The Influence of *Harmonia axyridis* on Wine Composition and Aging

GARY J. PICKERING, YONG LIN, ANDREW REYNOLDS, GEORGE SOLEAS, ROLAND RIESEN, AND IAN BRINDLE

ABSTRACT: This study sought to further characterize the effects of *Harmonia axyridis* (HA) on white and red wine quality, including determining the influence of bottle aging on the composition and sensory attributes of HA-affected wines and examining the hypothesis that methoxyypyrazines are responsible for the characteristic sensory profiles of these wines. Vinification in the presence of HA beetles had little effect on basic physical and chemical attributes of white and red wine, either at bottling or after 10-mo of aging. 2-Isopropyl-3-methoxyppyrazine (IPMP) was detected at relatively high concentrations and at levels above sensory threshold in wines fermented in the presence of HA. In addition, significant positive correlations were found between IPMP concentration in wines and sensory attributes that characterize HA “taint.” After aging, the aroma and flavor profiles of HA-treated wines were similar to those of 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 fruit and floral 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 increased.

Keywords: Multicolored Asian Lady Beetle, ladybug, wine chemistry, 2-Isopropyl-3-methoxyppyrazine, taint

**Introduction**

*Harmonia axyridis* (HA) (Coleoptera: Coccinellidae) (Multicolored Asian Lady Beetle) is an introduced species to North America and has been used as a biocontrol agent for aphids and other insect pests since the late 1970s (Chapin and Brou 1991; Nalepa and others 1996). Its distribution now includes the northeastern United States, eastern Canada, and parts of the western United States (Hoebeke and Wheeler 1996; Nalepa and others 1996).

Pickering and others (2004b) noted anecdotes from the North American winemaking community and news media of an association between atypical aroma and flavor in some wines and the observation of high numbers of HA beetles in source vineyards during harvest. Coccinellids possess a reflex bleeding response of hemolymph when stressed (Abassi and others 1998; Laurent and others 2001; Koch 2003), and hemolymph contains volatile compounds of known olfactory significance to humans (Rothschild and Moore 1987). Specifically, 2-isopropyl-3-methoxyppyrazine (IPMP) has been identified in the hemolymph of *Coccinella septempunctata* (Al Abassi and others 1998). The human olfactory threshold for methoxyppyrazines is extremely low, in the order of 2 ng/L in water (Buttery and others 1996; Seifert and others 1970) and has been reported to be lower in white compared with red wine (Allen and Lacey 1998; Boubee and others 2000; Sala and others 2002). It is possible that HA are capable of influencing wine quality via transfer of hemolymph onto grapes or directly into juice or must if the beetles become incorporated into the harvested fruit.

Pickering and others (2004b) showed that the inclusion of HA beetles during the fermentation of white and red musts significantly modifies wine aroma and flavor. Higher sensory intensity scores were observed for peanut, bell pepper, and asparagus aromas and flavors in white wines, and peanut, asparagus/bell pepper, and earthy/herbaceous aromas and flavors in red wines. Many of these descriptors are consistent with those that have been previously ascribed to methoxyppyrazines, which include green, earthy, herbaceous, vegetative, and bell pepper (Allen and others 1994, 1998; Boubee and others 2000; Buchbauer and others 2000; Sala and others 2002). Pickering and others (2004b) also reported that sweet, acid, and bitter tastes were affected in red wines, and a general trend of decreasing fruit and floral intensities as the number of beetles added increased was observed for both white and red wines.

There are a number of important questions that have not been addressed in the literature pertaining to this new wine taint. These include identification of the causal compound(s) in affected wine, determining whether the taint characteristics change with bottle aging and evaluation of the efficacy of potential remedial treatments for affected juice and wine. The objectives of this current study were to as follows: (1) to determine the influence of HA on the chemical composition and fermentation properties of affected wine; (2) to examine the hypothesis that methoxyppyrazine(s) are responsible for the characteristic aroma and flavor of affected wines; and (3) to describe the effects of bottle aging on the sensory characteristics of affected wines.

**Materials and Methods**

**Chemical composition and fermentation properties.**

\textbf{Wine preparation.} Wines were prepared as described in Pickering and others (2004b). Two commercial juice concentrates from South American grapes were used: White Bourgeron™ and Red Bergamais™ (both Vinco Int., St Catharines, Ont., Canada). The concentrates were rehydrated according to manufacturers’ directions. Basic composition of the rehydrated juices was as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
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</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>10 g/L</td>
</tr>
<tr>
<td>Sugar</td>
<td>20 g/L</td>
</tr>
<tr>
<td>pH</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**Wine fermentation.** Wines were fermented as described in Pickering and others (2004b). In brief, 10 g/L of HA beetles were added to the juice concentrates before inoculation with *Saccharomyces cerevisiae* and fermentation was performed under anaerobic conditions at 28°C.
White Bourgeron: °Brix, 21.9; pH, 3.14; and titratable acidity (TA), 5.9 g/L.

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

Beetles were sourced from the local area and screened for identity. Identification of HA was based on the morphological criteria detailed by Chapin and Brou (1991), and in particular on the presence of the characteristic dark M-shaped mark on the pronotum, extending to the anterior margin (Chapin and Brou 1991; Oi and Foshee 2002). Live HA beetles were then added to rehydrated juice in 20-L glass carboys at rates of 0 (control), 1, or 10 beetles per liter of juice. Three 20-L replicates of each of the beetle treatments and 4 20-L replicates of the control juice (no beetle) were thus prepared and processed separately. Juices were then inoculated with a rehydrated freeze-dried preparation of Saccharomyces bayanus (EC-1118®, Lallemand Inc., Montreal, Canada) at 5 to 6 x 10^6 cells/mL, according to manufacturer’s directions.

Fermentations were conducted at 18 °C and fermented to dryness. They were then racked (including removal of beetles), sulfited, cold stabilized, and bottled without filtration following standard microvinification protocol (Pickering and others 1999). Wines were stored in a temperature- and humidity-controlled wine cellar at 14 °C until required.

**Chemical and spectrophotometric analysis**

The following measurements were made on both white and red wines at bottling and after 10 or 11 mo of aging: pH, TA, and volatile acidity were assessed following the methods of Zoecklein and others (1995). Ethanol and acetic acid were measured using gas chromatography (GC) after Nurgel and others (2004). Free and total SO2 were assessed using the aspiration method described by Iland and others (2002). Free and total acidity were assessed following the methods of Zoecklein and others (1995). Live HA beetles were added to rehydrated juice in 20-L glass carboys at rates of 0 (control), 1, or 10 beetles per liter of juice. Three 20-L replicates of each of the beetle treatments and 4 20-L replicates of the control juice (no beetle) were thus prepared and processed separately. Juices were then inoculated with a rehydrated freeze-dried preparation of Saccharomyces bayanus (EC-1118®, Lallemand Inc., Montreal, Canada) at 5 to 6 x 10^6 cells/mL, according to manufacturer’s directions.

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**Sample preparation.** Solid-phase extraction was performed by 1st washing the caridges (3 mL containing 100 mg of endcapped C18 bonded porous silica, Diagnostix, Oakville, Ont., Canada) with 0.5 mL of ethyl acetate followed by 0.5 mL of 95% ethanol and finally with 0.5 mL of 10% v/v ethanol; 25 mL of the wine sample was passed through the cartridge, which was then dried for 10 min using a vacuum pump at 20-mm mercury vacuum. The absorbed pyrazines were eluted by adding 0.4 mL of dichloromethane and collecting the eluent in a calibrated vial and making up to 0.5 mL with ethyl acetate. This represented a 50-fold dilution of the original sample.

**GC-MSD (Mass Selective Detector) procedure.** An Agilent 5990 GC coupled to an Agilent 5973 MSD (Agilent, Mississauga, Ont., Canada) was fitted with a DB-5 MS column (30 m long x 0.25-mm inner dia, 0.25-mm thickness; J&W Scientific, Folsom, Calif., U.S.A.). A helium carrier gas flow of 1 mL/min was used. The temperature program was as follows: initial temperature, 70 °C, held for 0.5 min; 10 °C/min to 230 °C, held for 0.5 min; 30 °C/min to 250 °C, held for 2 min. Injector and detector temperatures were 200 °C and 230 °C, respectively. Injection of 2 mL of sample was in splitless mode. The MSD was set in selective ion monitoring mode, and each compound was quantitated based on the response of peak area using different ions: m/e (mass-to-charge ration) 124, 137, and 152 for isopropylpyrazine and m/e 124 and 152 for isobutylpyrazine. Concentration of the compounds was determined by external standard using a 3-point calibration curve. Calibration standards were prepared by dilution of stock standard solution in a pyrazine-free wine extract. The calibration standards were compared against a separate standard made up in ethyl acetate. Fresh standards were prepared for each analysis run.

The following measurements were made on both white and red wines after 10 mo of aging: Spectrophotometric analysis of color and phenolic content was performed after Iland and others (1988). Free volatile terpenes (FVT) and potentially volatile terpenes (PVT) were measured using the method of Dimitriadis and Williams (1984) as modified by Reynolds and Wardle (1989).

**Sensory evaluation**

After 10 mo of bottle age, wines produced from the treatments described previously were assessed using descriptive analysis. This descriptive panel also assessed samples that had been subjected to potential remedial treatments; data from these wines will be presented elsewhere.

**Panel recruitment and training**

The panel was recruited from Brock Univ. staff and student. A questionnaire was used to screen prospective panelists for anosmias or other conditions that might limit their suitability. Further selection was based on their interest and availability. The final panel consisted of 7 females and 1 male aged between 21 and 53 y.

Seven training sessions of 1-h duration each were held over 4 wk. A minimum of information on the nature of the study was provided to reduce potential bias. Samples were always presented blind, in coded International Standard Organization (ISO) wine glasses, and were expectorated. In the 1st and 2nd sessions, the panel was introduced to all white and red wine samples. The reference standards and descriptive terms used by Pickering and others (2004b) (that is, the standards/terms that were used to define these wines at bottling) were provided to the panel to assist in the development of an appropriate lexicon. Panelists were also encouraged to generate new, appropriate descriptors for the aroma and flavor of each wine. The panel leader facilitated the process of discussing all terms and looking for overlap and redundancy among the descriptors. New terms were added to the reference lists when panel consensus permitted.

In subsequent training sessions, new reference standards were developed as required, refined for each of the terms, and evaluated for suitability by reference to specific wine samples from the study. Line scales, 15 cm in length, were developed for each descriptor, with the scale ends indented 1 cm to avoid endpoint effects (Lawless and Heymann 1998). The left end of each scale was anchored with the phrase “absent” at the 1-cm indent mark, and the right end with “very high” at the corresponding 1-cm indent mark. The panel gained experience with rating the intensities of all wines for each of the descriptors. By panel consensus, the intensity of each of the reference standards was deemed to correspond to the “very high” anchor of respective line scales. The final training session consisted of an orientation to the computer program and sensory laboratory that would be used for collecting data, and as a “practice run” under experimental conditions.

Table 1 and 2 give the final lexicon of descriptive terms along with reference standard composition for the white and red wines, respectively. The panel did not note any differences between samples for appearance attributes (hue, density, and clarity), so these attributes were not included in the final lexicons. All terms used to profile the wines at bottling (Pickering and others 2004b) were also deemed appropriate by panel consensus for inclusion in this study, and the reference standard composition for these descriptors was identical to that used in the prior study.

**Data collection**

Formal assessment of the wines took place over 6 sessions. The evaluations were conducted in individual white booths with red
Harmonia axyridis and wine composition.

| Table 1—White wine aroma and flavor descriptors with corresponding reference standards |
|------------------|-------------------------------------------------|
| Descriptor       | Reference compositiona                           |
| Melon            | 2 tsp fresh honeydew melon juice                |
| Citrus           | 1 tsp fresh grapefruit juice + 1/2 tsp fresh lime juice |
| Floral           | 5 drops of mixture of: 10 mL ‘Green/herbaceous’ (nr 8947) + 10 mL ‘Geranium leaf’ (nr 9077) (both Wine Awakenings Inc®) + 10 mL linalool (Sigma Aldrich) in 20 mL distilled water |
| Asparagus        | 1 tsp canned asparagus juice (Equality™)       |
| Bell pepper      | 10 mm square of fresh bell pepper heated with naked flame for 20 s soaked in base wine for 20 min |
| Peanut           | 8 whole raw white peanuts crushed and soaked in 60 mL base wine for 30 min |
| Humus            | 50 g dried plant material (primarily bark) sourced from 2 cm below soil surface. Presented in plastic container without base wine |
| SO2              | 700 mg/L aqueous solution of potassium metabisulfite (Fisher Scientific) without base wine |
| Diesel           | 120 mg (148 mL) Isomyl and 300 mg (375 mL) isobutyl alcohol in 300 mL base wine |
| Oak              | 0.3 L French oak chips (Winemaster™, Vin Bon-Brew, St. Catharines, Ont., Canada) in base wine |
| Sweet            | 12.5 g/L sucrose in aqueous solution            |
| Acid             | 1.5 g/L tartaric acid in aqueous solution       |
| Bitter           | 12 mg/L quinine sulfate in aqueous solution     |

aData all standards made up 1 to 2 h before tasting in control white wine, unless otherwise indicated. All standards presented as 30 mL samples in ISO wine glasses unless otherwise indicated. Standards represent the “very high” anchor term at the far right end of the respective line scales (15 cm).

| Table 2—Red wine aroma and flavor descriptors with corresponding reference standards |
|------------------|-------------------------------------------------|
| Descriptor       | Reference compositiona                           |
| Red berry        | 2 to 3 fresh whole blackberries heated in microwave oven for 20 s + 1/3 tsp strawberry jam |
| Cherry           | 10 mL cherry cocktail (DelMonte Quality™) + 1/2 tsp canned cherry juice (E.D. Smith™) |
| Plum             | 2 tsp plum jam (S&F™)                            |
| Asparagus/bell pepper | 1/2 tsp of canned asparagus juice (Equality™) + 1.5 x 10 mm strip of fresh bell pepper heated with naked flame for 20 s |
| Cheesy           | 1g ripe Château Versailles™ brie cheese          |
| Peanut           | 8 whole raw white peanuts crushed and soaked in 60 mL base wine for 20 min |
| Earthy/Herbaceous| 50 g dried plant material (primarily bark) sourced from 2 cm below soil surface. Presented in plastic container without base wine |
| Vanilla          | One drop vanilla extract (Chardonnay kit, Wine Awakening Inc.™) in 60 mL base wine |
| Diacetyl         | 0.1g/L Diacetyl (Sigma®) in base wine            |
| Oak              | 0.3 L French oak chips (Winemaster™, Vin Bon-Brew, St. Catharines, Ontario) in mL base wine per 60 mL + 0.5 mL ‘Art toast (smoke) flavor, nr 8038-9138’, Wine Awakening Inc.™ |
| SO2              | 700 mg/L aqueous solution of potassium metabisulfite (Fisher Scientific) without base wine |
| Sweet            | 12.5 g/L sucrose in aqueous solution             |
| Acid             | 1.5 g/L tartaric acid in aqueous solution        |
| Bitter           | 12 mg/L quinine sulfate in aqueous solution      |

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Data analysis

Data for white and red wines were examined separately. Wine sensory attribute by treatment scores were assessed using ANOVA, with judge and session fitted as random effects, and all 2-way interactions included in the model. If the probability of the treatment F value was <0.05, the Bonferroni test was applied to separate means using the General Linear Model (GLM) procedure of SPSS (v. 11.0; SPSS Inc., Chicago, Ill., U.S.A.). Chemical and spectrophotometric data were also analyzed using GLM, with Fisher’s Protected LSD0.05 (least significant difference) as the means separation test.

Results and Discussion

Fermentation observations

All wines completed fermentation within 24 h of each other. Daily Brix and hydrometer readings indicated no differences between treatments with respect to initiation or rate of fermentation (data not shown). No visual differences were observed during fermentation, except for the presence of intact beetles on the wine surface in the corresponding treatments. Toward the end of fermentation, an atypical aroma was noted in the beetle treatments, which has been fully characterized in the finished wines by Pickering and others (2004b).
Basic chemical composition and spectrophotometric analysis

The basic chemical composition of the wines at bottling were not affected by the presence of *Harmonia axyridis* beetles in the fermenting musts (ANOVA, p [F] > 0.05; data not shown). The range of mean values across all treatments for white and red wines, respectively, were as follows: pH, 3.27 to 3.28 and 3.34 to 3.36; TA (g/L), 6.65 to 6.79 and 7.52 to 7.66; ethanol (% v/v), 12.4 to 12.7 and 12.3 to 12.7; residual sugar (g/L), 4.4% to 6.6% and 4.1% to 6.7%; total phenolics [A 280] (a.u.), 47.87a to 63.90a and 57.47a to 59.17a; flavan-3-ol content [% of total phenolics], 12.4 to 12.9 and 12.3 to 12.7; hue [A 420/A 520], 0.83a to 0.94a and 0.84a to 0.95a; total red pigments (a.u.), 24.19a to 32.60a and 22.14a to 28.19a; SO2 resistant pigments (a.u.), 2.44a to 2.60a and 1.00a to 1.50a; and 2-isopropyl-3-methoxypyrazine (2-IPMP) concentration at bottling, treatment means with different letters are significantly different (LSD after significant F-value from ANOVA, a = 0.05).

Differences were observed between white wine treatments after 10 mo of aging for A 280, A 320, and A 350 (Table 3). Absorbance at these wavelengths provides an estimate of hydroxycinnamate concentration (Somers and Vérette 1988), browning (Zoecklein and others 1990), and pinking (Zoecklein and others 1990), respectively.

Interestingly, the 1 beetle/L treatment shows higher absorbances for all 3 values compared with the control and 10 beetles/L wines. Although extraction of unknown *HA*-derived compounds absorbing at these wavelengths would appear a possible explanation, it does not explain the lower values observed in the 10 beetles/L wines. Visualy, there was no difference observed between these wines for appearance (hue, density, or clarity) at bottling (Pickering and others 2004b) or after 10 mo of aging.

Among the various color and phenolic measurements performed on the red wines after 10 mo of aging, only the estimate of total phenolic concentration differs, with the control wines showing slightly higher values than the beetle treatments (Table 3). Specifically, A 280 values were 3.6% higher than the 1 beetle/L treatment and 4.4% higher than the 10 beetles/L treatment. This may have been due to absorbance of phenolic material directly onto the beetle cells (which were subsequently removed during racking) or complexing of wine phenolics with beetle-derived compounds (such as protein) and subsequent precipitation during or after fermentation. Supporting the spectrophotometric assessment of color presented here, no differences between treatments were noted by sensory panels for any appearance attributes either at bottling (Pickering and others 2004b) or after 10 mo of aging.

Terpenes

Terpenes are important aromatic components in many wines and can be influenced by a range of viticultural and enological factors. In addition, and of particular interest, HA has been reported to release mono- and sesqui-terpenes (Aldrich and Riddick 2002), where they may act as pheromones. However, the introduction of HA did not influence the concentrations of either free volatile or potentially free volatile terpenes in these wines (ANOVA, p [F] > 0.05; data not shown). The range of mean values across treatments for white and red wines, respectively, were as follows: FVT (mg/L), 0.92 to 1.27 and 1.27 to 1.64; PVT (mg/L), 1.05 to 1.23 and 1.46 to 1.94.

Methoxypyrazine concentration

The limit of quantitation for IPMP and IBMP was approximately 4 to 5 ng/L. When both replicate injections gave values in or above this range, the mean value is reported here (Table 4). IPMP concentration at bottling showed a pattern of increase with number of beetles added to the juice. The 10 beetles/L treatments had a higher concentration of IPMP than either the 0 or 1 beetle/L treatments.
1 beetle/L treatments for both white and red wines (LSD). In addition, a trend of higher IPMP concentration in white wines is suggested. For the beetle treatments, this may suggest binding of IPMPs to polyphenolic material in the red wines, and subsequent precipitation during vinification. After 11 mo of bottle aging, the concentration of IPMP appears to decrease in all treatments. T tests were performed on the 10 beetles/L wines; the suggested change in concentration was significant (t < 0.05) for red but not white wines. The latter result may be related to the high standard deviation of the bottling data. The mechanism underlying the general reduction in concentration with aging is not clear. The presence of a quantifiable—albeit low—concentration of IPMP in both control wines is curious. The juice concentrates used to produce these wines were purchased as proprietary blends and may have included grape varieties known to contain IPMP such as Sauvignon Blanc and Cabernet Sauvignon (Allen and Lacey 1998).

In white wines, IBMP at bottling was detected at low levels and only in the 10 beetles/L treatment. In the red wines, low concentrations were also found in the 1 beetle/L and 10 beetles/L treatments. At levels close to the limit of quantitation, such as here, some caution should be exercised in interpreting the results. However, taken overall, the data suggest that IBMP is also produced by HA and is retained in the wine through to bottling. IBMP was not detected in wines from any treatment after 11 mo of bottle aging. As the concentrations of IBMP reported here are close to the human detection threshold, and that threshold is higher in wine for IBMP than for IPMP (Allen and Lacey 1998), it is likely that IBMP plays at best a nominal role in the characteristic HA-related wine taint.

IPMP and taint characteristics

In white wines, IPMP concentrations were linearly correlated with bell pepper aroma, asparagus aroma, humus aroma, and humus flavor (Figure 1). Similarly, in red wines, IPMP concentrations were linearly correlated with earthy/herbaceous aroma, asparagus/ bell pepper aroma, peanut aroma, and cheesy flavor (Figure 2). These are among the sensory attributes that best characterize “HA taint” (Pickering and others 2004b) and are generally consistent with descriptors that have previously been associated with IPMP in wine (Allen and others 1994, 1998; Boubee and others 2000; Buchbauer and others 2000; Sala and others 2002). Taken overall, these results implicate IPMP as a key aroma-active compound responsible for the distinctive characteristics of “HA-affected wines.” Corroborating this, Pickering and others (2004a) showed very similar sensory profiles between white and red wines produced from HA-affected juices and control wines spiked with IPMP.

Sensory properties after aging

There were no significant differences between the control (0 beetles/L) and 1 beetle/L aged white wines for any attribute (Figure 3). This contrasts with the data from the newly bottled wine where increased peanut aroma and flavor was found in the 1 beetle/L wine compared with control (Pickering and others 2004b). There was, however, a general trend of higher intensities in the 1 beetle/L wine of some beetle-derived aroma attributes, specifically peanut, bell pepper, and asparagus, and decreased intensity of fruity and floral aroma and flavor attributes. The most marked differences in the profiles were seen between the 10 beetles/L and control wines. For aroma, citrus, floral, diesel, and oak intensities were lower in the high beetle wines, whereas peanut, bell pepper, and asparagus aroma intensity were higher. Flavor intensities for peanut, bell pepper, and asparagus were also higher in the 10 beetles/L wines compared with control wines, and melon flavor was lower.

Figure 1—Sensory intensity scores and isopropyl-methoxypyrazine concentration for 12 experimental white wines [only sensory attributes with a significant [ANOVA, P < 0.05] linear correlation with 2-isopropyl-3-methoxypyrazine (IPMP) are shown. IPMP values under the limit of quantitation [4 to 5 ng/L] are considered to be zero for these models].

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URLs and E-mail addresses are active links at www.ift.org
In aged red wines at 1 beetle/L, only the intensity of plum flavor was affected (decreased) when compared with the control wine ($P = 0.019$) (Figure 4). At bottling, the only attribute that was different was bitterness, which was higher in the 1 beetle/L red wines (Pickering and others 2004b). At a rate of 10 beetles/L, however, a number of attributes were impacted compared with the control wines, particularly for aroma. Red berry, cherry and diacetyl aroma and plum aroma and flavor intensities were decreased, whereas peanut and asparagus/bell pepper aroma intensities were greatly increased in the 10 beetles/L wines.

All terms used to profile the wines at bottling (Pickering and others 2004b) were also included in this study, and reference standard composition for these descriptors was identical to that used in the prior study; this allows for a direct comparison of the data with those obtained at bottling (data not shown). A 2-tailed t-test was used to assess whether there were differences in the intensity scores of the wines after aging. Only 2 attributes were different; in white wines, floral aroma in the control wine and melon aroma in the 1 beetle/L wine had higher intensity scores after aging ($P = 0.015$ and $0.040$, respectively). This indicates reasonable stability in the sensory profiles of the respective wines after 10 mo of bottle aging.

However, the data suggested some general trends with respect to many of the attributes associated with HA taint (for example, peanut, asparagus, and bitterness). At 1 beetle/L, white and red wines showed no or relatively little change in these attributes. However, these taint descriptors tended toward higher intensity scores after aging for the high beetle treatment, particularly in the white wines. This may be attributable to small differences between the methodologies and particularly the panels used in the 2 studies. Interestingly, the decrease in IPMP and IBMP content of the wines during aging is not reflected in lower intensity scores for the sensory attributes typically associated with methoxypyrazines.

**Conclusions**

Vinification in the presence of HA had little effect on the basic physical, chemical, and spectrophotometric measures of white and red wines. 2-isopropyl-3-methoxypyrazine was detected at relatively high concentrations and at levels above sensory threshold in these wines. This result, in addition to the significant positive correlations between IPMP concentration and specific aroma and flavor attributes, support the conclusion that IPMP is the key aroma-active compound responsible for the distinctive sensory characteristics of HA-affected wines. This does not exclude possible contribution from other methoxypyrazine species. Isobutylmethoxypyrazine was detected at low amounts in most beetle treatments but was not detected after aging.

After 10 mo of bottle aging, the aroma and flavor profiles of HA-treated wines were similar to those of newly bottled wines. White wines showed a trend as beetle numbers increased of higher intensities of peanut, bell pepper, and asparagus aroma and flavor attributes and lower scores for fruit and floral descriptors. Red wines showed a trend of higher scores for peanut and asparagus/bell pepper aroma intensity and lower scores for fruit attributes with increasing number of beetles, particularly at the 10 beetles/L level.

This study verifies the effect of HA on wine quality. Research is now required to investigate and evaluate remedial treatments aimed at reducing or eliminating the impact of these beetles. Such remediation of juice or wine would ideally target and exploit the specific chemistries of IPMP. Excluding or removing HA from the vineyard before harvest would be desirable, and work has commenced to investigate appropriate cultural strategies toward this end.

Quantification of trace compounds in the low ng/L range, such as MPs in this study, is fraught with technical challenge. Nonetheless,
the reliable measurement of MPs, and particularly IPMP, down to at least the human sensory threshold is critical to fully characterize the role of MPs in HA-related juice and wine taint and in implementing and evaluating prevention/remedial strategies. Further work is therefore encouraged in the development and refinement of analytical methods, including solid-phase microextraction stable isotope dilution GC-MS, to improve on instrumental sensitivity for MPs. GCO (Gas Chromatography Olfactometry)-MS may also be an appropriate tool to further investigate the role of MPs in HA-affected wine.

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