not used in wine. Grapes are typically processed without washing (Cavazza et al., 2007) since wild yeast occurring on grape skin can affect wine quality (Egli et al., 1998) and contribute towards distinct regional characteristics (e.g., Oregon Pinot noir versus New Zealand Pinot noir) (Mateo et al., 1991, Khan et al., 2000). Washing grapes can also add significant water, thereby diluting wine. Thus, washing the grapes is not a control option.

Sorting methods may provide an alternative to washing since they were effective at separating grapes from lady bugs (Martinson, 2004). Grapes can be sorted either manually or mechanically to remove non-grape materials like leaves, stems and insects (Jackson, 2008). Options include manual, optical and vibration sorting. However, there is no information on the effectiveness of any sorting method on BMSB removal. A number of challenges can be anticipated in the use of sorting

methods to combat BMSB. For example, sorting before destemming may be less effective since BMSB can hide inside grape clusters. Sorting after destemming may prove to be more effective but no results have been reported. Sorting method are also labor-intensive, time consuming and expensive (Jackson, 2008; Martinson 2004). Finally, the use of sorting methods may stress the insect into releasing additional taint compounds, as discussed in the next section.

Thus, it seems feasible that BMSB can enter wine processing, either dead or alive. It therefore becomes very important to understand the impact of BMSB on wine during every stage of processing and in particular, its impact on wine sensory and consumer preference.

BMSB compounds in wine: Previous research shows that when disturbed, BMSB tends to release a range of volatile compounds referred to as "BMSB taint" (Fiola, 2011; Tomasino, 2013). This chemical response is similar to "lady bug taint" (Pickering et al., 2004; Pickering et al., 2007a, 2007b). BMSB taint compounds serve as allomones, alarm pheromones, aggregation pheromones and kairomones and are mainly released from the dorsal abdomen glands in nymphs and paired metathoracic glands in adults (Baldwin et al., 2014; Basnet, 2014; Mohekar et al., 2015a; Solomon et al., 2013). Multidimesional Gas Chromatography Mass Spectrometry (MDGC-MS) analysis of stressed BMSB, adults and nymphs, shows more than 39 compounds in their volatile secretions (i.e., "BMSB taint") (Mohekar et al., 2015a). The main

compounds in the taint were tridecane, dodecane, trans-2-decenal and trans-2-undecen-1-ol. Other studies showed similar results and additionally reported that tridecane and trans-2-decenal (Figure 1.4) together constitute at least 70% of BMSB taint (Baldwin et al., 2014; Solomon 2013).

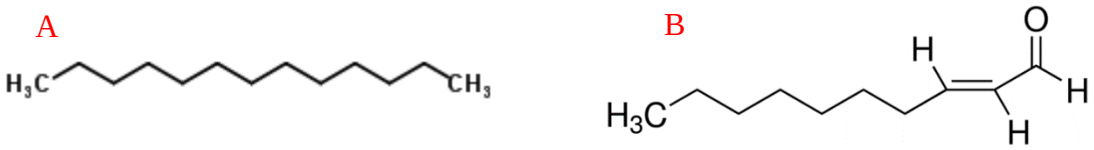

Figure 1.4. Chemical structure of tridecane (A: PubChem Substance ID 24889487) and trans-2-decenal (B: PubChem Substance ID 24858347)

After the outbreak of BMSB in Virginia in 2010, "stink bug taint" has been reported in grape juice (Basnet, 2014). The presence of the pest in grape clusters can also result in BMSB taint appearing in the finished wine (Tomasino et al, 2013). Again, this mirrors the situation with "lady-bug" taint which also appeared in the finished wine (Pickering et al, 2007b). The work by Tomasino et al. (2013), serves as the starting point for this thesis and is briefly described below. Pinot noir was made from grapes with no BMSB, one BMSB per four clusters and one BMSB per two cluster. The finished wines were analyzed using MDGC-MS. The results showed that, even in wine, BMSB taint compounds like trans-2-decenal, trans-2-octenal, trans-2-hexenal, tridecane, dodecane and tetradecane are present. The effect of BMSB taint on wine sensory was evaluated using discrimination analysis. A significant difference was found between control wine and wine made with one BMSB per two cluster.

However, this sensory test, only looked for the existence of a difference and the exact nature of the impact was not evaluated. Trans-2-decenal is typically described as "cilantro" and "green" (Eriksson et al. 2012; Lara et al., 2016). These descriptors are typically associated with low quality wine and therefore presence of trans-2-decenal may be detrimental to wine quality. Due to their negative effect, these taint compounds can also be used to establish AT and ET for pests like BMSB, which can contaminate harvested berries (Isaacs et al., 2012). A relationship between sensory perception of "lady bug taint" by human subjects and densities of lady beetle in grape clusters has been used to determine AT and ET for this pest in the vineyard (Pfeiffer et al., 2012). The goal is to apply appropriate measures in the field to keep taint levels in the wine below their sensory threshold (Galvan et al., 2007).

Effect of wine processing on BMSB taint release: Wine processing involves a number of steps, often specific to a winemaking style. The main winemaking steps include destemming, crushing, pressing, maceration, alcoholic and malolactic fermentation, post-fermentative correction, aging and bottling (Jackson, 2008). Winemakers typically perform some or all of these steps, using modifications in order to achieve a particular sensory profile in wine (Styger et al., 2011). Thus, it becomes important to understand the impact of each wine processing step on BMSB taint compounds.

Winemaking steps such as destemming, crushing, pressing involve noise, rotation, shaking and high pressure. These steps seem likely to stress BMSB, triggering taint release. The pressure applied during pressing may crush the insect, potentially releasing volatile compounds from its body and causing death. This been observed to occur for ladybugs (Kögel et al., 2012; Pickering et al., 2007). Pressing is also the last step when any additional taint can be introduced since most particulate matter (i.e. grape pomace) is removed during pressing (Jackson, 2008).

Winemaking also involves many steps where chemical reactions occur, including alcoholic and malolactic fermentation, maturation, and aging. (Costantini et al., 2009; Panighel and Flamini, 2015; Pérez-Coello and Díaz-Maroto, 2009; Polášková et al., 2008; Zamora, 2009) . During these reactions, BMSB compounds may change their form (e.g., aldehyde to alcohol) or could react with other compounds in wine. It is important to understand the chemical changes taking place and relating these to changes in wine sensory.

Overall, it is important to understand how much taint is introduced or removed by each processing step, either mechanically (e.g., pressing) or chemically (e.g., fermentation). This has large implications for winemaking protocols which is different for red and white wines. In red wine, fermentation occurs in the presence of grape pomace and is followed by pressing which means that the pest is present during fermentation. In white wine, pressing occurs before alcoholic fermentation which

means that no additional taint is introduced during fermentation. Finally, other steps like punch-downs during fermentation, maceration and malolactic fermentation are more common in red wine than white wine. Understanding the impact of each step on BMSB taint levels is critical to modeling its presence in the finished fine.

Overview of the methodology

The primary methods used in this thesis fall into two categories: a) sensory analysis via human subjects, and b) chemical analysis of wine.

Sensory Analysis

Sensory analysis consists of techniques that evoke, measure, analyze, and interpret human responses to products. The key data measured in sensory analysis are one or more of the five perceptions: sight, smell, touch, taste, and hearing (Stone and Sidel, 2004). The main application of sensory analysis is its ability to relate product composition data to consumer liking, which has direct applications to product development, targeted marketing, quality control, etc. (Lawless and Heymann, 2010; Meilgaard et al., 2006). Examples of sensory methods include discrimination testing, threshold testing and descriptive analysis. The choice of method depends on the specific study objective and multiple methods may be performed to gain additional insight. For example, a common sensory analysis task is to check if a consumer can discriminate between product versions having different compositions. If a change is detected, then the next task is to find which composition is preferable. Finally,

descriptive analysis, with trained subjects, can be used to uncover the product attributes driving the preference. In general, each sensory analysis is task-specific and requires careful choice of objective definition, method selection, panel selection and data analysis.

Sensory Thresholds

Sensory thresholds specify the lowest concentration of a target compound, above which a subject's sensory response changes. Examples of thresholds include detection, recognition, difference, terminal and rejection (Lawless and Heymann, 2010; Meilgaard et al., 2006). Applications of sensory thresholds include determining control limits on off-flavors and odors (Viswanathan et al. 1983; Stocking et al. 2001), flavor potency, and quality limits (Punter, 1983), comparing sensitivity of a subject or group of subjects (Amoore, 1971; 1979). The first choice in the design of sensory threshold estimation is deciding the type of threshold. This is determined by the experimental question being asked. In this thesis, the detection threshold (DT) and consumer rejection threshold (CRT) are the most applicable since trans-2-decenal is considered to be a taint compound in wine.

DT versus CRT: DT is one of the oldest sensory threshold methods (Engen, 1972; Harwood et al, 2012). It is defined as the lowest concentration of a target compound at which the compound is perceived. DT has been used extensively in the past to provide control limits on different compounds such as chemical contaminants

(geosmin) in water (Young et al., 1996), dimethyl trisulfide in whey protein isolate (Drake, 2007), and ladybug taint in wine (Pickering et al., 2007a). The procedure involves comparing a blank/control sample with a sample containing target compound, in a sequence of discrimination tests.

Despite its widespread use, DT has a serious shortcoming in that it does not provide any information about consumer preference, which is often of greater interest than mere detection (Prescott et al., 2005a) . This motivates a need for a consumer rejection threshold (CRT) which determines the lowest concentration of a target compound above which consumer preference is affected. The procedure to determine CRT also involves a series of sets containing blank/control as well as sample containing target compound. However, the focus is on comparing a subject's preference between two samples. In previous work, DT has been found to be lower than CRT, indicating that detection may not always lead to rejection (Harwood et al. 2012; Prescott et al. 2005; Ross et al., 2014). Therefore control measures defined based on CRT can be less expensive than DT. Since its introduction in 2005, CRT has been applied widely in the food and wine industry (Blackman et al., 2010; Campo et al., 2012; Harwood et al., 2013b, 2012a, 2012b; Methven et al., 2016a; Ross et al., 2014; Weekes et al., 2010; Yoo et al., 2012) .

Choice of discrimination test: Given the type of threshold, the next choice is the type of discrimination task to be performed. The choice of discrimination test is an

active area of research and depends on the question being asked, product being tested, target compound, the desired sensitivity in determining the existence of difference. Common choices here include 2-AFC, 3-AFC, triangle test, duo-trio and A-not A. (Lawless and Heymann, 2010; Meilgaard et al., 2006). Most of these are "forced choice" which removes subject bias (Lawless and Heyman, 2010; Perry and Hayes, 2016). The AFC (alternative forced choice) is applicable when samples differ in a single specific attribute and the test question focuses on that particular attribute (Lawless and Heymann, 2010; Meilgaard et al., 2006). For example, in 3-AFC, the subject may be given three samples, two of which are identically sweet while the third is much sweeter. The subject is asked to identify which of the three is the sweetest. In contrast, the triangle test does not identify a particular sensory attribute. Rather, it requires the subject to only identify the different sample in the set of three where again, two are identical and one is different. The triangle test is required when the experimenter does not know what attribute to look for (e.g. trans-2-decenal).

Method of threshold testing: A number of different testing methods are available to collect responses in threshold measurement such as method of constant stimuli and method of limits (Lawless and Heyman 2010). Each of these varies in how a single response is generated from a subject. Method of limits with ascending series, which is used in this study, is the most common choice (Eisele and Semon, 2005; Pickering, 2007a; Jaeger et al., 2014; Martineau et al., 1995; Meilgaard, 1993; Peng et al., 2012a). The "ascending series" has the benefit of low subject fatigue and prevents

adaptation from higher concentration which may occur in "descending series" methods (Lawless and Heyman, 2010).

Choice of concentration range: The concentrations to be tested must also be well chosen. The range should be large enough to cover the full range of sensitivities of the population. That is, the highest concentration tested should be large enough so that it can be detected/rejected by most, if not all of the subjects. On the other hand, the lowest concentration should be low enough so that it does not reduce a subject's sensitivity to subsequent tests. However, finding a concentration range that can cover the full range of panel sensitivity often involves a trade-off between concentration range and the number of sets. A large number of sets can cause panel fatigue whereas large steps in concentration can result in loss of precision. In order to reduce fatigue at high concentration, especially in subjects with high sensitivity, one may use "stopping rules" that permit a subject to stop responding after a certain number of correct responses have been recorded (Lawless and Heyman 2010). However, this can increase false positives at high concentration (Lawless and Heyman 2010).

Choice of base: A final design decision is the base (or "matrix") in which the target compound is tested. If, for example, inferences are to be made in wine, it may not be appropriate to test the target compound in water or ethanol solution. The main problem is that other components present in the product can interact with the target compound, which can influence compound's threshold (Villamor et al., 2013). The

effect of such interactions are lost in other bases. Therefore studying the target compound in its actual base (e.g., trans-2-decenal in wine instead of trans-2-decenal in water or alcohol) often has higher practical value (Perry and Hayes, 2016).

Estimating a threshold from panel responses: Given the preference data gathered using the previous methods, the next task is to estimate the value of the desired threshold. This is far from trivial due to a number of sources of variation, including but not limited to, differences between subjects, variability within a single subject and even the estimation method used. Method for threshold estimation include, best estimate threshold (BET) (E-679-04, ASTM, 2008a) and dose-response curve (Lawless, 2010). Thurstonian modelling has recently been applied since it improves significantly over the other methods, as described next.

Dose-response curve versus Thurstonian modeling: Dose-response curve is one of the most widely used methods of threshold measurement. In this method, at each concentration level, the proportion of correct responses by the panel (eg. proportion of correct detection in DT) is recorded. These proportions are then plotted against the log of concentrations. The threshold is then estimated by fitting a curve (linear or sigmoidal) and selecting a concentration based on some criteria (Figure 1.5) (Harwood et al., 2012a; Lawless, 2010; Lawless and Heyman, 2010; Prescott et al., 2005). A commonly used criteria is chance-corrected proportion of 50% (either

correct detection of sample containing target compound or correct rejection of control) (Lawless, 2010; Lawless and Heyman, 2010).

However, a key shortcoming of this commonly used method is that the threshold estimate is a function of the probability of correct responses by chance which changes from one discrimination test to the next (e.g., 0.5 in 2-AFC vs 0.33 in 3-AFC). Furthermore, the threshold estimate can also change depending on the exact question or task given to the subject (3-AFC versus triangle) (Bi et al., 1997; Ennis, 1993; Lawless, 2013; Frijters, 1979). These are serious disadvantages since the threshold should ideally be independent of the method used, or at least, comparable across methods. In this thesis, a more sophisticated technique, based on Thurstonian modeling, is used to estimate thresholds without the aforementioned issues.

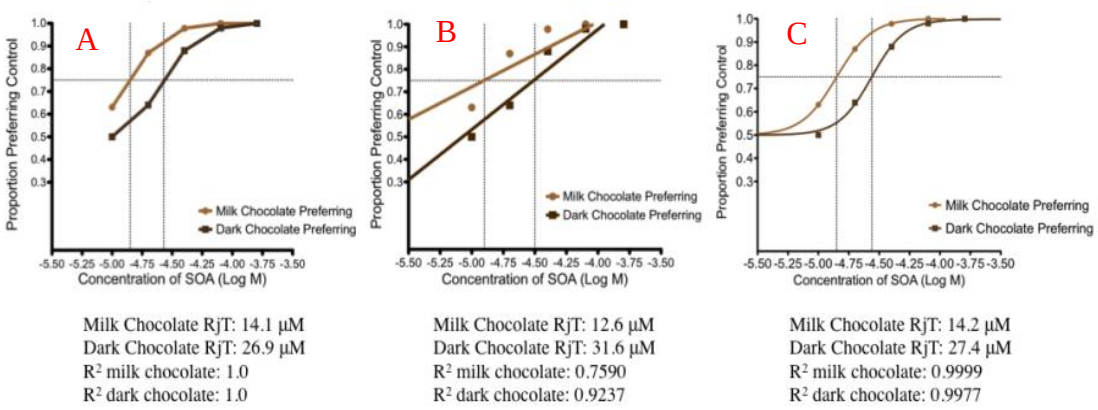

Figure 1.5. Dose-response curve fitted using A) segmental (piecewise linear), B) linear and C) sigmoidal fit. Threshold is calculated at chance corrected proportion of 50% (Adapted from Harwood, 2013a)

Thurstonian modeling (Thurstone, 1927) generalizes the definition of threshold using signal detection theory (Green & Swets, 1966; Klein, 2001; Macmillan & Creelman, 1991). The primary assumption in this method is that the sensory response of a single subject on a particular sample is normally distributed (Green & Swets, 1966). The subject's response to both the control sample as well as the sample containing target compound are assumed to be normally distributed with different means but identical standard deviations, as shown in Figure 1.6 (Thurstone, 1927).

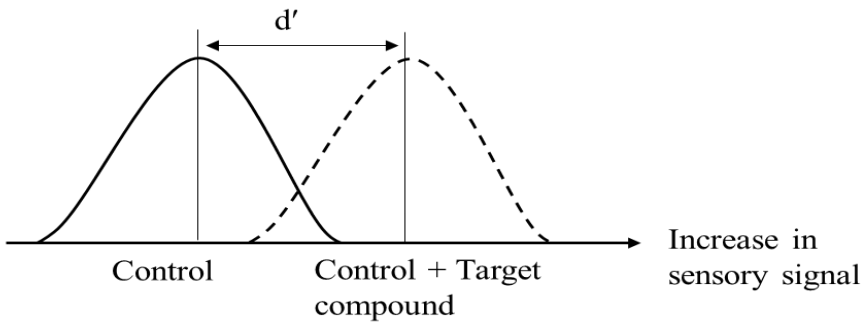

Figure 1.6. d′ in a discrimination testing (Adapted from Lawless and Heymann, 2010)

The key concept is a parameter, d', which is an estimate of the difference between the two means. The computation of d' depends on, both the proportion of correct responses as well as the particular discrimination test used (Bi 2006; Ennis, 1993; Frijters, 1979; Lawless, 2013). Therefore, d′ is method independent and solely based on subject's sensory response. d' is zero when no difference exists and is a large positive value when the two responses are far apart (Lawless and Heyman, 2010). This allows a threshold test to be defined where a difference is said to exist above some chosen value of d' (e.g., one) (Peng et al., 2012; Rousseau, 2015; Klein 2001) or if it deviates significantly from zero (used in this study).

Descriptive Analysis (DA)

In DA, the goal is to obtain an overall description of the sensory attributes of a product and determine the nature of sensory differences among products. The outcome of DA is typically a better understanding of the factors driving consumer acceptance or rejection of a product (Lawless and Heyman 2001; Meilgaard et al., 2006). Therefore, DA is typically performed after discrimination analysis and/or threshold testing, once products have been found to be different. Previous applications of DA include identifying key attributes underlying wine styles from different region, wines made using different protocols, and wines containing taint compounds (Cadot et al., 2010; Francis et al., 1992; Gutiérrez Afonso, 2002; Reynolds et al., 1996; Tomasino, 2011; Weekes et al. 2009).

During DA, various product attributes are rated by trained subjects, resulting in a profile of that particular product, allowing products to be compared. Unlike threshold methods, DA requires subjects trained extensively on various attributes of the product or experts (Lawless and Heyman 2001; Meilgaard et al., 2006; Parr et al., 2007). It also requires relatively few but well-trained subjects, compared to other sensory tests. The disadvantages of DA are primarily that it is time consuming and expensive. Training requires a long-term commitment from the subjects and recruitment of such individuals can be difficult.

A recent trend has been the use of wine experts or wine professionals in place of trained subjects. Wine experts can often discriminate, recognize and describe different wines, due to their extensive product knowledge, vocabulary of attributes and knowledge of the varietal characteristics (Hughson and Boakes, 2002, 2001; Parr et al., 2002; Perrin et al., 2007; Zamora and Guirao, 2004). Prior work has shown that experts with more than 15 years of experience are better at replicating attributes for a given wine as well as differentiating among wine samples, compared to trained subjects with less experience in wine tasting (Zamora and Guirao, 2004) . Wine professionals also tend to have a similar vocabulary of wine attributes (Ballester et al., 2008) and produce consistent results that are comparable to results obtained from trained sensory panel (Hopfer and Heymann, 2014; Perrin et al., 2007; Zamora and Guirao, 2004)

Wine chemical Analysis (Aroma compounds)

Although the sensory analysis methods described in previous sections provide significant details about products, additional information and insight can be obtained from chemical analysis. Specifically, chemical analysis reveals the key compounds in a product which can then be related to the sensory profiles of the product. For example, aroma analysis provides the concentration of 3-Isobutyl-2-methoxypyrazines in Sauvignon blanc which is strongly responsible for “bell-pepper” aroma (Styger et al., 2011a). Relating chemical analysis data to sensory analysis data is extremely challenging and is an active topic of research. The ability to predict

impact to sensory attributes given only the protocols and compositions as input has large commercial applications, especially in product design and reformulation.

In this thesis, aroma analysis is used to measure the concentration of BMSB taint compounds in wine. The main steps in aroma analysis include extraction, separation and identification. The most common method used to analyze volatile aroma compounds in grape and wine is Headspace Solid Phase Microextraction Multidimensional Gas Chromatography Mass Spectroscopy (HS-SPME-MDGC-MS) (Mendes et al., 2012; Siebert et al., 2005). The method involves extraction of volatile aroma compounds from wine by HS-SPME, separation of a mixture of volatiles on GC and identification of each compound by MS. The suitability of this method for the analysis of volatile and semi-volatile compounds present in wine as well as volatile secretions of BMSB has been shown in previous studies (Baldwin et al., 2014; Cai et al., 2007; Câmara et al., 2006; Culleré et al., 2011; Díaz-Maroto et al., 2004; Francioli et al., 1999; Lona-Ramirez et al., 2016; Rebière et al., 2010; Siebert et al., 2005; Solomon et al., 2013; Song et al., 2015; Tomasino et al., 2013; Muñoz- González 2011, Wiman and Tomasino 2015 *in review*; Panighel 2014).

The extraction method used here, HS-SPME, has numerous advantage over other available methods such as liquid-liquid extraction and stir-bar extraction. In this method, aroma compounds from the headspace of a sample vial are adsorbed onto a fused-silica fiber that has been coated with a polymeric material(s) (Ebeler, 2001;

Panighel and Flamini, 2015). The method of HS-SMPE is rapid, highly sensitive, reproducible and effective. Other benefits include lack of solvent for extraction, small sample volume, cost efficiency and simultaneous analysis of multiple sample (Muñoz- González 2011; Panighel and Flamini, 2015; Polášková et al., 2008). The method has gained widespread application as an extraction technique in food and wine (Mendes et al., 2012).

In a complex mixture like wine, one GC column may not be sufficient to achieve efficient separation of different compounds (Legrum et al., 2014; Marriott et al., 2012). Therefore, a heart-cut multidimensional gas chromatographic analysis which utilizes two or more GC columns connected in series can be utilized (Robinson et al., 2011). In this method, compounds of interest eluting from first GC can be separated further on the second GC column. Columns often use different polarity as well as different temperature program for separation (Muñoz-González et al. 2011).

For quantitative analysis, HS-SPME-MDGC-MS is often coupled with a method of internal standard addition. This method can compensate for variation due to sample preparation, drifts in instruments and matrix effect (Robinson et al., 2009) when compared to a method of external standard addition. In the method of internal standard addition, samples are prepared by adding a known amount of standard compound that is structurally similar but not identical to the target compound. Deuterated analogs of the target compounds are the ideal choice as they are inert but

their availability and cost can create challenges (Muñoz-González et al., 2011). Overall, HS-SPME-MDGC-MS in combination with method of internal standard addition can provide a rapid, cost effective and efficient aroma analysis.

Objective and contributions

In this thesis, we leverage data from wine sensory, wine processing and wine aroma to gain a deeper understanding about the impact of BMSB taint on wine quality and consumer preference.

The objectives of this thesis are as follows:

Objective 1: To determine the impact of trans-2-decenal on Pinot noir and Merlot wine sensory

Objective 2: To determine the critical stages of red and white wine processing responsible for BMSB taint secretion and threshold limits of BMSB in the vineyard

Objective 3: To determine the efficacy of commonly used winemaking procedures for the removal of BMSB taint compounds

Contributions of this thesis:

- Establishes consumer rejection threshold (CRT) of trans-2-decenal in Pinot noir and Merlot. This will allow the wine industry to establish control limits for trans-2-decenal in red wines.
- Introduces a segmentation criterion to create consumer groups with different CRT for trans-2-decenal in red wine. Relates consumer groups to consumer variables such as involvement, knowledge and experience, thereby uncovering some of the drivers of consumer segmentation. This relationship may be

useful in order to identify and target the appropriate consumer group for BMSB-impacted wines.

- Estimates the impact of each wine processing step in terms of BMSB taint release in red and white wine. This will help winemakers design winemaking protocols that minimize taint levels in finished wine.
- Relates the threshold density of BMSB in grape clusters to the CRT of trans-2-decenal in wine. This will assist vineyard managers in designing effective control measures for BMSB in the vineyard.
- Finally, evaluates the effectiveness of fining agents and reverse osmosis in reducing BMSB-taint levels in finished wine. This information will be useful in minimizing the impact of BMSB taint on wine quality.

Taken together, this thesis constitutes a detailed study into the impact of BMSB on the wine industry, from the winery, through post-harvest processing and ending with consumer preferences via sensory analysis.

CHAPTER 2

Brown Marmorated Stink Bug taint in Pinot noir: Detection and Consumer Rejection Thresholds of trans-2-decenal and effect to wine quality

Pallavi Mohekar[1], Trina J Lapis[1], Nik G Wiman[2], Juyun Lim[1] and Elizabeth Tomasino[1,*]

[1]Oregon State University, Department of Food Science & Technology, 100 Wiegand Hall, Corvallis, Oregon 97331

[2]Oregon State University, Department of Horticulture, 4017 Agriculture and Life Sciences Building, Corvallis, Oregon, 97331

[*] elizabeth.tomasino@oregonstate.edu, (T) (541)-737-4866, (F) (541)-737-1877

- **Acknowledgments**: the authors would like to thank Oregon State University and the Oregon Wine Research Institute for funding. Additional support came from USDA-NIFA-SCRI #2011-51181-30937. The participation of all sensory panelists is acknowledged and we would like to thank Firesteed winery (Rickreall, OR) for donation of untainted wine.

Short version of title: BMSB taint in Pinot noir

As seen in American Journal of Enology & Viticulture (2017): Volume 68, Issue 1

ABSTRACT

Brown marmorated stink bug contamination in grape clusters results in the addition of an aroma compound, trans-2-decenal, in wine. Described as a green, musty aroma, it is considered detrimental to wine quality. The main focus of this study was to estimate the detection and consumer rejection thresholds of trans-2-decenal in Pinot noir, determine its impact on wine quality and explore potential consumer segmentation. Thresholds were measured using an ascending forced choice method of limits applied to a series of triangle and paired comparison tests. Thresholds were estimated by a psychometric function with significance based on binomial distribution as well as d′ values based on Thurstonian models. The method of quantification resulted in different threshold levels. The detection threshold of the panel was estimated to be 0.51µg/L from a psychometric fit and between 1.92 and 4.80µg/L based on Thurstonian scaled values. Similarly for consumer rejection threshold, psychometric function resulted in a threshold of 13µg/L and d′ values between 4.80 and 12.00µg/L. Wine containing trans-2-decenal above consumer rejection threshold was described as green, musty and less fruity by wine professionals. When potential consumer segmentation was explored, based on the detection and consumer rejection threshold data, there was no direct link between sensitivity and preference. Based on such findings, the use of consumer rejection threshold is recommended when establishing consumer tolerance levels of trans-2-decenal in wine. Additionally, the use of d′ which provides a more sensitive method of threshold estimation seems more appropriate for compounds that negatively impact wine quality, such as trans-2-decenal.

Keywords: consumer rejection threshold, Thurstonian model, Pinot noir, sensory, BMSB

Introduction

Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is an exotic pest believed to have arrived in the United States in the mid-1900s from Asia. This pest has caused severe crop damage to fruits and vegetables in the Mid-Atlantic region of the United States (Pfeiffer et al. 2012). If BMSB are harvested with wine grapes, they can contaminate the juice and final wine (Tomasino et. al., 2013). Moreover, the movement of high numbers of these insects to buildings, including wineries, can provide another opportunity for BMSB to effect wine.

The chemical defenses of BMSB cause contamination of wine due to the release of trans-2-decenal and other compounds when the insect is "stressed" during winemaking (Tomasino and Wiman 2015, *in review*). Trans-2-decenal has been found in wine made from stink bug containing grapes (Tomasino et al 2013). The defense compound of BMSB, trans-2-decenal is not normally found in wine and there is no information regarding the impact of this compound on wine quality or consumer acceptability in wine.

Detection threshold (DT) is the traditional metric for determining the concentration at which a stimulus becomes perceivable. While DT provides useful information regarding the lowest concentration that evokes a sensation (Marin et al. 1991), it does not provide information on whether the sensation is considered positive or negative.

The limits obtained using DT are very low and do not provide a concentration above which consumer preference may be affected (Prescott et al. 2005).

For the food and wine industry, the main application of DTs is to define control limits of taints and off-flavors (Lawless and Heymann 2010, Meilgaard et al. 2007). But if consumers still find the product acceptable at DT levels, then a different threshold provides more valuable information. The consumer rejection threshold (CRT) is the level where a consumer will reject or dislike a product (Prescott et al. 2005). Previous studies have shown that CRT is normally higher than DT (Prescott et al. 2005, Saliba et al. 2009, Yoo et al. 2012). Therefore, products tainted at levels below CRT can be considered acceptable to consumers. Additionally, products containing taint levels below CRT can still be marketed while corrective measures are developed to reduce the taint (Lesschaeve 2007).

Because there is not an overwhelming negative or positive association for trans-2-decenal in wine, simply estimating the DT may not provide complete information regarding the nature of its effect on wine quality. The aroma of the pure compound does suggest that it will be detrimental to wine quality, as green, musty aromas are not considered favorable for red wine quality. Green aromas are associated with unripe characteristics and mustiness is associated with oxidation. Therefore, a consumer response toward wines containing various levels of trans-2-decenal is desired. The absence of this compound in Pinot noir wine makes it an excellent model to study both DT and CRT thresholds.

Consumers have shown a distinctive divided response to the green aroma of trans-2-decenal (Donega et al. 2013), specifically for the herb, coriander (cilantro), of which trans-2-decenal is a major component (Mauer 2011). Individuals either strongly like or strongly dislike the coriander aroma (Knaapila et al. 2012), and these preferences may be linked to genetics (Eriksson et al. 2012). Prior liking or disliking of trans-2-decenal may influence the preference for wine containing trans-2-decenal. This can potentially segment panelists into various groups with different threshold levels for trans-2-decenal in wine.

Because of the current factors limiting successful control of BMSB in the field and the inability to manage BMSB without an intensive chemical program, there is a need to determine post-harvest effects of this pest. But first control levels are required. The objectives of this study were: 1) to measure DT and CRT of trans-2-decenal in Pinot noir wine, 2) to investigate the effect of trans-2-decenal on perceived wine quality, 3) to investigate any potential segmentation that occurs for DT and CRT of trans-2-decenal.

Material and Methods

DT and CRT Analysis

Participants A total of 72 participants (41 F, 31 M) between 21 and 63 years of age (mean = 34 years) were recruited at Oregon State University campus and the surrounding area. Participants had no previous formal training on sensory evaluation of foods or wine. Participant inclusion criteria were individuals who 1) were older

than 21 years of age; 2) had no mouth sores and/or oral disorders; 3) were free from allergies to wine or wine components; and 4) consumed red wine at least once a week. The majority of the panel (72%) consisted of Pinot noir consumers, whereas the remaining 28% were distributed among Merlot and Cabernet Sauvignon drinkers. Participants were told that the study was a test of difference (for DT) and preference (for CRT) for wines. No information was collected pertaining to knowledge of BMSB taint as this is a new problem and is not yet widely recognized. The experimental protocol was approved by the Oregon State University Institutional Review Board. Study participants gave written informed consent and were compensated for their participation.

Stimuli A commercial Pinot noir wine (2010, Oregon) was selected as the base wine and determined to be free of trans-2-decenal (using GCMS analysis, data not shown). The wine was purchased directly from the winery and stored at 12°C until use.

Trans-2-decenal (95%, CAS # 3913-81-3, Sigma-Aldrich co.) was added to the base wine at increasing concentrations: 0 (blank), 0.05, 0.12, 0.31, 0.77, 1.92, 4.80, 12.00, and 30.00 µg/L. These concentrations increased by a factor of 2.5 and were chosen based on published threshold values for trans-2-decenal in beer (Meilgaard 1993) as well as preliminary studies (E. Tomasino, unpublished data). The same concentration series was used for both DT and CRT measurements. Two stock solutions (1.5 and 50 mg/L) of trans-2-decenal were prepared in 14% ethanol and stored at 18°C. The 1.5 mg/L solution was used for the first five concentrations and the 50 mg/L solution for

the last three concentrations. The two stock solutions were used to ensure that the ethanol content of the wine remained consistent in all levels (14%), as ethanol content is known to influence perception of aroma compounds (Goldner et al. 2009). Trans-2-decenal was added to the base wine an hour prior to the tasting sessions and the test samples (15 mL) were poured five min prior to tasting to prevent any potential loss of volatiles. Samples were served in clear INAO wineglasses (*INAO*, 2008) at room temperature (21 ± 2°C) and covered with a watch glass.

Threshold Procedures All sessions were conducted in the afternoon and under similar light and temperature conditions (21 ± 2 °C). DT and CRT sessions were scheduled at least two weeks apart. About half (53%) of the panel participated in DT tests first and the rest participated in CRT tests first. The counterbalancing of the order of tests was done to ensure that CRT was not impacted by previous DT tests, as repeated testing results in a decrease in threshold values (Pierce et al. 1996).

Detection threshold was measured using a series of eight triangle tests corresponding with trans-2-decenal levels in ascending order (Lawless 2010). Each set contained two blank samples and one trans-2-decenal added sample. Participants were instructed to place the entire sample (15 mL) in their mouth, swirl it around for few seconds, expectorate, and then identify the different sample on a paper ballot. Therefore differences presumably are based on retronasal aroma, as trans-2-decenal is not known to influence taste or mouthfeel perception.

All samples were coded with three-digit random numbers and the position of the altered sample was randomized across each set through the whole concentration series. A one minute break occurred after each set and a five-minute break after the fourth set to reduce potential fatigue and adaptation. During each break, participants were instructed to rinse their palate with water and eat an unsalted cracker to minimize any carry-over effect (Colonna et al. 2004). Participants were allowed to terminate their test series when they made three successive correct identifications (Peng et al. 2012). Note that the probability of making correct identification in three consecutive triangle tests by chance is below 5%.

CRT testing followed the same procedure as DT test except for two factors;

(1) Rejection threshold was measured using paired preference tests (Prescott et al. 2005), corresponding with trans-2-decenal levels in ascending order. Each pair consisted of one sample of base wine (blank) and one trans-2-decenal added wine. Participants were asked to select the sample of their preference on a paper ballot.

(2) Participants were allowed to terminate their test series when they reported preference of control sample four times successively. Again, the chance of reporting preference to the control sample without recognizing the difference between samples four times in-a-row (i.e., 1/16) is about 5%.

Data Analysis Two different methods were used to calculate thresholds as it is evident from many studies that using different methods results in very different concentrations (Lawless 2010, Peng et al. 2012). The first method for calculating

thresholds was based on binomial distribution tables (Lawless and Heymann 2010, Meilgaard et al. 2007) for triangle and paired comparisons tests, respectively. The second method utilized d′ values that were calculated as a function of proportion correct for DT tests and proportion preferring control for CRT tests (Ennis 1993). Thurstonian model was chosen as it is not confounded with a response bias and because d′ varies on an equal-interval scale and does not have the same boundaries as traditional accuracy measurements (Stanislaw and Todorov 1999). Significance at each trans-2-decenal concentration for both DT and CRT was then calculated for triangle and 2-AFC tests (Bi et al. 1997). The d′ values were compared with $d' = 0$ following Bi et al. (1997), and DT and CRT was defined as the minimum concentration at which d′ values were significantly different from zero (p-value < 0.05).

Descriptive Analysis

Participants A professional panel was used to determine the effect of trans-2-decenal on wine quality. Eighteen wine professionals (10-M, 8-F) were recruited from the Oregon wine industry. Each panelist had more than 10 years of experience tasting Pinot noir. All other inclusion criteria were conserved from the threshold tests. Participants were told that they were evaluating Pinot noir aroma. Study participants gave written consent and the experimental protocol was approved by the Oregon State University Internal Review Board.

Stimuli A 2010 Oregon Pinot noir wine that was free of BMSB taint was used as the base wine, the same wine used for the threshold study. Trans-2-decenal concentrations added to the base wine were 0, 0.2, 5, 12 and 30 µg/L. The concentration levels were chosen to determine the impact of trans-2-decenal on wine quality at levels above and below the calculated DT and CRT. Trans-2-decenal was added to the wine one hour prior to the tasting session.

Procedure Three, 2-hour, tasting sessions were conducted. Two sessions occurred in the morning and one session in the afternoon. All sessions were conducted in the same room under similar light and temperature conditions (21 ± 2 °C). All samples were evaluated in duplicate. 30 mL of each wine sample was served in INAO clear glasses (*INAO*, 2008) with random three-digit numbers. Wines were served in two different flights, each flight containing five wines. Panelists evaluated 10 orthonasal (aroma) and 3 retronasal (in mouth flavor) attributes for each wine. Orthonasal aroma attributes included: dark fruit, red fruit, earthy, musty, herbal, fresh green, spice, jam, floral, vegetal. Retronasal aroma attributes included: fruit density/concentration, green, and spice. Orthonasal aromas were evaluated before retronasal for each wine. These attributes were chosen from preliminary tastings (data not shown) and previously reported descriptors associated with BMSB in wine. To prevent the dumping effect, panelists were also given several categories in which they could write in their own descriptors. The intensity of each attribute was reported on a 100 mm visual analog scale with indented word anchors, none and extreme at 10 mm. The data was collected on a paper ballot. To reduce any fatigue and carry over effects,

panelists were given a one-minute break between each wine and each flight was separated by a 15-minute break. Panelists rinsed their mouth with water after each wine sample and were required to rinse with water and eat an unsalted cracker during the 15-minute break.

Data Analysis Intensity ratings were quantified using a number between zero and 100 that corresponded to the distance on the VAS scale from the left end to the written mark. Canonical variate analysis (CVA) was carried out with the trans-2-decenal concentration as the classification variable. Data was standardized prior to analysis. CVA analysis was carried out using XLStat (Addinsoft, New York, NY, USA).

Results

Detection threshold A method of extrapolation from the point at which the proportion correct reached the criterion for binomial significance resulted in a detection threshold of 0.51 µg/L (Figure 1). The calculated detection threshold using signal detection theory is between 1.92 and 4.8 µg/L (Table 1).

Consumer rejection threshold Two CRT's, 0.05 and 13.00 µg/L, were estimated by extrapolating from a point at which proportion preferring control reached a binomial significance (Figure 2). The CRT curve in Figure 2 is unusual as it is "U" shaped, and does not resemble more traditional threshold curves (Prescott et al. 2005). Thurstonian model also predicted two calculated thresholds for CRT, at < 0.05 µg/L

and between 4.80 and 12.00 μg/L (Table 1). The first of the two thresholds calculated using extrapolation and signal detection theory are below the calculated detection thresholds.

Descriptive Analysis Due to panel inconsistencies for herbal and spice aromas (data not shown), these two attributes were removed from the analysis. Significance was found for the first two variates (α=0.05) and accounted for 51 and 34% of the total variance (Figure 3). Clear separation was noted for wines with different concentrations of trans-2-decenal (Figure 3), resulting in three clear groupings; control wine (no trans-2-decenal), wines with lower concentrations of trans-2-decenal (0.2 and 5 μg/L) and wines with higher concentrations of trans-2-decenal (12 and 30 μg/L). The loadings of sensory terms suggest that wines without trans-2-decenal have more intense floral aromas. Wines with trans-2-decenal concentrations near the calculated DTs are described as possessing more intense spice flavor, jam, dark fruit and earthy aromas. Wines with trans-2-decenal levels near or above CRTs were found to have more intense green aromas and flavors (i.e., musty, herbal, fresh green, vegetal and green). The positioning of the sensory loadings, with the green associated aromatics opposite the fruit flavor aromatics, show a relationship with less fruit based aromas and more green aromatics as concentrations of trans-2-decenal increase.

Segmentation based on sensitivity and preference Due to the unusual results for CRT, namely the calculation of two different rejection thresholds, a further look into participants' sensitivity and preference was conducted. First, sensitivity to trans-2-

decenal was investigated by grouping individuals based on their sensitivity, as differences between participants' individual thresholds can be as wide as 100-fold (Liacopoulos et al. 1999). Based on the individual responses gathered over the eight sets in DT, participants were categorized into three groups defined as:

- Group 1: correct detection for spiked sample in at least six out of eight trans-2-decenal levels
- Group 2: correct detection for at least three out of eight trans-2-decenal levels/ remaining participants
- Group 3: correct detection for at most two out of eight trans-2-decenal levels

The criterion for group 3 was based on the random probability for triangle test across eight sets (i.e., 2.64). Because trans-2-decenal acts as a taint in wine, the more conservative level of rounding down was chosen as the chance level. These grouping criteria were then applied when evaluating both DT and CRT.

Participants showed different sensitivity to trans-2-decenal (Table 2a). The most sensitive (Group 1) detected trans-2-decenal between 0.05 and 0.12 µg/L, mid sensitive (Group 2) between 1.92 and 4.80 µg/L and the least sensitive (Group 3) could not detect trans-2-decenal at any given concentration. The distribution of participants in each group also varied (Table 2a) with the greatest portion of participants in Group 2.

Sensitivity does not appear to be related to preference of trans-2-decenal as trends for DT did not hold for CRT data. Specifically Group 1 did not reject the trans-2-decenal

spiked wines at low concentrations (Table 2b). Interestingly, all groups had the same rejection threshold at 30.00 µg/L. Group 2 also rejected the trans-2-decenal spiked wines at 0.05 µg/L, but not from 0.12 to 12 µg/L. Therefore sensitivity to trans-2-decenal concentration did not provide an explanation for the low and high concentration calculated CRTs.

To further explore data for CRT of trans-2-decenal in Pinot noir, individuals were grouped based on their CRT data (Table 3). Grouping criteria were similar to DT criteria but replaced detection with preference. Individuals who preferred the control wine for at least six out of eight tests were in Group 1, preference for control over trans-2-decenal added wine for three to five out of eight in Group 2 and preference for at most two out of eight in Group 3. In Group 1, constituting 28% of the population, preferred the control over the spiked wines at all levels, with CRT below 0.05 µg/L. Group 2, constituting 65% of the population, did not reject trans-2-decenal containing wine until the concentration exceeded 12 µg/L whereas in Group 3, constituting 7% of the population, the CRT was above 30µg/L. As with DT data in which participants can be segmented based on sensitivity, participants can also be segmented based on preference.

Discussion

DT and CRT of trans-2-decenal in Pinot noir wine

Calculation of DT using binomial distribution resulted in a traditional detection curve, while the U shaped curve for CRT was more puzzling. Two thresholds for CRT were

also found when calculating CRT using Thurstonian modeling. It is known that aroma compounds found at concentrations below their perception threshold may alter aroma perception (Ferriera 2007) and trans-2-decenal may have been altering sensory perception at both low and high concentrations. However the segmentation of consumers based on preference shows a good explanation for the two calculated thresholds. These results suggests that for CRT other information, such as preference, may need to be applied to the calculation of CRT. Previous research in wine has shown various consumer groups (Bindon et al. 2014) and it is most likely that to clearly understand preference based measurements for many food products, consumer segmentation should be investigated.

Descriptive Analysis: Effect of trans-2-decenal on wine quality

The impact of trans-2-decenal on wine quality was of particular interest due to the possible influence the compound may have on aroma. Compounds may act as aroma enhancers at low concentrations and then impart their own aroma at higher concentrations (Ferreira 2007) and the aroma activity of trans-2-decenal in wine was unknown. Of the three groupings found (Figure 3) it was at the two highest concentrations (12 and 30 µg/L) that aroma descriptors associated with lower quality wines, specifically green and musty aromas, were noted. While the aromatics of the wine did change at low concentrations, relating to the lower calculated population CRTs, the increase in fruit and spice aromatics are considered to be positive changes for red wine quality. The change in aromatics at low concentrations does not explain

the lower consumer rejection thresholds, as typically consumers "like" fruit aromas in their red wine (Bruwer et al. 2011).

Consumer segmentation

Consumer segmentation based on sensitivity and/or preference may explain the calculated CRTs. Conventionally, CRT is at higher concentrations than DT (Prescott et al. 2005, Saliba et al. 2009, Yoo et al. 2012). However, this study and that of Harwood et al. (2012), did not show a relationship between DT and CRT. Therefore sensitivity does not drive preference for trans-2-decenal and an alternative explanation must be considered.

Trans-2-decenal is one of the main aroma compounds associated with coriander (Potter 1995). Eriksson et al. (2012) have shown that a cluster of olfactory genes, OR6A2, are responsible for detection of aldehyde compounds found in coriander. Presence of these genes may be linked to perception of a strong soapy smell associated with coriander. Individuals who perceive coriander as soapy, rather than the traditional green aroma, have a strong "disliking" to the flavor. It is possible that the individuals who did not prefer trans-2-decenal in wine at all levels may have OR6A2 and therefore already have a strong disliking for the aroma of this compound. Another possible explanation for the lack of relationship between sensitivity and preference may be due to cultural factors, culture being linked to development of preferences over time (Prescott and Bell 1995, Tu et al. 2010). Preference of trans-2-decenal has not been studied prior to our work but disliking of coriander has shown to vary among different ethno-cultural groups

(Mauer and El-Sohemy 2012). There may be ethno-cultural implications that are driving the segmentation shown in this study. Incorporation of genetic testing for coriander preference or additional questions on ethno cultural information and preference for coriander may correlate with the consumer segmentation for CRT of trans-2-decenal in Pinot noir, although it was not included in this study. This would be of interest to include in future work on trans-2-decenal.

The results from segmentation and the differences in sensitivity and preference of trans-2-decenal have implications for food and wine production. Specifically, CRT can be used to indicate when the wine industry needs to be concerned about wine quality. Much effort is currently aimed at improving controls for BMSB and other stink bugs that produce trans-2-decenal in crops (Bergh 2013; Biddinger et al. 2012). However, despite these efforts BMSB continues to expand worldwide into important wine producing areas. The use of DT, a metric normally used for wine spoilage issues, does not accurately reflect sensory impact. This study has shown that trans-2-decenal concentrations at DT and the low CRT have a positive influence on Pinot noir aroma. It is only at high concentrations that negative aromatics occur. This phenomenon has also been shown for other wine spoilage compounds such as dimethyl sulfide (Segueral et al. 2004) and may be the case for other compounds associated with wine flaws or food spoilage associated compounds. Therefore, CRT is a more appropriate measurement when evaluating negatively associated aromas and flavors. The segmentation information and determination of the causes of CRT

segmentation are important tools for food producers and winemakers to use to market their products to the corresponding consumer group.

Conclusions

Detection and consumer rejection thresholds (DT and CRT) were determined for trans-2-decenal in Pinot noir wine. Different threshold levels were found depending on calculation method. A Thurstonian scaled value is preferred over the more traditional methods as it is more sensitive, particularly as trans-2-decenal in Pinot noir can be considered a flaw. Segmentation of the data also showed three different consumer groups for both DT and CRT. CRT of trans-2-decenal in wine is important for the wine industry as it provides a metric to ensure that wine quality is not affected by pests such as BMSB. For this and other wine spoilage associated compounds, we suggest using CRT as the level of concern for wine quality over DT. CRT has more direct implications for economic impact than DT. Future research must link the CRT level with pest density in order to appropriately establish economic thresholds for vineyard management activity. The CRT also provides a threshold that can be used by industry when considering remedial treatments for wine tainted by BMSB.

Literature Cited

Bergh, J.C. 2013. Single insecticides targeting brown marmorated stink bug in apple. Arthropod Manage. Tests. 38: A2

Biddinger, D., J. Tooker, A. Surcica, and G. Krawzyk. 2012. Survey of native biocontrol agents of the brown marmorated stink bug in Pennsylvania fruit orchards and adjacent habitat. Pa. Fruit News 42: 47–54.

Bi, J., D.M. Ennis, and M. O'Mahony. 1997. How to estimate and use the variance of d′ from difference tests. J. Sens. Stud. 12(2): 87–104.

Bindon, K., H. Holt, P.O. Williamson, C. Varela, M. Herderich, and L. Francis. 2014. Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 2. Wine sensory properties and consumer preference. Food Chem. 154: 90-101

Bruwer, J., A. Saliba, and B. Miller. 2011. Consumer behaviour and sensory preference differences: implications for wine product marketing. J Consum Mark. 28(1): 5-18

Colonna, A.E., D.O. Adams, and A.C. Noble. 2004 Comparison of procedures for reducing astringency carry-over effects in evaluation of red wines. Aus. J. Grape. Wine. Res. 10: 26-31

Donega, M. A., S.C. Mello, R.M. Moraes, and C.L. Cantrell. 2013. Nutrient uptake, biomeass yield and quantitative analysis of aliphatic aldehydes in cilantro plants. Ind Crop Prod. 44: 127–131.

Ennis, D.M. 1993. The power of sensory discrimination methods. J Sens Stud. 8(4): 353–370.

Eriksson, N., S. Wu, C.B. Do, A.K. Kiefer, J.Y. Tung, J.L. Mountain, D.A. Hinds, and U. Francke. 2012. A genetic variant near olfactory receptor genes influences cilantro preference. Flavour 1(1): 22.

Ferreira, V., A. Escudero, E. Campo, and J. Cacho. 2007. The chemical foundations of wine aroma- a role game aiming at wine quality, personality and varietal expression. Proceedings of the 13th Australian Wine Industry Technical Conference, Adelaide South Australia. Australian Wine Industry Technical Conference, Inc: 142–150.

Goldner, M. C., M.C. Zamora, P. di Leo Lira, H. Gianninoto, and A. Bandoni. (2009), Effect of ethanol level in the perception of aroma attributes and the detection of volatile compounds in red wine. Journal of Sensory Studies, 24: 243–257.

Knaapila, A., L.D. Hwang, A. Lysenko, F.F. Duke, B. Fesi, A. Khoshnevisan, R.S. James, C.J. Wysocki, M. Rhyu, M.G. Tordoff, A.A. Bachmanov, E. Mura, H. Nagal, and D.R. Reed. 2012. Genetic analysis of chemosensory traits in human twins. Chem Senses. 37(9): 869–881.

Lawless, H. T. 2010. A simple alternative analysis for threshold data determined by ascending forced-choice methods of limits. J Sens Stud. 25(3): 332–346.

Lawless, H.T., and H. Heymann. 2010. Sensory Evaluation of Food - Principles and Practices. 2nd ed. Springer US.

Lesschaeve, I. (2007) Sensory evaluation of wine and commercial realities: Review of current practices and perspectives. Am. J. Enol. Vitic. 58(2): 252-258

Liacopoulos, D., D. Barker, P.R. Howland, D.C. Alcorso, A.P. Pollnitz, G.K. Skouroumounis, K.H. Pardon, H.J. McLean, R. Gawel, and M.A. Sefton. 1999. Chloroanisole taint in wines. In Proceedings of the 10th Australian Wine Industry Technical Conference, 224–26. Sydney. pp224-226

Marin, A.B., J. Barnard, R.B. Darlington, and T.E. Acree. (1991) Sensory thresholds: Estimation from dose-response curves. J Sens Stud. 6: 205-225

Mauer, L., and A. El-Sohemy. 2012. Prevalence of cilantro (Coriandrum sativum) disliking among different ethnocultural groups. Flavour. 1(1), 1-5

Meilgaard, M.C. 1993. Individual differences in sensory threshold for aroma chemicals added to beer. Food Qual Prefer. 4(3): 153–167.

Meilgaard, M.C., G.V. Civille, and B.T. Carr. 2007. Sensory Evaluation Techniques. Boca raton, FL: CRC Press.

Peng, M., S.R. Jaeger, and M.J. Hautus. 2012. Determining odour detection thresholds: Incorporating a method-independent definition into the implementation of ASTM E679. Food Qual Prefer. 25: 995–1004.

Pfeiffer, D.G., T.C. Leskey, and H.J. Burrack. 2012. Threatening the harvest: The threat from three invasive insects in late season vineyards. In Arthropod Management in Vineyards, 449–474.

Pierce Jr, J.D., R.L. Doty, and J.E. Amoore. 1996. Analysis of position of trial sequence and type of diluent on the detection threshold for phenyl ethyl alcohol using a single staircase method. Percept Motor Skill. 82(2): 451-458

Potter, T. 1996 Essential oil composition of cilantro. J. Ag. Food Chem. 44(7): 1824-1826

Prescott, J, and G. Bell. 1995. Cross-cultural determinants of food acceptability: recent research on sensory perceptions and preferences. Trends Food Sci Tech. 6: 201–205.
Prescott, J., L. Norris, M. Kunst, and S. Kim. 2005. Estimating a 'consumer rejection threshold' for cork taint in white wine. Food Qual Prefer. 16: 345–349.

Saliba, A.J., J. Bullock, and W.J. Hardie. 2009. Consumer rejection threshold for 1,8-Cineole (eucalyptol) in Australian red wine. Food Qual Prefer. 20: 500–504.

Segurel, M.A., A.J. Razungles, C. Riou, M. Salles, and R.L. Baumes. 2004. Contribution of dimethyl sulfide to the aroma of Syrah and Grenache Noir wines and estimation of its potential in grapes of these varieites. J,. Ag. Food Chem. 52(23): 7084-7093

Stanislaw, H., and N. Todorov. 1999. Calculation of signal detection theory measures. Behav Res Meth Instr. 31(1): 137–149.

Tomasino, E., P. Mohekar, T. Lapis, V. Walton, and J. Lim. 2013. Effect of brown marmorated stink bug wine - impact to Pinot noir quality and threshold determination of taint compound trans-2-decenal. The 15th Australian Wine Industry Technical Conference, Sydney, Australia, July

Tu, V.P., D. Valentin, F. Husson, and C. Dacremont. 2010. Cultural differences in food description and preference: contrasting Vietnamese and French panellists on soy yogurts. Food Qual Prefer, 21(6): 602–610.

Yoo, Y.J., A.J. Saliba, P.D. Prenzler, and D. Ryan. 2012. Total phenolic content, antioxidant activity, and cross-cultural consumer rejection threshold in white and red wines functionally enhanced with catechin-rich extracts. J Agr Food Chem. 60(1): 388–393.

Table 2.1. Consumer panel (n=72) d′ values associated with proportion correct in detection threshold (DT) and consumer rejection threshold (CRT) of different concentrations of trans-2-decenal in Pinot noir wine

Trans-2-decenal (µg/L)	DT	CRT
0.05	0.0	0.5*
0.12	0.39	0.25
0.31	0.99	0.25
0.77	1.16	0.00
1.92	0.99	0.15
4.80	1.54***	0.34
12.00	1.68***	0.4*
30.00	2.18***	1.08***

*; $p < 0.05$ **; $p < 0.01$, ***; $p < 0.001$

Table 2.2a. Segmentation of consumer panel (n=72) for sensitivity of trans-2-decenal concentrations in Pinot noir wine for d′ values associated with proportion correct for detection thresholds (DT)

	Trans-2-decenal (µg/L)	Group 1	Group 2	Group 3
		N_1 =10 (14%)	N_2 = 41 (57%)	N_3 = 21 (29%)
DT	0.05	0.88	0.60	0.00
	0.12	2.50*	0.80	0.00
	0.31	4.03***	0.98	0.00
	0.77	4.03***	1.13	0.00
	1.92	4.03***	0.30	0.00
	4.80	Inf***	1.78**	0.00
	12.00	Inf***	1.90***	0.00
	30.00	Inf***	2.54***	0.00

*; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.001$

Table 2.2b. Segmentation of consumer panel (n=72) for sensitivity of trans-2-decenal concentrations in Pinot noir wine for d′ values associated proportion preference for control for consumer rejection threshold (CRT)

	Trans-2-decenal (µg/L)	Group 1	Group 2	Group 3
		N_1 =10 (14%)	N_2 = 41 (57%)	N_3 = 21 (29%)
CRT	0.05	0.36	0.77**	0.08
	0.12	1.19	0.30	0.00
	0.31	0.00	0.30	0.25
	0.77	0.00	0.04	0.00
	1.92	0.36	0.04	0.25
	4.80	0.00	0.48	0.43
	12.00	0.77	0.48	0.08
	30.00	1.80**	0.98***	1.01**

*; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.001$

Table 2.3. Segmentation of consumer panel (n=72) based on preference for trans-2-decenal in Pinot noir wine for d′ values associated with proportion preference for control in consumer rejection threshold (CRT)

Trans-2-decenal levels (µg/L)	CRT-Group 1	CRT-Group 2	CRT-Group 3
	N_1 = 20 (28%)	N_2 = 47 (65%)	N_3 = 5 (7%)
0.05	1.19**	0.42	0.00
0.12	1.81***	0.00	0.36
0.31	1.19**	0.00	0.00
0.77	0.95*	0.00	0.00
1.92	0.95*	0.04	0.00
4.80	Inf***	0.00	0.00
12.00	1.46**	0.19	0.00
30.00	Inf***	0.84**	0.00

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

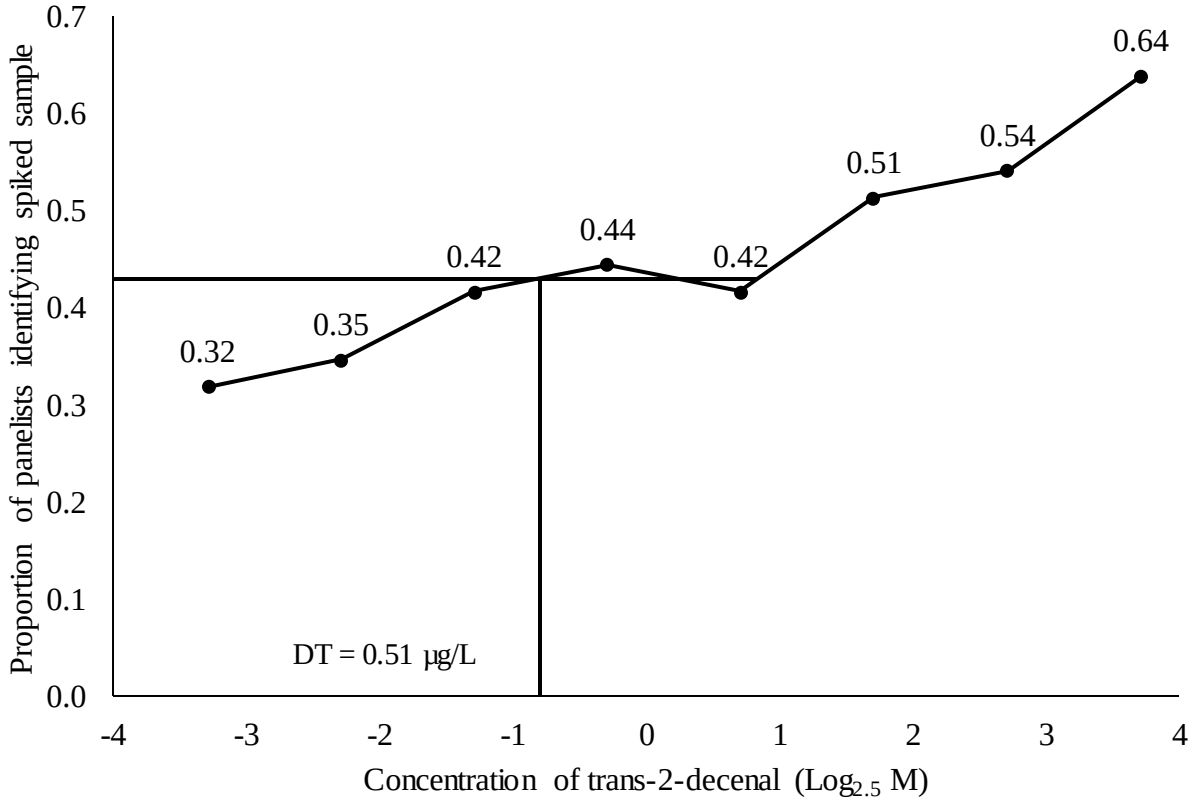

Figure 2.1. Group detection threshold (DT). Proportions of panelists correctly identifying the wine spiked with trans-2-decenal at each concentration level in Pinot noir. The solid line (y = 0.43) indicates 5% significance criterion based on binomial distribution for triangle tests (N=72).

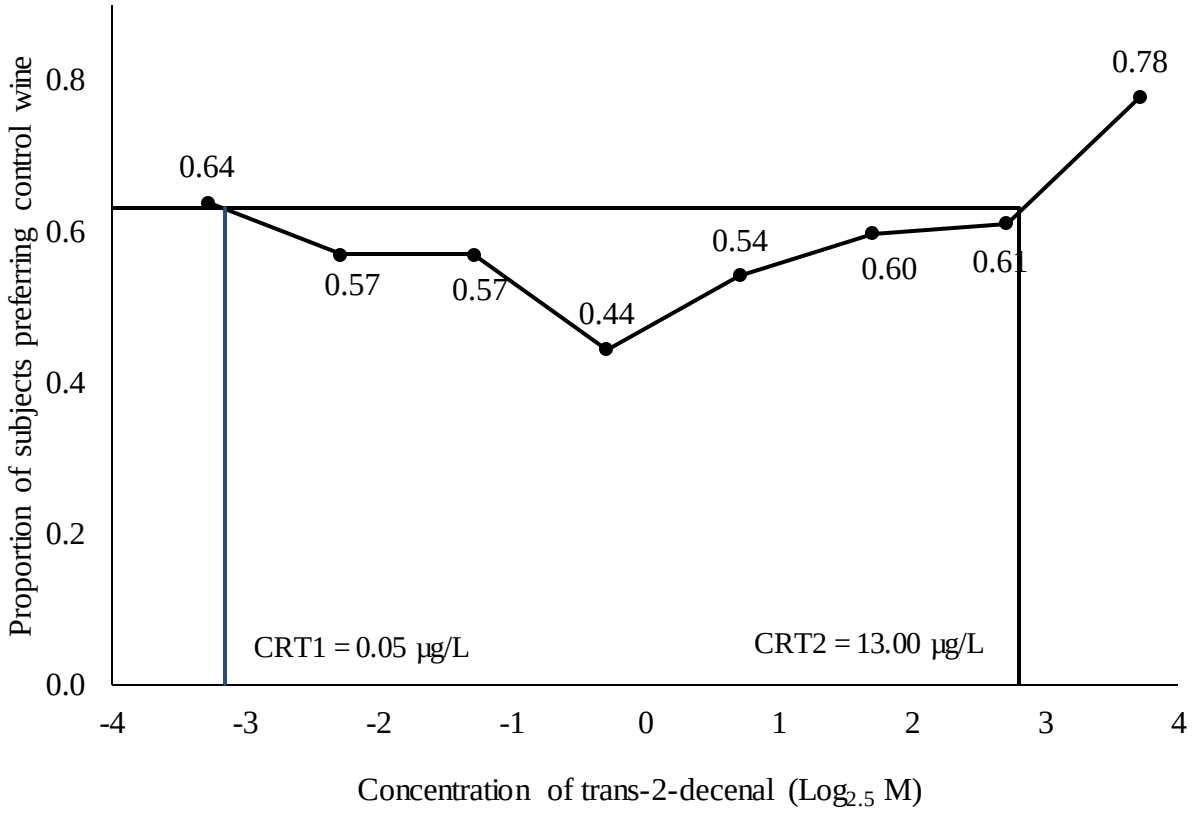

Figure 1.2. Group consumer rejection threshold (CRT). Proportions of panelists preferring control wine, at each trans-2-decenal concentration in Pinot noir. The solid line indicates the 5% significance criterion (y = 0.63) based on binomial distribution for preference tests (N=72).

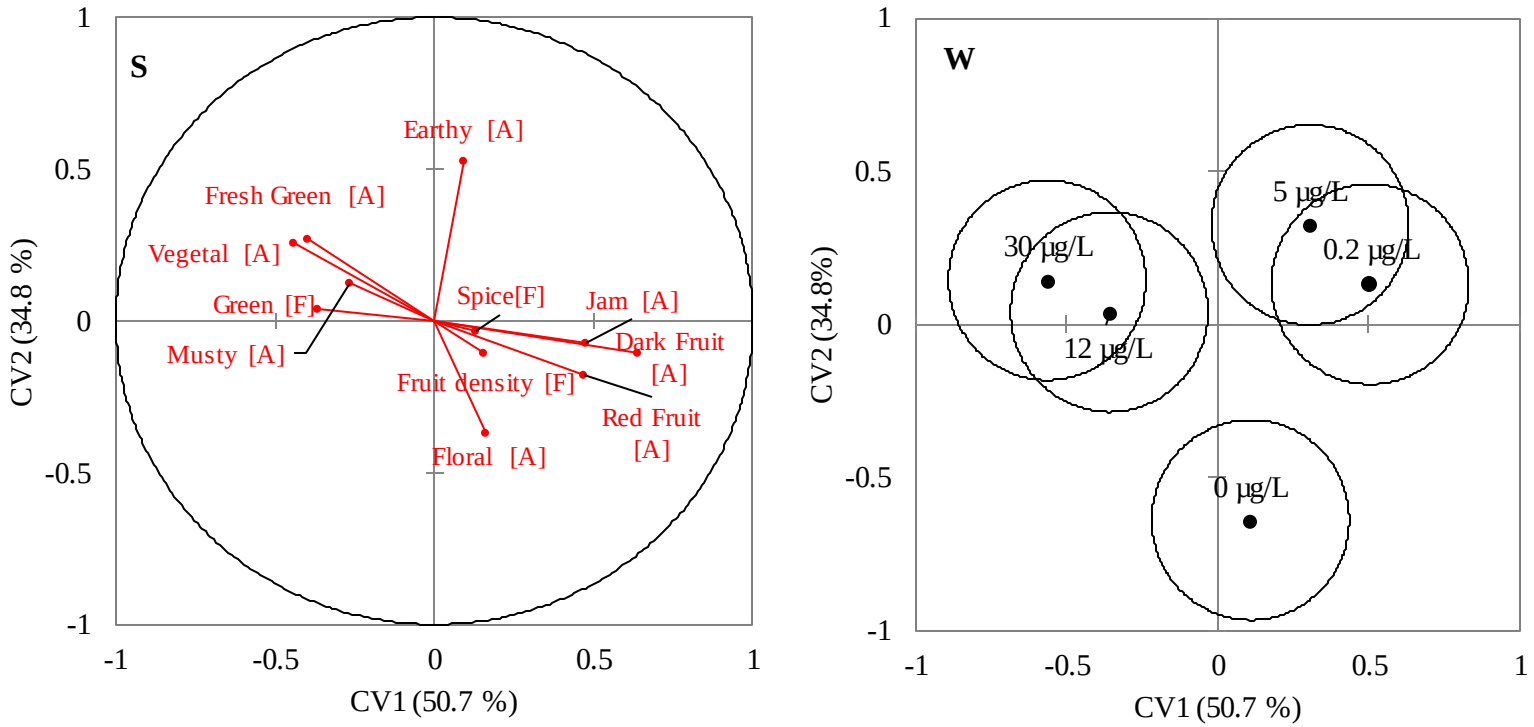

Figure 2.3. Separation of Pinot noir wines by trans-2-decenal concentration. Wines are positioned using the centroids. Circles represent 95% confidence intervals surrounding the wine means. Vectors for sensory terms (A=aroma, F= in mouth flavor) are in S and scores for wines are in W. Significant differences for wines are for circles that do not touch in W.

CHAPTER 3

Investigating drivers of wine consumer segmentation for trans-2-decenal rejection threshold

P. Mohekar[1], S. Emerson[2], E. Tomasino[1*]

[1]Oregon State University, Department of Food Science & Technology, 100 Wiegand Hall, Corvallis, Oregon 97331, USA

[2]Oregon State University, Statistics Department, 263 Weniger Hall, Corvallis, Oregon 97331, USA

[*]Corresponding Author: elizabeth.tomasino@oregonstate.edu, (T) (541)-737-4866, (F) (541)-737-1877

(prepared for submission to *Food Quality and Preference*)

ABSTRACT

Relationships between panel responses to paired preference tests for consumer rejection threshold (CRT) and consumer variables like involvement, knowledge, and experience on Merlot wine tainted with trans-2-decenal were investigated. Trans-2-decenal is a compound associated with stink bug taint in wine and clear consumer segmentation has been shown towards preference for this compound. Knowledge of these relationships are important in the marketing of wine towards a specific consumer segment. In this study, panel was segmented based on their consumer variables and responses for paired preference tests in CRT. Segments formed using the consumer variables showed different CRTs. Furthermore, groups segmented based on their paired preference data differed in their subjective knowledge, involvement and wine experience. However, only a weak relationship was found between consumer variables (wine involvement, subjective knowledge, and experience) and preference data. A failure to find stronger relationship may be a result of low variation in consumer variable scores. Future work with highly varied consumers may provide better insight into the relationship between consumer preference for trans-2-decenal and consumer variable. These results are important in designing control measures and appropriate marketing of wines containing trans-2-decenal.

Keywords: trans-2-decenal, brown marmorated stink bug, merlot, consumer rejection threshold, segmentation

Highlights:

- Trans-2-decenal CRT in Merlot was estimated to be 4.8µg/L
- Preference data from CRT resulted in consumer segments
- Subjective knowledge, involvement, experience showed a weak relationship with CRT data

Introduction

Consumer rejection threshold (CRT) is a sensory method that determines the lowest concentration of a compound at which consumer preference is affected. Since its introduction (Prescott et al., 2005), it has found widespread use in the food and wine industry, investigating methoxypyrazine content of grape juice (Weekes et al., 2010), eucalyptus in red wine (Saliba et al., 2009), citric acid in orange juice, salt in beef broth (Lee et al., 2008) and many others.

CRT has also found application in the study of consumer segmentation of a number of food products like chocolate milk, orange juice and wine (Blackman et al., 2010; Harwood et al., 2012a; Methven et al., 2016; Yoo et al., 2012). For example, Mohekar et al (2016)/Chapter 2 used the responses in paired preference tests to segment the panel. Doing so helped explain a surprising result that had occurred using traditional threshold calculations. However, segments can also be created using "consumer variables" and then analyzed using CRT. For example, a panel was segmented based on each subject's self-declared liking for either milk or dark chocolate. The resulting segments were able to explain differences in CRT for bitterness in chocolate milk (Harwood et al., 2013, 2012a, 2012b). Similarly, consumers segmented based on their nationally (Australian and Korean) were seen to differ in their CRT's for green tea and grape seed extract in wine (Yoo et al., 2012). Segmentation based on consumer variable is advantageous since it is far easier to measure consumer variables (e.g., via questionnaire) which permits the use of statistical methods for predicting preference.

In this study, CRT is used to analyze consumer preference and segmentation of wine tainted by trans-2-decenal. This compound is introduced into wine by an invasive pest, *Halyomorpha halys* (Stål)/brown marmorated stink bug (BMSB), whose taint primarily consists of trans-2-decenal (Fiola, 2011; Mohekar et al., 2015; Tomasino et al., 2013). Trans-2-decenal can impart "green", "herbal", and "vegetable" aroma characteristics (Mohekar et al., 2016/Chapter 2), often associated with low quality wine. Current management techniques for BMSB removal from grapes are not effective and therefore control levels in the wine are needed. CRT determines the minimum taint level above which control measures are required to maintain wine quality. It also allows the wine industry to better predict consumer preference of BMSB-tainted wine and therefore its marketing. The knowledge of trans-2-decenal CRT in wine has a direct economic impact to the wine industry.

Consumer segments that differ in their trans-2-decenal CRT have been reported before (Mohekar et al. 2016/Chapter 2). However, the drivers of such consumer segmentation are unclear. Mohekar et al. 2016/Chapter 2 showed that the perception of trans-2-decenal in wine is not responsible for these differences in the CRTs. The current hypothesis is that consumer liking or disliking for "cilantro" may be responsible for trans-2-decenal segmentation since it is a primary component of cilantro (Potter, 1995) and can be perceived as "soapy" or "green" based on genetic variation (Eriksson et al. 2012). This difference in perception leads to difference in cilantro liking. That is, individuals who perceive trans-2-decenal as "soapy" have strong dislike for cilantro. Such consumer variables (eg. "cilantro liking") due to their

ability to explain differences in consumer segments can assist with target marketing and therefore carry significant economic importance. Examples of other consumer variables driving food and wine preferences include age (Tuorila, 2015, Thach and Olsen, 2006), nationality (Campo et al., 2012; Lockshin et al., 2001), wine involvement (Lockshin et al., 2001, 1997, Hoe, 2007), wine knowledge (Blackman et al., 2010; Frøst and Noble, 2002) and product experience (Blackman et al., 2010). Consumers with high wine knowledge were found to prefer red wine (Taylor et al., 2008) whereas high involvement was associated with preference for wines from France and Italy (Bruwer and Buller, 2013). Dry wine preference was observed in consumers with more than 10 years wine consumption experience, were highly knowledgeable (Blackman et al., 2010), and highly involved in the type of wine they consumed (Dodd et al., 2010). In addition to wine preference, the above variables have also seen to influence wine purchasing behavior (Dodd et al., 2005; Johnson and Bastian, 2007).

Overall, these consumer variables have shown success in characterizing consumer segments with varying preferences. Therefore, these consumer variables (knowledge, involvement, experience, cilantro liking etc.) were selected to analyze their effect in explaining differences in consumer segments produced via preference data of trans-2-decenal in Merlot. The main objectives of this paper are to determine trans-2-decenal CRT in Merlot, the effect of consumer variable in creating groups of different CRT and to relate consumer variables to preference data. The estimate of trans-2-decenal CRT in Merlot will assist the wine industry with the design of control measures,

given the increasing densities of BMSB in the vineyard. Establishing a relationship between consumer variables and preference data will help predict consumer preferences using easily measured variables. Finally, knowledge of the drivers of consumer preference has applications to target marketing for wine containing trans-2-decenal.

Materials and Methods

Sensory Analysis

Subjects Non-smoking and reportedly healthy individuals willing to consume "alcoholic beverage" were recruited from the Corvallis campus of Oregon State University and nearby areas. During recruitment, subjects were told that this study will focus on evaluating wine quality and understanding of human perception. There was no mention of trans-2-decenal or its flavor (cilantro) until the end of the tasting session.

The panel was comprised of 93 (38 male, 55 female) regular red wine consumers, where "regular" was defined as anyone with consumption frequency of at least once a week. In order to gather a panel of typical wine consumers, subjects from the department of food science and technology were excluded from this study. Individuals with allergies or medical conditions restricting their alcohol consumption were also excluded. All subjects were reimbursed with a gift card from a local store

for their participation. The study was approved by the institutional review board at Oregon State University.

Fifty three percent of the panel was below the age of 40, 34% between the age of 41-50 years and 13% of the subjects were the age of above 60. A higher percent of younger participants could be a result of the bias introduced by the university. About half of the panel (58%) consumed red wine at least once a week and the remaining at least two or more times a week.

Stimuli A 2012 California Merlot was used as the base wine. Trans-2-decenal was added to this wine to achieve the concentrations of 0.05, 0.12, 0.31, 0.77, 1.92, 4.80, 12.00 and 30.00 µg/L. This range was selected based on previous work on trans-2-decenal threshold in Pinot noir (Mohekar et al., 2016/Chapter 2) and preliminary testings (data not shown). The compound was added at least 30 minutes before the session. To minimize the influence of ethanol on wine flavor (King, Dunn, & Heymann, 2013; Styger, Prior, & Bauer, 2011), two different trans-2-decenal standards, 1mg/ml and 50mg/L in 14% ethanol were used. The 1mg/L solution was used to achieve the first four concentrations and the 50mg/L solution for the last four concentrations. Standards were prepared as in Mohekar et al. 2016/Chapter 2.

CRT procedure CRT was measured using the protocol described by Prescott et al. (2005). It combines the technique of forced choice paired comparison with threshold

method of limits. Eight wine pairs consisting of base Merlot and trans-2-decenal added Merlot, were served to the panel, starting with the lowest concentration and ending with the highest. Each sample was coded with a three digit random number and the sample order in each pair was randomized. The subjects were instructed to taste each sample by placing the entire content in their mouth, swirling it around for few seconds and then expectorating. After tasting each pair, subjects were asked to select the sample that they preferred.

All wine samples, 15ml serving size, in INAO (Institut National des Appellations d'Origine) standard clear wine glasses at room temperature were poured 15 minutes before their evaluation. Subjects were given a one minute break after each set and five minute break after four sets to reduce any fatigue. During each break, subjects were asked to eat a cracker and rinse their palate with water to reduce any carry-over effect. The data was collected using Qualtrics (Qualtrics LLC, Utah, US) over a total of ten 60-minute sessions.

Consumer variables to explain segmentation

During each session, the panel was asked to complete a questionnaire to measure their product involvement (INV), subjective (SK) and objective knowledge (OK). Additional variables of interest included panel's wine experience (LEN), their red wine consumption frequency (RED), whether they consumed Merlot wine or not (MER), their liking for cilantro (CIL), a trans-2-decenal descriptor, and willingness to

try cilantro-flavored beverages in the future (WC). This information was later used for panel segmentation.

Consumer involvement (INV) was measured using a seven-point likert scale anchored between "strongly disagree (1)" and "strongly agree (7)" applied to four questions (Supplementary Table 1) (Bruwer and Buller, 2013; Hollebeek et al., 2007; Laurent and Kapferer, 1985; Lockshin et al., 2001, 1997; Michaelidou and Dibb, 2006; Mittal and Lee, 1989). These questions were selected based on the high reliability (i.e. high Cronbach's alpha) of the "involvement" construct (Bruwer and Buller, 2013).

Subjective knowledge (SK) was also measured on a seven-point likert scale applied to three questions (Supplementary – Table 2) (Johnson and Bastian, 2007; Taylor et al., 2008). Objective knowledge (OK) was estimated using eight multiple choice questions (Supplementary – Table 3), based on "Wine Trivia Quiz" by Frøst & Noble, 2002; "Australian Wine Questionnaire" by Johnson & Bastian, (2007); and a questionnaire developed by Dodd et al. (2005). This combination allowed us to test diverse facets of wine knowledge across difficulty levels.

Panel experience was measured in terms of their wine consumption length (LEN). Cilantro liking (CIL) and panel inclination for trying cilantro-flavored beverages (WC) was measured using a nine point hedonic scale and seven point likert scale respectively. Cilantro-related questionnaire were tested at the end of the wine tasting

session to avoid their influence on panel's sensory response. Finally, red wine consumption frequency (RED) and whether subjects were Merlot consumers (MER) or not was also measured.

Data Analysis

CRT estimation CRT was estimated using Thurstonian model as described in (Mohekar et. al, 2016/Chapter 2). The model relates the proportion of subjects preferring control wine to d′, a measure of sensory signal. The d′ value and its associated variance was calculated using R-package SensR (Brockhoff and Christensen, 2010). CRT was defined as the concentration above which d′ values were significantly different from zero (*p-value < 0.05*). This is in contrast to previous studies where CRT is defined using an absolute value of d` (= 1.0) which corresponds to 75% proportion correct in 2-AFC. In this work, the lower value of d` was used since trans-2-decenal is considered negative to wine quality.

The above procedure for computing CRT can be applied to any panel segment (i.e., group of subjects) allowing for group CRTs to be used for comparing different segmentation procedures. That is, a "good" segmentation criteria will produce groups with large differences in their CRT values whereas a "bad" segmentation criteria will produce groups with similar CRT values. In the section of panel segmentation, different segmentation criteria are analyzed and evaluated in terms of their ability to produce segments with different CRT's. This permits the partitioning of consumers

into groups that differ substantially in terms of their preferences for trans-2-decenal in Merlot.

Internal consistency and correlation in consumer variables The internal consistency of the questionnaire in measuring variables of interest was calculated using Cronbach's alpha value (Tavakol and Dennick, 2011). Correlation coefficients among involvement, subjective and objective knowledge were calculated and tested with Spearman correlation test. The analysis was conducted using R-studio (Version 3.2, Boston, MA, USA)

Panel segmentation

Different panel segmentation criteria are explored. These can be roughly described in two broad categories: a) Panel segmentation using responses on paired preference tests and b) Panel segmentation using consumer information.

Preference-based segmentation The criteria used to segment the panel was adapted from Mohekar et al. (2016)/Chapter 2. Specifically, subjects that preferred the control wine in at least six out of eight paired preference tests in CRT estimation were placed in Group 1 whereas subjects that preferred the control wine in three or fewer tests were placed in Group 3. The remaining subjects, preferring the control in either four or five tests were placed in the intermediate Group 2. The only difference between this and the criteria used in Mohekar et al. (2016)/Chapter 2 is the definition of Group

3, in which two or fewer tests were used. The definition used in this paper produces more balanced segments. Further discussion about the choice of the criteria using only preference data is given in discussion and future work of this Chapter.

Segmentation based on consumer variables Each of the six panel variables (INV, EXP, SK, OK, CIL, WC) were analyzed separately. Three groups were created by assigning approximately 25% of subjects with lowest scores to one group (low), approximately 50% to a second group (medium), and the highest scoring 25% to a third group (high) for variables, INV, SK, OK, WC (Frøst and Noble, 2002). For EXP, segmentation was based on the length of their wine consumption and groups were defined as: *low*, with 0-5 years wine drinking experience; *medium*, with 5-15 years wine drinking experience and *high*, with more than 15 years wine drinking experience. In CIL, subjects with hedonic score of 5 (corresponds to: neither like nor dislike) or less were categorized as "cilantro dislikers" and hedonic score of above 5 (corresponding to slight to strong liking) as "cilantro likers". Similarly, for WC, subjects with score of 4 (corresponds to: neutral regarding willingness to try cilantro) or less were categorized as "Low WC" and score of above 5 (corresponding to "somewhat - very likely" to try cilantro) as "High WC".

Wine consumption behavior was also taken into account for CRT differences. The panel was segmented based on their frequency of red wine consumption frequency and on their consumption of Merlot wines (Merlot group) or other red wines (Non-

Merlot group). Subjects who did not provide their preference for any of the red wines were excluded from the analysis since we could justify placing them in either group.

Relating consumer variable with preference based segments A linear regression analysis was performed to relate consumer variables to preference data (i.e., number of tests where the control is preferred). All the consumer variables were treated as continuous and normalized before fitting a linear regression function using R-studio (Version 3.2, Boston, MS, USA).

Results

Merlot CRT

Figure 3.1 shows that an increasing trans-2-decenal concentration results in an increase in the proportion of subjects preferring the control wine. The population CRT of trans-2-decenal in Merlot was estimated to be above 4.8 µg/L (Figure 3.1). This value is identical to previously reported CRT for trans-2-decenal in Pinot noir (Mohekar et al. 2016, Chapter 2), indicating a low impact of wine matrix on trans-2-decenal preference. The CRT value also shows that concentration levels above 4.8 µg/L have a perceived negative effect on wine quality.

Internal consistency in consumer variable questionnaire

The consumer variables INV and SK showed high internal consistency ($\alpha = 0.77$ and $\alpha = 0.66$ respectively) whereas the questionnaires for OK showed low α value of 0.23.

The low internal consistency for OK is unsurprising given that the OK questionnaire was intentionally designed to test knowledge across a range of concepts and difficulty levels. For example, the entire panel answered the question "Which wine should be served at room temperature?" correctly, indicating that the question may have been too easy.

Correlation in consumer variable questionnaire

A weak correlation was observed between SK and OK scores (correlation coefficient 0.15). This suggests that consumer knowledge cannot be measured using any single variable and corresponds with previous work where subjective knowledge is shown to be a poor representation of objective knowledge (Dodd et al., 2005). Consumer involvement (INV) was moderately correlated with SK (correlation coefficient: 0.56) and poorly correlated with OK (correlation coefficient: 0.12). This is in agreement with previous work which shows low correlation between OK and INV whereas a high correlation has been found between SK and INV for products like wine (Park and Moon, 2003). It is possible that consumers who are highly involved with their product consider themselves knowledgeable (Dodd et al., 2005), resulting in higher correlation between SK and INV. However, this may not translate into what consumers actually know about the product due to the difference in how the product information is perceived (House, 2004).

A weak correlation (correlation coefficient: 0.44) was observed between cilantro liking (CIL) and panel willingness to try cilantro-flavored beverages (WC). This indicates that cilantro preference may not always extrapolate to food products where this flavor is considered atypical or uncommon. Additionally, subjects may have been confused by the question for WC since "cilantro" is not a common flavor in beverages even though "cilantro" itself is well known. Another factor could be food neophobia (reluctance to/avoidance of novel foods) which has previously been shown to decrease the degree of product liking in novel products (Henriques et al., 2009).

Preference-based segmentation

Figure 3.2 shows the proportion of subjects preferring the control wine in each preference-based group as a function of trans-2-decenal concentration. As shown in previous work by Mohekar et al., 2016/Chapter 2, preference-based criterion creates consumer segments with high (group 1), mid (group 2) and low (group 3) preference for control wine (without trans-2-decenal).

Segmentation based on consumer variables

Impact of knowledge (SK, OK), experience (EXP), Involvement (INV) INV and OK have similarly shaped histograms and segment sizes (Figures 3.3a and 3.3b). The CRTs of the segments for both variables also demonstrated the same trend. Specifically, CRT decreased after objective knowledge (alternatively involvement) increased past a certain level (11 for OK and 23 for INV). This suggests that

consumers may need to gain a certain level of expertise before their preference is affected. Surprisingly, groups based on SK and EXP scores showed a different trend (Figure 3.3c and 3.3d). An initial increase in SK and EXP scores resulted in decrease of group preference towards trans-2-decenal. However, the CRT of low EXP group should be interpreted with caution due to its low sample size. Contrary to our expectation, further increase in these variables (SK score > 12 and EXP, wine consumption length of > 15 years) caused an increase in CRT instead of lowering it further. Further investigation into the segments for SK and EXP showed that groups with lowest CRT (mid EXP and SK) contained a low proportion of subjects aged above 50, (23% in mid-SK group and 1% in mid-EXP group). High age is often associated with loss of sensory acuity (Kremer et al., 2007) which might explain the high CRT for the group with older subjects and vice versa. It also suggests that a panel segment with consumption length between 5-15 years may be a better representation of highly experienced consumer group, since the panel may have acquired a good understanding of wine without losing sensory acuity. Overall, these results suggest that the combination of high experience and moderate age are involved in the criteria that define a highly discriminative consumer segment with high sensory acuity.

Impact of wine consumption, Merlot (MER) and red wine (RED) Wine consumption behavior was also evaluated for its effect on panel segmentation. Panel segmentation based on red wine consumption frequency showed that subjects who consumed red wine once a week found trans-2-decenal less offensive (CRT: >

4.8ug/L) compared to more frequent red wine consumers (two or more times per week) (CRT: > 1.92ug/L) (Figure 3.4a). For Merlot-based segmentation, an additional processing step was included since the question on Merlot consumption frequency was not a forced choice. This resulted in 18 subjects not providing a response, and therefore, were excluded from the analysis since it was unclear how to group them. The remaining subjects were segmented based on their Merlot consumption and their CRT's and histograms are shown in Figure 3.4b. The CRTs of both groups is identical (< 12µg/L) indicating that Merlot consumption has very little impact on preference. However, it is unclear what, if any, impact this question error may have had and additional experimentation would make this clear.

Impact of cilantro (CIL, WC) The group of "cilantro likers" had trans-2-decenal CRT 2.5 times lower than "cilantro dislikers" (Figure 3.4c). This is surprising because cilantro-likers were expected to find the presence of trans-2-decenal less offensive and therefore possess higher CRT. No difference was found between CRTs when groups were segmented based on their willingness to try cilantro-flavored beverages (WC) (Figure 3.4d). One hypothesis is that trans-2-decenal in its purest form has a cilantro aroma but when added into wine, it may possess a different description, interacting with other aroma compounds. Previous work has shown an increase in "green" aroma intensity with increase in trans-2-decenal concentration (Mohekar et al., 2016/Chapter 2) but it is currently unknown if "green" relates to "cilantro". Previous work on trans-2-decenal suggest that people who dislike cilantro may perceive it as "soapy" rather than "green" due to genetic variation in their

olfactory receptors for trans-2-decenal, which is a key aroma compound in cilantro (Eriksson et al., 2012). Therefore, green/cilantro may not be an appropriate descriptor for such consumers. Furthermore, liking for cilantro may not be a good indicator of its preference in other foods and beverages. Support for this hypothesis comes from the low correlation (correlation coefficient of 0.44) between the willingness to try cilantro in other beverages (WC) and cilantro liking (CIL). However, one of the major limitations of these results is the low sample size of "cilantro dislikers" and "low WC" groups. Future work with larger sample size is needed to draw stronger conclusion regarding the effect of cilantro liking on trans-2-decenal preference.

Relationship between consumer preference and consumer variables

Preference-based groups and consumer variables: Analysis of Means Results show clear trends between preference-based groups and consumer variables, SK, OK, INV, EXP (Figure 3.5 and Figure 3.6). Group 1 demonstrated all the characteristics of an expert subject: high knowledge, involvement, length of wine consumption between 5-15 years and frequent red wine consumption. On the other hand, group 3 subjects had the smallest mean knowledge (OK, SK) and INV values. Group 3 also contains the highest proportion of subjects with long wine consumption length (> 15 years) and 40% of subjects above the age of 50. Group 1, on the other hand, showed lowest proportion of subjects with wine consumption experience > 15 and 25% subject with age above 50. This agrees with results from single variable segmentation where age proved to be a strong driver of segmentation.

However, the strength of the relationship was not strong. Specifically, ANOVA between the group means of each of the three variables (SK, OK and INV) either found no effect or very weak effect. SK and INV showed weak differences (*p-values* < 0.1) whereas OK did not show any statistically significant difference between its group means. The proportion of subjects with wine consumption length of more than 15 were also found to be significantly different between group 1 and 3 (Z-test of proportions, *p-value < 0.05*). However, no difference was found between proportion of subjects with wine consumption length of less than 5 years and 5-15 years. These results show a very weak link between consumer variables and preference data. This is a negative result in that a stronger relationship between preference and consumer variables was expected, given the success of these variables in previous studies (Blackman et al., 2010; Bruwer and Buller, 2013; Dodd et al., 2010; Taylor et al., 2008). Some reasons that may have prevented a stronger effect from being observed are discussed in the next section (discussion and future work).

Relating consumer variable with preference based segments Linear regression was used to predict the preferences (i.e., number of tests where the control is preferred) using the consumer variables as regression inputs. The model showed very little predictive power with the intercept (i.e., predicting the mean preference) dominating the prediction. The only consumer variable used by the regression model is the length of wine consumption, which corresponds with results seen in above section of preference based groups. None of the other variables showed any predictive power in linear regression. Additional analyses using different regression

and classification methods like regression trees, logistic regression, generalized linear models and multinomial logistic regression were also performed to determine a relation between preference data and consumer variables (data not shown). None of these resulted in any model that showed significant predictive power from the consumer variables. Experiments that varied the type and number of inputs (normalized, categorical, etc.) also did not produce any reduction in error. That is, in each and every case, leave-one-out cross-validation showed that the baseline (i.e., predicting the mean preference counts) is the best model, indicating a weak relationship between consumer variables and preference data.

Discussion and future work

Relation between consumer variables and preference

A key finding of this paper is that the consumer variables INV, SK and EXP showed (weak) differences among preference based groups. The fact that a relationship exists is unsurprising given that these three variables encode a subject's expertise about the product which, in turn, may influence preference. Furthermore, prior work has shown that these variables are closely related, particularly for pleasurable products like wine (Dodd et al., 2005; Park and Moon, 2003; Taylor et al., 2008).

However, their relationship to preference data was surprisingly weak. That is the consumer variables were seen to be poor predictors of preference. One reason for this may be that the consumer variables were not sufficiently discriminative for the

selected panel. The panel was chosen randomly and it may be that insufficient variation exists among the included subjects. An interesting experiment for future work would be to conduct the above experiment with a carefully selected panel with known differences in the above variables and large response ranges, compared to the tightly clustered responses observed in this work. This approach of predefining the groups can be time consuming, especially when many variables are involved. A good starting point would be to use a subset of INV, SK and EXP that were found to be weakly related to consumer preference.

The consumer variable selection in this study was based on their importance in wine marketing and explaining differences in wine liking between consumers and experts (Blackman et al., 2010; Bruwer and Buller, 2013; Dodd et al., 2010, 2005; Frøst and Noble, 2002; Hollebeek et al., 2007; Johnson and Bastian, 2007; King et al., 2012). Although more interesting consumer variables exist, these can be significantly more costly to collect. One particularly interesting variable is a subject's sensory knowledge, which measures a person's ability to recognize different aromas in wine. It seems possible that this variable might provide additional predictive power since variables like objective knowledge, used in this study, are known to be poorly correlated with sensory knowledge whereas sensory knowledge has been known to influence wine liking (Frøst & Noble, 2002; Johnson & Bastian, 2007).

Finally, it may be the case that the variance in consumer variables can be increased with more specific questions. This can be difficult since there is no standard set of

questions that are known to conclusively measure subjective or objective knowledge. Another option is to use a line scale with indented anchors since prior work shows that people tend to avoid extreme responses (Lawless & Heymann, 2010).

Preference-based segmentation criteria

The criteria used to segment subjects based on their preference data was derived from previous work by Mohekar et al. (2016)/Chapter 2. A number of other segmentation criteria can be considered. For example, segmentation using only the panel responses at the three highest concentrations. The intuition is that this criteria separates subjects with "clearly" low sensitivity from the rest. However, it has the disadvantage of ignoring more than 60% (i.e., 5 out of 8 responses) of the preference data. The resulting segments had smaller CRT differences and also did not show any effect on consumer variables.

In retrospect, it seems that the simple count-based segmentation criteria used in this paper finds a good balance between the sensitivity of the subjects while being relatively immune to noise in the preference responses. It produces the largest CRT differences between group 1 and 3 and also shows some effect on consumer variables. However, this criteria assigns equal importance to each correct response, irrespective of concentration. It seems feasible that a more complex criteria that incorporates concentration and the "consistency" of correct responses might achieve a better

separation of subjects. The choice of preference-based segmentation criteria is a very interesting topic and future work will investigate this in greater detail.

Conclusion

Consumer variables like involvement, subjective knowledge, objective knowledge, and experience were shown to segment wine consumers into groups with different trans-2-decenal CRTs. The results indicate that the consumer variables have little to no predictive power when dealing with preference-based segmentation. The data suggests that there is a weak trend in the consumer variables segmented by preference data. However, attempts to explain the preference-based segmentation using consumer variables were unsuccessful. Additional work is needed in developing methods that can use consumer variables for predicting preferences since that knowledge is vital to the marketing and sales of BMSB-impacted wines.

Literature cited:

Blackman, J., Saliba, A., Schmidtke, L., 2010. Sweetness acceptance of novices, experienced consumers and winemakers in Hunter Valley Semillon wines. Food Qual. Prefer., Eighth Pangborn Sensory Science Symposium - Wine Special Issue 21, 679–683. doi:10.1016/j.foodqual.2010.05.001

Brockhoff, P.B., Christensen, R.H.B., 2010. Thurstonian models for sensory discrimination tests as generalized linear models. Food Qual. Prefer., Sensometrics 2008 (TBC) 21, 330–338. doi:10.1016/j.foodqual.2009.04.003

Bruwer, J., Buller, C., 2013. Product involvement, brand loyalty, and country-of-origin brand preferences of Japanese wine consumers. J. Wine Res. 24, 38–58. doi:10.1080/09571264.2012.717221

Campo, E., Saenz-Navajas, M. p., Cacho, J., Ferreira, V., 2012. Consumer rejection threshold of ethyl phenylacetate and phenylacetic acid, compounds responsible for the sweet-like off odour in wines made from sour rotten grapes. Aust. J. Grape Wine Res. 18, 280–286. doi:10.1111/j.1755-0238.2012.00198.x

Dodd, T.H., Kolyesnikova, N., Wilcox, J.B., 2010. A matter of taste: Consumer preferences of sweet and dry wines. Presented at the 5th International Academy of Wine Business Research Conference, Auckland, New Zealand.

Dodd, T.H., Laverie, D.A., Wilcox, J.F., Duhan, D.F., 2005. Differential Effects of Experience, Subjective Knowledge, and Objective Knowledge on Sources of Information used in Consumer Wine Purchasing. J. Hosp. Tour. Res. 29, 3–19. doi:10.1177/1096348004267518

Fiola, J.A., 2011. Brown Marmorated Stink Bug (BMSB) Part 3 - Fruit Damage and Juice/Wine Taint. Timely Vitic.

Frøst, M.B., Noble, A.C., 2002. Preliminary Study of the Effect of Knowledge and Sensory Expertise on Liking for Red Wines. Am. J. Enol. Vitic. 53, 275–284.

Harwood, M.L., Loquasto, J.R., Roberts, R.F., Ziegler, G.R., Hayes, J.E., 2013. Explaining tolerance for bitterness in chocolate ice cream using solid chocolate preferences. J. Dairy Sci. 96, 4938–4944. doi:10.3168/jds.2013-6715

Harwood, M.L., Ziegler, G.R., Hayes, J.E., 2012a. Rejection thresholds in chocolate milk: Evidence for segmentation. Food Qual. Prefer. 26, 128–133. doi:10.1016/j.foodqual.2012.04.009

Harwood, M.L., Ziegler, G.R., Hayes, J.E., 2012b. Rejection Thresholds in Solid Chocolate-Flavored Compound Coating. J. Food Sci. 77, S390–S393. doi:10.1111/j.1750-3841.2012.02889.x

Henriques, A.S., King, S.C., Meiselman, H.L., 2009. Consumer segmentation based on food neophobia and its application to product development. Food Qual. Prefer., European Conference on Sensory Science of Food and Beverages 2006 20, 83–91. doi:10.1016/j.foodqual.2008.01.003

Hollebeek, L.D., Jaeger, S.R., Brodie, R.J., Balemi, A., 2007. The influence of involvement on purchase intention for new world wine. Food Qual. Prefer. 18, 1033–1049. doi:10.1016/j.foodqual.2007.04.007

Johnson, T.E., Bastian, S.E. p., 2007. A preliminary study of the relationship between Australian wine consumers' wine expertise and their wine purchasing and consumption behaviour. Aust. J. Grape Wine Res. 13, 186–197. doi:10.1111/j.1755-0238.2007.tb00249.x

King, E.S., Johnson, T.E., Bastian, S.E.P., Osidacz, P., Francis, I.L., 2012. Consumer liking of white wines: segmentation using self-reported wine liking and wine knowledge. Emerald Insight 24, 33–46. doi:http://dx.doi.org/10.1108/17511061211213774

Kremer, S., Bult, J.H.F., Mojet, J., Kroeze, J.H.A., 2007. Food Perception with Age and Its Relationship to Pleasantness. Chem. Senses 32, 591–602. doi:10.1093/chemse/bjm028

Laurent, G., Kapferer, J.-N., 1985. Measuring Consumer Involvement Profiles. J. Mark. Res. 22, 41–53. doi:10.2307/3151549

Lawless, H.T., Heymann, H., 2010. Sensory Evaluation of Food: Principles and Practices. Springer Science & Business Media.

Lee, Y.-M., Prescott, J., Kim, K.-O., 2008. PROP taster status and the rejection of foods with added tastants | NOVA. The University of Newcastle's Digital Repository, PROP taster status and the rejection of foods with added tastants.

Lisa House, J.L., 2004. Objective and Subjective Knowledge: Impacts on Consumer Demand for Genetically Modified Foods in the United States and the European Union. The Journal of Agrobiotechnology Management 7.

Lockshin, L., Quester, P., Spawton, T., 2001. Segmentation by Involvement or Nationality for Global Retailing: A Cross-national Comparative Study of Wine Shopping Behaviours. J. Wine Res. 12, 223–236. doi:10.1080/09571260120106848

Lockshin, L.S., Spawton, A.L., Macintosh, G., 1997. Using product, brand and purchasing involvement for retail segmentation. J. Retail. Consum. Serv. 4, 171–183. doi:10.1016/S0969-6989(96)00048-3

Methven, L., Xiao, C., Cai, M., Prescott, J., 2016. Rejection thresholds (RjT) of sweet likers and dislikers. Food Qual. Prefer. 52, 74–80. doi:10.1016/j.foodqual.2016.03.012

Michaelidou, N., Dibb, S., 2006. Product involvement: an application in clothing. J. Consum. Behav. 5, 442–453. doi:10.1002/cb.192

Mittal, B., Lee, M.-S., 1989. A causal model of consumer involvement. J. Econ. Psychol. 10, 363–389. doi:10.1016/0167-4870(89)90030-5

Mohekar, P., Wiman, N.G., Osborne, J., Walton, V., Tomasino, E., 2015. Brown Marmorated Stink Bug (Halyomorpha halys): Impact on wine quality and management techniques. Presented at the 2015 Annual Meeting of the Entomological Society of America.

Park, C.-W., Moon, B.-J., 2003. The relationship between product involvement and product knowledge: Moderating roles of product type and product knowledge type. Psychol. Mark. 20, 977–997. doi:10.1002/mar.10105

Prescott, J., Norris, L., Kunst, M., Kim, S., 2005. Estimating a "consumer rejection threshold" for cork taint in white wine. Food Qual. Prefer. 16, 345–349. doi:10.1016/j.foodqual.2004.05.010

Saliba, A.J., Bullock, J., Hardie, W.J., 2009. Consumer rejection threshold for 1,8-cineole (eucalyptol) in Australian red wine. Food Qual. Prefer. 20, 500–504. doi:10.1016/j.foodqual.2009.04.009

Tavakol, M., Dennick, R., 2011. Making sense of Cronbach's alpha. Int. J. Med. Educ. 2, 53–55. doi:10.5116/ijme.4dfb.8dfd

Taylor, C.D., Dodd, T.H., Barber, N., 2008. Impact of Wine Education on Developing Knowledge and Preferences: An Exploratory Study. J. Wine Res. 19, 193–207. doi:10.1080/09571260902891175

Tomasino, E., P. Mohekar, T. Lapis, V. Walton, and J. Lim. 2013. Effect of brown marmorated stink bug wine - impact to Pinot noir quality and threshold determination of taint compound trans-2-decenal. The 15th Australian Wine Industry Technical Conference, Sydney, Australia, July

Tuorila, H., 2015. From sensory evaluation to sensory and consumer research of food: An autobiographical perspective. Food Qual. Prefer., Tenth Pangborn Sensory Science Symposium 40, Part B, 255–262. doi:10.1016/j.foodqual.2014.05.006

Weekes, L.N., Walsh, D., Ferguson, H., Ross, C.F., 2010. Impact of Multicolored Asian Lady Beetles on the sensory properties of Concord and Niagara grape juice. J. Food Sci. 75, S68-73. doi:10.1111/j.1750-3841.2009.01423.x

Yoo, Y.J., Saliba, A.J., Prenzler, P.D., Ryan, D., 2012. Total Phenolic Content, Antioxidant Activity, and Cross-Cultural Consumer Rejection Threshold in White and Red Wines Functionally Enhanced with Catechin-Rich Extracts. J. Agric. Food Chem. 60, 388–393. doi:10.1021/jf203216z

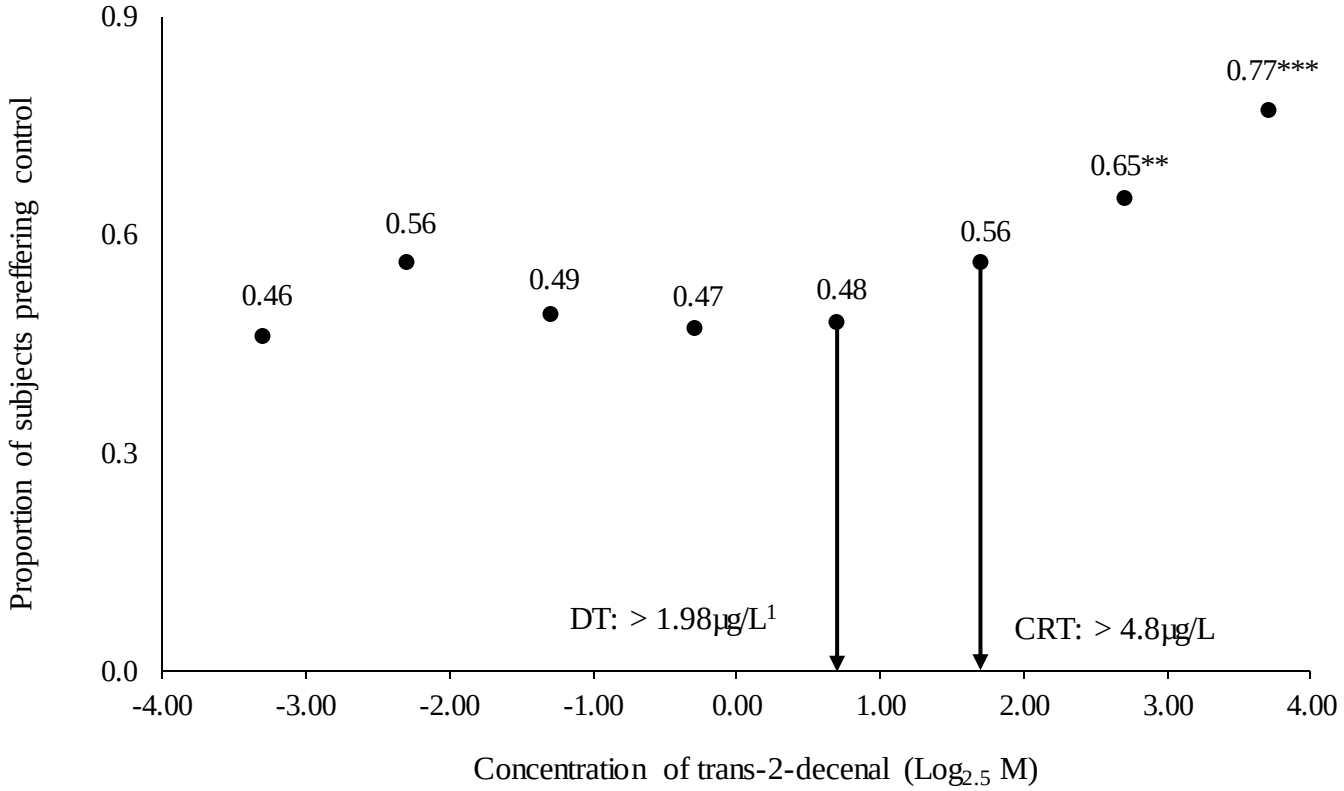

Figure 3.1. Group consumer rejection threshold (CRT). Proportion of subjects preferring control wine, at each trans-2-decenal concentration in Merlot. Significance is based on if the d′ values at a corresponding proportion is significantly different from d′ = 0.

*, *p*-value < 0.05; **, *p*-value < 0.01; ***, *p*-value < 0.001

[1]: Detection threshold of trans-2-decenal in Pinot noir (Mohekar et al., 2016/Chapter 2)

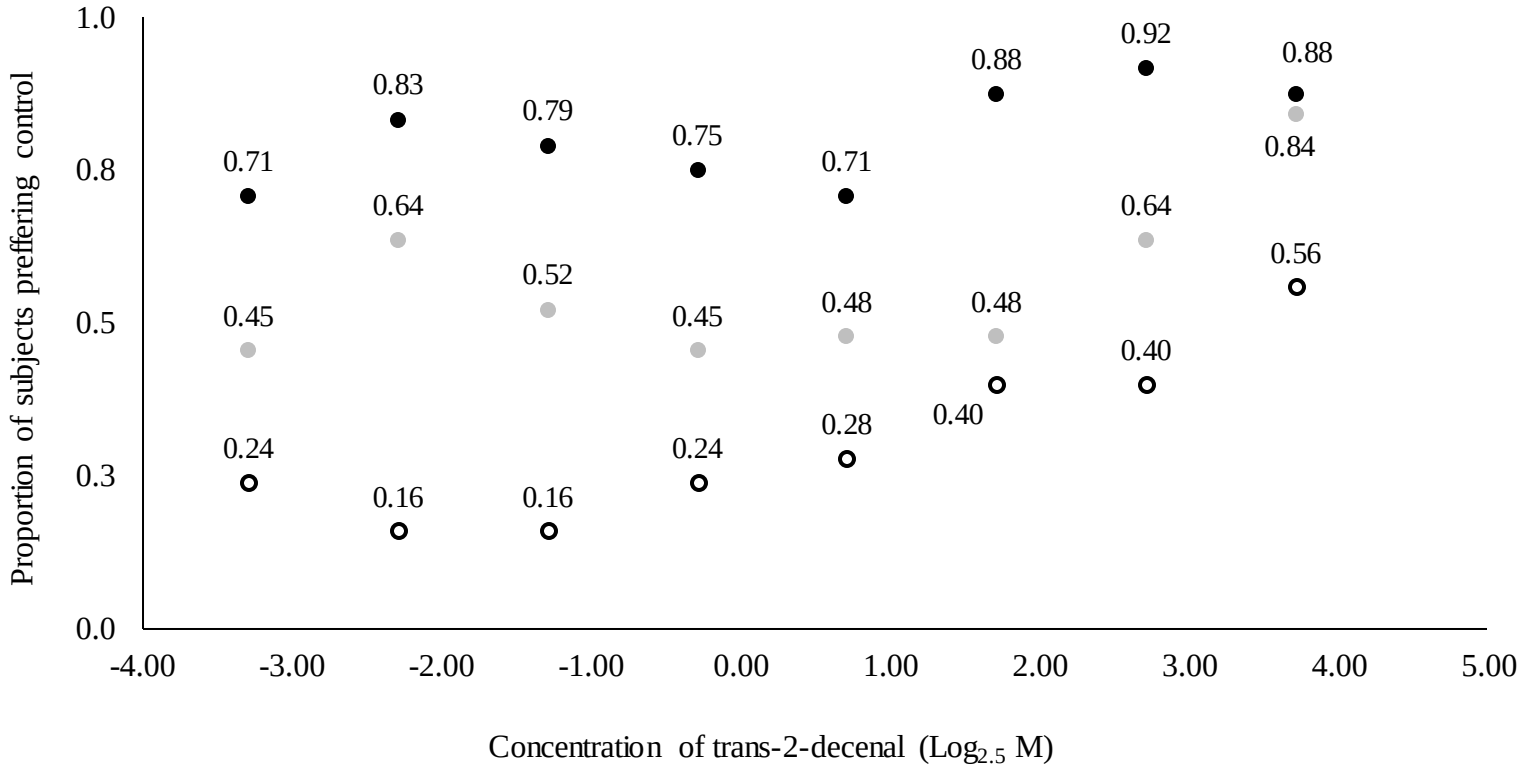

Figure 3.2. Relationship between preference for control and trans-2-decenal concentration in each preference-based group i.e. groups segmented based on their response in paired preference test. (■), Group 1 (N = 24); (■), Group 2 (N = 44); (□), Group 3 (N = 25).

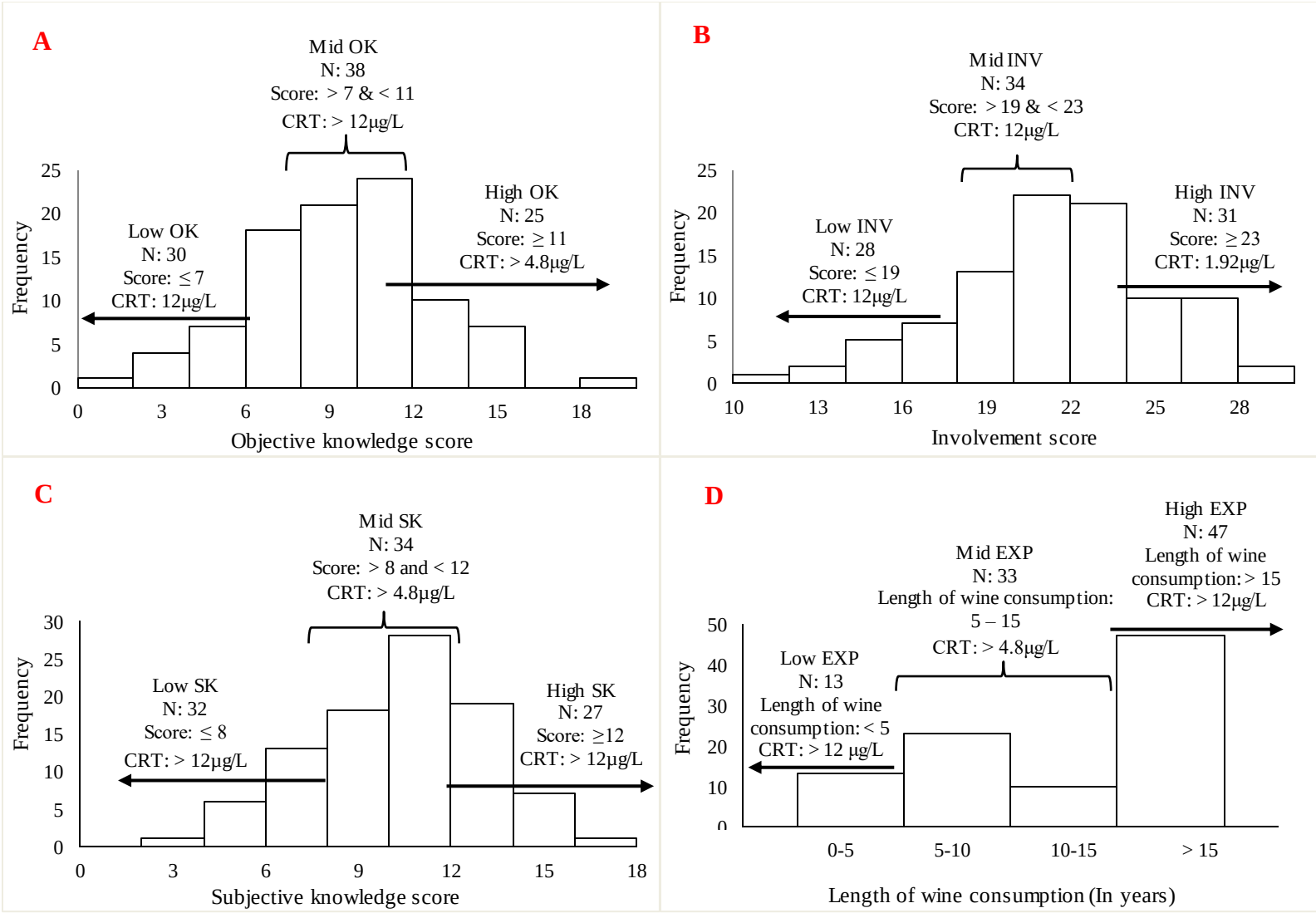

Figure 3.3. Distribution of consumer variable scores in the panel (N = 93). Segmentation based on A: Objective knowledge (OK), B: Involvement (INV), C: Subjective knowledge (SK), D: Experience (EXP) along with consumer rejection threshold (CRT) and sample size (N) for each group

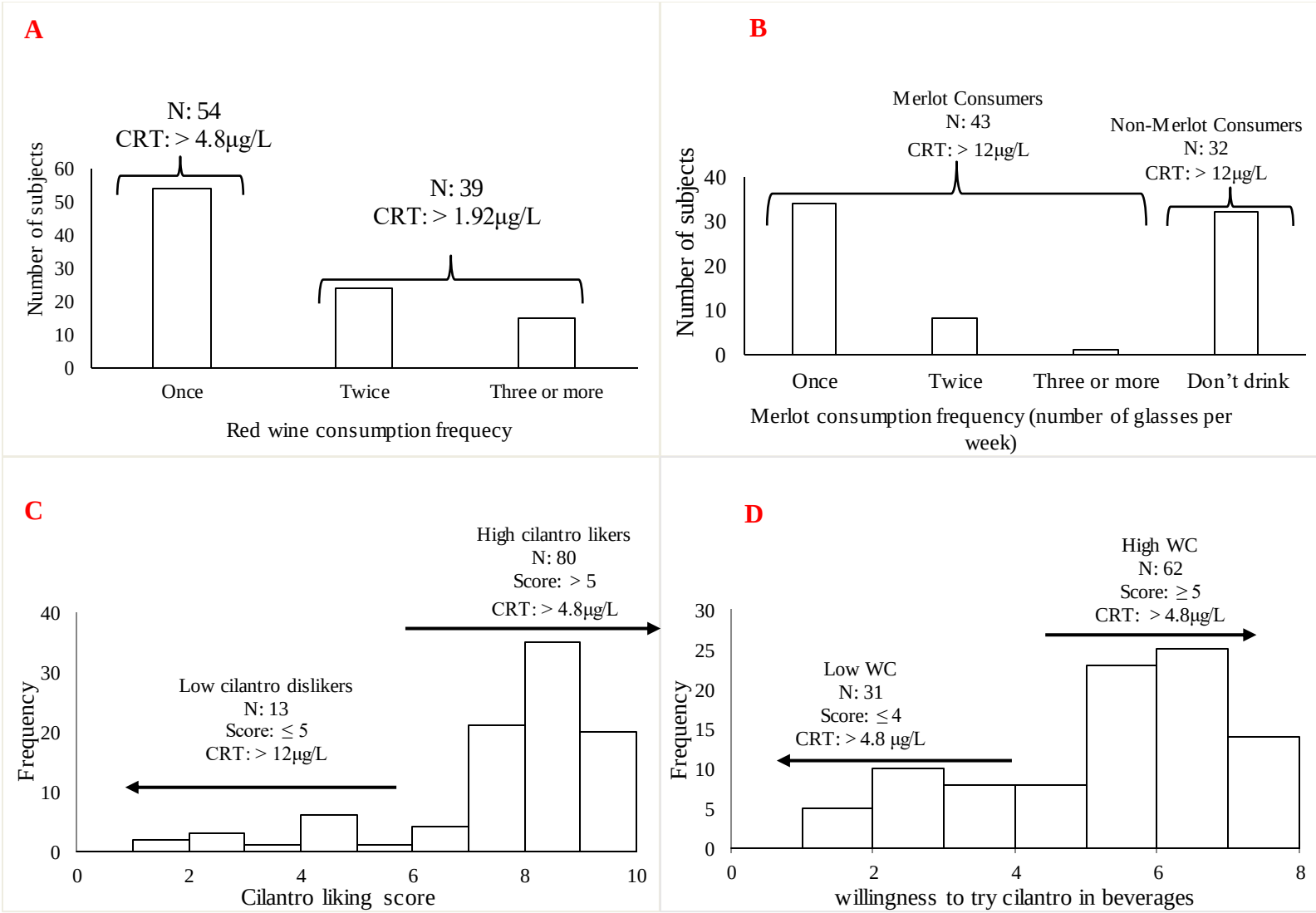

Figure 3.4. Distribution of consumer variable scores in the panel (N = 93). Segmentation based on A: Red wine consumption frequency (RED), B: Merlot consumer or not (MER), C: Cilantro liking (CIL), D: their willingness to try cilantro in beverages (WC) along with consumer rejection threshold (CRT) and sample size (N) for each group

1

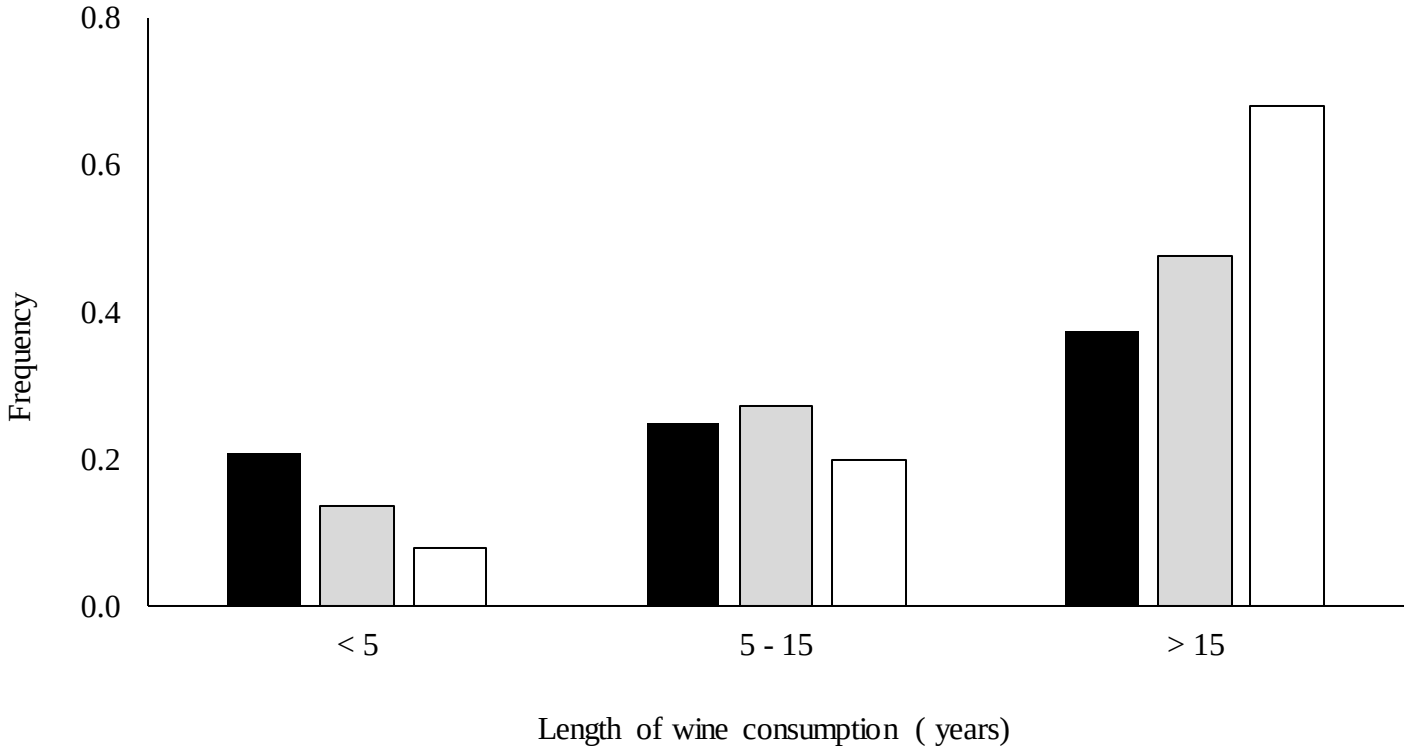

Figure 3.5. The difference in length of wine consumption (EXP) in preference-based groups. (■), Group 1; (■), Group 2; (□), Group 3

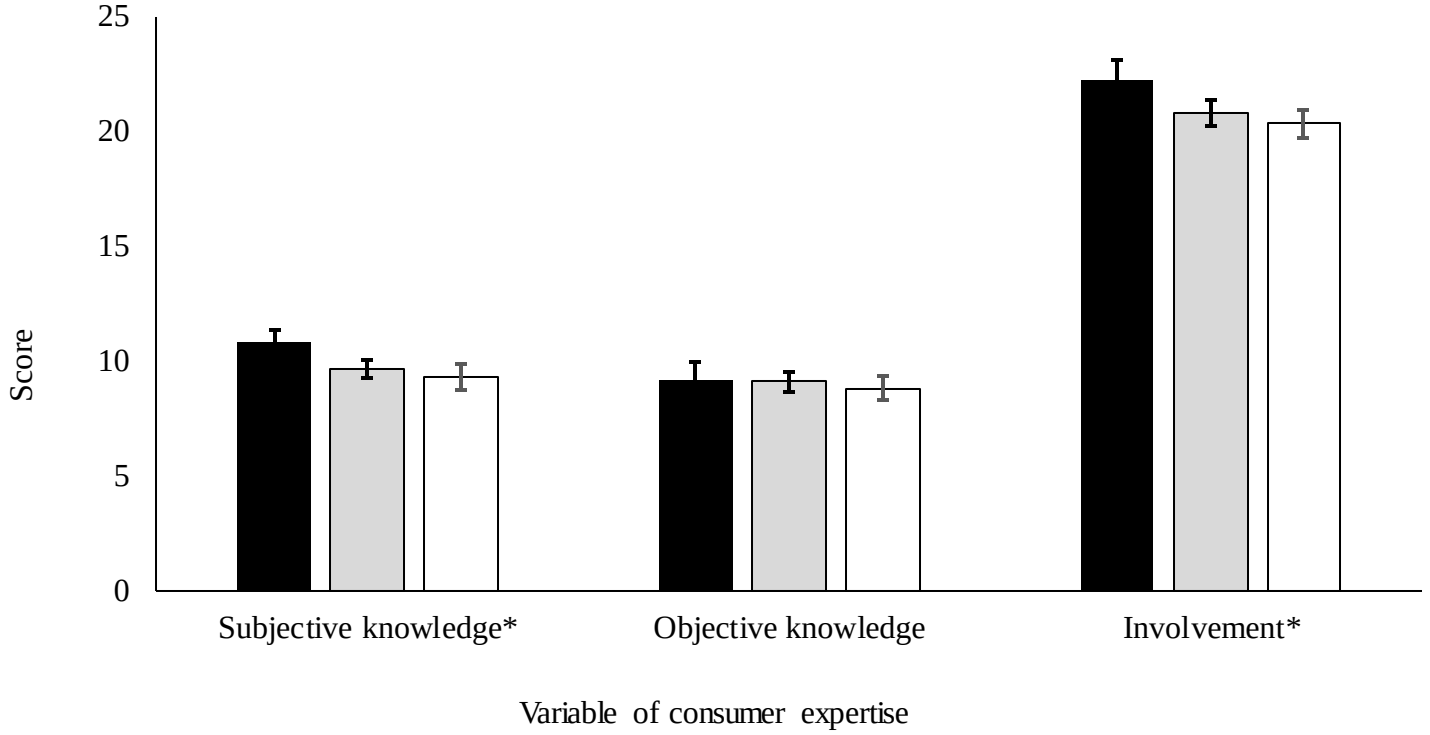

Figure 3.6. The difference in mean subjective knowledge, objective knowledge and involvement scores between preference-based groups. (■), Group 1; (■), Group 2; (□), Group 3

*Subjective knowledge and involvement were found to be significantly different at $p < 0.1$

Supplementary Tables

Table 1: Involvement questionnaire

Involvement	Type of involvement	References
1. I have a strong interest in wine	Interest/product	Hollebeek et al., 2007; Laurent & Kapferer, 1985; Lockshin et al., 2001; Lockshin et al, 1997; Mittal and Lee, 1989
2. Wine is important to me	Product	Mittal and Lee, 1989
3. Drinking wine gives me pleasure	Pleasure	Laurent and Kapferer, 1985; Michaelidou and Dibb, 2006
4. I choose my wine very carefully	Risk/Brand decision	Hollebeek et al., 2007; Lockshin et al., 2001; Lockshin et al., 1997

Table 2: Subjective knowledge questionnaire

Subjective knowledge	References
1. I feel very knowledgeable about wine. 2. Compared to my friends and acquaintances (most other people), I know more about wine? 3. Compared to wine experts, I know LESS about wine?	Johnson and Bastian, 2007; Taylor et al., 2008

Table 3: Objective knowledge questionnaire

1.	Which of the following characteristics (from sensory perspective) is commonly associated with wine Merlot. Answer choices: A. Black cherry, berries, plum, chocolate, herbal, spice; B. Apple, pineapple, melon, peach, lemon; C. Aromatic, herbaceous, laurel leaf, fresh hay, citrus; D. None of the above Difficulty level: Medium Score: +2 for correct (A), -1 for wrong (Others)
2.	Which of the following is one aromatic characteristic identified in / Pinot Noir Answer choices: A. Herbaceous; B. Berries; C. Ginger; D. None of the above Difficulty level: Medium Score: +2 for correct (B), -1 for wrong (Others)
3.	Most table wines have an alcohol content of Answer choices: 1. 3-5%; 2. 5-9%; 3. 9-14%; 4. 17-21%; 5. More than 21% Difficulty level: Easy Score: +1 for correct (C), -1 for wrong (Others)
4.	Which of the following grape varieties make red wines (Indicate all / that apply) Answer choices: 1. Syrah; 2.Cabernet Franc; 3. Viognier; 4. Gamay; 5. Riesling; 6. Gewurztraminer Difficulty level: Easy Score: +1 for correct (A, B, D), -1 for wrong (Others)
5.	Tannin is normally associated with what type of wine? Answer choices: 1. White; 2. Red; 3. Rose; 4. Sparkling Difficulty level: Easy Score: +1 for correct (B), -1 for wrong (Others)
6.	Which wine should be served at room temperature? Answer choices: 1. Red; 2. Rose; 3. Sparkling; 4. White Difficulty level: Easy Score: +1 for correct (A), -1 for wrong (Others)

7. Which of the following grape varieties are used for the famous / wines from Bordeaux? (Indicate all that apply)
   Answer choices: 1. Pinot Noir; 2. Gamay; 3. Cabernet Sauvignon; 4. Grenache; 5. Merlot; 6. Sangiovese

   Difficulty level: Difficult
   Score:
   Cabernet Sauvignon and Merlot: 5 points
   Cabernet Sauvignon alone: 2 points
   Cabernet Sauvignon or Merlot and Any other option: 1 point
   Answers that did not include Cabernet Sauvignon or Merlot: 0 point

8. What variety is used to make white wine with the label Fume Blanc
   Answer choices: 1. Chardonnay; 2. Sauvignon Blanc; 3. Cabernet Franc; 4. Semillon; 5. Pinot Blanc;
   6. Pinot Gris

   Difficulty level: Difficult
   Score: +3 for correct (B), -1 for wrong (Others)

Table 4: Consumer rejection threshold (CRT), sample size (N) for panel segments based on their consumer variables: objective knowledge (OK), involvement (INV), subjective knowledge (SK), length of wine consumption (EXP), Red wine consumption frequency (RED), Merlot consumer or not (MER), Cilantro liking (CIL), panel willingness to try cilantro in beverages (WC).

Variable		Cut off/Criterion	N	CRT (μg/L)
Objective Knowledge (OK)	Low OK	≤ 7	30	> 12
	Mid OK	> 7 & < 11	38	> 12
	High OK	≥ 11	25	> 4.8
Involvement (INV)	Low INV	≤ 19	28	> 12
	Mid INV	> 19 & < 23	34	> 12
	High INV	≥ 23	31	> 1.92
Subjective Knowledge (SK)	Low SK	≤ 8	32	> 12
	Mid SK	> 8 & < 12	34	> 4.8
	High SK	≥ 12	27	> 12
Experience (EXP)	Low EXP	< 5	13	> 12
	Mid EXP	5 - 15	33	> 4.8
	High EXP	> 15	47	> 12
Merlot Consumption (MER)		Merlot consumers	43	> 12
		Non-Merlot consumers	32	> 12
Red Wine Consumption (RED)		Once a week	54	> 4.8
		Two or more times a week	39	> 1.92
Cilantro Liking (CIL)		≤ 5	13	> 12
		> 5	80	> 4.8
Willingness to try cilantro in a beverage (WIL)		≤ 4	31	> 4.8
		> 4	62	> 4.8

CHAPTER 4

Effect of red and white wine processing on Brown Marmorated Stink Bug, *Halyomorpha haly* taint production

P. Mohekar[1], J. Osborne[1], N. G. Wiman[2], V. Walton[2], E. Tomasino[1]*

[1]Oregon State University, Department of Food Science & Technology, 100 Wiegand Hall,
Corvallis, Oregon 97331, USA

[2] Oregon State University, Department of Horticulture, 4017 Agriculture and Life Sciences Building, Corvallis, Oregon 97331, USA

*elizabeth.tomasino@oregonstate.edu, (T) (541)-737-4866, (F) (541)-737-1877

(prepared for submission to *Food Research International*)

ABSTRACT

Brown marmorated stink bug (BMSB) release taint compounds that have been found to effect final wine quality. This taint is a result of BMSB presence in grape clusters during wine processing. Tridecane, an odorless compound, and trans-2-decenal, a green, coriander-like aroma compound, are the main components of this taint. This study focuses on determining the effect of wine processing on trans-2-decenal and tridecane release in both red and white wine. Wines were produced by adding BMSB into grape clusters at densities 0, 0.3, 1 and 3 per cluster. Taint concentrations were quantified after each processing step. For red wines, taint compounds were released during destemming, decreased through alcoholic fermentation and increased after pressing, with the highest taint concentrations at pressing. Pinot noir made from 3 BMSB per cluster showed trans-2-decenal levels above its detection threshold but below its consumer rejection threshold. This density is therefore recommended for devising control measures in the vineyard. Due to the importance of pressing, different options were investigated in relation to BMSB taint production. Wines processed from press fractions showed higher levels of taint compared to free run. The type of press, bladder versus basket, was also seen to impact taint release. BMSB taint for white wines was not found to be problematic to wine quality as pressing occurs prior to fermentation and the final wine contained low levels of tridecane and no trans-2-decenal. The outcomes of this study will assist winemakers in devising appropriate modifications to minimize BMSB taint in wine.

Keywords: *Halyomorpha halys*, brown marmorated stink bug, trans-2-decenal, tridecane, Pinot noir, Pinot gris

Graphical Abstract

Taint compounds released by BMSB:
- Trans-2-decenal
- Tridecane

BMSB

+

Grape cluster

Wine processing of grapes containing BMSB

- *What wine processing steps are responsible for taint release by BMSB during wine making?*
- *What BMSB density in grapes will produce wine with taint compounds exceeding their sensory threshold*

BMSB tainted wine shows low consumer preference

Highlights

- Pressing and its parameters have the greatest impact to BMSB taint in red wine.
- BMSB taint is not an issue for white wines.
- Tridecane was present in red wine made from grape containing dead BMSB.
- BMSB threshold limit for red wine was estimated to be 3 bugs per cluster.

Chemical compounds studied in this article

Tridecane (PubChem CID: 12388); Trans-2-decenal (PubChem CID: 5283345)

Introduction

Brown marmorated stink bug (BMSB) (*Halyomorpha halys*, Hemiptera: Pentatomidae) is an economic threat to agricultural crops, including grapes and wine. The potential crop loss and impact to wine quality is of a concern to the wine industry (Kelly, 2012; Smith, 2014; Leskey et al., 2012a; Mohekar et al., 2015b). In 2010, increase in BMSB populations caused major damage to the agricultural crop in the Mid-Atlantic States. This resulted in low yields (as little as 10%) and left a significant economic impact on the agricultural industry in this region (Leskey et al., 2012a; Leskey et al., 2012b; Nielsen and Hamilton, 2009). Additionally, BMSB populations have been spreading rapidly across the country. As of June 2016, BMSB was found in 43 states, (USDA-NIFA SCRI, www.stopbmsb.org) including important wine growing states such as Oregon, California, Washington (Belisle 2012; Lara et al., 2016), Virginia (Basnet et al., 2015; Day et al., 2009) and New York (Smith, 2014). Outside of the United States, BMSB has also been detected in France (Callot and Brua, 2013), Switzerland (Gariepy et al., 2013; Wermelinger et al., 2008), Germany (Heckmann, 2012), Hungary (Vétek et al., 2014), Greece (Milonas and Partsinevelos, 2014), Canada (Fogain and Graff, 2011; Gariepy et al., 2013), Liechtenstein (Arnold, 2009), Italy (Cesari et al., 2014; Maistrello et al., 2014) and New Zealand (Harris, 2010). A rapid spread of BMSB on a global level has caused certain countries to implement strict regulations on imports to avoid BMSB introduction (Australian Government, Dept. of Agriculture and Water resources, http://www.agriculture.gov.au/import/industry-advice/ian/15/03-2015).

Currently, limited options are available for BMSB control in the field. The effectiveness of current field management techniques are variable, with no one practice completely removing BMSB. Most insecticides that are effective, possess major drawbacks. They can harm beneficial insects (such as anthropods) (Leskey et al., 2012b; Pfeiffer, 2012) and only work for a short time (Leskey et al., 2014). Even though monitoring tools have improved over the years, currently available commercial traps have less than optimal results in BMSB detection and control (Morrison et al., 2015). Further challenges in BMSB management arise due to their strong scattering ability and lack of natural enemies (Basnet, 2014; Leskey et al., 2012a; Hamilton, 2009; Pfeiffer, 2012).

Recent work on BMSB illustrates the damaging effect of this pest on grapes. In vineyards, BMSB can lower both, grape cluster yield and quality through feeding damage to berries. This damage can further lead to secondary pest attacks or infection by other pathogens (Basnet et al., 2015; Leskey et al., 2012a) and result in fruit collapse due to progressive necrosis (Lee, 2015; Leskey et al., 2012a). Direct injury to grape berries at veraison and pre-harvest have been found in Virginia vineyards (Basnet et al., 2015). Additional damage by BMSB occurs through grape contamination, a topic little explored, and the major focus of this study.

When BMSB populations are not successfully managed in the vineyard, the insects are carried into the winery within the grape clusters. BMSB are easily harvested with grape clusters due to their small size and coloring, allowing them to hide and remain

unnoticed. Additionally, BMSB numbers in the vineyard are known to increase dramatically during the harvest time (August-September) (Nielsen and Hamilton, 2009a, 2009b; Nielson 2011), resulting in a greater chance of BMSB grape contamination. The presence of BMSB during wine processing can taint juice and finished wine through a release of "BMSB taint" compounds (Baldwin et al., 2014; Fiola, 2011; Tomasino et al, 2013).

Tridecane and trans-2-decenal are the major components of BMSB taint as they make up more than 70% of the released taint compounds (Baldwin et al., 2014; Mohekar et al., 2015a). Tridecane, the largest component of BMSB taint is an odorless compound which may have an indirect impact on wine's quality. To the best of our knowledge there is no information regarding the effect of tridecane on wine sensory. Trans-2-decenal on the other hand is an aromatic compound with strong green, coriander (or cilantro) and musty-like aromas, typically considered undesirable in wine. Trans-2-decenal concentration above 4.8 µg/L (consumer rejection threshold, CRT) has been shown to cause a significant drop in consumer preference, indicating a negative effect of this compound on wine quality (Mohekar et al., 2014). Due to its direct association with consumer preference, CRT can provide a good estimate at which management techniques for BMSB and its taint are warranted.

The presence of BMSB taint in wine is considered detrimental to wine quality and therefore should be minimized. However, there is no information regarding the effect of wine processing on BMSB taint release. This knowledge can be very important to

winemakers in devising quality control measures. Wine processes can be changed to minimize BMSB taint levels in contaminated grape clusters. The objective of this study is to determine the wine processing steps responsible for "BMSB taint" release in both red and white wine, and to determine when levels of contamination in grape clusters reach CRT of trans-2-decenal (4.8 µg/L). Additionally, the effect of wine processing on presence of dead BMSB was also investigated. The impact of BMSB taint was measured in terms of *tridecane*, the largest component of stink bug taint and *trans-2-decenal*, the main compound responsible for the BMSB taint odor.

Methods

Brown Marmorated Stink Bug

BMSB were collected from August to September of 2013 and 2014 from various locations throughout Oregon. The following key characteristics were used to identify and distinguish *Halyomorpha halys* from similar species: light bands on the antennae, smooth "shoulders", and banding patterns on abdominal margins (Hoebeke and Carter, 2003). Insects were stored in plastic cages at 20 °C and fed organic food and water. BMSB densities of 0.3, 1 and 3 per grape cluster were used to produce different wine treatments (Table 4.1).

Grapes

Vitis vinifera L. cv. Pinot noir, Pinot gris and Merlot grapes were hand-harvested from a small research vineyard, owned and managed by Oregon State University (Monroe, OR). Pinot noir, Pinot gris and Merlot wines were produced in 2013 while,

Pinot noir was also made in 2014. Grapes were inspected after harvest and found to be free of BMSB. Fruit was stored for 24 – 48 hours at 4°C after harvest.

Vinification

Information regarding different winemaking treatments in this study is given in Table 4.1. Treatments include contamination with live BMSB, dead BMSB, press type, and press fractions. The number of BMSB per cluster was based on the current BMSB levels found in vineyards (Basnet et al., 2015; Smith et al., 2014). Standard winemaking protocol were used to make white wine (Pinot gris, Figure 4.1) and red wine (Pinot noir and Merlot, Figure 4.2). Cold soak was not a part of Merlot processing since this step is more typical for Pinot noir winemaking rather than Merlot.

Grape clusters were pre-weighed according to their treatment and replicate (Table 4.1). Pre-counted BMSB were mixed with grape clusters by scattering them throughout the grapes. Shaking and agitation was minimized to reduce any unwanted release of "BMSB taint" not related to the treatments. Pinot gris and Pinot noir grapes went through a destemmer (Velo DPC 40, Altivole, Italy) whereas Merlot grapes went through both destemming and crushing. This step was followed by a careful inspection of the destemmer to ensure all bugs went with the grapes into the must instead of with stems or left behind. Treatments were processed from low to high bug numbers. Any leftover bugs inside the destemmer were removed and added to the must to maintain the bug/cluster number. The crusher/destemmer was rinsed with

water in between treatments. White and red wine parameters for the processing steps following destemming are given below.

White wine Pinot gris must was pressed using a bladder press (Willmes model 6048 pneumatic press, 118 Lorsch, Germany) immediately after destemming. A pressure of 1 bar and 1.5 bar was used for soft press and hard press respectively. Each press cycle lasted for two minutes and the pomace inside the press was broken by hand between each cycle. Juice was cold settled at 4°C for 24 hours and racked off solids. Clarified juice was removed from cold storage and left at room temperature overnight before yeast inoculation. Alcoholic fermentation was conducted at 13°C with *Saccharomyces cerevisiae* strain EC 1118 (Lalvin). Yeast nutrients, diammonium phosphate (DAP) at the rate of 0.5g/L and Fermaid K at the rate of 0.25g/L (Lallemand, Montreal, Canada), were added 24 hours after yeast inoculation. Fermentation progress was monitored through Brix and temperature measurement using Anton-Paar DMA 35N Density Meter (Graz, Austria). Measurements were taken every two days. Fermentation was terminated by adding SO2 at 2° Brix. Wines were chilled at 4°C till clear and then racked to remove sediments and yeast lees. SO2 was added until 30mg/L free SO2 was reached. Wines were hand bottled into 375 mL bottles and sealed with screw caps (Stelvin, Amcor Limited, CA, USA).

Red wine After destemming, Pinot noir must went through a five day cold soak at 8°C. As stated previously, Merlot wines did not go through a cold soak or malolactic fermentation. Alcoholic fermentation for both wines was carried out by

Saccharomyces cerevisiae (BGY, Endoferm Burgundy) at 25 ± 2 ˚C. Yeast nutrient, diammonium phosphate (DAP) at the rate of 0.5g/L and Fermaid K at the rate of 0.25g/L (Lallemand, Montreal, Canada) was added 24 hours after inoculation. During fermentation, punch downs occurred once daily along with brix and temperature measurement using an Anton-Paar DMA 35N Density Meter (Graz, Austria). Wines were pressed once all treatments reached dryness (brix < 0.2^{o}). Two different press types were used (Table 4.1). A custom made basket press with a pressure gauge was operated at 2 bar for one minute and a bladder press (Willmes model 6048 pneumatic bladder press, Lorsch, Germany) was used at 1 bar for two minutes. BMSB left the wine system after pressing. Treatments then went through malolactic fermentation (MLF) carried out by *Oenococcus oeni* (Vinoflora, Chr. Hansen) at 28 ± 2^{o}C. Free SO_2 levels were adjusted to 30 mg/L when malic acid concentration dropped below 100 mg/L (i.e. end of MLF). Wines were then racked and bottled into 375ml bottles with screw caps (Stelvin, Amcor Limited, CA, USA).

Sampling Must and wine samples were collected after each processing step as indicated in Figure 4.1 and 4.2. A 40ml sample was collected in 50ml centrifuge tubes (VWR International) during wine processing for "BMSB taint analysis". Samples were centrifuged at 4200 rpm for 10 minutes (Allegra–22 Centrifuge, Beckman Coulter Inc.) and stored in 40ml amber vials with PTFE lined caps (Sigma Aldrich) at −18 ^{o}C.

Additional wine treatments

Effect of pressing: Differences in press types and press fractions were compared to determine their impact on taint levels in the finished wines. Taint concentration in Pinot noir processed using basket press (2bar for 1 min) and a bladder press (1bar for 2 min) were compared. To determine the effect of press fraction, Merlot wine processed using a bladder press (1bar for 2 min) was divided into its free run and first press fraction. One third of the free run was processed separately from the pressed fraction. The taint levels in the finished wine made from the press fraction was calculated based on the final volume before removing the free run.

Presence of dead BMSB Grape clusters that have been treated for pests may contain dead BMSB. These dead insets may not be able to produce taint compounds but may still contain them in their body which can also affect wine. Pinot noir wine was made as per red wine vinification protocol but using 0.3 dead BMSB per cluster in place of live (Table 4.1).

Chemical Analysis

Standard Wine Parameters Wine samples were analyzed for alcohol, pH, titratable acidity, reducing sugar, SO_2 (free and total) and malic acid using the methods described by (Iland, 2004). Alcohol percentage from 2013 and 2014 wines were measured using an ebulliometer and density meter (Anton Paar DMA 4500 M-EC) respectively.

BMSB taint analysis

Headspace-Solid Phase Micro-Extraction-Multidimensional Gas Chromatography-Mass Spectrometry (HS-SPME-MDGC-MS) method was developed to measure trans-2-decenal and tridecane concentration in the wine samples.

Reagents Trans-2-decenal, 3-octanol, tridecane, and D-30 tetradecane were purchased from Sigma Chemical Co. (St. Louis, MO, USA) (Table 4.2). D30-tertradecane and 3-octanol were used as internal standards for tridecane and trans-2-decenal analysis respectively. Milli-Q water was obtained from a Millipore Direct-Q® 5 UV-R water purification system (EMD-Millipore, Billerica, MA, USA). Absolute ethanol (200-proof) was obtained from Pharmco-AAPER (Vancouver, WA, USA) and sodium chloride from VWR/JT Baker. All chemicals used were HPLC/ACS grade. Commercial wines used for calibration curves and to check standards were free of BMSB taint and include, 2010 Oregon Pinot noir, 2012 California Merlot and 2010 Oregon Pinot gris.

Sample preparation Stock standard solutions of all aroma chemicals were prepared in absolute ethanol and stored at -18°C in 40ml amber vials with PTFE lined caps. Stock standards were prepared fresh prior to the analysis. Diluted standards for sample preparation were made in 14% ethanol from stock solutions. Tridecane and trans-2-decenal diluted standards were prepared as a composite and internal standards were kept separate. Diluted standards were then transferred into 2ml amber vials and stored at -18 °C until use. Each 2 mL subsamples was used only once.

Before each analysis, a working standard was prepared by adding 130µL of diluted standard into 8.07 ml of a base wine (Pinot noir, Merlot or Pinot gris). The required volume of a working standard (0 to 1.8 ml), base wine (1.8 – 0 ml), 7.04 ml of Milli-Q water, 80 µL of 3-octanol standard solution, and 80 µL of D30-tetradecane standard solution were placed into 20 ml amber glass vials (22.5 x 75.5 mm), followed by (4.5 - 5) g of sodium chloride addition and closed tightly using PTFE-lined caps.

Sample preparation for wines and must All samples were prepared in a 20ml amber vials, 22.5 x 75.5 mm. 7.04ml of Milli-Q water and 1.8ml of thawed wine sample was added to the vial to achieve a 5X dilution. For juice samples, water level was reduced to 5.24ml and alcohol level was adjusted by adding 1.8 ml of 14% (for red wine) or 11% (for white wine) ethanol solution, to ensure the ethanol concentration was the same in all vials. Sample dilution was followed by 80µL of each internal standard and 4.5g sodium chloride addition. Vials were tightly capped with PTFE-lined caps and placed in a stack cooler attached to the Combi-Pal autosampler (CTC-Analytics, Zwingen, Switzerland). Vial temperature was maintained at 8°C prior to sample extraction.

SPME fibre, conditioning and sample extraction Headspace solid phase microextraction (HS-SPME) using a stableflex DVB/CAR/PDMS combination SPME fiber (p/n 57348-U, 50/30 µm thick, 2cm long, 24 Ga) was used for the

extraction of BMSB taint compounds from must/wine. All new fibers were incubated for 30min at 250°C before their first use.

For taint extraction, sample vials were incubated at 40°C and agitated simultaneously at 500 rpm (5s on, 2s off) for 10 min. SPME fiber was then inserted into the vial headspace for the absorption of sample volatiles for 60min at 40°C with no agitation. The fiber was then injected into the first GC and compounds were desorbed at 250°C for 10min. The fiber was conditioned further in an NDL heater for 10min at 250°C.

MDGC-MS Shimadzu GC-2000 plus with a split/splitless injector coupled to a Shimadzu QP 2010 GC-mass spectrometer by a heart-cutting dean's switch (Shimazdu North America, Pleasanton, CA, USA) was used to determine tridecane and trans-2-decenal levels in wines. The instrument was equipped with CTC Combi-Pal autosampler (CTC-Analytics AG, Switzerland). Compound separation occurred on two columns: 1) RTX-wax, 30.0m length, 0.25mm ID and 0.5μm film thickness (Crossbond® Carbowax®polyethylene glycol, Restek Corporation, Bellefonte, PA, USA) in the first GC-FID and 2) Rxi-5MS, 15 m x 0.25 mm ID x 0.5 m (100% dimethyl polysiloxane, Restek Corporation, Bellefonte, PA, USA) in the second GCMS.

A temperature program was used for both columns which started simultaneously. First GC was operated under following parameters: FID injector temperature at 230 °C. The column oven was initially held at 35°C for 5min, and raised to 100°C at a rate of 10°C/minute and held at this temperature for 10min, then further increased to

180°C at a rate of 4°C/minute, and finally raised temperature to 250°C at a rate of 10°C, at which the temperature was kept constant for 10min. The first GC system was operated under splitless mode. Carrier gas (Helium) flow rate was controlled using a constant pressure of 169.5kPa. The sample travelled through a Deans switch connected to the second GCMS. The heart-cutting windows were: 12.5 − 15.5 min, 17.0 − 19.3 min, 20.0 − 25.0 min, 31.5 − 34.5 min. The switching pressure was 135kPa. The temperature program for second GC was as follows: The column oven was held at 35°C for 10min, and then increased to 70°C at a rate of 3°C/min and held at this temperature for 8min, then further increased to 115°C at a rate of 3°C/min and at which the temperature was kept constant for 6min, and finally raised temperature to 250°C at a rate of 15°C and held at this temperature for 6min. The total flow rate was controlled using a constant pressure of 86.9kPa. The total program time per sample was 65.67 minutes.

Compound separation on second GC was followed by identification and quantification on MS. The ion source and interface temperatures were set at 200 °C and 250 °C. The MS detector was operated in electron impact mode (EI, 70eV) under a scan mode (m/z 33–303 Da). A variable detector gain between 0.8 to 1.35 was used for different compounds. Compound identification was based on comparison of pure standards and NIST11 (National Institute of Standards and Technology) mass spectra library. Retention times, target ions and qualifier ions for each compound are reported in Table 4.3.

Quantification, Limit of Detection, Limit of Quantification An eight point standard calibration curve was used to quantify trans-2-decenal and tridecane levels in wine samples. The concentration range is given in Table 4.3. Standard curves were obtained by plotting the peak versus concentration area ratio of the reference standard to the corresponding internal standard. All wine samples and calibration curve samples were analyzed in triplicate. Two separate standard curves, one for white wine and one for red wine were calculated for each compound. Pinot gris was used as a base wine for the white wine standard curve. The standard curve for red wine was an average of reference standards measured in Pinot noir and Merlot. Concentration of taint compounds was measured using a linear regression equation from the calibration curve.

Limit of Detection, Limit of Quantification, Accuracy, Repeatability Calculations for limit of detection (LOD) and limit of quantification (LOQ) were based those used by (Callejón et al., 2008). Recoveries of standard addition were estimated by running red and white base wines with reference standards added. Percent recoveries were then used to estimate method accuracy and any matrix effects. During each run, a standard with trans-2-decenal concentration of 3.22 µg/L and tridecane concentration of 6.45 µg/L was analyzed to determine the stability of the internal standard.

Statistical Analysis

All statistical analysis was run using XLSTAT-Pro 2014 (Addinsoft, New York, NY, USA). Significant differences among BMSB wine treatments were measured using one way Analysis of Variance (ANOVA), student's t-test and Tukey's HSD.

Results and discussion

Basic chemical composition

Table 4.3 shows the alcohol content, pH, titratable acidity and residual sugar content of wines. Wine chemical composition demonstrated small variation among treatments but none were based on the BMSB addition. All wines reached dryness or desired brix level without any difficulty. Our results indicate that the presence of BMSB has no effect on wine's basic oenological parameters. This is similar to what has been observed previously for multicolored asian lady beetles (Pickering et al., 2007b).

Tridecane and trans-2-decenal analysis

The estimates of LOD and LOQ for trans-2-decenal are below the levels at which trans-2-decenal can impact wine's flavor (Table 4.4) (Mohekar et al., 2014). Tridecane showed a low percent recovery in both red and white wine (Table 4.4). Therefore a factor of 4.94 was applied for its quantification in Pinot gris and of 2.17 in Pinot noir and Merlot. A low solubility of tridecane in water and therefore in ethanol solution could be responsible for its low percent recovery. Future work should focus on improving these parameters for tridecane quantification. Trans-2-decenal measurements did not require any adjustments as percent recovery was well within an acceptable range of 80-120% (Table 4.4) (Sinha et al., 2011).

BMSB taint in white wine

Taint levels during Pinot gris processing varied (Figure 4.3). At each processing step, tridecane concentration was found to be higher than trans-2-decenal. This was

expected as tridecane is the largest component of BMSB secretions (Baldwin et al., 2014; Mohekar et al., 2015a). Taint release occurred during destemming and pressing. Similar results have been reported for lady bugs where crushing/destemming and pressing were found to be the critical steps of processing (Pickering et al., 2007b; Kögel et al., 2012). All treatments released more taint during hard pressing compared to soft pressing. The exception to this was trans-2-decenal release in T1 (0.25 BMSB/cluster) treatment. Higher pressure was applied during hard pressing compared to soft pressing and thus is likely to have resulted in higher amount of BMSB taint release. However, alcoholic fermentation appears to remove most of the taint compounds which is likely due to the taint compounds being driven off by carbon dioxide or reactions with other wine volatile or non-volatile compounds (Morakul et al., 2010; Robinson et al., 2009). This is in agreement with previous work by Fiola (2011) who also reported loss of BMSB taint post fermentation. No trans-2-decenal was found in any of the finished wines and only low levels of tridecane, 9.69 µg/L in T2 (1 BMSB/cluster). Therefore, the risk of BMSB contamination in white wine grapes is low as both wine processing steps responsible for taint release occur before alcoholic fermentation.

Trans-2-decenal and tridecane in red wine

Trans-2-decenal and tridecane concentration during red wine processing can be found in Figure 4.4a and 4.4b respectively. As anticipated, the concentration of both compounds increased with an increase in BMSB density. Trans-2-decenal was not found in any of the finished wine treatments except for 2014 Pinot noir at high BMSB

density, 3 BMSB per cluster (T3). This treatment contained 2µg/L of trans-2-decenal and 614.4 µg/L of tridecane. Similar to white wine, destemming and pressing significantly impacted the amount of BMSB taint. However, because in red wine; pressing occurs after alcoholic fermentation the levels of BMSB taint in the wines were significantly higher than that seen in the white wines. Furthermore, the presence of high BMSB taint in the red wines after pressing is of concern given the minimal decrease of the taint compounds in the wine post-pressing and the low reactivity of BMSB taint compounds in the wine matrix. For example, trans-2-decenal levels decreased by only a small amount after pressing in comparison to a large reduction after destemming. Other insects, such as lady beetles have shown similar behavior where red wine pressing is seen to be more problematic than destemming (Kögel et al., 2012). Due to the high significance of pressing for BMSB taint in red wine the study was expanded to specifically investigate different pressing options.

The effect of press type At a density of 3BMSB per cluster, tridecane level in Pinot noir processed using bladder press was observed to be higher than its level in Pinot noir processed using basket press. (Figure 4.5). A similar result was observed at BMSB density of 0.3 per cluster (data not shown). These preliminary findings suggest that taint levels in the finished wine can be minimized by using a softer press (basket press). However, given that the basket and bladder press were operated at different pressures and times additional research is needed to confirm the findings reported in the present study. In addition, the basket press used in the present study was a small scale custom made press that may not accurately represent a larger commercial sized

basket press. The use of similar processing parameters and press types that are better representative of commercial presses used in the winery are required in order to better understand the effect of press type on BMSB taint.

The effect of press fraction Press fractions in red wine making are often kept separate to achieve a desired phenolic profile. Certain wine styles are produced from free run, without any press fraction. Therefore, the levels of BMSB taint in free run versus press fraction after alcoholic fermentation was of interest. Our results show that wines made from free run contained significantly less tridecane compared to corresponding wine from press fractions ($\alpha = 0.05$) (Figure 4.5). This was anticipated since pressing tends to contribute a significant portion of wine taint. Therefore, the use of free run and elimination of pressing step is recommended for highly contaminated grape clusters.

The impact of dead BMSB Tridecane levels from wines made with dead BMSB at the density of 0.3 per cluster are shown in Figure 4.4b. Trans-2-decenal levels for this treatment were found to be below the LOD except at the end cold soak. Finished wine contained 7.74 µg/L of tridecane which was lower than the tridecane level (26.88 µg/L) in Pinot noir made from live BMSB at the same density. The result of this treatment indicates that killing BMSB in the field may not be enough to get rid of BMSB taint compounds from the wine. Although the impact of tridecane to final wine quality has not yet been determined. To completely remove BMSB taint all BMSB (dead or alive) must be removed from the grape clusters prior to processing.

BMSB threshold in grape clusters

BMSB threshold in grapes corresponding to trans-2-decenal CRT of red wine was determined. We did not calculate this threshold for white wine since BMSB taint does not appear to be problematic. A single threshold limit is reported for both Pinot noir and Merlot since trans-2-decenal CRT in these wines is similar (Mohekar et al., 2015b). A conservative approach has been adopted for reporting BMSB threshold density due to the negative effect of its taint compound on wine quality, the variation caused by press type which can be as large as 10-fold, and the non-linear relationship between the amount of taint released and BMSB densities in grape clusters. BMSB tolerance limit is estimated to be 3 bugs per cluster, resulting in wine with 2µg/L trans-2-decenal which is lower than known CRT of trans-2-decenal in red wine. We realize that wines made in this study were produced on a small scale at a research winery, likely to result in low taint secretions. Higher taint levels are expected during industrial pressing operation which utilizes heavier press type and longer pressing time. Therefore, an upper threshold of 3 BMSB per cluster should maintain trans-2-decenal level in wine below its CRT. A previous study has shown that 28% of the consumer panel exhibits low preference towards trans-2-decenal and therefore could reject Pinot noir containing trans-2-decenal below 0.05µg/L. Wines targeted towards such consumers could require lower BMSB thresholds in the vineyard (Mohekar et al., 2014).

The BMSB threshold levels from this study are higher than previously reported threshold of 10-20 BMSB per 25 lb lugs (0.1-0.2 per cluster) (Fiola, 2011, 2013).

This disparity is most likely due to differences in methodology and sensory panelists. Threshold limits by Fiola (2011, 2013) were based on taint levels in must as opposed to finished wine. We have clearly shown that the BMSB taint in must decreases during fermentation and that it is pressing after red wine fermentation that is the critical control point. This resulted in low BMSB threshold since must tends to contain higher concentrations of taint compounds.

Sensory analysis by Fiola (2011) was performed by wine experts who were familiar with BMSB taint. Expertise and familiarity are known to increase panel sensitivity, resulting in low threshold levels (Blackman et al., 2010; Pickering et al., 2007a). The CRT levels however were determined by regular wine consumers who were not familiar with the taint, providing a more realistic sensory threshold. This study provides the first estimates of the BMSB threshold limits estimated in the finished wine. BMSB threshold levels can be applied to guide a pesticide application program in the vineyard. Additionally, it will provide a valuable reference point for future research on this taint.

Conclusion

While it is preferred to make wine free of BMSB and the resulting BMSB taint, this may prove to be difficult in the future due to the increasing prominence of BMSB in vineyards. Should contaminated grapes be brought into the winery it is possible to reduce/remove this taint through winemaking processes. We have shown that the critical step for BMSB taint in wines are those that occur after fermentation,

specifically at pressing. Therefore we do not see any BMSB taint problems associated with white or rose wines, since pressing occurs before fermentation.

Taint levels in finished wine can be reduced by carefully modifying the red wine making protocol. Winemakers are advised to use free run or a lighter press to reduce BMSB taint levels in wine. This is particularly important when BMSB densities in the vineyard exceed one per cluster. To maximize the yield while keeping taint levels below sensory threshold, we recommend blending free run and press fraction following malolactic fermentation. Lastly, the minimum density of BMSB in grapes clusters relating to sensory threshold of its taint compound was estimated to be 3 or more BMSB per cluster. It provides a reference point above which control measures in the vineyard are warranted. However, this density level should be followed with caution when wine is made with heavier industrial press, higher pressure and longer press cycle.

Overall, the outcome of this study will allow winemakers to make an informed decision regarding their winemaking protocol to minimize the BMSB taint levels. This is particularly important if fining treatments are unable to reduce BMSB taint in the finished wine. Additionally, vineyard manager will be able to devise necessary control measures in the field based on BMSB threshold densities in grapes relating to trans-2-decenal CRT.

Acknowledgement:

Funding: This work was supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number SCRI#2011-51181-30937 and the U.S. Department of Agriculture Northwest Center for Small Fruits under award number OWB# 4040-0001.

Literature cited:

Arnold, K., 2009. Halyomorpha halys (Stål, 1855), eine für die europäische Fauna neu nachgewiesene Wanzenart (Insecta: Heteroptera: Pentatomidae: Cappaeini).

Mitteilungen des Thüringer Entomologenverbandes 16, 19.

Baldwin, R.L., Zhang, A., Fultz, S.W., Abubeker, S., Harris, C., Connor, E.E., Van Hekken, D.L., 2014. Hot topic: Brown marmorated stink bug odor compounds do not transfer into milk by feeding bug-contaminated corn silage to lactating dairy cattle. J. Dairy Sci. 97, 1877–1884. doi:10.3168/jds.2013-7545

Basnet, S., 2014. Biology and pest status of brown marmorated stink bug (Hemiptera: Pentatomidae) in Virginia vineyards and raspberry plantings (Thesis). Virginia Polytechnic Institute and State University, Blacksburg, VA.

Basnet, S., Kuhar, T.P., Laub, C.A., Pfeiffer, D.G., 2015. Seasonality and Distribution Pattern of Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) in Virginia Vineyards. J. Econ. Entomol. tov124. doi:10.1093/jee/tov124

Blackman, J., Saliba, A., Schmidtke, L., 2010. Sweetness acceptance of novices, experienced consumers and winemakers in Hunter Valley Semillon wines. Food Qual. Prefer., Eighth Pangborn Sensory Science Symposium - Wine Special Issue 21, 679–683. doi:10.1016/j.foodqual.2010.05.001

Callejón, R.M., González, A.G., Troncoso, A.M., Morales, M.L., 2008. Optimization and validation of headspace sorptive extraction for the analysis of volatile compounds in wine vinegars. J. Chromatogr. A 1204, 93–103. doi:10.1016/j.chroma.2008.07.064

Callot, H., Brua, C., 2013. Halyomorpha halys (Stål, 1855), the marmorated stink bug, new species for the fauna of France (Heteroptera Pentatomidae). L'Entomologiste 69, 69–71.

Cesari, M., Maistrello, L., Ganzerli, F., Dioli, P., Rebecchi, L., Guidetti, R., 2014. A pest alien invasion in progress: potential pathways of origin of the brown marmorated stink bug Halyomorpha halys populations in Italy. J. Pest Sci. 88, 1–7. doi:10.1007/s10340-014-0634-y

Day, E.C., McCoy, T., Miller, D., Kuhar, T.P., Pfeiffer, D.G., 2009. Brown Marmorated Stink Bug | Publications and Educational Resources | Virginia Tech [WWW Document]. Va. Coop. Ext. URL https://pubs.ext.vt.edu/2902/2902-1100/2902-1100.html (accessed 3.16.16).

Fiola, J.A., 2011. Brown Marmorated Stink Bug (BMSB) Part 3 - Fruit Damage and Juice/Wine Taint. Timely Vitic.

Fiola, J. A. 2013. The BMSB - A "new" and very important pest of grapes - and wine. http://www.pawinegrape.com/uploads/PDF files/Meeting Presentations/2013 ipm/Fiola_BMSB update grape for IPM 0313.pdf.

Fogain, R., Graff, S., 2011. First records of the invasive pest, Halyomorpha halys (Hemiptera: Pentatomidae), in Ontario and Quebec. J. Entomol. Soc. Ont. 142, 45–48.

Gariepy, T.D., Haye, T., Fraser, H., Zhang, J., 2013. Occurrence, genetic diversity, and potential pathways of entry of Halyomorpha halys in newly invaded areas of Canada and Switzerland. J. Pest Sci. 87, 17–28. doi:10.1007/s10340-013-0529-3

Harris, A.C., 2010. Halyomorpha halys (Hemiptera: Pentatomidae) and Protaetia brevitarsis (Coleoptera: Scarabeidae: Cetoniinanae) intercepted in Dunedin. The Weta 40, 42–44.

Heckmann, R., 2012. First evidence of Halyomorpha halys (Stål, 1855) (Heteroptera: Pentatomidae) in Germany. Heteropteron H 36, 17–18.

Hoebeke, E.R., Carter, 2003. Halyomorpha halys (Stål) (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. P Entomol Soc Wash. Proc. Entomol. Soc. Wash. 105, 225–237.

Iland, P., 2004. Chemical Analysis of Grapes and Wine: Techniques and Concepts. Patrick Iland Wine Promotions PTYLTD.

Jeffrey R Smith, S.P.H., 2014. Potential Impact of Halyomorpha halys (Hemiptera: Pentatomidae) on Grape Production in the Finger Lakes Region of New York. J. Entomol. Sci. 49, 290–303.

Kögel, S., gross, jurgen, Hoffmann, C., 2012. Sensory thresholds of ladybird taint in “Riesling” and “Pinot Noir” under different fermentation and processing conditions. Vitis -Geilweilerhof- 51, 27–32.

Lara, J., Pickett, C., Ingels, C., Haviland, D.R., Grafton-Cardwell, E., Doll, D., Bethke, J., Faber, B., Dara, S.K., Hoddle, M., 2016. Biological control program is being developed for brown marmorated stink bug. Calif. Agric. 70, 15–23. doi:10.3733/ca.v070n01p15

Lee, D.-H., 2015. Current status of research progress on the biology and management of Halyomorpha halys (Hemiptera: Pentatomidae) as an invasive species. Appl. Entomol. Zool. 50, 277–290. doi:10.1007/s13355-015-0350-y

Leskey, T.C., Hamilton, G.C., Nielsen, A.L., Polk, D.F., Rodriguez-Saona, C., Bergh, J.C., Herbert, D.A., Kuhar, T.P., Pfeiffer, D., Dively, G.P., Hooks, C.R.R., Raupp,

M.J., Shrewsbury, P.M., Krawczyk, G., Shearer, P.W., Whalen, J., Koplinka-Loehr, C., Myers, E., Inkley, D., Hoelmer, K.A., Lee, D.-H., Wright, S.E., 2012a. Pest Status of the Brown Marmorated Stink Bug, Halyomorpha Halys in the USA. Outlooks Pest Manag. 23, 218–226. doi:10.1564/23oct07

Leskey, T.C., Short, B.D., Butler, B.R., Wright, S.E., 2012b. Impact of the Invasive BrownMarmorated Stink Bug, Halyomorpha halys (St°al), inMid-Atlantic Tree Fruit Orchards in the United States: Case Studies of CommercialManagement. Hindawi Publ. Corp. 1–14. doi:10.1155/2012/535062

Maistrello, L., Costi, E., Caruso, S., Vaccari, G., Bortolotti, P., Nannini, R., Casoli, L., Montermini, A., Guidetti, R., 2014. Halyomorpha halys in Italy: first results of field monitoring in fruit orchards., in: ResearchGate. Presented at the IOBC-WPRS Working Group - Integrated Plant Protection in Fruit Crops, Sub Groups Pome fruit arthropods and Stone fruits.

Milonas, P.G., Partsinevelos, G.K., 2014. First report of brown marmorated stink bug Halyomorpha halys Stål (Hemiptera: Pentatomidae) in Greece. EPPO Bull. 44, 183–186. doi:10.1111/epp.12129

Mohekar, P., Lim, J., Lapis, T., Tomasino, E., 2014. Consumer rejection thresholds of trans-2-decenal in Pinot noir: Linking wine quality to threshold segmentation. Presented at the Institute of Food Technology Annual Meeting, New Orleans, LA.

Mohekar, P., Tomasino, E., Wiman, N.G., 2015a. Defining defensive secretions of brown marmorated stink bug, Halyomorpha halys. Presented at the 2015 Annual Meeting of the Entomological Society of America, Minneapolis, MN.

Mohekar, P., Wiman, N.G., Osborne, J., Walton, V., Tomasino, E., 2015b. Brown Marmorated Stink Bug (Halyomorpha halys): Impact on wine quality and management techniques. Presented at the 2015 Annual Meeting of the Entomological Society of America.

Morakul, S., Athes, V., Mouret, J.-R., Sablayrolles, J.-M., 2010. Comprehensive Study of the Evolution of Gas−Liquid Partitioning of Aroma Compounds during Wine Alcoholic Fermentation. J. Agric. Food Chem. 58, 10219–10225. doi:10.1021/jf101890y

Nielsen, A.L., Hamilton, G.C., 2009a. Seasonal Occurrence and Impact of Halyomorpha halys (Hemiptera: Pentatomidae) in Tree Fruit. J. Econ. Entomol. 102, 1133–1140. doi:10.1603/029.102.0335

Nielsen, A.L., Hamilton, G.C., 2009b. Life History of the Invasive Species Halyomorpha halys (Hemiptera: Pentatomidae) in Northeastern United States. Ann. Entomol. Soc. Am. 102, 608–616. doi:10.1603/008.102.0405

Pfeiffer, D., 2012. Brown Marmorated Stink Bug, in: Arthropod Management in Vineyards: Pests, Approaches, and Future Directions. Springer, pp. 450–457.

Pickering, G. J., Karthik, A., Inglis, D., Sears, M., Ker, K., 2007a. Determination of Ortho- and Retronasal Detection Thresholds for 2-Isopropyl-3-Methoxypyrazine in Wine. J. Food Sci. 72, S468–S472. doi:10.1111/j.1750-3841.2007.00439.x

Pickering, G.J., Ker, K., Soleas, G.J., 2007b. Determination of the critical stages of processing and tolerance limits for Harmonia axyridis for "ladybug taint" in wine. VITIS - J. Grapevine Res. 46, 85–90.

Robinson, A.L., Ebeler, S.E., Heymann, H., Boss, P.K., Solomon, P.S., Trengove, R.D., 2009. Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. J. Agric. Food Chem. 57, 10313–10322. doi:10.1021/jf902586n

Sinha, S.N., Bhatnagar, V.K., Doctor, P., Toteja, G.S., Agnihotri, N.P., Kalra, R.L., 2011. A novel method for pesticide analysis in refined sugar samples using a gas chromatography–mass spectrometer (GC–MS/MS) and simple solvent extraction method. Food Chem. 126, 379–386. doi:10.1016/j.foodchem.2010.10.110

Smith, J.R., Hesler, S.P., Loeb, G.M., 2014. Potential Impact of Halyomorpha halys (Hemiptera: Pentatomidae) on Grape Production in the Finger Lakes Region of New York. J. Entomol. Sci. 49, 290–303. doi:10.18474/0749-8004-49.3.290

Styger, G., Prior, B., Bauer, F.F., 2011. Wine flavor and aroma. J. Ind. Microbiol. Biotechnol. 38, 1145–1159. doi:10.1007/s10295-011-1018-4

Tomasino, E., Wiman, N., Osborne, J., Hedstrom, C., Walton, V., 2013. Impact of Brown Marmorated Stinkbug on Pinot noir Wine Quality. Presented at the 64th ASEV National Conference, Monterey, CA

Tomasino, E., Mohekar, P., Lapis, T., Wiman, N., G., Walton, V., Lim, J., 2013. Effect of Brown Marmorated Stink Bug on Wine - Impact to Pinot noir Quality and Threshold determination of taint compound trans-2-decenal. Presented at the 15th Australian Wine Industry Technical Conference, Sydney, Australia.

Vétek, G., Papp, V., Haltrich, A., Rédei, D., 2014. First record of the brown marmorated stink bug, Halyomorpha halys (Hemiptera: Heteroptera: Pentatomidae), in Hungary, with description of the genitalia of both sexes. Zootaxa 3780, 194–200.

Wermelinger, B., Wyniger, D., Foster, B., 2008. First records of an invasive bug in Europe: Halyomorpha halys Stål (Heteroptera: Pentatomidae), a new pest on woody ornamentals and fruit trees? Mitteilungen Schweizerische Entomol Gesellschaft. Mitteilungen Schweiz. Entomol. Ges. 81.

Table 4.1. Batch size, number of replicates and Brown Marmorated Stink Bug (BMSB) density of the wine treatments produced during 2013 and 2014.

Year	Wine Style	Number of BMSB per replicate (per Kg of grapes)				Number of replicates	Batch size (Kg)	Press type
		Control (0 BMSB/ cluster)	T1* (0.25 or 0.3 BMSB/ cluster)	T2 (1 BMSB/ cluster)	T3 (3 BMSB/ cluster)			
2013	Pinot noir	0	3	8	30	3	2.5	Basket
2013	Pinot gris	0	2.5	10	–	3	6.1	Bladder
2013	Merlot	0	–	–	–	3	3.0	Basket
2013	Merlot (press fraction)		–	6.6	–	2	18.0	Bladder
2013	Merlot (free run)		–	6.6	–	2	18.0	Bladder
2014	Pinot noir	0	3	–	30	2	30.0	Bladder
2014	Pinot noir with dead BMSB		3			3	30.0	Bladder

*In Pinot gris, T1 corresponds to 0.25 BMSB per cluster. In rest of the wine treatments T1 corresponds to 0.3 BMSB per cluster.

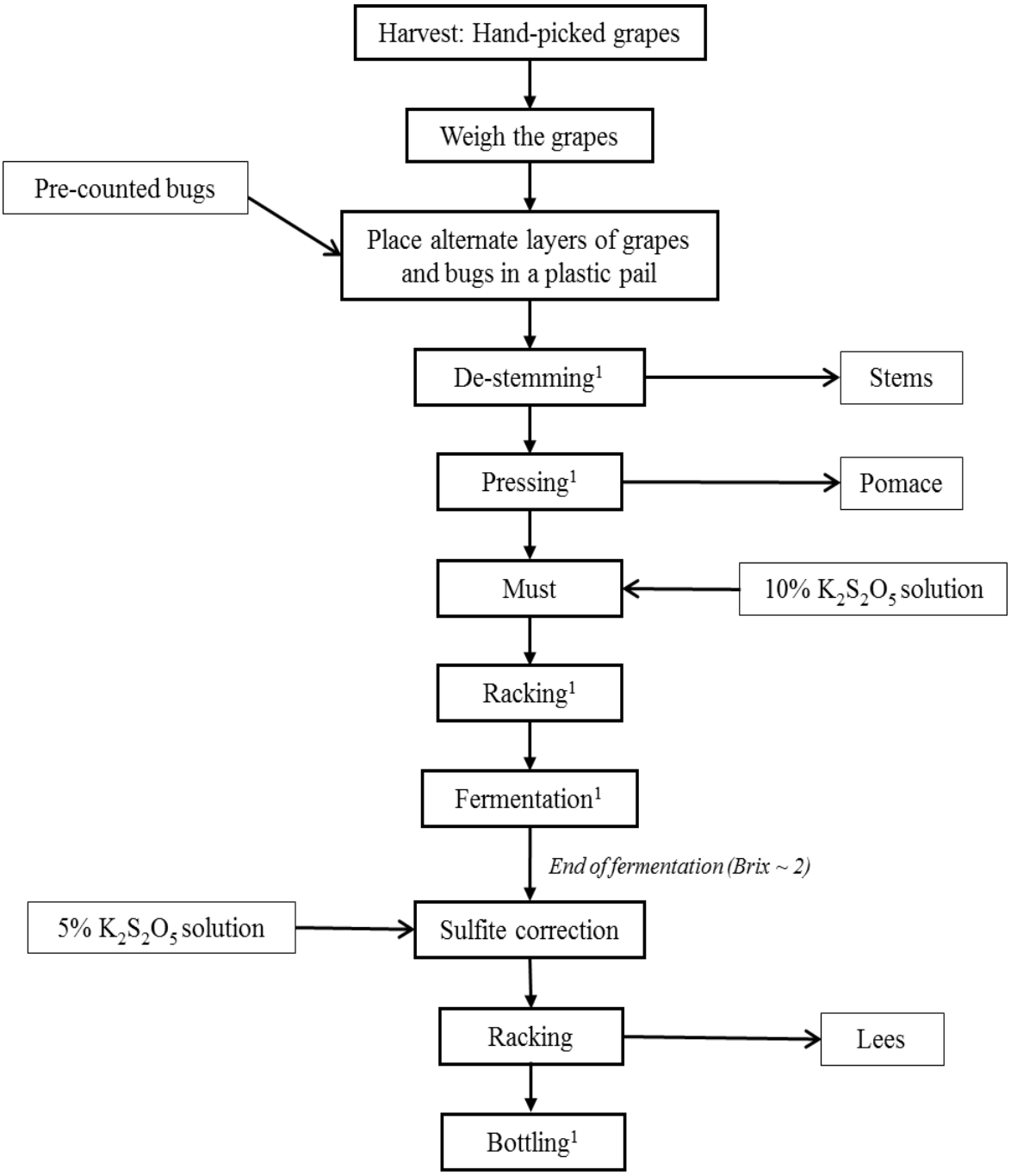

Figure 4.1. Flowchart of white wine (Pinot gris) processing

[1]Wine/must samples were collected after each of these processing steps for BMSB taint analysis

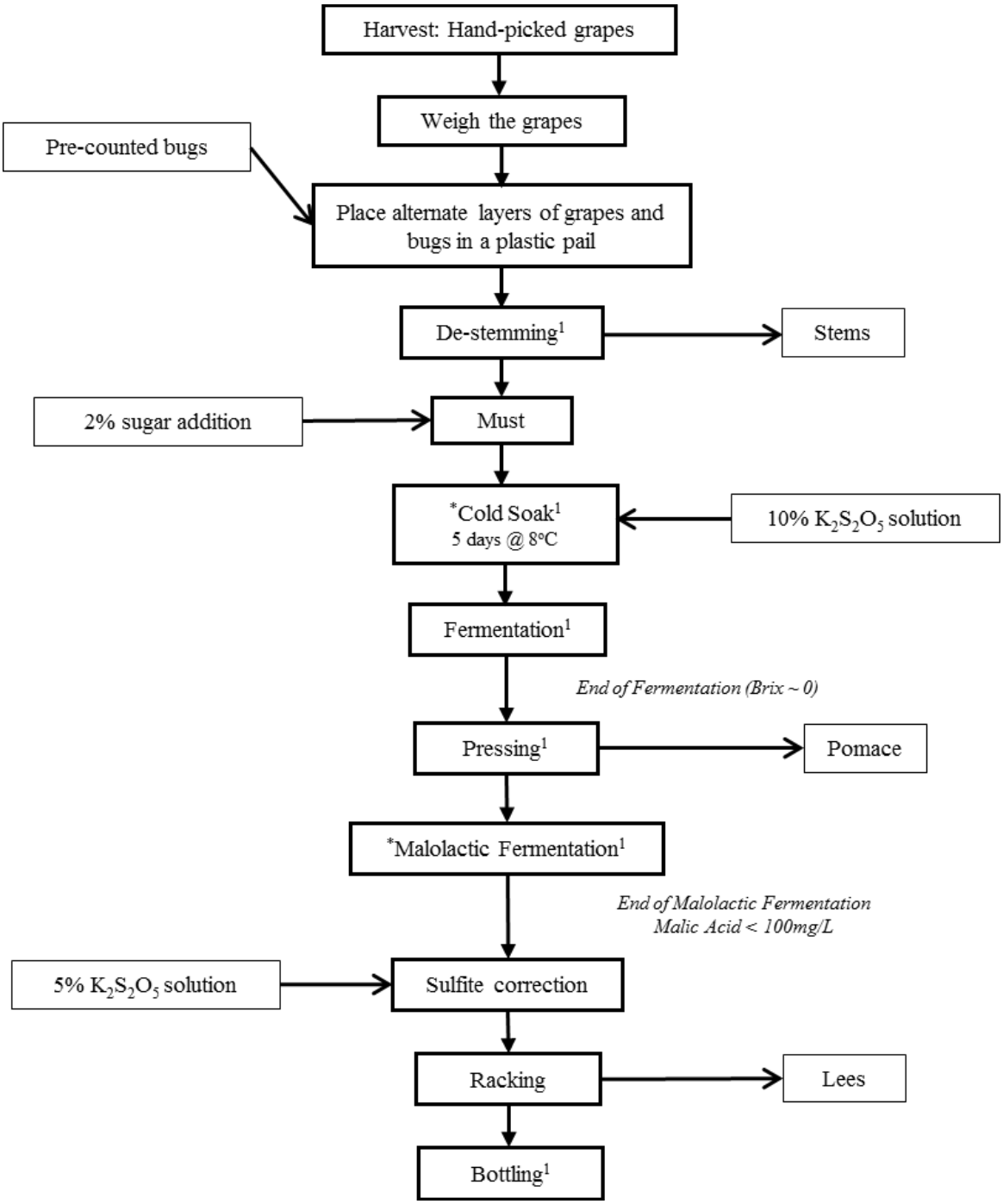

Figure 4.2. Flowchart of red wine (Pinot noir and Merlot) processing

[1]: Wine/must samples were collected after each of these processing steps for BMSB taint analysis

*: Merlot wines did not go through cold soak or malolactic fermentation

Table 4.2. Quantification parameters for the headspace-solid phase microextraction-GC-MS analysis of Tridecane and Trans-2-decenal

Analyte	CAS No.	Purity (%)	Retention time (min)	Target Ions *m/z*	Confirming ions *m/z*	Calibration range (µg/L)	Standard curve (R^2) Red Wine	Standard curve (R^2) White Wine
Tridecane	629-50-5	99.0	37.67	57	43, 71, 85	0 – 38.73	99.32	99.85
Trans-2-decenal	3913-81-3	95.0	40.28	55	70, 98	0 – 19.37	99.79	98.86
3-Octanol	20296-29-1	99.0	25.05	59	55, 83			
D30-Tetradecane	204244-81-5	98.5	42.24	66	50, 82			

Table 4.3. Alcohol, titratable acidity, pH and residual sugar in finished Pinot gris, Pinot noir and Merlot wines made from grapes in presence and absence of BMSB

	Alcohol (% v/v)	Titratable Acidity (g/L)	pH	Residual Sugar (g/L)
2013 PG Control	11.4 ± 0.2^{a}	7.5 ± 0.1^{a}	3.1 ± 0.0^{a}	21.5 ± 1.5^{a}
2013 PG Treatment 1 (0.25 BMSB/ cluster)	11.8 ± 0.4^{a}	7.3 ± 0.0^{a}	3.1 ± 0.0^{a}	17.5 ± 3.9^{a}
2013 PG Treatment 2 (1 BMSB/ cluster)	11.8 ± 0.1^{a}	7.0 ± 0.2^{b}	3.1 ± 0.0^{a}	22.2 ± 0.3^{a}
2013 PN Control	14.0 ± 0.2^{b}	5.2 ± 0.3^{a}	3.7 ± 0.0^{a}	1.6 ± 0.1^{a}
2013 PN Treatment 1 (0.3 BMSB/ cluster)	14.5 ± 0.5^{a}	5.0 ± 0.1^{a}	3.7 ± 0.0^{b}	1.7 ± 0.2^{a}
2013 PN Treatment 2 (1 BMSB/ cluster)	14.8 ± 0.2^{a}	5.1 ± 0.1^{a}	3.7 ± 0.0^{a}	1.7 ± 0.4^{a}
2013 PN Treatment 3 (3 BMSB/ cluster)	14.5 ± 0.1^{a}	5.3 ± 0.3^{a}	3.7 ± 0.0^{a}	1.4 ± 0.4^{a}
2013 Merlot Control	14.4 ± 0.5^{a}	6.3 ± 0.1^{a}	3.5 ± 0.0^{a}	2.0 ± 0.1^{ab}
2013 Merlot Press Treatment 1 (1 BMSB/ cluster)	12.9 ± 0.0^{b}	6.5 ± 0.1^{a}	3.5 ± 0.0^{a}	1.9 ± 0.1^{b}
2013 Merlot Free Run Treatment 1 (1 BMSB/ cluster)	12.9 ± 0.0^{b}	6.2 ± 0.3^{a}	3.5 ± 0.1^{b}	2.1 ± 0.0^{ab}
2014 PN Control	13.5 ± 0.2^{bc}	6.0 ± 0.1^{a}	3.8 ± 0.1^{a}	1.1 ± 0.1^{ab}
2014 PN Treatment 1 (0.3 BMSB/ cluster)	13.7 ± 0.1^{ab}	5.8 ± 0.3^{a}	3.6 ± 0.0^{b}	1.4 ± 0.1^{a}
2014 PN Treatment 3 (3 BMSB/ cluster)	13.1 ± 0.1^{c}	6.1 ± 0.3^{a}	3.7 ± 0.0^{ab}	1.8 ± 0.6^{b}
2014 PN Treatment (0.3 Dead BMSB per cluster)	14.1 ± 0.1^{a}	6.0 ± 0.2^{a}	3.8 ± 0.0^{a}	1.3 ± 0.0^{a}

Means with the same letter are not significantly different from each other (Tukey's HSD, α: 0.05)

Table 4.4. Limit of detection (LOD), limit of quantification (LOQ) and recovery for tridecane and trans-2-decenal

	Trans-2-decenal			Tridecane		
	LOD (µg/)	LOQ (µg/L)	Recovery (%)	LOD (µg/)	LOQ (µg/L)	Recovery (%)
White wine	0.02	0.07	90.60	0.02	0.06	20.24
Red wine	0.01	0.03	94.52	0.01	0.03	46.00

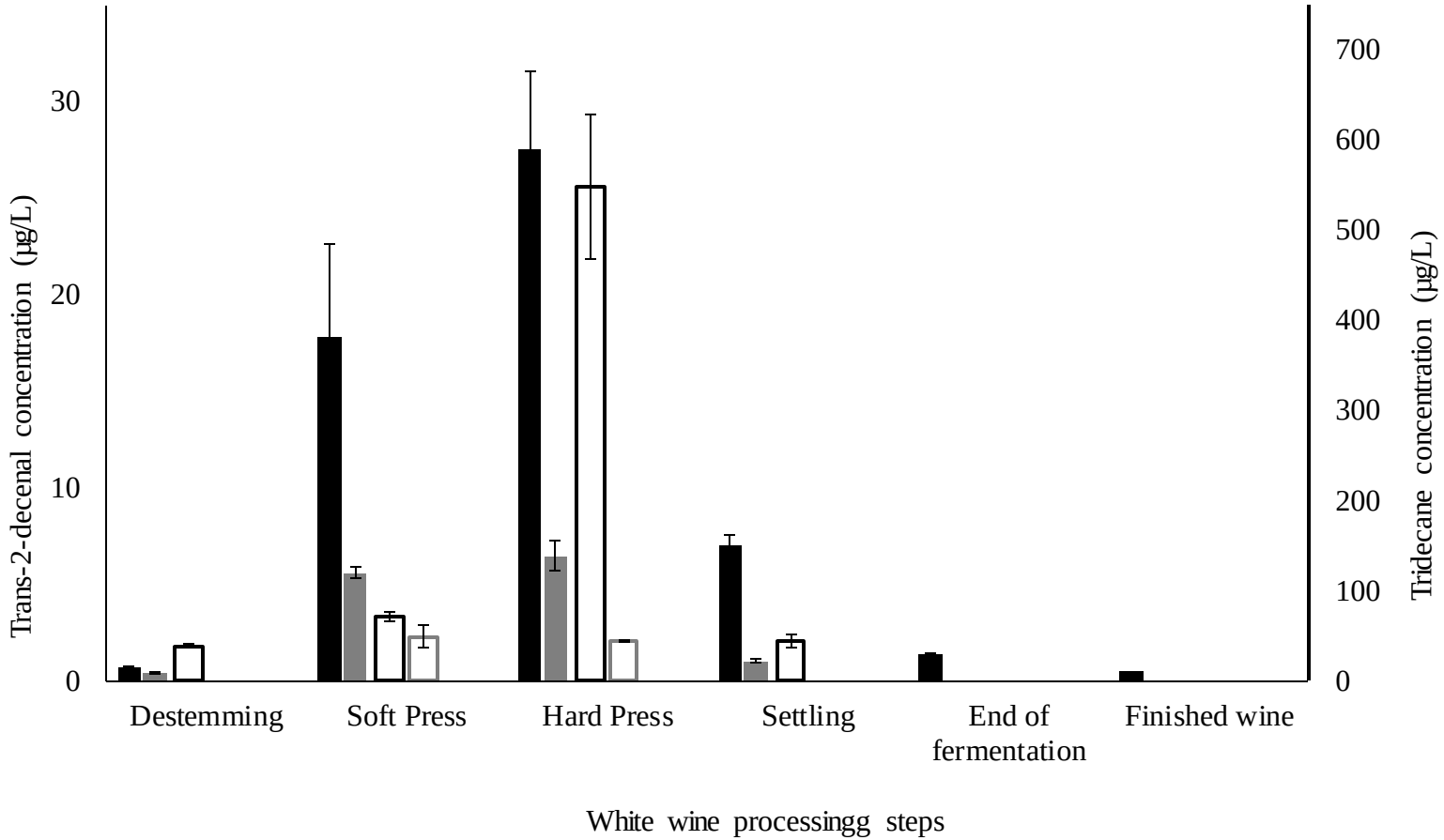

Figure 4.3. BMSB taint concentration during Pinot gris processing, a) Trans-2-decenal (□) in T2 and (□) in T1; b) Tridecane (■) in T2 and (■) T1

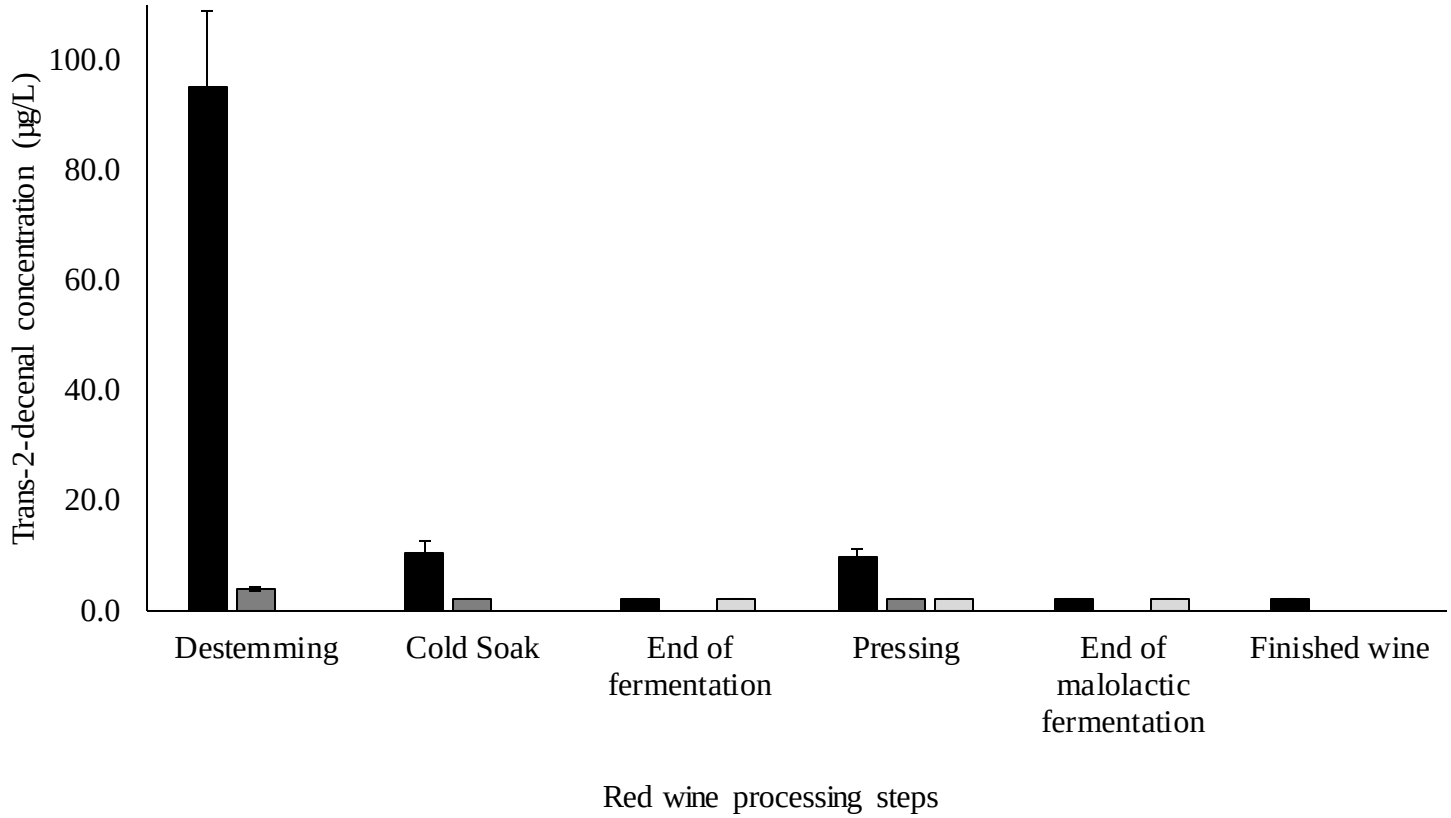

Figure 4.4a: Trans-2-decenal concentration during Pinot noir processing, (■) in 2014 T3, (■) in 2014 T1, and (■) in 2013 T3 at each processing step

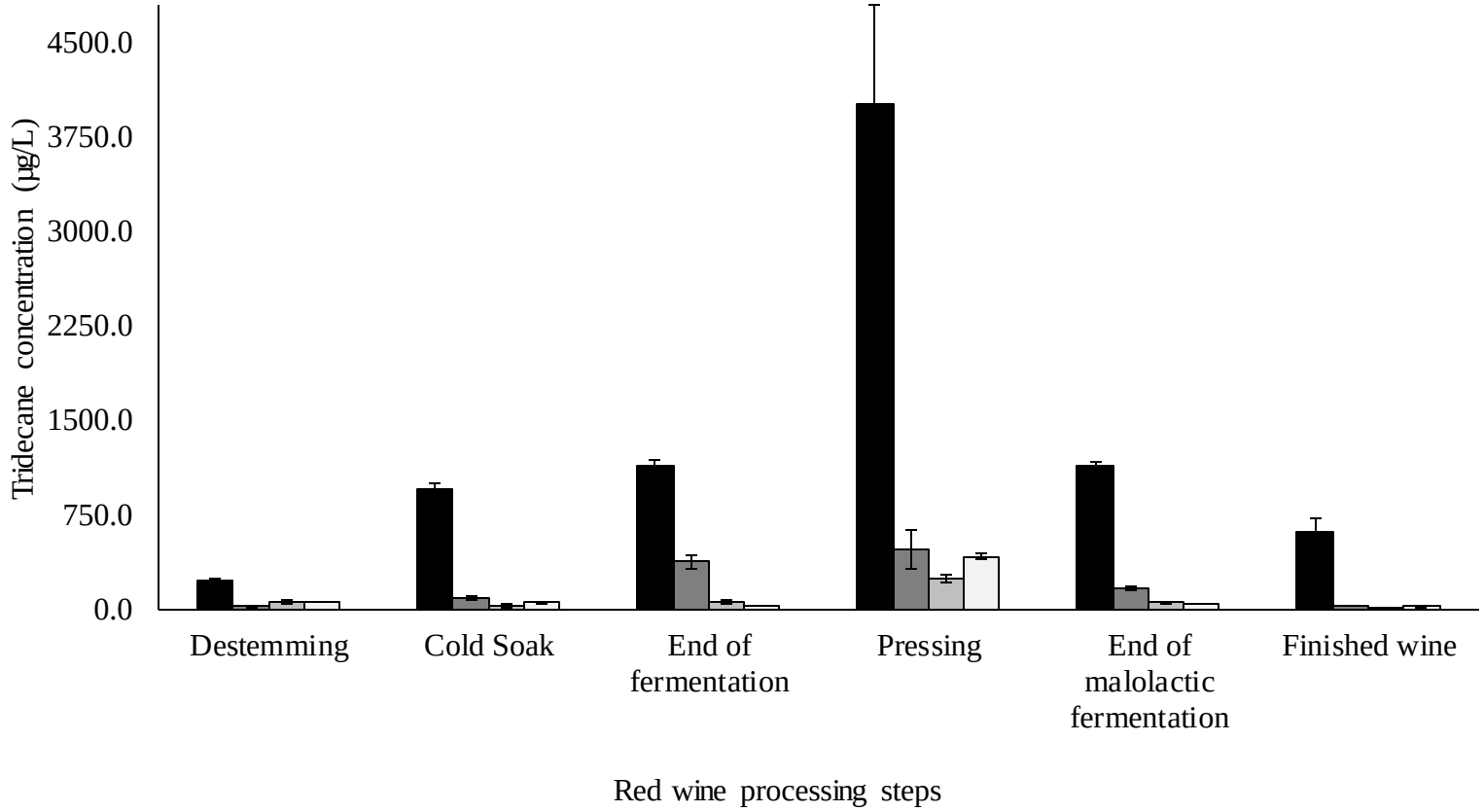

Figure 4.4b. Tridecane concentration during Pinot noir processing (■) in 2014 T3, (■) in 2014 T1, (■) in 2014 PN with dead BMSB T1, (■) in 2013 T2 at each processing step

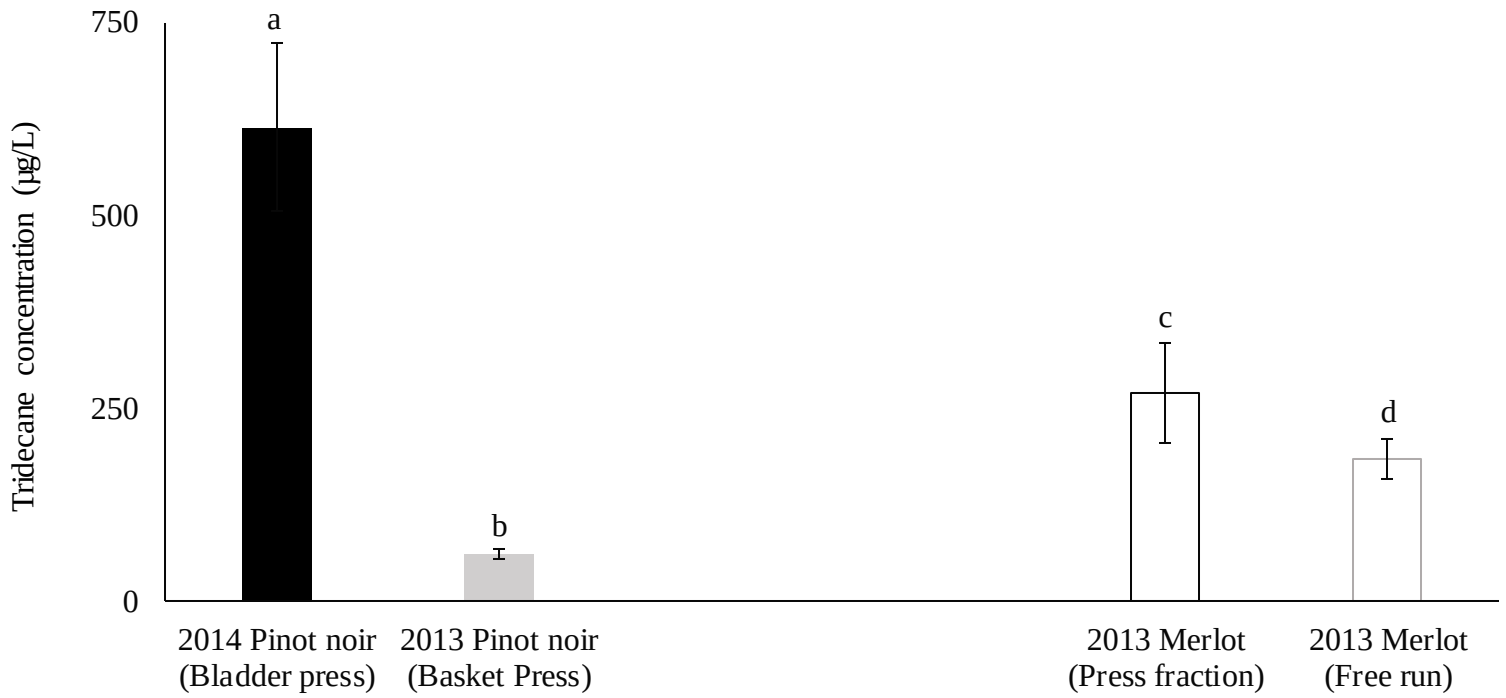

Figure 4.5. Difference in tridecane concentration in 2014 Pinot noir T3 using (■) bladder press and in 2013 Pinot noir T3 using (■) basket press, and in Merlot T2 using (□) press fraction and (□) free run. Merlot was processed using bladder press.

*Means with the same letter are not significantly different from each other (Student's t-test, *α:* 0.05)

Supplementary Table

Must composition for Pinot noir, Pinot gris and Merlot before and after sugar and acid adjustments made during year 2013 and 2014

Year	Wine	Brix (°) before adjustment	Brix (°) after adjustment	Titratable acidity (g/L) before adjustment	Titratable acidity (g/L) after adjustment	pH
2013	Pinot noir[1]	19.0	21.0	8.8		3.2
	Merlot[1]	19.0	21.0	8.7		3.1
	Pinot gris[2]	21.0	-	7.7		3.1
2014	Pinot noir[3]	21.0	-	6.4	6.9	3.4

[1]: Sugar adjustment only, No acid adjustment was performed
[2]: No sugar or acid adjustment was performed
[3]: No sugar but only acid adjustment was performed

CHAPTER 5

Effect of fining agents, reverse osmosis and wine age on Brown Marmorated Stink Bug, *Halyomorpha halys* taint in wine

P. Mohekar[1], J. Osborne[1], E. Tomasino[1]*

[1]Oregon State University, Department of Food Science & Technology, 100 Wiegand Hall, Corvallis, Oregon 97331, USA

*Corresponding Author: elizabeth.tomasino@oregonstate.edu, (T) (541)-737-4866, (F) (541)-737-1877

- **Acknowledgments:** the authors would like to thank Oregon State University, Oregon Wine Research Institute and USDA-NIFA-SCRI #2011-51181-30937 and USDA # 59-5358-4-016 for financial support. We would also like to thank Stanvin for providing oak samples, Winesecrets for running reverse osmosis. Finally, we would like to thank the wine consumers and Oregon wine professionals for their participation in sensory evaluation.

- **Authorship declaration:** E.T. and P.M. conceived and designed the experiments; P.M. performed the experiments and analyzed the data; P.M. and E.T. wrote the paper, with J.O. providing editing support.

Short version of title: BMSB taint management in wine

(prepared for submission to *Australian Journal of Grape and Wine Research*)

ABSTRACT

Background and aim: Trans-2-decenal and tridecane are compounds found in wine made from brown marmorated stink bug (BMSB) contaminated grapes. High concentrations of these compounds in wine can lower wine quality and affect consumer preference. In this paper, we evaluate the effectiveness of post-fermentation processes on reducing their concentration in finished wine and analyze their longevity during wine aging.

Methods: Red wines containing trans-2-decenal were treated with fining agents and put through reverse osmosis filtration. The efficacy of these treatments was estimated using chemical analysis (MDGC-MS) and sensory descriptive analysis. Tridecane and trans-2-decenal concentrations in red and white wine were determined at bottle aging durations of 0, 6, 12 and 24 months using MDGC-MS.

Results: Reverse osmosis was found to be partially successful in removing trans-2-decenal concentration from finished wine. With the exception of French oak, the fining agents used in this study were found to be ineffective in reducing trans-2-decenal levels. French oak was seen to mask the expression of BMSB related sensory characters. Both, tridecane and trans-2-decenal concentrations decreased during bottle aging.

Conclusions: Post-fermentative treatments are not effective at removing BMSB taint compounds. Therefore, BMSB densities in the grape clusters should be minimized.

Significance of the study: The efficacy of commonly used remedial methods against BMSB taint has been evaluated. The knowledge of techniques effective against

BMSB taint in finished wine is critical to maintaining its quality even under BMSB invasion in the vineyard.

Keywords: brown marmorated stink bug, reverse osmosis, fining, aging, wine

Introduction

Brown marmorated stink bug (BMSB) contamination in grape clusters has been previously shown to have a negative effect on wine quality ((Mohekar et al., 2014; Tomasino, 2013). BMSB is an invasive pest that is believed to have arrived into the United States from East-Asia and is currently detected in 43 states (Northeastern IPM, June 2016). Globally, it is also found in Canada, Italy, Hungary and Europe where wine has economic importance (Haye et al., 2015; Lee, 2015). When present in the vineyard, the pest can lower crop yield and effect quality. When present in grape cluster, it may enter wine processing where it can harm wine quality through a release of "BMSB taint". The chance of BMSB entering wine processing is increasing, as greater densities of BMSB are being observed in the vineyard (Basnet, 2014; Haye et al., 2015; Smith et al., 2014; Wiman et al, 2014). In order to maintain wine quality, techniques are needed to minimize BMSB taint concentration in finished wine.

BMSB primarily secretes tridecane and trans-2-decenal when stressed (Baldwin et al., 2014; Mohekar et al., 2015; Solomon et al., 2013). Tridecane is an odorless compound and its effect on wine quality is currently unknown. Trans-2-decenal is considered to be the main component of this taint due to its strong "green", "cilantro" like aroma (Fiola, 2011; Tomasino, 2013). It has been shown to have a negative effect on red wine quality, significantly decreasing consumer preference at a concentration as low as 4.8 μg/L (Mohekar et al., 2014). This concentration is the consumer rejection threshold (CRT) of trans-2-decenal in red wine. Above its CRT, trans-2-decenal can add green, musty, herbal characteristics to wine which are not desirable

(Mohekar et al. 2016/Chapter 2). Additionally a reduction in favorable attributes such as dark fruit, red fruit and floral characteristics has also been observed (Mohekar et al. 2016/Chapter 2). Due to this negative impact of BMSB taint on wine quality and consumer preference, efforts are needed to minimize the concentration of these taint compounds in finished wine.

It has been shown that as low as three BMSB per cluster in the vineyard can result in finished wine with 2.02 µg/L of trans-2-decenal (Mohekar et al. 2016/Chapter 4). The same bug density may also result in trans-2-decenal concentration at or above the CRT when winemaking causes stress to BMSB, resulting in higher secretion of taint compounds. Additionally, previous work suggests that the presence of dead BMSB can result in wine containing tridecane but no trans-2-decenal (Mohekar et al. 2016/Chapter 4).

Modifications in wine making protocol may reduce BMSB taint concentrations in final wine (Mohekar et al. 2016/Chapter 4). Alterations in harvesting, pressing and fermentation have shown potential in reducing BMSB taint in finished wine. However, modification of wine processing may not be always appropriate as it can restrict the style of wine made from BMSB contaminated grapes. Additionally, process modification may not be sufficient against high BMSB densities and finished wine may still contain trans-2-decenal or tridecane. Therefore, post-fermentative measures are required to be able to produce a desired wine style while minimizing taint levels.

The wine industry relies on fining agents to correct wine faults and to improve wine quality (Fudge et al., 2012; Jackson, 2008; Pickering et al., 2006). Wine sensory characteristics such as flavor, color, mouthfeel can be adjusted by fining agents (Cosme et al., 2012; Jackson, 2008; Threlfall et al., 1999). Fining agents are chosen for their affinity to unwanted compounds in wine through mechanisms such as hydrophobic interaction, hydrogen bonds, Vander Wall's interaction and electrostatic interaction (Braga et al., 2007; Fudge et al., 2012). The fining agent, along with unwanted or taint compounds, are then removed by racking, centrifugation or filtering. Commonly used fining agents such as bentonite, gelatin, casein and activated charcoal have previously been used on taint compounds from lady bug and smoke exposure (Fudge et al., 2012; Pickering et al., 2006). In these studies, oak was able to mask green aroma characteristics of lady beetle taint in red and white wine (Pickering et al., 2006) whereas activated charcoal and synthetic mineral were effective against smoke taint compounds.

In addition to fining agents, reverse osmosis filtration has also been explored as a viable option for taint removal (Fudge et al., 2011). In this process, selective taint removal can be achieved by carrying out filtration under pressure. Reverse osmosis is often combined with an adsorption or ion-exchange column to remove taint compounds more efficiently. This technique has been found to be successful in removing 4-ethylguaiacol and 4-ethylphenol from *Brettanomyces*-affected wine (Ugarte et al. 2005) using Amberlite XAD-16 HP resin and smoke taint compounds

(guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol) using a polystyrene based adsorbent resin (Fudge et al., 2011).

Another possible technique to reduce BMSB taint in wine is through aging. During aging, complex reactions occur that are known to change wine composition and sensory characteristics (Ebeler, 2001; Styger et al., 2011). Since most wines are aged for at least a year, it is important to understand how BMSB taint is modified during aging. This information is important in order to assess the quality of an aged wine. Currently, there is no technique known to be effective against BMSB taint in finished wine and reduction techniques are needed.

Materials and methods

Wines

Three different Pinot noir wines (PN1, PN2 and PN3) were used in this study. All wines were produced on a small scale at OSU research winery. PN1 and PN2 were produced from the same grapes sourced from a vineyard located in Oregon and under the same winemaking protocol. PN1 was made from grape that did not contain any BMSB. PN2 on the other hand, was made from grapes, to which BMSB was added at a density of three per cluster. PN2 therefore contained both taint compounds, tridecane and trans-2-decenal whereas PN1 was taint free. Both wines, PN1 and PN2 were made using a winemaking protocol given in Chapter 4. These wines were used to study the effect of aging and reverse osmosis filtration.

PN3 was also produced without any addition of BMSB but the grapes were sourced from a different vineyard in Oregon. Winemaking procedure used to make PN3 was similar to the protocol given in Mohekar et al. 2016. Trans-2-decenal was added to this wine to study the effect of fining treatment.

Fining agents

Fining agents and their dose level was selected based on a preliminary study (data not shown). In the end, five fining agents: gelatin (BBL, Div Becton Dicklnson & company), egg albumin, potassium caseinate (Laffort USA, Petaluma, CA), bentonite (), yeast lees (Laffort USA, Petaluma, CA) and French oak (StaVin Inc., Sausalito CA, USA) were tested. Egg albumin solution was prepared in 1% NaCl. Other fining agents were prepared in hot or cold water per manufacturer's instruction. The following dose levels were used for each fining agent: gelatin at 30mg/L, egg albumin at 67mg/L, potassium caseinate at 150mg/L, bentonite at 75mg/L and yeast lees at 150mg/L. The addition rate for French oak was based on manufacturer's instruction.

To run fining trials, Pinot noir with trans-2-decenal concentration of 30µg/L was prepared. This was done by adding trans-2-decenal standard (50µg/L made in 14% ethanol) into the base wine, PN3. The concentration of 30 µg/L was selected for fining treatment because it is significantly above trans-2-decenal CRT (4.8µg/L) and has been shown to add green sensory characteristics associated trans-2-decenal. Additionally, a significant proportion of consumers (78%) were seen to reject Pinot noir containing 30µg/L of trans-2-decenal (Mohekar et al., 2014).

Twenty four hours after trans-2-decenal addition into PN3, fining agents were added. PN3 containing trans-2-decenal and fining agent were then stored at 4°C for three days. At the end of three days, fining agents were removed by racking, wines were stored for their analysis and bottles were purged with nitrogen. Twenty hours after racking, wines were analyzed using sensory descriptive analysis. At the same time, 40ml sample of these racked wines was collected for trans-2-decenal quantification using MDGC-MS. Samples were stored in amber vials with PTFE lined caps (Sigma Aldrich) at -18°C until their analysis.

Reverse osmosis

Two wines, PN1 and PN2 went through reverse osmosis filtration that was conducted by WineSecrets Corp. (Sebastopol CA, USA). PN2 was made from BMSB contaminated grapes and therefore contained trans-2-decenal whereas PN1 was found to be free of BMSB taint. The purpose for PN1 was to determine the effect of reverse osmosis on aroma compounds not typically associated with BMSB. Therefore, both wines were treated in exactly the same manner. Reverse osmosis was performed on a lab scale Memstar unit with a CBC-5 carbon block filter cartridge (Pentair Pentek, Milwaukee, WI, USA) attachment. Feeding pump pressure was maintained between 1700-1800kPa and total sample flow rate at 50ml per minute.

Effect of aging

Tridecane and trans-2-decenal concentration during bottle aging was measured using HS-SPME-MDGC-MS (Mohekar et al. 2016/Chapter 4). PN2 was analyzed after 0.5 and 1 year of bottling.

Chemical Analysis

A previously described method of Headspace-Solid Phase Micro-Extraction-Multidimensional Gas Chromatography-Mass Spectrometry (HS-SPME-MDGC-MS) was used to quantify trans-2-decenal and tridecane in wine treatments (Mohekar et al. 2016/Chapter 4).

Descriptive Analysis

Descriptive analysis was used to determine the effect of fining treatment and reverse osmosis on wine sensory characteristics. Sixteen wine professionals (12 M, 4 F) from the Oregon wine industry participated in this study. Each panelists had more than 10 years of experience tasting wines. Consent was obtained from all panelists and the study was approved by Oregon State University's Internal Review Board (IRB).

Descriptive analysis data was collected over three tasting sessions, each lasting two hours. Each of the three sessions was conducted in the morning. The third session was conducted in a different room but under similar light and temperature ($21 \pm 2^{\circ}C$) conditions. Wines were served in INAO black glasses (International Organization for

Standardization 1977) to remove any influence of color (Jackson, 2009). All samples were coded with a three digit random numbers and served in a random order.

At the start of each session, panelists were given a set of three wine samples; control, Pinot noir with trans-2-decenal at its CRT (4.8µg/L), and Pinot noir with trans-2-decenal above its CRT (30µg/L). This was done to familiarize panelists with the taint compound and its effect on Pinot noir aroma and flavor. These wines were prepared an hour before the tasting session by adding a trans-2-decenal standard (50µg/L prepared in 14% ethanol). Wines were served in three sets containing five samples each. To avoid the effect of fatigue, panelists were given a one minute break after each wine and five minutes after each set. Panelists were requested to rinse their palate and eat a cracker during each break to minimize any carryover effect.

Samples were evaluated for ten aromas (dark fruit, earthy, herbal, musty, red fruit, floral, fresh green, spice) and three flavors (fruit density, green, and spice). These attributes have been used previously to evaluate the effect of trans-2-decenal on Pinot noir quality (Mohekar et al. 2016/Chapter 2). Each attribute was rated on a 100mm visual analog scale with indented word anchors, none and extreme. Panelists were allowed to rate any other attribute they thought was relevant to describe these samples, to avoid any dumping effect (Lawless and Heymann, 2010).

Statistical Analysis

Any differences in trans-2-decenal concentration between PN3, with and without fining agents was analyzed using one-way ANOVA and Dunnett's test. Descriptive analysis data was analyzed using mixed model ANOVA to determine consensus among the assessors for each attribute and wine. The fixed effect was wine and the random effects were panelists and replication. Canonical variate analysis (CVA) was used to explore the separation between wine treatments (Heymann and Noble, 1989; Peltier et al., 2015). Significant differences during aging were analyzed using one way Analysis of Variance (ANOVA) and Tukey's HSD. All analyses was conducted using XLSTAT-Pro 2015 (Addinsoft, New York, NY, USA).

Result and discussion

Chemical Analysis Trans-2-decenal concentration in PN3, after the addition of trans-2-decenal but before fining treatment, was found to be 23.88µg/L (Figure 1). After fining, no significant difference was found between trans-2-decenal concentration in base wine and fined wines ($p < 0.05$, Dunnett's). These results suggest that fining agents evaluated in this study are incapable of removing trans-2-decenal. Additional work is needed to determine the underlying factors for this result. One potential reason may be that the fining agents have only weak binding ability with trans-2-decenal. Alternatively, the fining agents may have higher affinity for phenolics or other aroma compounds compared to trans-2-decenal. This explanation seems likely given that most fining agents are known to bind with non-volatile components in wine such as proteins and phenolic compounds (Braga et al., 2007; Cosme et al.,

2012; Jackson, 2008; Reynolds, 2010). Their interaction with volatile compounds is considered to be a secondary binding action and an undesirable effect that can be exploited for taint reduction (Fudge et al., 2012; Jackson, 2008; Reynolds, 2010; Sanborn et al., 2010).

The treatment of reverse osmosis reduced the trans-2-decenal concentration in wine from 2.02µg/L to 1.82µg/L (t-test, *p-value* < 0.05). This slight reduction of 0.2µg/L is minimal and would only be effective in changing sensory perception if the final concentration after fining was near CRT. However, this result indicates the ability of reverse osmosis to reduce trans-2-decenal, which was not found with other treatments. Therefore, with additional improvements reverse osmosis may prove to be a viable option for BMSB taint management. The use of other semipermeable membranes, adsorption/ion exchange column, pressure and flow rate may be optimized to remove greater amounts of trans-2-decenal.

Chemical analysis of the aged wines showed a decrease in BMSB taint (trans-2-decenal and tridecane) during bottle aging. Trans-2-decenal level in PN2 after six months was found to be below the limit of detection. A significant drop in tridecane level was also observed in PN2, wine made from BMSB containing grapes (Figure 5.1). A longer aging period may be preferable in wines containing BMSB taint since aging process appears to naturally decrease their levels. A decrease in BMSB taint post bottling is likely to be a result of aging related reactions occurring in wine such as hydrolysis, component degradation, condensation and reduction reaction (Ebeler,

2001; Styger et al., 2011; Ugliano, 2013). These reactions can modify existing compounds or generate new ones.

Aldehydes such as trans-2-decenal are highly reactive and can bind with a number of different compounds such as SO2 or phenolics (Barker et al., 1983; Chatonnet and Dubourdieu, 1998; Flamini et al., 2002; Jackson, 2008). Prior work shows that reductive conditions during bottle aging can cause aldehydes to decrease in wines, changing to their corresponding alcohol (Jackson, 2008; Spillman et al., 1998). However, if wines are matured under oxidative condition, acetals can form as result of reaction between aldehydes and alcohol (Culleré et al., 2007; Jackson, 2008). At this time, the underlying reason for the absence of trans-2-decenal or tridecane is unclear, although it is thought that trans-2-decenal is changed to, decenol, its related alcohol. The experimental work on the impact of aging on BMSB taint is at a very preliminary stage and additional research is needed to better understand the underlying chemistry of these compounds during maturation and post-bottling.

Furthermore, the corresponding sensory impact of BMSB taint during aging also needs to be evaluated. This will estimate the effect after compounds released by BMSB have undergone aging related changes such as trans-2-decenal conversion to decenol which is considered to have a different sensory threshold.

Sensory Analysis The effect of fining agent on reducing sensory impact of trans-2-decenal was evaluated using eight wines. Six wines relating to the different fining

treatments were evaluated. The last two were PN3, with and without trans-2-decenal. A significant interaction between panel and wine treatment was observed for spice flavor. This indicates inconsistency in the use of intensity scale or interpretation of this attribute by wine professionals (Lawless and Heymann, 2010). The effect of such interaction is unavoidable due to inherent anatomical differences in panelists. There is also the possibility of term "spice" being too generic and therefore being interpreted differently by each panelist. Given that the interaction exists, spice flavor was excluded from CVA analysis. Significant differences in aroma intensity between wine treatments were found in ANOVA. However, no difference in trans-2-decenal related attributes such as green, musty and earthy were found. Rather, the observed differences (a change in red fruit aroma) are typically attributed as a side effect of the fining agents themselves (Bamforth and Ward, 2014).

CVA analysis (Figure 5.2) showed a clear separation between the wines. Three distinct groups were visible: 1) PN3 without trans-2-decenal, 2) French oak treatment and 3) remaining six wines (PN3 with trans-2-decenal and the other five fining treatments). PN3 without trans-2-decenal was mainly characterized by fruity aroma and flavor. Wine with French oak treatment showed a strong spice aroma but no trans-2-decenal related attributes. Since the chemical analysis of oak treatment did not show any reduction in trans-2-decenal level, the effect of oak is most likely a masking effect. Previous work on lady bug taint has also reported a similar making effect of oak addition (Pickering et al., 2006). PN3 with trans-2-decenal and the five other wines that went through fining treatment were characterized as fresh green, earthy,

musty and herbal. These attributes are associated with trans-2-decenal (Mohekar et al. 2016/Chapter 2). More importantly, in CVA, no separation was observed between PN3 with trans-2-decenal and these five fining treatments. This suggests that fining agents may not be able to reduce the impact of trans-2-decenal on wine sensory. Overall, the results of sensory evaluation agree with the conclusion of the chemical analysis data, namely, that fining agents failed to remove trans-2-decenal from wine.

The two wines from reverse osmosis (RO) were analyzed separately. Additionally, PN3 with and without trans2-decenal were included in the analysis for comparison. Thus, in the second CVA analysis, a total of four wines were analyzed: 1) PN1 (made from BMSB free grapes) after RO, 2) PN2 (wine made from BMSB added grapes) after RO, 3) PN3 with trans-2-decenal and 4) PN3 without trans-2-decenal. The last two wines were included in the analysis in order to provide a means to compare the effect of reverse osmosis filtration and high levels of trans-2-decenal on wine aromatics.

A significant interaction was observed between panel and wine for green flavor. Using the same justification as before, green flavor was removed from CVA analysis. The results of CVA analysis are shown in Figure 5.3. Three groups were seen on a CVA plot: 1) PN3 with trans-2-decenal, 2) PN3 without trans-2-decenal and 3) PN1 and PN2 after RO. The first group, consisting of PN3 with trans-2-decenal, is separated from the second group, which consists of PN3 without trans-2-decenal, indicating separation as a result of trans-2-decenal. RO filtration could be a basis of

separation between Group 3 and PN3 (both with and without trans-2-decenal) since the main difference between these groups was the treatment of reverse osmosis which was applied to group 3 wines.

Once again, PN3 without trans-2-decenal was perceived as fruity and floral. The addition of trans-2-decenal brought out green, musty herbal characteristics in the same wine. None of these negative characteristics were found in PN1 and PN2 treated with RO. After reverse osmosis, PN2 was mainly characterized by earthy notes but none of the more pronounced attributes associated with trans-2-decenal such as green, musty and herbal were observed. However, it is not clear if this is a result of RO treatment or low concentration of trans-2-decenal. In previous work by Mohekar 2016, Pinot noir containing 5µg/L of trans-2-decenal has also been described as earthy (Mohekar et al. 2016/Chapter 2). Therefore, the effect reverse osmosis on wine sensory is currently unclear.

Conclusion

Corrective measures are of significant importance for wine containing BMSB taint. They provide the last option for winemakers to correct wine faults. Taking this into consideration, this study evaluated the effectiveness of reverse osmosis, commonly used fining agents and bottle aging on BMSB taint compound. The results indicate reverse osmosis to be a viable option with additional improvements. Reverse osmosis was able to reduce trans-2-decenal concentration in wine by 10%, which resulted in limited improvements in its sensory characteristics. In addition to reverse osmosis,

oak addition can also be considered while dealing with BMSB taint. It was found to be successful in masking green characteristics of trans-2-decenal in wine. However, this option is only appropriate for wines where spice aroma and flavor characteristics are desirable. Finally, winemakers can age the wine containing BMSB taint compounds as both, trans-2-decenal and tridecane decreased post bottling. Future work is recommended to evaluate additional fining agents (eg. Charcoal), since none of fining agents tested in this were found to be effective in reducing BMSB taint.

Taken together, wine containing BMSB can be treated by one of three options, addition of French oak, reverse osmosis or aging. Overall, the outcome of this study provides means of dealing with BMSB taint in finished wine. This information is important to minimize the impact of BMSB taint and maintain wine quality.

Literature cited:

Baldwin, R.L., Zhang, A., Fultz, S.W., Abubeker, S., Harris, C., Connor, E.E., Van Hekken, D.L., 2014. Hot topic: Brown marmorated stink bug odor compounds do not transfer into milk by feeding bug-contaminated corn silage to lactating dairy cattle. J. Dairy Sci. 97, 1877–1884. doi:10.3168/jds.2013-7545

Bamforth, C.W., Ward, R.E., 2014. The Oxford Handbook of Food Fermentations. Oxford University Press.

Barker R L, Gracey D F, Irwin A J, Pipasts P, Leiska E 1983 Liberation of staling aldehydes during storage of beer. J Inst Brew 89 411È415.

Basnet, S., 2014. Biology and pest status of brown marmorated stink bug (Hemiptera: Pentatomidae) in Virginia vineyards and raspberry plantings (Thesis). Virginia Polytechnic Institute and State University, Blacksburg, VA.

Braga, A., Cosme, F., Ricardo-da-Silva, J.M., Laureano, O., 2007. Gelatine, Casein, and Potassium Caseinate as distinct wine fining agents; different effects on colour, phenolic compounds and sensory characteristics. J. Int. Sci. Vigne Vin 41, 203–214.

Chatonnet, P., Dubourdieu, D., 1998. Identification of substances responsible for the "Sawdust" aroma in oak wood. Journal of the Science of Food and Agriculture 76(2):179-188

Cosme, F., Capão, I., Filipe-Ribeiro, L., Bennett, R.N., Mendes-Faia, A., 2012. Evaluating potential alternatives to potassium caseinate for white wine fining: Effects on physicochemical and sensory characteristics. LWT - Food Sci. Technol. 46, 382–387. doi:10.1016/j.lwt.2011.12.016

Culleré, Cacho, J., Ferreira, V., 2007. An Assessment of the Role Played by Some Oxidation-Related Aldehydes in Wine Aroma. J. Agric. Food Chem. 55, 876–881. doi:10.1021/jf062432k

Ebeler, S.E., 2001. Analytical Chemistry: Unlocking the Secrets of Wine Flavor. Food Rev. Int. 17, 45–64. doi:10.1081/FRI-100000517

Fiola, J.A., 2011. Brown Marmorated Stink Bug (BMSB) Part 3 - Fruit Damage and Juice/Wine Taint. Timely Vitic.

Flamini, R., Luca, G.D., Stefano, R.D., 2015. Changes in carbonyl compounds in Chardonnay and Cabernet Sauvignon wines as a consequence of malolactic fermentation. VITIS - J. Grapevine Res. 41, 107.

Fudge, A. l., Ristic, R., Wollan, D., Wilkinson, K. l., 2011. Amelioration of smoke taint in wine by reverse osmosis and solid phase adsorption. Aust. J. Grape Wine Res. 17, S41–S48. doi:10.1111/j.1755-0238.2011.00148.x

Fudge, A. l., Schiettecatte, M., Ristic, R., Hayasaka, Y., Wilkinson, K. l., 2012. Amelioration of smoke taint in wine by treatment with commercial fining agents. Aust. J. Grape Wine Res. 18, 302–307. doi:10.1111/j.1755-0238.2012.00200.x

Haye, T., Gariepy, T., Hoelmer, K., Rossi, J.-P., Streito, J.-C., Tassus, X., Desneux, N., 2015. Range expansion of the invasive brown marmorated stinkbug, Halyomorpha halys: an increasing threat to field, fruit and vegetable crops worldwide. J. Pest Sci. 1–9. doi:10.1007/s10340-015-0670-2

Heymann, H., Noble, A.C., 1989. Comparison of Canonical Variate and Principal Component Analyses of Wine Descriptive Analysis Data. J. Food Sci. 54, 1355–1358. doi:10.1111/j.1365-2621.1989.tb05991.x

Jackson, R.S., 2009. Wine Tasting: A Professional Handbook. Academic Press.

Jackson, R.S., 2008. Wine Science: Principles and Applications. Academic Press.

Lawless, H.T., Heymann, H., 2010. Sensory Evaluation of Food: Principles and Practices. Springer Science & Business Media.

Lee, D.-H., 2015. Current status of research progress on the biology and management of Halyomorpha halys (Hemiptera: Pentatomidae) as an invasive species. Appl. Entomol. Zool. 50, 277–290. doi:10.1007/s13355-015-0350-y

Mohekar, P., Lim, J., Lapis, T., Tomasino, E., 2014. Consumer rejection thresholds of trans-2-decenal in Pinot noir: Linking wine quality to threshold segmentation. Presented at the Institute of Food Technology Annual Meeting, New Orleans, LA.

Mohekar, P., Tomasino, E., Wiman, N.G., 2015. Defining defensive secretions of brown marmorated stink bug, Halyomorpha halys. Presented at the 2015 Annual Meeting of the Entomological Society of America, Minneapolis, MN.

Peltier, C., Visalli, M., Schlich, P., 2015. Comparison of Canonical Variate Analysis and Principal Component Analysis on 422 descriptive sensory studies. Food Qual. Prefer., Tenth Pangborn Sensory Science Symposium 40, Part B, 326–333. doi:10.1016/j.foodqual.2014.05.005

Pickering, G., Lin, J., Reynolds, A., Soleas, G., Riesen, R., 2006. The evaluation of remedial treatments for wine affected by Harmonia axyridis. Int. J. Food Sci. Technol. 41, 77–86. doi:10.1111/j.1365-2621.2005.01039.x

Reynolds, A., 2010. Managing Wine Quality: Oenology and Wine Quality. Elsevier.

Sanborn, M., Edwards, C.G., Ross, C.F., 2010. Impact of Fining on Chemical and Sensory Properties of Washington State Chardonnay and Gewürztraminer Wines. Am. J. Enol. Vitic. 61, 31–41.

Smith, J.R., Hesler, S.P., Loeb, G.M., 2014. Potential Impact of Halyomorpha halys (Hemiptera: Pentatomidae) on Grape Production in the Finger Lakes Region of New York. J. Entomol. Sci. 49, 290–303. doi:10.18474/0749-8004-49.3.290

Solomon, D., Dutcher, D., Raymond, T., 2013. Characterization of Halyomorpha halys (brown marmorated stink bug) biogenic volatile organic compound emissions and their role in secondary organic aerosol formation. J. Air Waste Manag. Assoc. 1995 63, 1264–1269.

Spillman, P.J., Pollnitz, A.P., Liacopoulos, D., Pardon, K.H., Sefton, M.A., 1998. Formation and Degradation of Furfuryl Alcohol, 5-Methylfurfuryl Alcohol, Vanillyl Alcohol, and Their Ethyl Ethers in Barrel-Aged Wines. J. Agric. Food Chem. 46, 657–663. doi:10.1021/jf970559r

Styger, G., Prior, B., Bauer, F.F., 2011. Wine flavor and aroma. J. Ind. Microbiol. Biotechnol. doi:10.1007/s10295-011-1018-4

Threlfall, R. t., Morris, J. r., Mauromoustakos, A., 1999. Effects of fining agents on trans-resveratrol concentration in wine. Aust. J. Grape Wine Res. 5, 22–26. doi:10.1111/j.1755-0238.1999.tb00147.x

Tomasino, E., 2013. Impact of Brown Marmorated Stinkbug on Pinot noir Wine Quality.

Ugarte, P., Agosin, E., Bordeu, E. and Villalobos, J.I., 2005 Reduction of 4-ethylphenol and 4-ethylguaiacol concentration in red wines using reverse osmosis and adsorption. American Journal of Enology and Viticulture 56, 30–36.

Ugliano, M., 2013. Oxygen Contribution to Wine Aroma Evolution during Bottle Aging. J. Agric. Food Chem. 61, 6125–6136. doi:10.1021/jf400810v

Wiman, N., Rondon, S., Walton, V., and P.W. Shearer. 2014. Distribution of brown marmorated stink bug in Oregon and risk for specialty crops. Research Report, 72nd Annual PNW Insect Management Conference, p. 42.

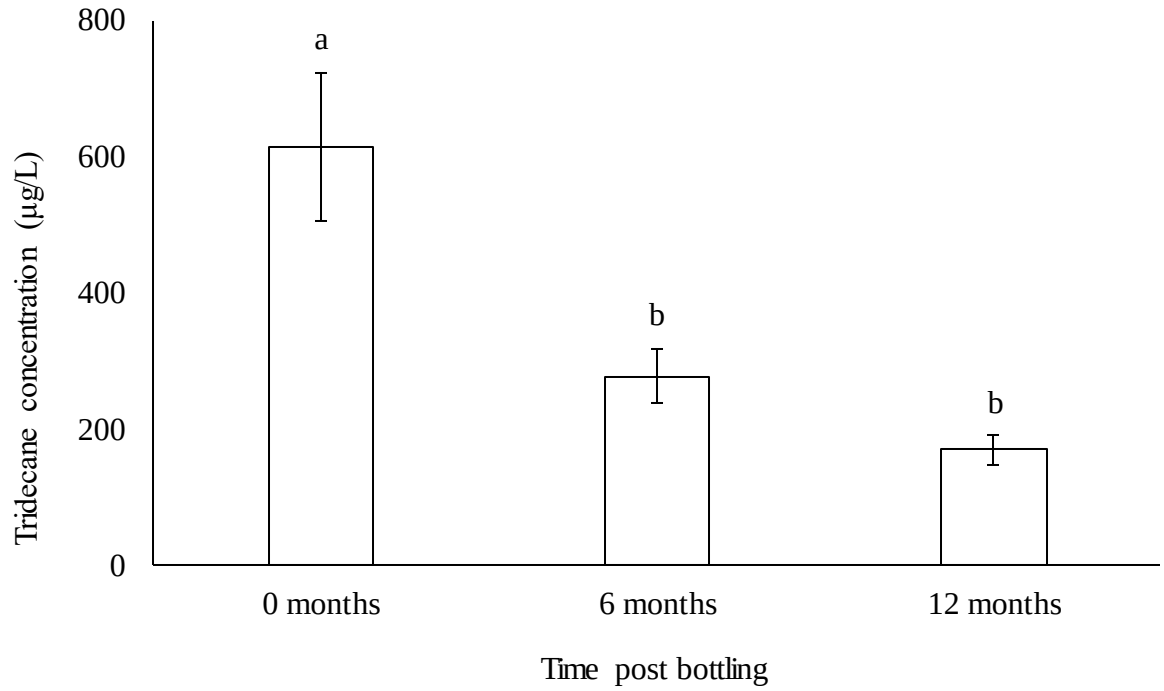

Figure 5.1. Tridecane concentration in Pinot noir made from BMSB containing grapes, PN2 at 0, 6 and 12 months of bottle aging (n = 3)

Means with the same letter are not significantly different from each other (Tukey's HSD, α: 0.05)

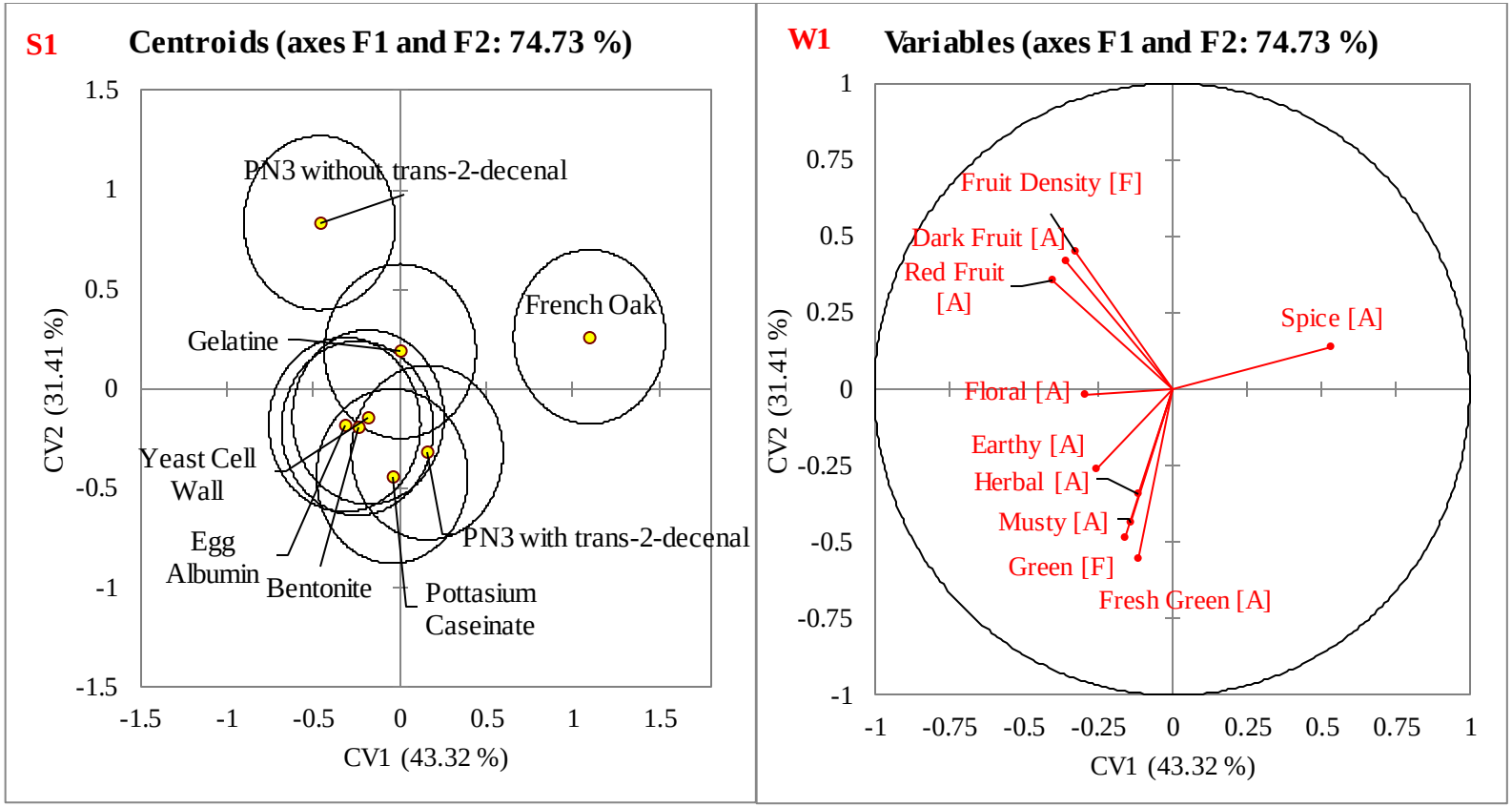

Figure 5.2. Separation between PN3 without trans-2-decenal, PN3 with trans-2-decenal (23.88µg/L) and PN3 containing trans-2-decenal (23.88µg/L) when treated with fining agents (Gelatin, Bentonite, Yeast cell wall, Potassium caseinate, Egg albumin, French oak).

Wines are positioned using the centroids. Circles represent 95% confidence intervals surrounding the wine means. Vectors for sensory terms (A=aroma, F= in mouth flavor) are in W1 and scores for wines are in S1. Significant differences for wines are for circles that do not touch in S1.

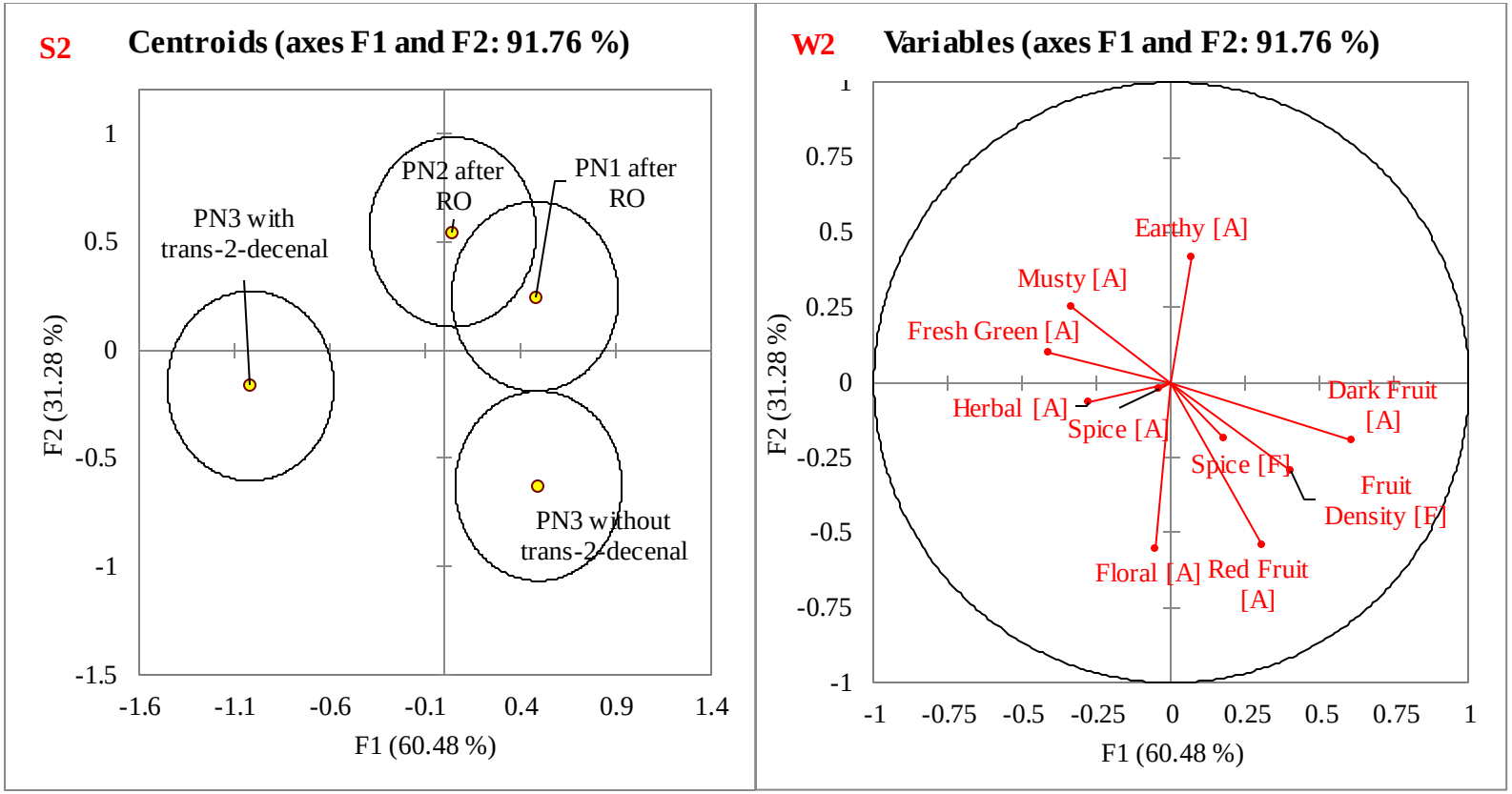

Figure 5.3. Separation among wines that went through reverse osmosis (RO) filtration: PN1 (wine made from *BMSB free* grapes), PN2 (wine made from BMSB containing grapes and contains 1.82 µg/L of trans-2-decenal) and wines that did not go through reverse osmosis (RO) filtration: PN3 without added trans-2-decenal, PN3 with trans-2-decenal (23.88µg/L) Wines are positioned using the centroids. Circles represent 95% confidence intervals surrounding the wine means. Vectors for sensory terms (A=aroma, F= in mouth flavor) are in W2 and scores for wines are in S2. Significant differences for wines are for circles that do not touch in S2.

CHAPTER 6

CONCLUSION AND FUTURE WORK

Conclusion

This thesis focused on evaluating and analyzing the impact of BMSB in red and white wine due to its growing presence in vineyards. The outcome of this study provides important information about the impact of brown marmorated stink bug taint on wine sensory, steps in wine processing responsible for taint introduction and techniques to minimize their levels in wine either through wine processing modification or corrective measures. This knowledge is highly significant and has direct implication to grape and wine industry.

The sensory data showed that BMSB taint compounds negatively affect wine quality. The main aroma compound in BMSB taint, trans-2-decenal, was detected at 1.92µg/L (DT) in Pinot noir. Further increase in trans-2-decenal concentration caused negative effect on consumer preference. Specifically, Pinot noir and Merlot containing trans-2-decenal at and above 4.8µg/L (CRT) were rejected by consumers. Above this concentration, trans-2-decenal imparted "green", "herbal", "vegetal" aroma in Pinot noir, that is associated with low quality wine. It also suppressed fruit characteristic in this wine. Overall trans-2-decenal was found to be detrimental to wine quality and therefore its level in wine should be minimized.

Next, consumers were segmented using their detection response data, effectively separating subjects into groups with different DT. However, all three segments, despite their different DTs, had the same CRT for trans-2-decenal (> 30μg/L). This is an important result as it shows detection of trans-2-decenal in Pinot noir is not related to its rejection. Therefore, CRT is a better metric to design control measures for taint compounds since it accurately reflects consumer preference and is likely to be more economical.

Given these findings, it becomes important to understand which consumer variables drive preference. Our results showed that wine involvement, subjective knowledge, objective knowledge, and experience can be used to segment consumers into groups with different CRT. However, the results also indicate that the same variables cannot be easily used to directly predict preference response. Additional work is needed in identifying predictive consumer variables as well as developing methods that can better predicting preferences.

Due to the negative effect of trans-2-decenal on wine sensory and consumer preference, it becomes critical to identify how BMSB taint levels change during wine processing. This knowledge can help winemakers adjust their protocols in order to keep taint levels low in the finished wine. Our result show pressing to be the critical wine processing step that introduced significant BMSB taint in wines. In red wine, taint compounds were released during destemming, decreased through alcoholic

fermentation and increased after pressing, with the highest taint concentrations at pressing. Taint released during pressing did not decrease significantly during subsequent steps, resulting in red wine with 2µg/L of trans-2-decenal and 615µg/L of tridecane. We do not see any BMSB taint problems associated with white or rose wines, since pressing occurs before fermentation. More generally, these results indicate that winemakers can minimize BMSB levels in red wine by adjusting their winemaking protocol. Winemakers are advised to use free run or a lighter press to reduce BMSB taint levels in wine. Additional reduction could be achieved through malolactic fermentation, a typical processing step in most red wines post-pressing. In order to maximize the yield while keeping taint levels below sensory threshold, blending of free run and press fraction following malolactic fermentation is recommend.

Besides wine processing, BMSB action threshold (AT) was determined by relating BMSB density in grape clusters to the sensory threshold of trans-2-decenal in the finished wine. AT provides a reference point above which control measures in the vineyard are warranted. The results showed that as few as 3 BMSB per grape clusters were sufficient to reach the DT and remained below CRT of trans-2-decenal in wine. However, this value of BMSB density depends on the particular processing steps used and may turn out to be a conservative estimate if wine is made with heavier industrial press, higher pressure and longer press cycle, all of which can release much more BMSB taint into the wine. This study will permit winemakers to make more informed decisions regarding their winemaking protocols in order to minimize the BMSB taint

levels. Vineyard managers will also be able to take appropriate measures in the field based on the BMSB threshold limits given here.

Finally, the effectiveness of reverse osmosis and commonly used fining agents in reducing BMSB taint in the finished wine was analyzed. The longevity of the BMSB taint during bottle aging was also evaluated. Reverse osmosis was able to reduce trans-2-decenal concentration in wine by 10%, producing small improvements in sensory characteristics. However, tuning the process may further reduce trans-2-decenal levels in wine. Most fining agents showed poor efficacy in binding with trans-2-decenal and were unable to remove the sensory characteristics associated with this compound. Oak addition was found to be successful in masking green characteristics of trans-2-decenal in wine but it also imparted significant spice aroma and flavor characteristics. Aging had a positive effect on BMSB taint where concentration of both, trans-2-decenal and tridecane decreased post bottling. The outcome of this study will allow winemakers to apply appropriate corrective measures to manage BMSB taint in wine and minimize its impact on wine quality.

Taken together, this thesis advances the understanding of BMSB's impact on red and white wines. More generally, it provides a framework for understanding the impact of taint compounds by relating sensory analysis to wine chemistry and processing steps.

Future Work

There are three main directions in which this work can be extended, as discussed below.

The first task is to identify a stronger relationship between consumer variables and preference data. In this thesis, consumer variables showed limited capability in consumer segmentation for trans-2-decenal in wine. A stronger relationship between consumer variables and consumer segments would be preferable. A useful technique may be to recruit consumer groups with well-defined differences in their knowledge, involvement and experience. This may reveal the impact of these variables on segmentation. Additionally, it may be useful to consider additional consumer variables such as sensory knowledge, which measures a person's ability to discriminate, recognize and describe different aromas in wine. Although this variable is somewhat harder to measure, it has previously been shown to affect wine liking (Frost and Noble 2002). Finally, a better segmentation of subjects may be achieved by using a more complex criteria that incorporates both, concentration as well as the subject's consistency in providing correct responses. Preference-based segmentation criteria and its relationship to consumer variables is an extremely important topic due to its large economic significance in terms of providing insight into the drivers of consumer preference in wine.

The second interesting line of future work involves wine processing. The work in chapter 4 focused on some of the processing steps involved in BMSB taint release. However, relatively little is known about the impact of other processes such as sorting, industrial pressing or oxidative aging on taint levels. Given the impact of pressing and aging seen in chapter 4 and 5, it seems very likely that these steps will also affect BMSB taint levels in finished wine. Pressing was found to be the largest contributor of the taint in wine, indicating the need to understand this process in detail. A number of different options are available while performing pressing which may affect the amount of taint released by BMSB. For example level of taint released during pressing may differ based on the press fractions, time and pressure used during pressing etc. This knowledge will allow winemakers to further modify this step to minimize BMSB taint in finished wine. Finally, an important issue meriting further investigation is the effectiveness of sorting which may have the potential to physically remove BMSB at an early stage. Given that taint compounds decrease during fermentation, methods that minimize BMSB presence at the time of pressing are likely to be extremely effective.

The current study has provided preliminary results on the effect of wine aging on BMSB taint. Much work needs to be done to improve current understanding of the underlying chemistry of this process. Examples of open questions include the effect of reductive versus oxidative aging, effect of different types of aging on wine sensory, etc. A decrease in BMSB taint during bottle aging is likely to be a result of aging-related reactions occurring in wine such as hydrolysis, component degradation,

condensation and reduction reaction (Ebeler, 2001; Styger et al., 2011; Ugliano, 2013). At this time, the underlying reason for the absence of trans-2-decenal or tridecane is unclear. The most likely reason is that trans-2-decenal is changed to, decenol, its related alcohol but this hypothesis needs to be tested.

Finally, future work should investigate the role of other BMSB taint compounds besides trans-2-decenal and tridecane. In this study, tridecane and trans-2-decenal were used as markers to study the effect of BMSB taint in wine, due to their high composition in BMSB taint. However, the pest can release more than 39 volatile compounds which may or may not be present in wine. A number of these compounds like trans-2-hexenal, trans-2-octenal etc. possess strong aroma such as green, musty etc. (Wiman and Tomasino 2015 *in review*) and therefore, may affect wine sensory. Very little is known about these compounds in wine, their concentration levels or effect on wine sensory.

Bibliography:

Acebes, A.L., 2016. Host plant effects on the biology, behavior and ecology of brown marmorated stink bug, Halyomorpha halys (Stål) (Hemiptera: Pentatomidae).

American/Western Fruit Grower. 2011. Brown Marmorated Stink Bug Causes $37 Million in Losses to mid-Atlantic Apple Growers. April 14, 2011. http://www.growingproduce.com/fruits/apples-pears/brown-marmorated-stink-bugcauses-37-million-in-losses-to-mid-atlantic-apple-growers/. Accessed June 30, 2016

Amoore, J. E. 1971. Olfactory genetics and anosmia. In: L. M. Beidler (ed.), Handbook of Sensory Physiology. Springer, Berlin, pp. 245–256.Amoore, J. E. 1979. Directions for preparing aqueous solutions of primary odorants to diagnose eight types of specific anosmia. Chemical Senses and Flavor, 4, 153–161.

Arnold, K. 2009 *Halyomorpha halys* (Stål, 1855), a stink bug species newly detected among the European fauna (Insecta: Heteroptera, Pentatomidae, Pentatominae, Cappaeini). itteilungen des Thuringer Entomologenverbandes, e.V. 16:10

ASTM. 2008. Standard practice for determining odor and taste thresholds by a forced-choice ascending concentration series method of limits, E -679-04. Annual Book of Standards, Vol. 15.08. ASTM International, Conshocke n, PA, pp. 36–42.

Bakken, A.J., Schoof, S.C., Bickerton, M., Kamminga, K.L., Jenrette, J.C., Malone, S., Abney, M.A., Herbert, D.A., Reisig, D., Kuhar, T.P., Walgenbach, J.F., 2015. Occurrence of Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) on Wild Hosts in Nonmanaged Woodlands and Soybean Fields in North Carolina and Virginia. Environ. Entomol. 44, 1011–1021. doi:10.1093/ee/nvv092

Baldwin, R.L., Zhang, A., Fultz, S.W., Abubeker, S., Harris, C., Connor, E.E., Van Hekken, D.L., 2014. Hot topic: Brown marmorated stink bug odor compounds do not transfer into milk by feeding bug-contaminated corn silage to lactating dairy cattle.

Ballester, J., Patris, B., Symoneaux, R., Valentin, D., 2008. Conceptual vs. perceptual wine spaces: Does expertise matter? Food Qual. Prefer. 19, 267–276. doi:10.1016/j.foodqual.2007.08.001

Basnet, S., 2014. Biology and pest status of brown marmorated stink bug (Hemiptera: Pentatomidae) in Virginia vineyards and raspberry plantings (Thesis). Virginia Polytechnic Institute and State University, Blacksburg, VA.
Bi, J., Ennis, D.M., O'Mahony, M., 1997. How to estimate and use the variance of d' from difference tests. J. Sens. Stud. 12, 87–104. doi:10.1111/j.1745-459X.1997.tb00055.x

Bi, J. 2006. Sensory Discrimination Tests and Measurements: Statistical Principles, Procedures and Tables. Blackwell Publishing Professional, Ames, IA.

Blackman, J., Saliba, A., Schmidtke, L., 2010. Sweetness acceptance of novices, experienced consumers and winemakers in Hunter Valley Semillon wines. Food Qual. Prefer., Eighth Pangborn Sensory Science Symposium - Wine Special Issue 21, 679–683. doi:10.1016/j.foodqual.2010.05.001

Bult, J.H.F., de Wijk, R.A., Hummel, T., 2007. Investigations on multimodal sensory integration: Texture, taste, and ortho- and retronasal olfactory stimuli in concert. Neurosci. Lett. 411, 6–10. doi:10.1016/j.neulet.2006.09.036

Buntin, G.D. 1996. Economic Thresholds for Insect Management, In Higley, L.G., Pedigo, L.P. Economic Thresholds for Integrated Pest Management. University of Nebraska Press. pp. 128–150

Cadot, Y., Caillé, S., Samson, A., Barbeau, G., Cheynier, V., 2010. Sensory dimension of wine typicality related to a terroir by Quantitative Descriptive Analysis, Just About Right analysis and typicality assessment. Anal. Chim. Acta, A Selection of papers presented at the 6th Symposium in Vino Analytica ScientiaIn Vino 2009 660, 53–62. doi:10.1016/j.aca.2009.10.006

Cai, L., Koziel, J.A., O'Neal, M.E., 2007. Determination of characteristic odorants from Harmonia axyridis beetles using in vivo solid-phase microextraction and multidimensional gas chromatography–mass spectrometry–olfactometry. J. Chromatogr. A 1147, 66–78. doi:10.1016/j.chroma.2007.02.044

Callot. H, Brua, C. 2013 Halyomorpha halys (Stål, 1855), the marmorated stink bug, new species for the fauna of France (Heteroptera Pentatomidae). L'Entomologiste 69:69–71

Câmara, J.S., Alves, M.A., Marques, J.C., 2006. Multivariate analysis for the classification and differentiation of Madeira wines according to main grape varieties. Talanta 68, 1512–1521. doi:10.1016/j.talanta.2005.08.012

Campo, E., Saenz-Navajas, M. p., Cacho, J., Ferreira, V., 2012. Consumer rejection threshold of ethyl phenylacetate and phenylacetic acid, compounds responsible for the sweet-like off odour in wines made from sour rotten grapes. Aust. J. Grape Wine Res. 18, 280–286. doi:10.1111/j.1755-0238.2012.00198.x

Cavazza, A., Franciosi, E., Pojer, M. [et al, 2007. Washing the grapes before crushing: Effects on contaminants and fermentation. Bull. OIV, Sonoma County Wine Library.

Costantini, A., García-Moruno, E., Moreno-Arribas, M.V., 2009. Biochemical Transformations Produced by Malolactic Fermentation, in: Moreno-Arribas, M.V., Polo, M.C. (Eds.), Wine Chemistry and Biochemistry. Springer New York, pp. 27–57.

Culleré, L., Ferreira, V., Cacho, J., 2011. Analysis, occurrence and potential sensory significance of aliphatic aldehydes in white wines. Food Chem. 127, 1397–1403. doi:10.1016/j.foodchem.2011.01.133

Dhami, M.K., Dsouza, M., Waite, D.W., Anderson, D., Li, D., 2016. Real-Time PCR Assay for the Identification of the Brown Marmorated Stink Bug (Halyomorpha halys). Front. Mol. Biosci. 3. doi:10.3389/fmolb.2016.00005

Díaz-Maroto, M.C., Sánchez-Palomo, E., Pérez-Coello, M.S., 2004. Fast Screening Method for Volatile Compounds of Oak Wood Used for Aging Wines by Headspace SPME-GC-MS (SIM). J. Agric. Food Chem. 52, 6857–6861. doi:10.1021/jf049032m

Drake, M.A., 2007. Invited Review: Sensory Analysis of Dairy Foods. J. Dairy Sci. 90, 4925–4937. doi:10.3168/jds.2007-0332

Ebeler, S.E., 2001. Analytical Chemistry: Unlocking the Secrets of Wine Flavor. Food Rev. Int. 17, 45–64. doi:10.1081/FRI-100000517

Eisele, T.A., Semon, M.J., 2005. Best Estimated Aroma and Taste Detection Threshold for Guaiacol in Water and Apple Juice. J. Food Sci. 70, S267–S269. doi:10.1111/j.1365-2621.2005.tb07201.x

Egli, C. M., Edinger, W. D., Mitrakul, C. M., and Henick-Kling, T. (1998) Dynamics of indigenous and inoculated yeast populations and their effect on the sensory character of Riesling and Chardonnay wines. J. Appl. Microbiol. 85, 779–789.

Engen, T. 1972. Psychophysics. Woodworth & schlosberg's experimental psychology. J. W. Kling & L. A. Riggs. New York, Holt, Rineh art and Winston, Inc.

Ennis, D.M., 1993. The Power of Sensory Discrimination Methods. J. Sens. Stud. 8, 353–370. doi:10.1111/j.1745-459X.1993.tb00225.x

Fiola, J.A., 2011. Brown Marmorated Stink Bug (BMSB) Part 3 - Fruit Damage and Juice/Wine Taint. Timely Vitic.

Fogain, R., Graff, S. 2011. First records of the invasive pest, Halyomorpha halys (Hemiptera: Pentatomidae), in Ontario and Quebec. J Entomol Soc Ontario 142:45–48

Francioli, S., Guerra, M., López-Tamames, E., Guadayoi, J.M., Caixach, J., 1999. Aroma of Sparkling Wines by Headspace/Solid Phase Microextraction and Gas Chromatography/Mass Spectrometry. Am. J. Enol. Vitic. 50, 404–408.

Francis, I.L., Sefton, M.A., Williams, P.J., 1992. A Study by Sensory Descriptive Analysis of the Effects of Oak Origin, Seasoning, and Heating on the Aromas of Oak Model Wine Extracts. Am. J. Enol. Vitic. 43, 23–30.

Frijters, J. E. R. 1979. Variations of the triangular method and the relationship of its unidimensional probabilistic model to three-alternative forced choice signal detection theories. British Journal of Mathematical and Statistical Psychology, 32, 229–241.

Galvan, T.L., Burkness, E.C., Vickers, Z., Stenberg, P., Mansfield, A.K., Hutchison, W.D., 2007. Sensory-Based Action Threshold for Multicolored Asian Lady Beetle-Related Taint in Winegrapes. Am. J. Enol. Vitic. 58, 518–522.

Genovese, A., Piombino, P., Gambuti, A., Moio, L., 2009. Simulation of retronasal aroma of white and red wine in a model mouth system. Investigating the influence of saliva on volatile compound concentrations. Food Chem. 114, 100–107. doi:10.1016/j.foodchem.2008.09.022

Gordon, J., 2016. U.S. Wine Sales Total $38 billion [WWW Document]. Wines Vines. URL http://www.winesandvines.com/news/163380 (accessed 6.20.16).

Green, D. M., & Swets, J. A. 1966. Signal detection theory and psychophysics. Huntington, NY: Robert E. Krieger Publishing Co.

Gutiérrez Afonso, V. l., 2002. Sensory Descriptive Analysis Between White Wines Fermented With Oak Chips and In Barrels. J. Food Sci. 67, 2415–2419. doi:10.1111/j.1365-2621.2002.tb09563.x

Hamilton G. C., 2009. Brown marmorated stink bug. Am Entomol 55:19–20

Harwood, M.L., 2013a. A novel method to assess consumer acceptance of bitterness in chocolate products. The Pennsylvania State University.

Harwood, M.L., Ziegler, G.R., Hayes, J.E., 2013b. Tolerance for High Flavanol Cocoa Powder in Semisweet Chocolate. Nutrients 5, 2258–2267. doi:10.3390/nu5062258

Harwood, M.L., Ziegler, G.R., Hayes, J.E., 2012. Rejection thresholds in chocolate milk: Evidence for segmentation. Food Qual. Prefer. 26, 128–133. doi:10.1016/j.foodqual.2012.04.009

Haye, T., Abdallah, S., Gariepy, T., Wyniger, D., 2014. Phenology, life table analysis and temperature requirements of the invasive brown marmorated stink bug, Halyomorpha halys, in Europe. J. Pest Sci. 87, 407–418. doi:10.1007/s10340-014-0560-z

Haye, T., Gariepy, T., Hoelmer, K., Rossi, J.-P., Streito, J.-C., Tassus, X., Desneux, N., 2015. Range expansion of the invasive brown marmorated stinkbug, Halyomorpha halys: an increasing threat to field, fruit and vegetable crops worldwide. J. Pest Sci. 1–9. doi:10.1007/s10340-015-0670-2

Heckmann, R. 2012. First evidence of Halyomorpha halys (Stål, 1855) (Heteroptera: Pentatomidae) in Germany. Heteropteron H 36:17–18

Higley, L.G., Pedigo, L.P. 1996. The EIL concept, In Higley, L.G., Pedigo, L.P. Economic Thresholds for Integrated Pest Management. University of Nebraska Press. pp. 9–21

Hoebeke, E.R., Carter, 2003. Halyomorpha halys (Stål) (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. P Entomol Soc Wash. Proc. Entomol. Soc. Wash. 105, 225–237.

Hopfer, H., Heymann, H., 2014. Judging wine quality: Do we need experts, consumers or trained panelists? Food Qual. Prefer. 32, Part C, 221–233. doi:10.1016/j.foodqual.2013.10.004

Hopfer, H., Nelson, J., Ebeler, S.E., Heymann, H., 2015. Correlating Wine Quality Indicators to Chemical and Sensory Measurements. Molecules 20, 8453–8483. doi:10.3390/molecules20058453

Hughson, A.L., Boakes, R.A., 2002. The knowing nose: the role of knowledge in wine expertise. Food Qual. Prefer. 13, 463–472. doi:10.1016/S0950-3293(02)00051-4

Hughson, A.L., Boakes, R.A., 2001. Perceptual and cognitive aspects of wine expertise. Aust. J. Psychol. 53, 103–108. doi:10.1080/00049530108255130

Isaacs, R., Saunders, M.C., Bostanian, N.J., 2012. Pest Thresholds in Vineyards, in: Bostanian, N.J., Vincent, C., Isaacs, R. (Eds.), Arthropod Management in Vineyards: Springer Netherlands, pp. 17–36.

Jackson, R.S., 2008. Wine Science: Principles and Applications. Academic Press.

Jaeger, S.R., de Silva, H.N., Lawless, H.T., 2014. Detection Thresholds of 10 Odor-active Compounds Naturally Occurring in Food Using a Replicated Forced-Choice Ascending Method of Limits. J. Sens. Stud. 29, 43–55. doi:10.1111/joss.12085

Jones, P.R., Gawel, R., Francis, I.L., Waters, E.J., 2008. The influence of interactions between major white wine components on the aroma, flavour and texture of model white wine. Food Qual. Prefer. 19, 596–607. doi:10.1016/j.foodqual.2008.03.005

Khan, W., Augustyn, O. P. H., Van der Westhuizen, T. J., Lambrechts, M. G., and Pretorius, I. S. 2000. Geographic distribution and evaluation of Saccharomyces cerevisiae strains isolated from vineyards in the warmer, inland regions of the Western Cape in South Africa. S. Afr. J. Enol. Vitic. 21, 17–31.

Klein, S. (2001). Measuring, estimating, and understanding the psychometric function: A commentary. Attention, Perception, & Psychophysics, 63 (8), 1421–1455.

Kögel, S., Gross, G., Hoffmann, C., 2012. Sensory thresholds of ladybird taint in "Riesling" and "Pinot Noir" under different fermentation and processing conditions. Vitis -Geilweilerhof- 51, 27–32.

Lara, J., Pickett, C., Ingels, C., Haviland, D.R., Grafton-Cardwell, E., Doll, D., Bethke, J., Faber, B., Dara, S.K., Hoddle, M., 2016. Biological control program is being developed for brown marmorated stink bug. Calif. Agric. 70, 15–23. doi:10.3733/ca.v070n01p15

Lawless, H.T., 2013. Quantitative Sensory Analysis: Psychophysics, Models and Intelligent Design. John Wiley & Sons.

Lawless, H.T., 2010. A Simple Alternative Analysis for Threshold Data Determined by Ascending Forced-Choice Methods of Limits. J. Sens. Stud. 25, 332–346. doi:10.1111/j.1745-459X.2009.00262.x

Lawless, H.T., Heymann, H., 2010. Sensory Evaluation of Food: Principles and Practices. Springer Science & Business Media.

Lee, D.-H., 2015. Current status of research progress on the biology and management of Halyomorpha halys (Hemiptera: Pentatomidae) as an invasive species. Appl. Entomol. Zool. 50, 277–290. doi:10.1007/s13355-015-0350-y

Legrum, C., Gracia-Moreno, E., Lopez, R., Potouridis, T., Langen, J., Slabizki, P., Weiand, J., Schmarr, H.-G., 2014. Quantitative analysis of 3-alkyl-2-methoxypyrazines in German Sauvignon blanc wines by MDGC–MS or MDGC–MS/MS for viticultural and enological studies. Eur. Food Res. Technol. 239, 549–558. doi:10.1007/s00217-014-2250-8

Leskey, T.C., Agnello, A., Bergh, J.C., Dively, G.P., Hamilton, G.C., Jentsch, P., Khrimian, A., Krawczyk, G., Kuhar, T.P., Lee, D.-H., Morrison, W.R., Polk, D.F., Rodriguez-Saona, C., Shearer, P.W., Short, B.D., Shrewsbury, P.M., Walgenbach, J.F., Weber, D.C., Welty, C., Whalen, J., Wiman, N., Zaman, F., 2015. Attraction of the Invasive Halyomorpha halys (Hemiptera: Pentatomidae) to Traps Baited with Semiochemical Stimuli Across the United States. Environ. Entomol. nvv049. doi:10.1093/ee/nvv049

Leskey, T.C., Hamilton, G.C., Nielsen, A.L., Polk, D.F., Rodriguez-Saona, C., Bergh, J.C., Herbert, D.A., Kuhar, T.P., Pfeiffer, D., Dively, G.P., Hooks, C.R.R., Raupp, M.J., Shrewsbury, P.M., Krawczyk, G., Shearer, P.W., Whalen, J., Koplinka-Loehr, C., Myers, E., Inkley, D., Hoelmer, K.A., Lee, D.-H., Wright, S.E., 2012a. Pest Status of the Brown Marmorated Stink Bug, Halyomorpha Halys in the USA. Outlooks Pest Manag. 23, 218–226. doi:10.1564/23oct07

Leskey, T.C., Short, B.D., Lee, D.-H., 2014. Efficacy of insecticide residues on adult Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) mortality and injury in apple and peach orchards. Pest Manag. Sci. 70, 1097–1104. doi:10.1002/ps.3653

Leskey, T.C., Wright, S.E., Short, B.D., Khrimian, A., 2012b. Development of Behaviorally-Based Monitoring Tools for the Brown Marmorated Stink Bug (Heteroptera: Pentatomidae) in Commercial Tree Fruit Orchards. J. Entomol. Sci. 47, 76–85. doi:10.18474/0749-8004-47.1.76

Lona-Ramirez, F.J., Gonzalez-Alatorre, G., Rico-Ramírez, V., Perez-Perez, M.C.I., Castrejón-González, E.O., 2016. Gas chromatography/mass spectrometry for the determination of nitrosamines in red wine. Food Chem. 196, 1131–1136. doi:10.1016/j.foodchem.2015.09.090

Luna, J. M., House, G. J. 1990. Pest Management in Sustainable Agricultural Systems, In Edwards, C.A., Lal, R., Madden, P, Miller, R.H., House G. Sustainable Agricultural Systems. CRC Press. pp. 157–173

Macmillan, N. A., & Creelman, C. D. 2005. Detection theory: A user's guide (2nd ed.). Mahwah, New Jersey: Lawrence Erlbaum Associates.

Marriott, P.J., Chin, S.-T., Maikhunthod, B., Schmarr, H.-G., Bieri, S., 2012. Multidimensional gas chromatography. TrAC Trends Anal. Chem. 34, 1–21. doi:10.1016/j.trac.2011.10.013

Martineau, B., Acree, T.E., Henick-Kling, T., 1995. Effect of wine type on the detection threshold for diacetyl. Food Res. Int. 28, 139–143. doi:10.1016/0963-9969(95)90797-E

Martinson, Timothy. "Asian Lady Beetle Control Update." (2004).
Mateo, J. J., Jimenez, M., Huerta, T., and Pastor, A. (1991) Contribution of different yeast isolated from musts of Monastrell grapes to the aroma of wine. Intl J. Food Microbiol. 14, 153–160.

McPherson, J.E., McPherson, R., 2000. Stink Bugs of Economic Importance in America North of Mexico. CRC Press.

Meilgaard, M.C., 1993. Individual differences in sensory threshold for aroma chemicals added to beer. Food Qual. Prefer. 4, 153–167. doi:10.1016/0950-3293(93)90158-3

Meilgaard, M.C., Carr, B.T., Civille, G.V., 2006. Sensory Evaluation Techniques, Fourth Edition. CRC Press.

Mendes, B., Gonçalves, J., Câmara, J.S., 2012. Effectiveness of high-throughput miniaturized sorbent- and solid phase microextraction techniques combined with gas

chromatography–mass spectrometry analysis for a rapid screening of volatile and semi-volatile composition of wines—A comparative study. Talanta 88, 79–94. doi:10.1016/j.talanta.2011.10.010

Methven, L., Xiao, C., Cai, M., Prescott, J., 2016. Rejection thresholds (RjT) of sweet likers and dislikers. Food Qual. Prefer. 52, 74–80. doi:10.1016/j.foodqual.2016.03.012

Milonas, P. G., Partsinevelos, G. K., (2014) First report of brown marmorated stink bug Halyomorpha halys Stål (Hemiptera: Pentatomidae) in Greece. EPPO Bull 44:183–186

Mohekar, P., Tomasino, E., Wiman, N.G., 2015. Defining defensive secretions of brown marmorated stink bug, Halyomorpha halys. Presented at the 2015 Annual Meeting of the Entomological Society of America, Minneapolis, MN.

Morrison, W.R., Cullum, J.P., Leskey, T.C., 2015. Evaluation of Trap Designs and Deployment Strategies for Capturing Halyomorpha halys (Hemiptera: Pentatomidae). J. Econ. Entomol. 108, 1683–1692. doi:10.1093/jee/tov159

Muñoz-González, C., Rodríguez-Bencomo, J.J., Moreno-Arribas, M.V., Pozo-Bayón, M.Á., 2011. Beyond the characterization of wine aroma compounds: looking for analytical approaches in trying to understand aroma perception during wine consumption. Anal. Bioanal. Chem. 401, 1497–1512. doi:10.1007/s00216-011-5078-0

Nielsen, A.L., Hamilton, G.C., 2009a. Seasonal occurrence and impact of Halyomorpha halys (Hemiptera: Pentatomidae) in tree fruit. J. Econ. Entomol. 102, 1133–1140.

Nielsen, A.L., Hamilton, G.C., 2009b. Life History of the Invasive Species Halyomorpha halys (Hemiptera: Pentatomidae) in Northeastern United States. Ann. Entomol. Soc. Am. 102, 608–616. doi:10.1603/008.102.0405

Nielsen, A.L., Holmstrom, K., Hamilton, G.C., Cambridge, J., Ingerson-Mahar, J., 2013. Use of black light traps to monitor the abundance, spread, and flight behavior of Halyomorpha halys (Hemiptera: Pentatomidae). J. Econ. Entomol. 106, 1495–1502.

Panighel, A., Flamini, R., 2015. Solid Phase Extraction and Solid Phase Microextraction in grape and wine volatile compounds analysis. Sample Prep. 2.

Parr, W.V., Heatherbell, D., White, K.G., 2002. Demystifying Wine Expertise: Olfactory Threshold, Perceptual Skill and Semantic Memory in Expert and Novice Wine Judges. Chem. Senses 27, 747–755. doi:10.1093/chemse/27.8.747

Peiffer, M., Felton, G.W., 2014. Insights into the Saliva of the Brown Marmorated Stink Bug Halyomorpha halys (Hemiptera: Pentatomidae). PLOS ONE 9, e88483. doi:10.1371/journal.pone.0088483

Peng, M., Jaeger, S.R., Hautus, M.J., 2012. Determining odour detection thresholds: Incorporating a method-independent definition into the implementation of ASTM E679. Food Qual. Prefer. 25, 95–104. doi:10.1016/j.foodqual.2012.02.006

Pérez-Coello, M.S., Díaz-Maroto, M.C., 2009. Volatile Compounds and Wine Aging, in: Moreno-Arribas, M.V., Polo, M.C. (Eds.), Wine Chemistry and Biochemistry. Springer New York, pp. 295–311.

Perrin, L., Symoneaux, R., Maître, I., Asselin, C., Jourjon, F., Pagès, J., 2007. Comparison of Conventional Profiling by a Trained Tasting Panel and Free Profiling by Wine Professionals. Am. J. Enol. Vitic. 58, 508–517.

Perry, D.M., Hayes, J.E., 2016. Effects of Matrix Composition on Detection Threshold Estimates for Methyl Anthranilate and 2-Aminoacetophenone. Foods 5, 35. doi:10.3390/foods5020035

Pfeiffer, D.G., Leskey, T.C., Burrack, H.J., 2012. Threatening the Harvest: The Threat from Three Invasive Insects in Late Season Vineyards, in: Bostanian, N.J., Vincent, C., Isaacs, R. (Eds.), Arthropod Management in Vineyards: Springer Netherlands, pp. 449–474.

Pickering, G. J., Karthik, A., Inglis, D., Sears, M., Ker, K., 2007a. Determination of Ortho- and Retronasal Detection Thresholds for 2-Isopropyl-3-Methoxypyrazine in Wine. J. Food Sci. 72, S468–S472. doi:10.1111/j.1750-3841.2007.00439.x

Pickering, G.J., Ker, K., Soleas, G.J., 2007b. Determination of the critical stages of processing and tolerance limits for Harmonia axyridis for “ladybug taint” in wine. VITIS - J. Grapevine Res. 46, 85–90.

Pickering, G., Lin, J., Riesen, R., Reynolds, A., Brindle, I., Soleas, G., 2004. Influence of Harmonia axyridis on the Sensory Properties of White and Red Wine. Am. J. Enol. Vitic. 55, 153–159.

Punter, P. H. 1983. Measurement of human olfactory thresholds for several groups of structurally related compounds. Chemical Senses, 7, 215–235.

Polášková, P., Herszage, J., Ebeler, S.E., 2008. Wine flavor: chemistry in a glass. Chem. Soc. Rev. 37, 2478–2489. doi:10.1039/B714455P

Prescott, J., Norris, L., Kunst, M., Kim, S., 2005. Estimating a “consumer rejection threshold” for cork taint in white wine. Food Qual. Prefer. 16, 345–349. doi:10.1016/j.foodqual.2004.05.010

Rebière, L., Clark, A.C., Schmidtke, L.M., Prenzler, P.D., Scollary, G.R., 2010. A robust method for quantification of volatile compounds within and between vintages using headspace-solid-phase micro-extraction coupled with GC–MS – Application on Semillon wines. Anal. Chim. Acta, A Selection of papers presented at the 6th Symposium in Vino Analytica ScientiaIn Vino 2009 660, 149–157. doi:10.1016/j.aca.2009.10.029

Reynolds, A.G., Yerle, S., Watson, B., Price, S.F., Wardle, D.A., 1996. Fruit Environment and Crop Level Effects on Pinot noir. III. Composition and Descriptive Analysis of Oregon and British Columbia Wines. Am. J. Enol. Vitic. 47, 329–339.

Rice, K.B., Bergh, C.J., Bergmann, E.J., Biddinger, D.J., Dieckhoff, C., Dively, G., Fraser, H., Gariepy, T., Hamilton, G., Haye, T., Herbert, A., Hoelmer, K., Hooks, C.R., Jones, A., Krawczyk, G., Kuhar, T., Martinson, H., Mitchell, W., Nielsen, A.L., Pfeiffer, D.G., Raupp, M.J., Rodriguez-Saona, C., Shearer, P., Shrewsbury, P., Venugopal, P.D., Whalen, J., Wiman, N.G., Leskey, T.C., Tooker, J.F., 2014. Biology, Ecology, and Management of Brown Marmorated Stink Bug (Hemiptera: Pentatomidae). J. Integr. Pest Manag. 5, A1–A13. doi:10.1603/IPM14002

Roberts, D.D., Acree, T.E., 1995. Simulation of Retronasal Aroma Using a Modified Headspace Technique: Investigating the Effects of Saliva, Temperature, Shearing, and Oil on Flavor Release. J. Agric. Food Chem. 43, 2179–2186. doi:10.1021/jf00056a041

Robinson, A.L., Boss, P.K., Heymann, H., Solomon, P.S., Trengove, R.D., 2011. Development of a sensitive non-targeted method for characterizing the wine volatile profile using headspace solid-phase microextraction comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry. J. Chromatogr. A 1218, 504–517. doi:10.1016/j.chroma.2010.11.008

Robinson, A.L., Ebeler, S.E., Heymann, H., Boss, P.K., Solomon, P.S., Trengove, R.D., 2009. Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. J. Agric. Food Chem. 57, 10313–10322. doi:10.1021/jf902586n

Ross, C. f., Zwink, A. c., Castro, L., Harrison, R., 2014. Odour detection threshold and consumer rejection of 1,1,6-trimethyl-1,2-dihydronaphthalene in 1-year-old Riesling wines. Aust. J. Grape Wine Res. 20, 335–339. doi:10.1111/ajgw.12085

Rousseau, B., 2015. Sensory discrimination testing and consumer relevance. Food Qual. Prefer. 43, 122–125. doi:10.1016/j.foodqual.2015.03.001

Sáenz-Navajas, M.-P., Campo, E., Fernández-Zurbano, P., Valentin, D., Ferreira, V., 2010. An assessment of the effects of wine volatiles on the perception of taste and

astringency in wine. Food Chem. 121, 1139–1149. doi:10.1016/j.foodchem.2010.01.061

Sáenz-Navajas, M.-P., Fernández-Zurbano, P., Ferreira, V., 2012. Contribution of Nonvolatile Composition to Wine Flavor. Food Rev. Int. 28, 389–411. doi:10.1080/87559129.2012.660717

Segurel, M.A., Razungles, A.J., Riou, C., Salles, M., Baumes, R.L., 2004. Contribution of dimethyl sulfide to the aroma of Syrah and Grenache Noir wines and estimation of its potential in grapes of these varieties. J. Agric. Food Chem. 52, 7084–7093. doi:10.1021/jf049160a

Siebert, T.E., Smyth, H.E., Capone, D.L., Neuwöhner, C., Pardon, K.H., Skouroumounis, G.K., Herderich, M.J., Sefton, M.A., Pollnitz, A.P., 2005. Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. Anal. Bioanal. Chem. 381, 937–947. doi:10.1007/s00216-004-2992-4

Smith, J.R., Hesler, S.P., Loeb, G.M., 2014. Potential Impact of Halyomorpha halys (Hemiptera: Pentatomidae) on Grape Production in the Finger Lakes Region of New York. J. Entomol. Sci. 49, 290–303. doi:10.18474/0749-8004-49.3.290

Solomon, D., Dutcher, D., Raymond, T., 2013. Characterization of Halyomorpha halys (brown marmorated stink bug) biogenic volatile organic compound emissions and their role in secondary organic aerosol formation. J. Air Waste Manag. Assoc. 1995 63, 1264–1269.

Song, M., Xia, Y., Tomasino, E., 2015. Investigation of a Quantitative Method for the Analysis of Chiral Monoterpenes in White Wine by HS-SPME-MDGC-MS of Different Wine Matrices. Molecules 20, 7359–7378. doi:10.3390/molecules20047359

Spedding, D. J., and Raut, P. (1982) The influence of dimethyl sulphide and carbon disulphide in the bouquet of wines. Vitis 21, 240–246.

Stern, V. M., Smith, R.F., van den Bosch, R., Hagen, K. S. 1959. The integrated control concept. Hilgardia 29:81–101

Stone, H. and Sidel, J. L. 2004. Sensory Evaluation Practices, Third Edition. Academic, San Deigo.Talamas, E.J., Herlihy, M.V., Dieckhoff, C., Hoelmer, K.A., Buffington, M., Bon, M.-C., Weber, D.C., 2015. Trissolcus japonicus (Ashmead) (Hymenoptera, Scelionidae) emerges in North America. J. Hymenopt. Res. 43, 119–128. doi:10.3897/JHR.43.4661

Styger, G., Prior, B., Bauer, F.F., 2011. Wine flavor and aroma. J. Ind. Microbiol. Biotechnol. doi:10.1007/s10295-011-1018-4
Thurstone, L.L., 1927. A law of comparative judgment. Psychol. Rev. 34, 273–286. doi:10.1037/h0070288

Tomasino, E., P. Mohekar, T. Lapis, V. Walton, and J. Lim. 2013. Effect of brown marmorated stink bug wine - impact to Pinot noir quality and threshold determination of taint compound trans-2-decenal. The 15th Australian Wine Industry Technical Conference, Sydney, Australia, July

Tomasino, E. 2011. "Characterization of Regional Examples of New Zealand Pinot Noir by Means of Sensory and Chemical Analysis." New Zealand: Lincoln University.

Tomasino, E., Harrison, R., Sedcole, R., Frost, A., 2013. Regional Differentiation of New Zealand Pinot noir Wine by Wine Professionals Using Canonical Variate Analysis. Am. J. Enol. Vitic. ajev.2013.12126. doi:10.5344/ajev.2013.12126

Vétek, G., Papp, V., Haltrich, A., Rédei, D., 2014. First record of the brown marmorated stink bug, Halyomorpha halys (Hemiptera: Heteroptera: Pentatomidae), in Hungary, with description of the genitalia of both sexes. Zootaxa 3780, 194–200.

Villamor, R.R., Ross, C.F., 2013. Wine Matrix Compounds Affect Perception of Wine Aromas. Annu. Rev. Food Sci. Technol. 4, 1–20. doi:10.1146/annurev-food-030212-182707

Villamor, R.R., Evans, M.A., Mattinson, D.S., Ross, C.F., 2013. Effects of ethanol, tannin and fructose on the headspace concentration and potential sensory significance of odorants in a model wine. Food Res. Int. 50, 38–45. doi:10.1016/j.foodres.2012.09.037

Walton, V., Wiman, N., Daane, K., Zalom, F., Cooper, M.L., Varela, L.G., 2015. Brown Marmorated stink bug risk and impacts in western vineyards. California Department of Food and Agriculture, 2015 Research Progress Reports on Pierce's Disease and Other Pests and Diseases of Winegrapes.

Weekes, L.N., Walsh, D., Ferguson, H., Ross, C.F., 2010. Impact of Multicolored Asian Lady Beetles on the sensory properties of Concord and Niagara grape juice. J. Food Sci. 75, S68-73. doi:10.1111/j.1750-3841.2009.01423.x

Wermelinger B, Wyniger D, Forster B (2008) First records of an invasive bug in Europe: Halyomorpha halys Stål (Heteroptera: Pentatomidae), a new pest on woody ornamentals and fruit trees? MitSch Ges 81:1–8

Wiman, N., Rondon, S., Walton, V., and P.W. Shearer. 2014a. Distribution of brown marmorated stink bug in Oregon and risk for specialty crops. Research Report, 72nd Annual PNW Insect Management Conference, p. 42.

Xu, J., Fonseca, D.M., Hamilton, G.C., Hoelmer, K.A., Nielsen, A.L., 2014. Tracing the origin of US brown marmorated stink bugs, Halyomorpha halys. Biol. Invasions 16, 153–166. doi:10.1007/s10530-013-0510-3

Yang, Z.-Q., Yao, Y.-X., Qiu, L.-F., Li, Z.-X., 2009. A New Species of Trissolcus (Hymenoptera: Scelionidae) Parasitizing Eggs of Halyomorpha Halys (Heteroptera: Pentatomidae) in China with Comments on its Biology. Ann. Entomol. Soc. Am. 102, 39–47. doi:10.1603/008.102.0104

Yoo, Y.J., Saliba, A.J., Prenzler, P.D., Ryan, D., 2012. Total Phenolic Content, Antioxidant Activity, and Cross-Cultural Consumer Rejection Threshold in White and Red Wines Functionally Enhanced with Catechin-Rich Extracts. J. Agric. Food Chem. 60, 388–393. doi:10.1021/jf203216z

Young, W.F., Horth, H., Crane, R., Ogden, T., Arnott, M., 1996. Taste and odour threshold concentrations of potential potable water contaminants. Water Res. 30, 331–340. doi:10.1016/0043-1354(95)00173-5

Zamora, F., 2009. Biochemistry of Alcoholic Fermentation, in: Moreno-Arribas, M.V., Polo, M.C. (Eds.), Wine Chemistry and Biochemistry. Springer New York, pp. 3–26.

Zamora, M.C., Guirao, M., 2004. Performance Comparison Between Trained Assessors and Wine Experts Using Specific Sensory Attributes. J. Sens. Stud. 19, 530–545. doi:10.1111/j.1745-459X.2004.051404.x

APPENDIX 1

Effect of mechanical harvesting on Brown Marmorated Stink Bug, *Halyomorpha halys* taint release

Objective: To investigate the effect of mechanical harvesting on BMSB taint release.

Method: The effect of mechanical harvesting was simulated through agitation. The effect was tested on a Merlot wine made from grape clusters containing BMSB at a density of 1 per cluster. Before destemming and crushing, a mix of grapes and BMSB were rolled in a plastic pail for 45 seconds to mimic the movement and agitation as a result of mechanical harvesting (Pickering et al., 2007). Merlot winemaking protocol from Chapter 4 was followed here. A basket press (Allenair Crop. Model VH-SR-R) operated at 2 bar for one minute was used for pressing. Tridecane and trans-2-decenal concentrations were quantified after destemming-crushing and in finished wine.

Results:

Trans-2-decenal levels were found to be below the limit of detection. Therefore, the effect of agitation was studied in terms of tridecane concentration. Merlot with agitation produced significantly more tridecane at destemming and in finished wine compared to wine without agitation ($\alpha = 0.05$) (Figure 1). The increase in tridecane after destemming is not a concern since BMSB taint compounds decrease significantly during fermentation (Chapter 4). The higher tridecane levels in finished wine with agitation could be a result of pressing, which occurs after fermentation and

is the largest contributor of BMSB taint in wine. Since taint levels at pressing were not estimated, it remains to be seen if higher tridecane in finished wine is a result of pressing or agitation.

Future work should investigate the effect of mechanical harvesting at high BMSB level of 3 per cluster or above to determine its effect on BMSB causal compound, trans-2-decenal. Additionally, taint levels at pressing should also be estimated to differentiate between taint fractions in finished wine from pressing and agitation.

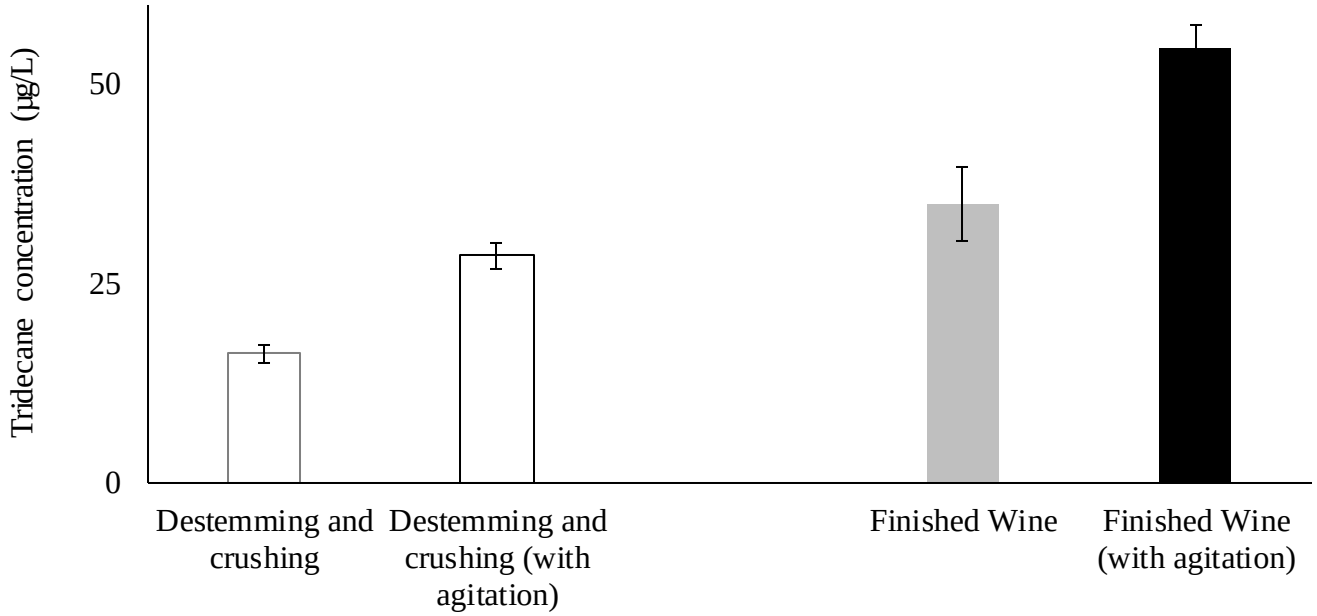

Figure 1: Difference in tridecane concentration in 2013 Merlot, after destemming and crushing without agitation (□), after destemming and crushing when agitation was included (□), finished wine without agitation (■), in finished wine when agitation was included (■)

*Trans-2-decenal is not shown as the measured levels were below LOD

APPENDIX 2

Sensory effect of bottle aging on Brown Marmorated Stink Bug, *Halyomorpha halys* taint

Introduction

This section evaluates the effect of decreasing BMSB taint concentration during bottle aging on wine's sensory. Pinot noir and Pinot gris made from 3 BMSB per cluster and 1 BMSB per cluster (Chapter 4) respectively were analyzed using MDGC-MS at different stages of bottle aging. In Pinot noir, both tridecane and trans-2-decenal levels were measured at 0, 6 and 12 months of aging. In Pinot gris, only tridecane was estimated at 0 and 24 months post-bottling, it did not contain trans-2-decenal at bottling (Chapter 4). The results of MDGC-MS analysis on aged wine are given in Chapter 5. These concentrations were used to determine sensory effect of aging.

Methods

Stimuli: Two base wines, Pinot noir and Pinot gris, free from any BMSB taint were used in this study. Pinot noir1 was made at OSU research winery and Pinot gris1 was purchased from a winery in Oregon. Trans-2-decenal and tridecane were added to these base wines at the concentration corresponding to different stages of aging (Table 1). These addition resulted in one Pinot gris and five Pinot noir treatments containing trans-2-decenal and/or tridecane. Single Pinot gris treatment corresponding to aging point 0 (bottling) was included in sensory evaluation since tridecane levels in Pinot gris were reported to be below the limit of detection after 24

months of aging. Taint containing Pinot noir and Pinot gris were then compared to their corresponding base wines (no taint compounds) using discrimination analysis (Table 1).

Two different tridecane standards, 61.8 mg/L and 1.23g/L were used to achieve desired concentration of tridecane in base wines. Tridecane standards were made in 100% ethanol due to their low solubility in 14% ethanol, particularly at high concentration. A desired trans-2-decenal concentrations in Pinot noir1 were achieved using a trans-2-decenal standard of 3.1 mg/L prepared in 14% ethanol. Both compounds were added to the base wine an hour before sensory evaluation.

Panel: A total of 31 regular red and white wine consumers participated in this study. A regular wine consumer was defined as someone who consumed on average at least one glass of red and white wine every week. Panel consisted of 15 males and 16 females aged between 25−69 (mean = 48). Subjects were told that they will be evaluating differences in wine quality and no information regarding BMSB taint was made available prior to their tasting. Subjects signed a consent form prior to their participation and were compensated for their participation in the form of gift cards from local shops. The study was approved by Oregon State University Internal Review Board (IRB).

Data collection: Data was collected over four, 45 minutes sessions. All sessions were conducted in the afternoon, in the same room, under similar temperature and light

condition. During each session, panel evaluated six triangle tests (five red and one white wine). White wines were evaluated at the beginning, followed by red wines with increasing concentration of tridecane and trans-2-decenal. Each session included a one minute break after each set and five minutes after first four sets to reduce fatigue. Panelists was asked to rinse their palate with water and eat a cracker during each break to minimize any carry over effect. Red wines were served at room temperature and white wine at 13 ± 2°C in INAO clear glasses (Institut National d'Appellation d'Origine). Samples were coded with a three digit random numbers and served in a random order. Subjects were asked to smell and taste the wine and identify the sample that was different.

Data analysis: A significant difference between base wine and same wine with BMSB taint compounds was determined using Thurstonian model (d' values) for triangle test. SensR package in r-studio was used to quantify d′ values and their significance at $\alpha = 0.05$ (Brockhoff & Christensen, 2010).

Result

The sensory impact of decreasing BMSB taint levels during bottle aging was evaluated using discrimination analysis. Pinot noir (no BMSB taint) was found to be significantly different from Pinot noir containing trans-2-decenal and tridecane at 2µg/L and 615µg/L respectively. These concentrations correspond to taint levels in Pinot noir made from 3BMSB per cluster at bottling (Table 1). Therefore, the above result indicates that at bottling, red wine perception can be altered by BMSB taint.

The effect of BMSB taint on wine perception decreased with aging and no significant difference was found between Pinot noir at 0 months and Pinot noir with taint compounds corresponding to 6 and 12 months of aging (Table 2).

In white wine, tridecane concentration at bottling did not show any effect on wine's sensory perception. This, in combination with lack of trans-2-decenal at bottling suggests that BMSB may not be an issue for white wines.

Conclusion

Aging had a positive effect on both, red and white wine composition as concentration of trans-2-decenal and tridecane decreased post bottling. At bottling, sensory perception of red wine was affected significantly by the presence of trans-2-decenal and tridecane, but their effect lessened as taint level decreased during aging. In white wine, tridecane showed no effect on wine's sensory perception indicating that BMSB may not be a concern for white wines.

Table 1: Concentration of tridecane and trans-2-decenal that was added into Pinot gris and Pinot noir to determine the effect of aging

Treatment No.	Wine	Trans-2-decenal concentration (μg/L)	Tridecane concentration (μg/L)	Corresponding to
1	Pinot gris		10	At bottling
2	Pinot noir		160	12 months aging
3	Pinot noir		279	6 months aging
4	Pinot noir		615	At bottling
5	Pinot noir	2		At bottling
6	Pinot noir	2	615	At bottling

Table 2: Proportion of correct responses and *d-prime* values from discrimination analysis between Pinot noir with added trans-2-decenal and/or tridecane and control (Pinot noir without tridecane and trans-2-decenal) and comparison between Pinot gris with added tridecane and control (Pinot gris without tridecane)
Each wine represents bottle-aged Pinot noir made from BMSB added grapes.

Treatment No.	Wine treatment	Proportion correct	d-prime
1	Pinot gris at bottling	0.45	1.20
2	Pinot noir at 12 months aging	0.42	1.00
3	Pinot noir at 6 months aging	0.29	0.00
4	Pinot noir at bottling (Tridecane alone)	0.48	1.38$^{\bullet}$
5	Pinot noir at bottling (Trans-2-decenal alone)	0.65	2.21***
6	Pinot noir at bottling (Tridecane + Trans-2-decenal)	0.61	2.04**

$^{\bullet}$: $p < 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$