

Vitis 47 (4), 227–230 (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 vineyard pest in many winemaking regions of the world due to its ability to taint juice and wine ('ladybug taint') through excretion or extraction of 2-isopropyl-3-methoxypyrazine (IPMP) when the beetles are incorporated with the grapes at harvest. A common vineyard 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 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⁻¹ juice, added either live or at 1, 3, 7, or 60-days post-mortem. A control wine with no added beetles was 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 insecticidal sprays, as well as the establishment of grape quality parameters.**

Key words: *Harmonia axyridis*, Multi-colored Asian Lady Beetle, MALB, IPMP, vineyard pest, wine aroma, wine flavour.

Introduction

Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae; the Multicolored Asian Lady Beetle; MALB) can be found throughout the USA and Canada (DAY *et al.* 1994, HOEBEKE and WHEELER 1996, NALEPA *et al.* 1996), and have also been identified in France, Switzerland, England, South America and other winemaking countries (PICKERING and LIN 2006). MALB can negatively affect wine quality when present with the fermenting juice, contributing peanut, bell pepper and asparagus aromas and flavours in white wine, and peanut, asparagus/bell pepper, and earthy/herbaceous aromas and flavours in red wine (PICKERING *et al.* 2004). This collection of undesirable sensory attributes constitutes 'ladybug taint' (LBT), which is stable after bottle ageing (PICKERING *et al.* 2005) and generally resistant to common wine fining agents (PICKERING *et al.* 2006 a). Thus, treating MALB in the vineyard has become an important

focus in preventing occurrence of LBT in wine. 2-isopropyl-3-methoxypyrazine (IPMP), a component of *Coccinellidae haemolymph*, has been identified as the main causal compound of this taint (PICKERING *et al.* 2005, 2006 b). For MALB, IPMP likely serves a dual function as both an alerting signal and an aggregation pheromone (AL ABASSI *et al.* 1998). In wine, this compound has a very low human olfactory threshold, ranging from 0.32 to 2.29 ng·l⁻¹, depending on wine style and evaluation mode (PICKERING *et al.* 2007 a). LBT develops when IPMP is transferred from MALB into juice or fermenting must, while transfer from MALB onto grapes prior to or during harvest, at concentrations that elicit LBT, does not appear likely (PICKERING *et al.* 2007 b). Tolerance limits for MALB at harvest, below which development of LBT is unlikely, have been estimated as approx. 1,250 and 1,550 beetles/tonne grapes for white and red wines respectively, although a more conservative limit of 200-400 beetles/tonne grapes has been suggested (PICKERING *et al.* 2007 b).

LBT has resulted in significant economic losses for vineyards and wineries (PICKERING *et al.* 2006 b) and further investigations aimed at improving understanding of the origins of this taint and development of preventative treatments have been encouraged. A practical concern for winegrowers, faced with MALB infestation in the vineyard, is that dead beetles are often incorporated in with the harvested grapes, as they often remain resident within berry clusters post-mortem. This incorporation makes it difficult to assess the ultimate efficacy of spraying and other interventions directed at MALB in preventing formation of LBT in the final wine. Determining the influence of MALB post-mortem should assist in decisions on the timing of insecticidal sprays, and will also be of interest to icewine/eiswein producers. Grapes for these latter wine styles are picked 2-3 months after the normal table wine harvest, and where nets and machine harvesting are employed, as is the case in much of Canada, dead MALB have been noted as components of the harvested 'fruit'. The main objective of this study, therefore, was to determine the influence of dead MALB on IPMP concentration in red wine.

Material and Methods

MALB: Live MALB were sourced from the biological control laboratory at University of Guelph as unmated

adults. To euthanise the beetles, they were transferred into a glass container sealed at the top with an air lock. Carbon dioxide gas was fed into the bottom of the chamber for approximately 5 min. The container was then sealed and left for 3 h. Dead beetles were transferred into a plastic Ziploc™ container, and the top was closed with nylon to allow for air circulation to partially reflect environmental conditions within berry clusters in a vineyard. The container was stored at room temperature (approx. 21 °C) until the beetles were added to the fermentation vessels.

Wine making and treatments: Commercial Cabernet Sauvignon juice concentrate (Californian Connoisseur, Vineco International Products Ltd., St. Catharines, ON) was used to make the base juice. 'Cabernet Sauvignon' was selected for the study because it is a late-ripening variety, which are more prone to LBT as MALB tend to migrate into vineyards later in the season, and because it is a variety that is grown in most winemaking regions of the world. The concentrate was rehydrated following manufacturer's instructions, and 7.5 l aliquots of juice were separated into 10 l glass carboys in preparation for inoculation. The rehydrated juice was then inoculated with yeast strain EC1118 (Lallemand Inc., Santa Rosa, California) at 300 mg·l⁻¹. MALB were added to each carboy at a rate of 10 beetles·l⁻¹ either as live beetles or 1, 3, 7, or 60 d post-mortem. A control condition followed the same protocol without beetles. All treatments were duplicated and fermentations were conducted in a temperature-controlled chamber at a constant temperature of 18 °C. They were monitored daily for °Brix and temperature and, at the completion of primary fermentation, wines were racked, sulfited, cold-stabilised, and stored at -2 °C until required for analysis.

Chemical and statistical analysis: pH (ATI Orion model 550 pH meter, Beverly, MA, USA), titratable acidity (TA; NaOH titration to end point of 8.2), °Brix, and ethanol (ebulliometry) were determined according to ILAND *et al.* (2004). The basic physicochemical composition of the juice and wine are given in the Table.

IPMP was determined using solid-phase microextraction coupled with gas chromatography mass spectrometry (HS-SPME-GC-MS) after KOTSERIDIS *et al.* (2008). IPMP was extracted from the headspace of juice and wine samples using SPME, and adsorbed onto a 2 cm 23 gauge

DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA). After extraction, IPMP was desorbed in the injector, and then onto an HP-5 column (30 m, 0.25 mm i.d., 0.25 µm film thickness) (J&W Scientific, Folsom, CA) in an Agilent 6890GC/5973MSD. IPMP detection used selected ion monitoring (SIM). Mass channels were *m/z* = 137 and 152 for IPMP and *m/z* = 140 and 155 for [²H₃] IPMP. Ions 137 and 140 were used for quantification, while ions 152 and 155 were used as qualifier ions. All samples were analyzed in triplicate. Quantitation was achieved using an internal deuterated (OD₃) IPMP standard ([²H₃] IPMP), and is described in full in KOTSERIDIS *et al.* (2008). All chemical data were analyzed using the ANOVA procedure within XLSTAT® version 7.5.2 (Addinsoft, 40, rue Damrémont, 75018 Paris, France). If *p*(F) was < 0.05, Fishers LSD was used as the means separation test.

Results and Discussion

No differences were observed for the pH of the wine samples, and small differences were noted in TA (Table). These data are in general agreement with PICKERING *et al.* (2005, 2007 b) who reported no or small changes in basic wine composition after MALB were added to and fermented with white grape juice. The 1-d-post-mortem treatment showed a higher final ethanol concentration than other wines (*p* < 0.05), a finding we are unable to account for. The Figure shows the absolute and corrected average IPMP concentrations for the wines. Fermentation in the presence of live MALB leads to substantially higher IPMP concentration in the finished wines. When beetles are added at 60 and 7 d post-mortem, there is no change in IPMP concentration beyond that derived from the grapes. Corrected IPMP concentrations decrease by approximately 21 % between the 1 and 3 d post-mortem treatments. Using the 1, 3 and 7 d post-mortem data points (linear regression: $y = -1.237x + 23.411$; $R^2 = 0.983$), IPMP concentration ± 1 SD returns to that of the control wine (no added beetles) after 6.5 d. The increase in IPMP concentration after fermenting with live MALB (45 ng·l⁻¹) is higher than previously reported. PICKERING *et al.* (2005), also using juice concentrate and a rate of 10 beetles·l⁻¹, reported an increase of 30 ng·l⁻¹ (white wine) and 23 ng·l⁻¹ (red wine). In that

Table

Basic physico-chemical composition of 'Cabernet Sauvignon' juice and wine¹

Analyte	Juice	Stage of <i>Harmonia axyridis</i> addition to wine ²					Control (no beetles)
		Live beetles	Dead 1 d	Dead 3 d	Dead 7 d	Dead 60 d	
°Brix	19.8 ± 0.1	-	-	-	-	-	-
pH	3.45 ± 0.03	3.54 ± 0.06	3.47 ± 0.08	3.49 ± 0.02	3.51 ± 0.07	3.57 ± 0.04	3.44 ± 0.04
Titratable acidity (g·l ⁻¹)	6.47 ± 0.02	6.24 ± 0.03	6.71 ± 0.04	6.74 ± 0.06	6.64 ± 0.05	6.21 ± 0.04	6.74 ± 0.03
Ethanol (% v/v)	na	10.9 ± 0.5	11.9 ± 0.4	11.4 ± 0.2	11.2 ± 0.3	10.8 ± 0.4	11.5 ± 0.2

¹ Data represents the mean value of triplicate measurements (juice) or triplicate measurement of duplicate fermentation replicates (wine) ± standard deviation; ² beetles added at 10 beetles/L juice.

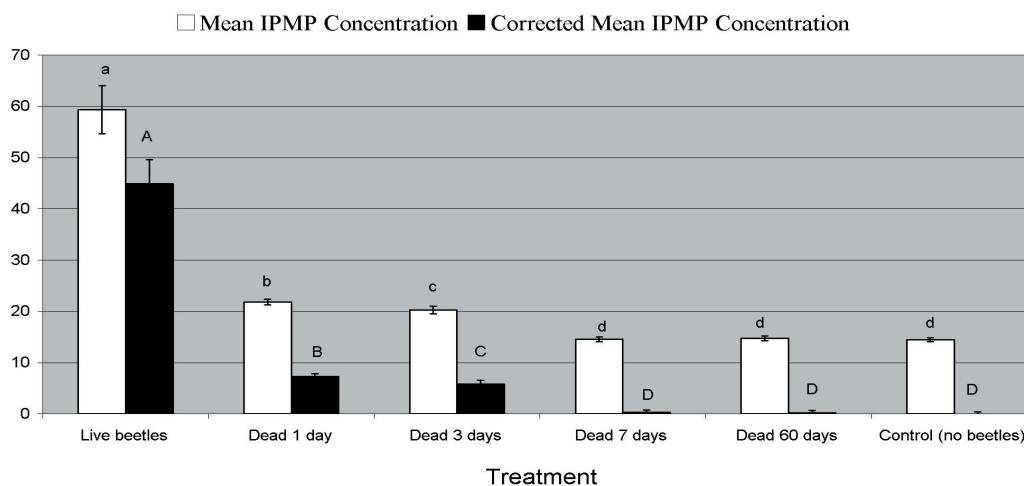


Figure: Absolute and corrected 3-Isopropyl-2-methoxy-pyrazine (IPMP) concentrations in 'Cabernet Sauvignon' wines produced with addition of 10 *Harmonia axyridis* beetles \cdot l $^{-1}$ juice. Corrected mean is total concentration minus initial IPMP juice concentration. Data represent mean values from duplicate measurements of duplicate fermentation replicates \pm SD. Treatment means identified with different letters are significantly different ($p(F) < 0.05$, followed by Fishers LSD).

study, the analytical method employed solid-phase liquid extraction and external standards, possibly leading to under-recovery of the total IPMP load. Alternatively, small differences in fermentation technique and/or length, or differences in the MALB populations sampled may account for the discrepancy. CAI *et al.* (2007) noted differences in the headspace concentration of IPMP between lightly and darkly colored beetles, possibly indicating sex-based variation.

Practical perspectives and conclusion: IPMP levels return to baseline at approximately 6.5 d post-mortem, after which time we speculate there is minimal risk of LBT developing in the subsequent juice or wine from the presence of dead beetles during processing. However, this should be corroborated with sensory data. Given that any MALB incorporated with grapes during icewine/eiswein harvest are likely to have been dead for many weeks, because of spraying, low ambient temperature or other factors, LBT seems unlikely to develop in these wine styles.

A high density of MALB was used in this study, and 'safe' post-mortem periods should be shorter where there are lower levels of vineyard/grape infestation. Additionally, exposure of dead beetles to environmental factors such as rain and wind may reduce the 'extractable' IPMP content of a beetle, and these factors are not accounted for in this experimental protocol. As various winemaking practices, including skin contact, juice clarification (KOTSERIDIS *et al.* 2008) and bottle aging (PICKERING *et al.* 2005) may affect IPMP concentration in the final wine, we recommend our results be used as a *guideline* to aid in decisions on grape quality control and vineyard interventions.

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 M. DROUIN, L. VAN ZUIDEN, G. HIGENELL, A. BLAKE, K. KER, Dr. T. HUDLICKY and Dr. A. REYNOLDS, Brock University, for technical assistance and comments. Dr. G. SOLEAS and his team from the Quality Assurance Dept. of the Liquor Control Board of Ontario are thanked for their input.

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Received October 8, 2007