Background

- **Harmonia axyridis** (Coleoptera: Coccinellidae) (**HA**) introduced into the US from Japan, the Republic of Korea, and the former USSR during the late 1970s and early 1980s as a biocontrol tool for aphids and other insect pests

- Now widespread throughout much of these regions and has also been recorded in some western areas of the United States and Canada

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Background

- HA have a reflex bleeding response of haemolymph when stressed
- Methoxypyrazines (low odor threshold – green, vegetal, bell pepper aroma) have been identified in HA
- Plausible that HA capable of influencing wine quality via transfer of haemolymph onto grapes, or directly into juice

Study 1: Impact of HA on the Sensory Properties of White and Red Wine

Acknowledgements

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Harmonia axyridis and Wine Quality

Source: (www.ncpest.com/ladybeetlefrom_usda.htm)

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Research objectives/questions

1. Quantify the impact of HA on wine quality
   - Sensory and chemical

2. How does this impact change with time?
   - Bottle ageing

Experimental Design

- White and red wines fermented in the presence of different levels of *Harmonia axyridis*
- Std microvinification procedures (from concentrate)
- Treatments:
  - No beetles (16L x 4, & 10L)
  - 1 beetle/L (16L x 3)
  - 10 beetles/L (16L x 3)
- Chemical analysis - methoxypyrazines (Soleas et al., 2003); other measurements — Iland (1988)
- Sensory - descriptive analysis (Lawless & Heymann, 1988)

Mean intensity scores for white wine aroma and FLAVOR attributes for control wine

Mean intensity scores for white wine aroma and FLAVOR attributes for control wine and 1 level of HA addition to juice

Mean intensity scores for white wine aroma and FLAVOR attributes for control wine and 2 levels of HA addition to juice

Mean intensity scores for red wine aroma and FLAVOR attributes for control wine
Chemical results

- No or minimal effect of beetles in white or red wines on:
  - rate of fermentation
  - pH
  - titratable acidity
  - ethanol
  - residual sugar
  - free and bound terpenes
  - hue, hue density, browning, packing, phenolic conc. (spectro)

- Small reduction in volatile acidity for both whites & reds with beetles at any level!

But are methoxypyrazines responsible for the HA character?

Concentration of IP and IB methoxypyrazines

<table>
<thead>
<tr>
<th>Wine</th>
<th>Isopropyl MP (ppt)</th>
<th>Isobutyl MP (ppt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White, No Beetle</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>White, 1 Beetle/L</td>
<td>26</td>
<td>&lt;5</td>
</tr>
<tr>
<td>White, 10 Beetle/L</td>
<td>157</td>
<td>6*</td>
</tr>
<tr>
<td>Red, No Beetle</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Red, 1 Beetle/L</td>
<td>7*</td>
<td>5*</td>
</tr>
<tr>
<td>Red, 10 Beetle/L</td>
<td>23</td>
<td>15*</td>
</tr>
</tbody>
</table>

(All measurements, GC-MS, LCBO)
Okay, but what happens with bottle age?

Mean intensity scores for white wine aroma and FLAVOR at bottling and after 8 months (10 HA beetle/L juice treatment)

Mean intensity scores for red wine aroma and FLAVOR at bottling and after 8 months (10 HA beetle/L juice treatment)
Chemical changes after 8 months

- Isopropyl MP lower in all beetle treatments for both white and red
  - For 10 beetle/L treatments:
    - 90% lower in white
    - 25% lower in red
  - For 1 beetle/L treatments:
    - ND (analytically)
- Isobutyl MP ND
- Little change with other parameters (pH, titratable acidity, ethanol, residual sugar, spectro. measures)

Study 2: Evaluation of Potential Remedial Treatments

Bench-tested range potential remedial treats, incl:

- Fining agents:
  - Bentonite: various levels
  - Issinglass
  - Bentonite + Issinglass
  - Activated charcoal: various periods
- Oak chips - various brands & levels + de-odorized chips
- Light sources:
  - UV: 254nm, 18.4W for different periods
  - Broad spectrum: halogen bulb, 120W for different periods
- (Irradiation: 90, 110, and 180 Gyr)

Settled on:

- Fining agents:
  - Bentonite: 1g/L, 3 days
  - Activated charcoal: 0.2g/L, 3 days
- Oak chips:
  - 'Normal': French medium toast chips @ 4g/L for 3 days
  - De-odorized: Per above but chips treated: 40% ethanol overnight, washed & boiling in water for 10 mins.
- Light:
  - UV: only for red wines, 254nm, 18.4W, for 5 min.
  - Broad spectrum: white wines only, halogen bulb 120W, 5 min.
- (Irradiation - 90 and 110 Gyr)

Experimental Design

- These wines treated and analyzed in duplicate as before:
  - Chemical analysis
    - Methoxypyrazines (Soleas et al., 2003)
    - Other measurements – Deal (1988)
  - Sensory
    - Descriptive analysis (Lawless & Heymann, 1988)

Results - summary

- Chemical: No to minimal change in isopropyl MP conc. for all treatments except….
  - Activated charcoal - 34% decrease in white wine
  - Activated charcoal - 11% decrease in red wine
- Sensory:
  - White wine:
    - 'Normal' oak chips significantly reduced HA-associated attributes (average 17% for aroma & 53% for flavor [masking])
    - Other treatments: less successful
  - Red wine:
    - 'Normal' oak chips significantly improved red wine aroma, but not with the other treatments used.
    - Decrease in both asparagus and earthy/herbaceous flavors in all treatments except for UV & irradiation.
    - 'Normal' oak chips (134%) & bentonite (133%) most effective across all HA-associated flavor attributes.
Study 3 — HA-related wine taint:
Identification of critical stages during wine processing & determination of sensory thresholds
(2003 vintage)

(With support from Wine Council of Ontario and Grape Growers of Ontario)

Experimental Plan

1. 690 kg of Riesling sourced from commercial vineyard believed to be free from HA & with a history of low-no occurrences of HA
2. Fruit carefully hand-sorted and any beetles (very few) carefully removed. Following treatments instituted using CCOVI’s teaching & research winery:
   i. Control (no beetles)
      HA beetles introduced at different stages:
   ii. Whole fruit – agitation treatment to simulate mechanical harvester (@ 3 beetles/kg fruit)
   iii. Crush/de-stemmer (@ 3 beetles/kg fruit)
   iv. Crush/de-stemmer (@ 0.3 beetles/kg fruit)
   v. Whole bunch press (@ 3 beetles/kg fruit)
   vi. Directly into wine (per 2001 study) (@ 3 beetles/kg fruit)

Analyses

- Methoxypyrazines (Soleas et al., 2003) (and other wine analyte – Hoad, 1988) measured at each stage of treatment & processing
  → an objective estimate of the influence of HA throughout the winemaking process
  → better inform preventative measures
- Sensory (triangle) tests to determine which treatments (if any) different from Control
- Sensory threshold tests conducted (ascending limits, forced-choice, paired comparison - Lawless and Heymann, 1998)
  - Data then related back to tolerance level for fruit (i.e. # of beetles/weight of grapes req’d to contribute detectable character)

Results - summary

Critical Stage of Processing ……

- Isopropyl methoxypyrazine < 5 ppt (LOQ) in all treatments except direct addition of beetles into wine (10 ppt)
  - In triangle test, only the direct addition of beetles into wine treatment was different from Control (p=0.001)
- Ethanol, TA, RS, spectrophotometric, and other quality parameters showed little or no change compared with Control

Sensory Threshold ……

- Using the established “50% above chance” criteria (i.e. 75% correct response), threshold is at 58.6% of the 3 beetles/kg grapes blend
  - Translates to 1758 beetles/kg grapes
  - Which equals 1758 beetles/ton of grapes
Results - summary
Sensory Threshold ……

Caution
➢ Assumptions: beetle behavior at the micro-processing level is the same as at commercial level
➢ Extrapolating to other wine styles (particularly reds with skin/beetle maceration) and alternative processing regimes
➢ Detection ≠ consumer rejection

Overall Summary & Conclusions
● Fermenting in the presence of *H. axyridis* had minimal impact on basic wine composition
● ↑ in IPMP conc. with ↑ # of beetles (greater in white wine)
● Sensory profile modified:
  – Beetle-derived aroma and flavor attributes
  – Decrease in ‘varietal’ attributes
  – Profile can now be used as tool for measuring consumer acceptance, effectiveness of remedial treatments, etc
● IPMP strongly implicated in taint, BUT data suggest relationship between conc. & sensory impact not simple
  – may not be the only culprit (isobutyl and sec-butyl MP have been detected in HA – [Brindle, unpublished data])
  – trained nose still the best instrument

Further work
● Stable-isotope-dilution method to quantify MP at lower levels than currently possible
● Other remedial juice/wine options, including ‘designer’ molecules to selectively bind MP
● Development & evaluation of exclusion practices in the vineyard and winery
  – e.g. sprays, deterrents, decoys, shaking sorting trays
● Determine threshold/tolerance levels in other wine styles, particularly red wines
  – how to estimate HA numbers in a bin of grapes
● Determine tolerance level of consumers for HA

Further information
➢ CCOVI, Brock University web site:
  ➢ Pickering lab MALB research:
    www.brocku.ca/ccovi/res/result6.html
  ➢ General MALB questions & answers: