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

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Abstract: The possible influence of *Harmonia axyridis* (the Multicolored Asian Lady Beetle) on the sensory properties of wine was investigated. *Harmonia axyridis* were added to white and red grape musts at a rate of 0, 1, or 10/L, and a trained panel evaluated the finished wines using flavor-profiling techniques. Significant modification of both wine aroma and flavor characteristics were observed in the 10 beetle/L treatments, with smaller effects noted at the 1 beetle/L rate. Vinification in the presence of *H. axyridis* gave higher intensity scores for peanut, bell pepper, and asparagus aromas and flavors in the white wines and for peanut, asparagus/bell pepper, and earthy/herbaceous aromas and flavors in the red wines. In addition, sweet, acid and bitter tastes were affected in red wines, and a general trend of decreasing fruit and floral intensities with increasing beetle rate was observed in both white and red wines.

Key words: wine quality, sensory evaluation, taint, *Harmonia axyridis*, lady beetle, ladybug, methoxypyrazine

Harmonia axyridis (Coleoptera: Coccinellidae) (HA) was introduced into the United States from Japan, the Republic of Korea, and the former USSR during the late 1970s and early 1980s (Nalepa et al. 1996) as a biocontrol tool for aphids and other insect pests (Chapin and Brou 1991). Its successful establishment in the northeastern United States and eastern Canada may have been accidental (Day et al. 1994), but it is now widespread throughout much of these regions (Hoebeke and Wheeler 1996) and has also been recorded in some western areas of the United States and Canada (Nalepa et al. 1996). Over the last decade there have been intermittent reports from the winemaker community in the eastern wine region of North America of an atypical aroma and flavor, reminiscent of crushed lady beetles, in some wines from the region. Typically, this has coincided with the observation of high numbers of HA beetles in vineyards and on the fruit at harvest, with as many as 20 to 50 beetles reported on some grape clusters (Martinson 2002).

Coccinellids possess a reflex bleeding response of haemolymph when stressed (Al Abassi et al. 1998). The chemical composition of haemolymph has been partially determined and includes volatile compounds of known olfactory significance to humans (Rothschild and Moore 1987). In the *Coccinella septempunctata* ladybird beetle, 2-isopropyl-3-methoxy-pyrazine has been identified in the effluent, where it is thought to serve both pheromonal and defensive functions (Al Abassi et al. 1998). The human olfactory thresholds for methoxypyrazines are extremely low and in the order of 2 ng/L in water (Buttery et al. 1969, Seifert et al. 1970). It would, therefore, seem plausible that HA are capable of influencing wine quality via transfer of haemolymph onto grapes or directly into juice should beetles become incorporated into the harvested fruit or associated material. Several winemakers have reported seeing significant numbers of lady beetles in grape musts after the harvest and processing of the fruit.

What influence, if any, the incorporation of HA beetles into grape juice might have on the quality of finished wines has not been established, although much anecdotal comment and news media speculation has been noted. The objective of this study was to characterize the sensory properties of white and red wine fermented in the presence or absence of HA. Elucidation of the effects of HA on the chemical composition of wine is in progress and will be reported at a later date.

Materials and Methods

Wine preparation. Two commercial juice concentrates from South American grapes were used: White Bourgeron and Red Bergamais (Vinco International, St Catharines, Ontario). The concentrates were rehydrated according to manufacturer's directions. Basic composition of the rehydrated juices was the following: White Bourgeron: 21.9 Brix, pH 3.14, and 5.9 g/L titratable acidity (TA); Red Bergamais: 22.7 Brix, pH 3.32, and 6.0 g/L TA.

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Lady beetles were sourced from the local area and screened for identity. Identification of *Harmonia axyridis* 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; F.M. Oi and W. Foshee, http://www.aces.edu/department/ipm/ladybugs.htm).

Live HA beetles were then added to rehydrated juice in 20-L glass carboys at rates of 0, 1, or 10 beetles/L of juice. Three 20-L replicates of each of the beetle treatments and four 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* (EC1118; Lallemand, 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, and cold-stabilized following standard microvinification protocol (Pickering et al. 1999). After four weeks, the individual replicates from each treatment were assessed by a small, experienced tasting panel (four faculty and senior students from Brock University's Cool Climate Oenology and Viticulture Institute [CCOVI]) and considered not to differ sensorially. The replicate wines within each treatment were then pooled and bottled, without filtration. Wines were stored in a cellar at 14°C until required. The basic composition of the finished wines is given in Table 1.

Panel recruitment and training. The sensory panel was recruited from Brock University staff and students. A questionnaire was used to screen prospective panelists for anosmias or other conditions which might limit their suitability. Further selection was based on interest and availability. The final panel consisted of six females between 21 and 63 years of age. The gender distribution reflected availability of suitable personnel rather than any target composition. All participants signed an Informed Consent Form, and the project was approved by the Brock University Research Ethics Board (file 01-290).

Table 1 Basic composition of wines made with three levels of *Harmonia axyridis* beetles added to the juice.

	Treatment (beetles/L)[a]					
	White wine			Red wine		
	0	1	10	0	1	10
pH	3.28	3.27	3.27	3.35	3.34	3.36
Titratable acidity (g/L)	6.79	6.71	6.65	6.80	6.78	6.83
Ethanol (% v/v)	12.50	12.70	12.40	12.65	12.30	12.60
Residual sugar (g/L)	4.70	4.70	5.90	5.37	6.20	4.10

[a]Mean values of duplicate measurements of triplicate fermentations (for the two beetle addition treatments) or four fermentations (0 beetle treatment).

Nine one-hour training sessions were held over five weeks. A minimum of information on the nature of the study was provided in order to reduce potential bias. During sessions 1 and 2 the panel was presented with samples of wine from all six treatments (three white and three red). Samples were consistently presented blind, in coded ISO wine glasses, and were expectorated. The panel was instructed to generate appropriate descriptors for the appearance, aroma, and flavor of each wine. The panel leader facilitated the process of discussing terms as a group and looking for overlap and redundancy among descriptors. Terms that were used by only one person were removed from the developing lexicon. By panel consensus, appearance attributes (hue, density, and clarity) were removed from further consideration, as wines within each style (white or red) did not appear to differ.

In subsequent training sessions, reference standards were developed and refined for each of the terms and evaluated for suitability by reference to specific wine samples from the study. For each descriptor, 15-cm line scales were developed, 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 both beetle-treated and control 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. The final lexicon of descriptive terms and the reference standard composition for the white and red wines are given in Tables 2 and 3, respectively.

Data collection and analysis. Formal assessment of the wines took place over three sessions. The evaluations were conducted in individual white booths with red lighting (130 volt, 100 W Haskellite red bulb covered with red cellophane) in the ventilated sensory lab at the CCOVI. The following six samples were evaluated in triplicate for the aroma and flavor intensity of predefined attributes (Tables 2 and 3) using a randomized complete block design, with order of presentation of samples randomized within each flight: (1) White Bourgeron fermented without *Harmonia axyridis* (HA); (2) White Bourgeron fermented with 1 HA beetle/L; (3) White Bourgeron fermented with 10 HA beetles/L; (4) Red Bergamais fermented without HA; (5) Red Bergamais fermented with 1 HA beetle/L; and (6) Red Bergamais fermented with 10 HA beetles/L.

The wines were assessed 10 weeks after bottling, and white and red wines were evaluated in separate sessions. In

Table 2 White wine aroma and flavor descriptors with corresponding reference standards.

Descriptor	Reference composition[a]
Melon	2 tsp fresh honeydew melon juice
Citrus	1 tsp fresh grapefruit juice + ½ tsp fresh lime juice
Floral	5 drops of mixture: 10 mL green/herbaceous (#8947) + 10 mL geranium leaf (#9077) (Wine Awakenings) + 10 mL linalool (Sigma Aldrich) in 20 mL distilled water
Asparagus	1 tsp canned asparagus juice (Equality)
Bell pepper	10 mm square fresh bell pepper flame-heated for 20 sec, soaked in base wine for 20 min
Peanut	8 whole raw white peanuts crushed and soaked in 30 mL base wine for 30 min
Humus	50 g dry plant material (primarily bark) from 2 cm below-soil surface; presented in plastic container without base wine
SO_2	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

[a]All standards made up 1-2 hr before tasting in 60 mL unoaked neutral Chardonnay base wine (CCOVI Pilot Winery) 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 3 Red wine aroma and flavor descriptors with corresponding reference standards.

Descriptor	Reference composition[a]
Red berry	2-3 fresh whole blackberries heated in microwave for 20 sec + ⅓ tsp strawberry jam
Cherry	10 mL cherry cocktail (Del Monte Quality) + ½ tsp canned cherry juice (E.D. Smith)
Plum	2 tsp plum jam (S&F)
Asparagus/ bell pepper	½ tsp of canned asparagus juice (Equality) + one 5 x 10 mm strip of fresh bell pepper flame-heated for 20 sec
Cheesy	1 g ripe brie cheese (Château Versailles)
Peanut	8 whole raw white peanuts crushed and soaked in 30 mL base wine for 20 min
Earthy/ herbaceous	50 g dry plant material (primarily bark) from 2 cm below-soil surface; presented in plastic container without base wine
SO_2	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

[a]All standards made up 1-2 hr before tasting in 60 mL unoaked neutral Chardonnay base wine (CCOVI Pilot Winery) 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).

addition, panelists were asked to list any additional descriptive terms they felt applicable. All data were collected using Compusense software (C5V4, Guelph, Ontario, Canada). Before each flight, panelists were instructed to refamiliarize themselves with each reference standard. The standards were also available during data collection for reference if required. All wines were presented as 30-mL samples in covered ISO tasting glasses coded with 3-digit random numbers at ambient temperature (21°C ± 1°C).

The aroma and flavor of each sample was assessed separately in order to reduce halo effects (Lawless and Heymann 1998). Two flights of three samples were evaluated for aroma first, with a minimum 30-sec break between samples and a 5-min break between flights. Following a 15-min break, the same two flights were represented to the panel (with changed 3-digit codes) and assessed for flavor under the same assessment protocol.

Panel performance was assessed using generalized Procrustes analysis (GPA) within S-Plus 6 for Windows (Insightful Corporation, Seattle, WA). 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 two-way interactions included in the model. If the p of the treatment F-value was <0.05, then the Bonferroni test was applied to separate means using the GLM procedure of SAS statistical software (version 8; SAS Institute, Cary, NC).

Results and Discussion

Panel performance. GPA was used to evaluate the performance of the panel overall and of individual panelists (Gower 1975). Object residuals were calculated for each treatment as a measure of panel agreement. For white wine, these were 0.43, 0.47, and 0.29 for 0, 1, and 10 beetles/L, respectively, and 0.45, 0.35, and 0.43 for red wines. These values are all relatively small and similar, indicating good agreement, with all treatments rated similarly by the panel. Analysis of panelist residuals showed good consensus, particularly with the white wines. No panelist appeared to differ significantly in judgment from others, with a range in panelist residual scores of 0.07 for white wines and 0.11 for red wines.

The Procrustes statistics were also calculated for each panelist after each stage of the analysis (data not shown) (Dijkersthuis and Gower 1991, Dijkersthuis 1995). Although similar values were seen for both white and red wines at the start of analysis, both translation and rotation of the data decreased the distance from consensus for the majority of panelists, indicating, respectively, variation in the part of the scale used and differences in the understanding of some of the attributes. Analysis of the correlation of each of the initial attributes with the first two consensus dimensions indicated that panelists varied most in their understanding of cheesy aroma and cheesy flavor in the red wines, humus aroma and melon flavor in the white wines, and sweet and SO_2 flavor in both wine types. The scaling step did not improve the consensus distance, indicating that the panelists were using similar score ranges on the scales.

Panel understanding of the peanut aroma and flavors is of particular interest. As shown later in this section, these attributes largely define the influence of HA on wine aroma and flavor, they are atypical characters in wine, and panel performance with respect to "taint" measurement has not been well characterized in the literature. Correlations with the first two consensus dimensions for peanut aroma and flavor for both the white and red wines are shown in Figure 1. The direction and angle of the individual panelist vectors shown are generally similar, indicating that the panel shares a common understanding of the attribute. Vectors appear more dispersed for peanut flavor compared with aroma, particularly in the red wine for one panelist, possibly reflecting the perceptually more complex task of isolating and assessing individual flavors, given the range of interactions and competing stimuli.

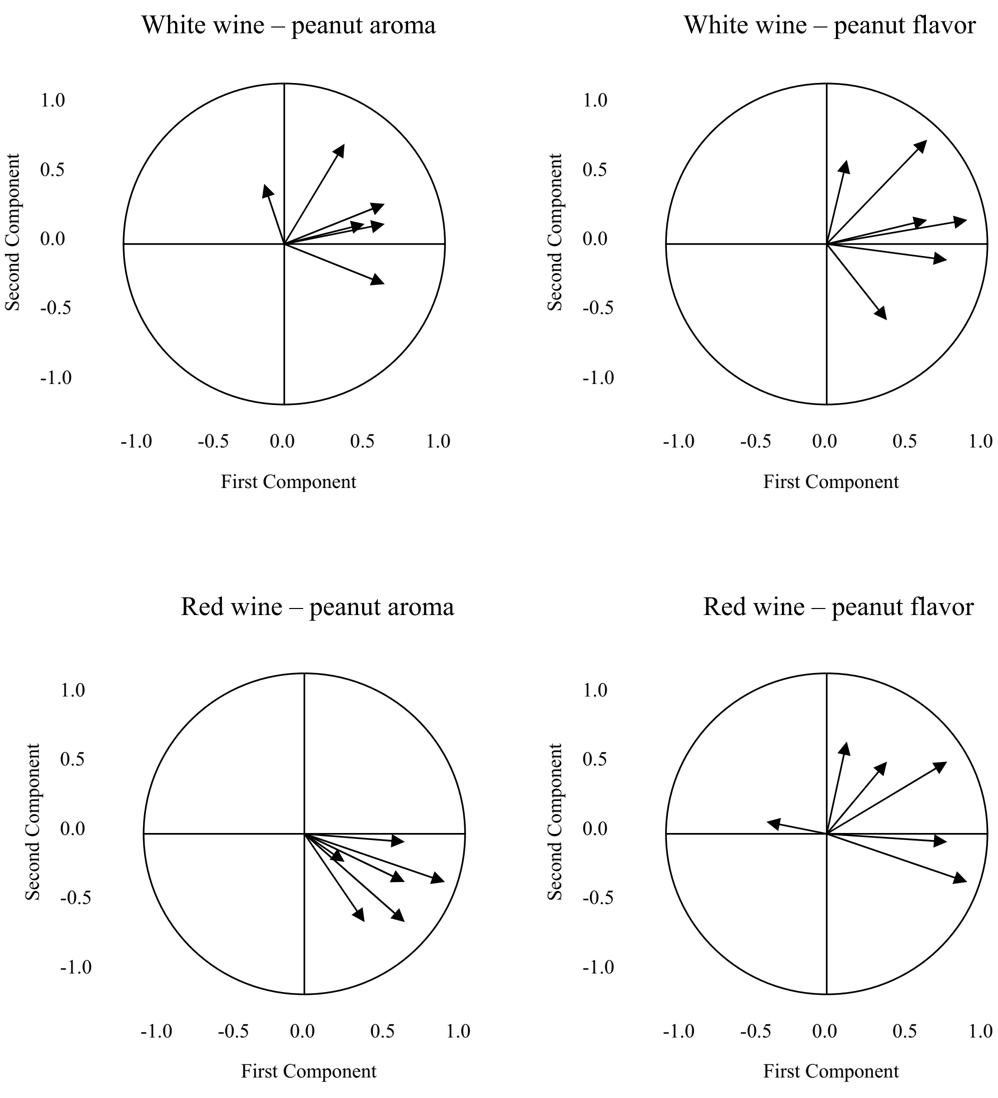

Figure 1 Correlations of peanut aroma and flavor with the first two consensus dimensions from generalized Procrustes analysis.

Treatment differences. The mean intensity scores for the 8 aroma and 11 flavor terms used to profile the white wines and the results of the Bonferroni means separation tests are shown in Figure 2. At 1 beetle/L, bell pepper aroma and peanut flavor increased in intensity with respect to the control wine (0 beetles/L), with no significant differences noted for the other attributes. At a rate of 10 beetles/L, however, several attributes were impacted compared with the control. As well as the increase in bell pepper aroma and peanut flavor noted above, peanut and asparagus aromas and bell pepper and asparagus flavors also increased.

Bell pepper and asparagus aroma and flavor intensities were also significantly higher in the 10 beetle/L treatments compared with the 1 beetle/L wines. The beetle-induced attributes (peanut, bell pepper, and asparagus) appear to be more intense when assessed ortho-nasally than retro-nasally, suggesting that smell may be more reliable than taste when assessing a white wine for potential HA influence. While not statistically significant, a trend of decreasing fruit and floral aroma intensities is suggested when beetle treatments as a whole are compared with the control wines, which would be consistent with a masking effect from the relatively strong aromatic components apparently introduced in the beetle-treated wines.

The mean intensity scores for the 8 aroma and 11 flavor terms used to profile the red wines and the results of the Bonferroni means separation tests are shown in Figure 3. At 1 beetle/L, only bitter intensity was affected (increased) when compared to the control wine (0 beetles/L), with no significant differences noted for any of the other attributes. At a rate of 10 beetles/L, however, several attributes were impacted compared with the control. Plum and cherry aroma and sweet flavor intensities decreased, while peanut, asparagus/bell pepper, and earthy/herbaceous aromas and flavors as well as acid and bitter intensities all increased.

Red berry, plum, and cherry aroma and red berry flavor intensities were significantly lower in the 10 beetle/L treatments compared with the 1 beetle/L wines, while asparagus/bell pepper and earthy/herbaceous aroma scores were higher. The result of no significant difference in red berry aroma and flavor at 10 beetle/L compared with control wines may be a consequence of the relatively low statistical power associated with a small panel. Interestingly, all three taste attributes were affected by the

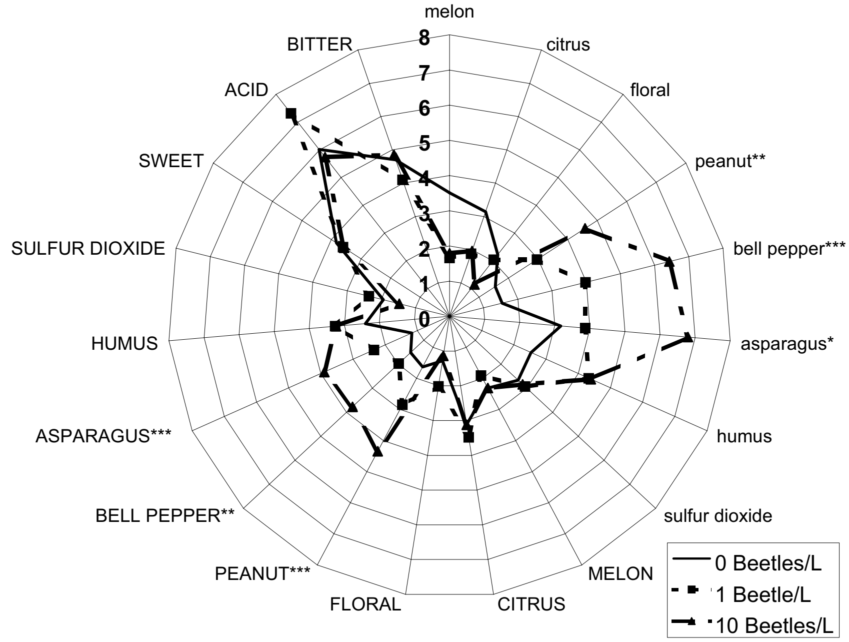

Figure 2 Cobweb diagram showing mean intensity scores for white wine aroma (lowercase) and flavor (uppercase) attributes for three levels of addition of *Harmonia axyridis* beetles to juice. Values are the mean ratings of six judges and triplicate assessments. *, **, and *** indicate significant differences at 0.05, 0.01, and 0.001, respectively (Bonferroni).

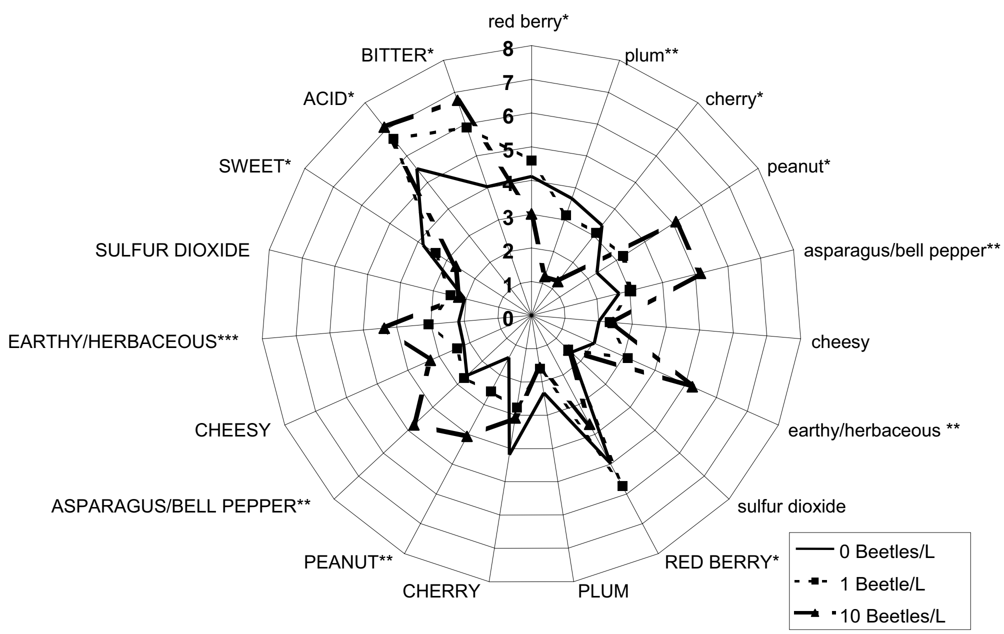

Figure 3 Cobweb diagram showing mean intensity scores for red wine aroma (lowercase) and flavor (uppercase) attributes for three levels of addition of *Harmonia axyridis* beetles to juice. Values shown are the mean ratings of six judges and triplicate assessments. *, **, and *** indicate significant differences at 0.05, 0.01, and 0.001, respectively (Bonferroni).

presence of beetles in the red wines assessed, while none were influenced in the white wines.

Further considerations. As previously noted, 2-isopropyl-3-methoxypyrazine has been identified in the effluent of the related species *Coccinella septempunctata* (Al Abassi et al. 1998). The ortho- and retro-nasal aroma descriptors shown here to characterize HA-affected wines are generally consistent with the known sensory properties of substituted pyrazines (Buchbauer et al. 2000) and how methoxyprazines are perceived in a wine medium (Allen et al. 1991). We therefore speculate that HA-derived methoxypyrazine(s) are the dominant aroma-active component(s) underlying the unique profile of these wines. The chemical origin of the increased bitterness observed in red wine fermented with beetles is less clear. To the authors' knowledge, bitterness is not an attribute that has been ascribed to methoxypyrazines. Alkaloid compound(s) are a possibility, as they are typically bitter and over 50 have been reported in Coccinellidae haemolymph (Daloze et al. 1995, King and Meinwald 1996).

While the panel size (n = 6) was at the lower end of the typical range used for flavor profiling (Lawless and Heymann 1998), several significant main effects have been observed. All members of the panel were female; it would be of interest to determine if any gender differences exist with respect to the relative flavor profiles derived for these wines, particularly given our result for bitterness and the increased sensitivity of females to bitterants reported in the literature (Duffy and Bartoshuk 1996, Bartoshuk 2000).

The altered sensory profiles shown here do not predict or indicate the direction or extent of consumer preference and acceptability of commercial wine that may be influenced by HA. Indeed, determination of consumer perception and purchase behavior toward these wines would be a logical extension of this study.

Conclusion

Significant modification of wine aroma and flavor characteristics was observed in both white and red musts fermented in the presence of 10 *Harmonia axyridis* (HA) beetles/L. Smaller effects were observed at a dosage rate of 1 beetle/L. A number of sensory attributes were enhanced in the beetle treatments: peanut, bell pepper, and asparagus aromas and flavors in white wine, and peanut, asparagus/bell pepper, and earthy/herbaceous aromas and flavors in red wine. In addition, sweet, acid, and bitter tastes were affected in red wine, and a general trend of decreasing fruit and floral intensities was observed with increasing beetle rate in both wines.

Taken overall, these results do indicate the potential for HA to influence wine quality. The effects were dose-dependent, and the external validity of the beetle addition rates employed here (that is, the concentration of HA or effluent 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 and wine-style dependent. For instance, in these trials the typicalness of the red wine appeared to be more adversely affected than that of the white wine.

Research is underway to fully characterize the influence of HA on the chemical composition of wine. Associated with this, we are testing the hypothesis that methoxypyrazines are the principal odor-active compounds in HA-affected wine. The possible influence on wine quality of reflex-bleeding directly onto grape should be investigated and the effect of bottle aging on the sensory properties shown here should be determined. It may also be appropriate to investigate the effectiveness of various remedial juice and wine treatments aimed at removing or reducing undesirable aroma and flavor contributions from HA. The sensory profiles developed here should serve as a useful baseline for this work.

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