Summary of recent MALB research findings from the Cool Climate Oenology and Viticulture Institute, Brock University

July 2nd, 2008

Introductory comments

The following pages summarize the results of the most recent projects from CCOVI concerned with the Multi-Colored Asian Ladybeetle (MALB) and ladybug taint in wine. While other interesting and potentially informative trials have been and are being conducted on this insect, we have limited this summary to findings that have appeared in the refereed literature or are under consideration there. This decision reflects the widely accepted principle that sound, reproducible and apolitical scientific enquiry is the only valid basis for truly understanding MALB behaviour and deriving effective strategies and technologies for addressing the problems they can cause. The rigors of the peer-review process should give us considerable confidence in the quality of the hypotheses, methods, results and conclusions of the research.

We will update these pages on a regular basis as current projects reach their conclusion and as new projects - funding permitting - are initiated. You are encouraged to contact the corresponding author(s) of these studies if you have any specific questions on the research or for a copy of the referenced papers. Finally, thank you to all our funding partners and industry collaborators for their on-going support of this research programme.

Prof Gary Pickering,

on behalf of the MALB team at Brock.

2008

Detection Thresholds for 2-Isopropyl-3-Methoxypyrazine in Niagara and Concord Grape Juice

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Summary

2-Isopropyl-3-methoxypyrazine (IPMP) is the compound responsible for ladybug taint, which can occur when Harmonia axyridis (MALB) beetles become incorporated with the grapes during juice processing. It is also a very important grape-derived constituent of juice flavor for some varieties. The objective of this study was to determine the orthonasal (ON, 'smell') and retronasal (RN, 'taste') detection thresholds for IPMP in juice. (The detection threshold refers to the minimum concentration of a stimulus that is required before it can be perceived). The ASTM E679 method of limits was used to determine detection thresholds in Concord and Niagara juices for 26 individuals. The average group best estimate threshold (BET) was 0.93 ng/L, and BETs were 50% and 21% higher in Concord than in Niagara juices for ON and RN evaluation, respectively. BETs for IPMP (ng/L) for Niagara were ON: 0.74 and RN: 0.84, and for Concord were ON: 1.11 and RN: 1.02. There were differences between many individuals in their detection thresholds, although familiarity with ladybug taint was not associated with individual threshold values. We conclude that humans are very sensitive to IPMP in juice, and that detection thresholds are more strongly influenced by the grape variety than by the evaluation mode (ON v RN). These results should assist juice producers in establishing tolerance levels for IPMP in juice affected by ladybug taint or derived from grapes of less than optimal ripeness.

Acknowledgements: The Cadbury Schweppes Beverages Plant, St. Catharines, Ontario is gratefully acknowledged for the donation of Concord and Niagara juice. We also thank John Pauls (Cadbury Schweppes) and Lynda Van Zuiden (Brock University) for technical assistance. We are very grateful for the financial support of the Natural Sciences and Engineering Research Council of Canada, the Wine Council of Ontario and the Grape Growers of Ontario. Finally, thank you to all the participants who took part in the sensory assessments.

Full reference: G. J. Pickering, A. Karthik, D. Inglis, M. Sears and K. Ker. (2008). Detection Thresholds for 2-Isopropyl-3-Methoxypyrazine in Concord and Niagara Grape Juice. *Journal of Food Science* (in press).

2008

Yeast strain affects 3-isopropyl-2-methoxypyrazine concentration and sensory profile in Cabernet Sauvignon wine

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Summary

3-isopropyl-2-methoxypyrazine (IPMP) is both a grape- and insect-derived trace compound found in wine that contributes green characteristics to the product. There has been renewed interest in examining how viticultural and oenological practices may mediate IPMP concentrations in wine since the recent concerns regarding ladybug taint (LBT) – an off-flavour due to IPMP extracted from Harmonia axyridis (MALB) when they are incorporated in and processed with harvested grapes. This study sought to determine the influence of commercial *Saccharomyces* yeast strains on IPMP concentration in Cabernet Sauvignon wines and to examine their sensory effect. Re-hydrated juice from Cabernet Sauvignon concentrate was spiked with 30ng/L IPMP and fermented (in triplicate) with Lalvin EC1118, Lalvin BM45, Lalvin ICV-D21 or Lalvin ICV-D80. A control wine was fermented with EC1118 from juice with no IPMP added. IPMP was determined using headspace solid-phase microextraction coupled with gas chromatography mass spectrometry, and was unchanged from juice levels in wine fermented by EC1118, D21 and D80, but increased by 11 ng/L in wine fermented by BM45. Addition of IPMP to juice resulted in higher intensity scores for earthy/peanut/musty aroma, green pepper aroma, canned green vegetable flavour and nutty/peanut flavour, all characteristics previously associated with LBT. Yeast strains differed in their sensory impact on wine made from IPMP-spiked juice for 5 aroma and 4 flavour attributes. **We recommend** caution in fermenting with D80 or BM45 in juices with high IPMP concentration, and suggest D21 as a desirable strain in these situations.

Acknowledgments: The authors wish to thank the Natural Sciences and Engineering Research Council of Canada, the Wine Council of Ontario and the Grape Growers of Ontario for their financial support of this research. Thank you to Melissa Drouin, Lynda Van Zuiden, Gail Higenell, Amy Blake, Kevin Ker, Dr Tomas Hudlicky, and Dr Andy Reynolds, Brock University, for technical assistance and comments. Dr George Soleas and his team from the Quality Assurance Dept. of the Liquor Control Board of Ontario are thanked for their input. Finally, we acknowledge the invaluable contribution of our sensory panel.

Full reference: manuscript in review with the *Australian Journal of Grape and Wine Research*

2008

Morbidity of *Harmonia axyridis* mediates ladybug taint in red wine

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Summary

Harmonia axyridis (Pallas) *(Coleoptera: Coccinellidae;* MALB) is a novel vineyard pest in many grape and wine regions of the world due to tainting of juice and wine from excretion or extraction of 2-isopropyl-3-methoxypyrazine (IPMP) when beetles are harvested with the grapes during processing. This complex of off-odors and flavors is known as 'ladybug taint' (LBT). Insecticidal sprays in the vineyard have been advocated to mitigate the problem, however the resulting dead beetles are often incorporated in with the grape bunches during harvesting operations. The main objective of this study was to quantify the impact of dead MALB on LBT in red wine. Duplicate wines were produced from Cabernet Sauvignon with the addition of 10 beetles/L juice, added either live or at 1, 3, 7, or 60-days post-mortem. A control wine with no added beetles was also included. Finished wines were evaluated using a trained sensory panel and descriptive analysis. The intensities of aroma and flavour attributes associated with LBT were highest in live beetle wines. Sensory data were inconclusive for the remaining wines, although they suggest that MALB did not affect wine quality after 3 days post-mortem and beyond. Concentrations of IPMP were strongly and positively correlated with 6 atypical aroma and flavour attributes (r=0.902 – 0.966), confirming the association between IPMP and LBT, and the intensity of LBT was linearly related to IPMP concentration (LBT = 3.059 + 0.048*IPMP; R^2 = 0.934). These results should help in establishing grape quality parameters and inform decisions regarding the use of insecticides in the vineyard.

Acknowledgements: The authors wish to thank the Natural Sciences and Engineering Research Council of Canada, the Wine Council of Ontario and the Grape Growers of Ontario for their financial support of this research. Thank you to Melissa Drouin, Lynda Van Zuiden, Gail Higenell, Amy Blake, Kevin Ker, Dr Tomas Hudlicky, and Dr Andy Reynolds, Brock University, for technical assistance and comments. Dr George Soleas and his team from the Quality Assurance Dept. of the Liquor Control Board of Ontario are thanked for their input. Finally, we acknowledge the invaluable contribution of our sensory panel.

Full reference: G. J. Pickering, M. Spink, Y. Kotseridis, I. D. Brindle, M. Sears and D. Inglis (2008). Morbidity of *Harmonia axyridis* mediates ladybug taint in red wine. *Journal of Food, Agriculture and Environment* (in press)

2008

The influence of *Harmonia axyridis* morbidity on 2-Isopropyl-3-methoxypyrazine in Cabernet Sauvignon wine

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Summary

Harmonia axyridis (Pallas) *(Coleoptera: Coccinellidae;* the Multicolored Asian Lady Beetle; MALB) is a pest in many viticultural regions of the world because of its ability to taint juice and wine ('ladybug taint') through excretion or extraction of 2-isopropyl-3-methoxypyrazine (IPMP) when the beetles are included with the grapes at harvest. A common intervention is the use of insecticidal sprays and the resulting dead beetles are often incorporated in with the harvested fruit. The main objective of this study, therefore, was to quantify the impact of dead MALB on IPMP concentrations in red wine. Duplicate Cabernet Sauvignon wines were produced with the addition of 10 beetles/L juice, added either live or at 1, 3, 7, or 60-days post-mortem, and a control wine with no added beetles was also included. IPMP concentration was substantially higher in live-beetle wines, and decreased to base-line levels at approximately 6.5 days post-mortem. These results should assist in decisions on viticultural interventions, such as timing of sprays, as well as the establishment of grape quality parameters.

Acknowledgements: The authors wish to thank the Natural Sciences and Engineering Research Council of Canada, the Wine Council of Ontario and the Grape Growers of Ontario for their financial support of this research. Thank you to Melissa Drouin, Lynda Van Zuiden, Gail Higenell, Amy Blake, Kevin Ker, Dr Tomas Hudlicky, and Dr Andy Reynolds, Brock University, for technical assistance and comments. Dr George Soleas and his team from the Quality Assurance Dept. of the Liquor Control Board of Ontario are thanked for their input.

Full reference: G. J. Pickering, M. Spink, Y. Kotseridis, I. D. Brindle, M. Sears and D. Inglis (2008). The influence of *Harmonia axyridis* morbidity on 2-Isopropyl-3-methoxypyrazine in Cabernet Sauvignon wine. *Vitis,* 46 (2) (in press)

2007

Determination of the critical stages of processing and tolerance limits for *Harmonia axyridis* for 'ladybug taint' in wine

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Summary

'Ladybug taint' (LBT) has been reported in some wines from North America, & is associated with 2-isopropyl-3-methoxypyrazine (IPMP), produced by *Harmonia axyridis* (the Multicolored Asian Lady Beetle - MALB) when they are incorporated into the winemaking process. It is not known when IPMP is transferred from MALB (e.g. in the vineyard onto grapes or during must processing) nor what MALB densities are required for production of LBT in the final wines. This study sought to clarify these issues through three trials. In the first, MALB were added to Riesling grapes or juice at different stages of processing (harvest, crush/destem, pressing or directly to juice), & the resultant wines were analysed chemically & by paired-comparison sensory difference tests. The presence of MALB during processing had minimal effect on the basic composition & the spectral properties of the wine. Concentrations of IPMP were < 5 $ng.l^{-1}$ for all wines except those produced after the direct addition of MALB to the juice (10.3 $ng.l^{-1}$). Sensorially, control wines (no added MALB) could be differentiated from wines made after MALB were added at crushing/destemming (at 3 beetles per kg grapes), whole bunch pressing & when added directly into the juice, but not when MALB were added & subsequently removed from a simulated harvest treatment or when added during crushing/destemming at 0.3 beetles per kg grapes. In trials 2 & 3, sensory detection thresholds for LBT were established for white & red wines produced with known densities of MALB. Estimates of 'tolerance limits' in the vineyard were then calculated using regression models, & correspond to 1530 & 1260 beetles/tonne grapes for white & red wines respectively. However, given the range of grape & wine processing options available to producers, many of which are not accounted for in this study, we recommend that a more conservative limit of 200-400 beetles/tonne grapes may be appropriate. These results should assist in directing appropriate interventions in the vineyard/winery, & provide baseline targets for reducing MALB density to avoid development of LBT.

Acknowledgements: The authors wish to thank Materials & Manufacturing Ontario, the Natural Sciences & Engineering Research Council of Canada, the Wine Council of Ontario & the Grape Growers of Ontario for their financial support of this research. We also thank the following individuals & organizations for their invaluable contributions: Lynda van Zuiden, James Lin, Mark Jen & Dr Debbie Inglis from the Cool Climate Oenology & Viticulture Institute, Brock University; Ker Crop Management Services & staff; our dedicated sensory panel; Steve Cater from the Liquor Control Board of Ontario; Glenlake Orchards & Vineyards, & our other industrial partners.

Full reference: Pickering, G. J., Ker, K & Soleas, G.J. (2007). Determination of the critical stages of processing & tolerance limits for *Harmonia axyridis* for 'ladybug taint' in wine. *Vitis*, 46 (2): 85-90.

2007

Determination of Ortho- and Retro-nasal Detection Thresholds for 2-isopropyl-3-methoxypyrazine in Wine

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Summary

2-Isopropyl-3-methoxypyrazine (IPMP) is a grape-derived component of wine flavor in some wine varieties as well as the causal compound of ladybug taint (LBT), which occurs when *Harmonia axyridis* (MALB) beetles become incorporated with the grapes during juice and wine processing. The main objective of this study was to obtain valid and robust estimates of the ortho-nasal (O-N) and retro-nasal (R-N) detection thresholds for IPMP in wines of differing styles. The ASTM E679 ascending forced choice method of limits was used to determine detection thresholds for 47 individuals in three different wines – Chardonnay, Gewürztraminer and a red wine blend of Baco Noir and Marechel Foch. The group best estimate thresholds (BETs) obtained for IPMP (ng/L) were: Chardonnay, O-N: 0.32; Gewürztraminer, O-N: 1.56, R-N: 1.15 and red wine blend, O-N: 1.03, R-N: 2.29. Significant variation in individual detection thresholds was observed. Familiarity with LBT was inversely correlated with detection thresholds for Gewürztraminer, and no difference in thresholds was observed between winemakers and non-winemakers. We conclude that the detection thresholds for IPMP in humans are extremely low and influenced significantly by evaluation mode and wine style. We recommend against the reporting of single threshold values for wine flavor compounds, and encourage the determination of consumer rejection thresholds for IPMP in wine.

Acknowledgements: The following wineries are gratefully acknowledged for supplying materials for this study: Andrés Wines, Grimsby, Ontario; Henry of Pelham Winery, St. Catharines, Ontario; Vincor International, Niagara Falls, Ontario. Lynda Van Zuiden, Brock University, is sincerely thanked for technical assistance. We are very grateful for the financial support of the Natural Science and Engineering Research Council of Canada, the Wine Council of Ontario and the Grape Growers of Ontario. Many thanks to Dr George Soleas, Quality Assurance Lab, LCBO, Ontario. Finally, thank you to all the participants who took part in the sensory assessments.

Full reference: G. J. Pickering, A. Karthik, D. Inglis, M. Sears and K. Ker. (2007). Determination of Ortho- and Retro-nasal Detection Thresholds for 2-isopropyl-3-methoxypyrazine in Wine. *Journal of Food Science*, 72(7), S468-S472.

2006

The evaluation of remedial treatments for wine affected by *Harmonia axyridis*

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Summary

This study evaluated the efficacy of a number of commercially available fining agents & other interventions on reducing 2-isopropyl-3-methoxypyrazine (IPMP) concentration & taint characteristics of white & red wine affected by *Harmonia axyridis* (MALB). The off-flavours associated with MALB are known collectively as 'ladybug taint' (LBT). The fining agents & other treatments were selected after a series of bench-tests to identify the most promising approaches. Wines fermented in the presence of MALB beetles were treated with activated charcoal, bentonite, oak chips, deodorized oak chips & either ultraviolet (red wine) or visible (white wine) light. Activated charcoal was successful at reducing IPMP concentration in white wine & deodorized oak in red wine, although this did not generally translate into lower intensity of the sensory attributes associated with LBT. Oak chips were successful in reducing the intensity of MALB-taint characteristics in both white & red wines, probably through a masking effect. Other processes investigated, including the non-traditional use of deodorised oak, UV & visible light, generally had no effect on white wine & limited effect on red wine. While research is on-going to investigate methods for preventing MALB beetles from entering the juice & winemaking process, further investigation is also required to develop appropriate technologies to remove LBT from juice & wine. A limiting factor in these approaches may be legislation restricting permissible additions to these products. A successful technology would ideally target & selectively bind IPMP, with subsequent removal of the complex from the juice or wine.

Acknowledgements: The authors wish to thank Materials & Manufacturing Ontario, the Natural Science & Engineering Research Council of Canada, the Wine Council of Ontario & the Grape Growers of Ontario for their financial support of this research. In addition, we would like to thank the following individuals & organizations for their invaluable contributions: Kevin Ker & the team from Ker Crop Management Services; our dedicated sensory panel; Lynda van Zuiden, Gail Higenell & Valerie Higenell from the Cool Climate Oenology & Viticulture Institute; Steve Cater from the Liquor Control Board of Ontario, & our various industrial partners.

Full reference: Pickering, G.J., Lin, J.Y., Reynolds, A., Soleas, G. & Riesen, R. (2006). The evaluation of remedial treatments for wine affected by *Harmonia axyridis*. *International Journal of Food Science and Technology*, 41, 77-86.

2005

The influence of *Harmonia axyridis* on wine composition and aging

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Summary

This study aimed to further characterize the effects of *Harmonia axyridis* (MALB) on white and red wine quality, including determining the influence of bottle aging on the composition and sensory attributes of MALB-affected wines and testing the hypothesis that methoxypyrazines are responsible for the characteristic sensory profiles of these wines. Vinification in the presence of MALB beetles had little effect on the basic chemical and physical attributes of white and red wine, either at bottling or after 10 months aging. 2-isopropyl-3-methoxypyrazine (IPMP) was detected at relatively high concentrations and at levels above sensory threshold in wines fermented with MALB. In addition, significant positive correlations were found between IPMP concentration in wines and sensory attributes that characterize MALB off-flavour (a.k.a. ladybug taint – LBT). These results support the conclusion that IPMP is the key aroma-active compound responsible for LBT, although they do not exclude possible contribution from other methoxypyrazine species. After aging, the aroma and flavor profiles of MALB-treated wines were similar to those of the newly bottled wines. White wines showed a trend, as beetle numbers increased, of higher intensities of peanut, bell pepper, asparagus and bitterness attributes and lower scores for floral and fruit descriptors. Red wines showed a trend of higher scores for peanut and asparagus/bell pepper aroma intensity, and lower scores for fruit attributes as the number of beetles added increased.

Acknowledgements: The authors wish to thank Materials and Manufacturing Ontario, the Natural Science and Engineering Research Council of Canada, the Wine Council of Ontario and the Grape Growers of Ontario for their financial support of this research. In addition, we would like to thank the following individuals and organizations for their invaluable contributions: Kevin Ker and the team from Ker Crop Management Services; our dedicated sensory panel; Lynda van Zuiden, Gail Higenell and Valerie Higenell from the Cool Climate Oenology and Viticulture Institute; Steve Cater from the Liquor Control Board of Ontario, and our various industrial partners.

Full reference: Pickering, G.J., Lin, J.Y., Reynolds, A., Soleas, G., Riesen, R. and I. Brindle. (2005). The influence of *Harmonia axyridis* on wine composition and aging. *Journal of Food Science* 70 (2): S128-S135.

2004

Influence of *Harmonia axyridis* on the Sensory Properties of White and Red Wine

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Summary

The possible influence of *Harmonia axyridis* (the Multicolored Asian Lady Beetle; MALB) on the sensory properties of wine was investigated. MALB beetles were added to white & red grape musts at a rate of 0, 1 or 10 per L, & a trained panel evaluated the finished wines using descriptive analysis techniques. Significant changes in wine aroma & flavor were found for both white & red musts fermented in the presence of 10 MALB beetles/L. Smaller effects were observed at a dosage rate of 1 beetle/L. A number of sensory attributes showed increased intensity in the beetle treatments; peanut, bell pepper & asparagus aromas & flavors in white wine, & peanut, asparagus/bell pepper, & earthy/herbaceous aromas & flavors in red wine. Additionally, acid, sweet & bitter tastes were affected in red wine, & a general trend of lower floral & fruit intensity scores was observed with increasing beetle rate in both wines.

Taken overall, these results indicate the potential for MALB to significantly influence wine quality. The effects were dose-dependent, & the 'external validity' of the beetle addition rates employed here (i.e. the density of MALB that might be incorporated into grape juice during commercial winemaking) requires further investigation. The relative impact of the beetles is also likely to be variety & wine-style dependent. Research is underway to fully characterize the influence of MALB on the chemical composition of wine. Associated with this, we are testing the hypothesis that methoxypyrazines are the principal odor-active compounds in MALB-affected wine. Research should now investigate the effectiveness of various remedial juice & wine treatments aimed at removing or reducing the undesirable aroma & flavor contributions of MALB. The sensory profiles developed here should serve as a useful baseline for this work.

***Acknowledgments*:** The authors wish to thank Materials and Manufacturing Ontario, the Natural Science and Engineering Research Council of Canada, the Wine Council of Ontario and the Grape Growers of Ontario for their financial support of this research. In addition, we would like to thank the following individuals and organizations for their invaluable contributions: Kevin Ker and the team from Ker Crop Management Services, our dedicated sensory panel, Gail Higenell and Lynda van Zuiden from the Cool Climate Oenology and Viticulture Institute, Steve Cater from the Liquor Control Board of Ontario, CreaScience, and our various industrial partners.

Full reference: Pickering, G.J., Lin, J.Y., Riesen, R., Reynolds, A., Brindle, I., and Soleas, G. (2004). Influence of *Harmonia axyridis* on the Sensory Properties of White and Red Wine. *Am. J. Enol. Vitic*., 55 (2), 153-159.