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

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Summary   This study evaluated the efficacy of a range of commercially available fining agents and other interventions on reducing 2-isopropyl-3-methoxypyrazine (IPMP) concentration and taint characteristics of white and red wine affected by *Harmonia axyridis* (*HA*). Wines fermented in the presence of *HA* beetles were treated with bentonite, activated charcoal, oak chips, deodorized oak chips and either ultraviolet (red wine) or visible (white wine) light. IPMP concentrations were lowered by activated charcoal in white wine and deodorized oak in red wine. Treatment with oak chips reduced the intensity of *HA*-associated sensory attributes ('ladybug taint') in both white and red wines, while other applications generally had no effect on white wine and limited effect on red wines.

Introduction

The ability of *Harmonia axyridis* (Coleoptera: Coccinellidae) (a.k.a. the Multicolored Asian Lady Beetle) (*HA*) to adversely affect wine quality if incorporated into fermenting juice has been established by Pickering *et al.* (2004, 2005), consistent with anecdotal evidence from the North American winemaking community and news media. *HA* is an introduced species to North America, and is now widespread throughout many areas, including the north-eastern United States, eastern Canada and parts of the western United States (Hoebeke & Wheeler, 1996; Nalepa *et al.*, 1996). Anecdotally, *HA* has been reported as contributing a taint to wine across an even wider range of winegrowing regions, including Europe.

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Harmonia axyridis influences wine quality when beetles are included with grapes during crushing/destemming and pressing operations or when added to juice and incorporated with the fermentation (Pickering *et al.*, 2004). In a commercial situation, the beetles are located in grape clusters and typically enter the production process when 'harvested' along with grape material. Coccinellids possess a reflex bleeding response of haemolymph when stressed (Al Abassi *et al.*, 1998; Laurent *et al.*, 2001; Koch, 2003) and haemolymph contains compounds of known olfactory significance to humans (Rothschild & Moore, 1987), including 2-isopropyl-3-methoxypyrazine (IPMP) (Al Abassi *et al.*, 1998). IPMP has been identified at concentrations of 9.7–37.7 ng L^{-1} in wine fermented in the presence of *HA* beetles (Pickering *et al.*, 2005), well above the human sensory threshold for methoxypyrazines, which is in the order of 2 ng L^{-1} (Buttery *et al.*, 1969; Seifert

et al., 1970). In a study that included both chemical and sensory trials, Pickering *et al.* (2005) concluded that IPMP was the principle compound responsible for the taint.

The unique sensory profile of *HA*-affected wines is characterized by peanut, bell pepper and asparagus aromas and flavours in white wines, and peanut, asparagus/bell pepper, and earthy/herbaceous aromas and flavours in red wines (Pickering *et al.*, 2004). Bitterness may also be enhanced, and a trend of decreasing fruit and floral intensities as the number of beetles added increased was also reported for both white and red wines. Pickering *et al.* (2005) showed that these sensory characteristics remain relatively stable during bottle ageing, and also reported that vinification in the presence of *HA* has little effect on basic physical, chemical and spectrophotometric measures of wine quality, as assessed both at bottling and after bottle aging.

The *HA*-taint is relatively new to the juice and wine industries, and research to date has been very limited. Pickering *et al.* (2004, 2005) stressed the need for the development and evaluation of potential remedial treatments for affected juice and wine. Fining agents such as bentonite, gelatin, isinglass and activated charcoal are commonly used in the wine industry to adjust and stabilize wine quality, including the prevention and/or removal of visual, olfactory and gustatory faults. Oak has been shown to absorb many wine aroma compounds of diverse chemical species (Ramirez *et al.*, 2001), and can be employed to 'hide' minor wine defects, particularly those of an olfactory nature. Aiken & Noble (1984) found that 'green' attributes were decreased after oak ageing of Cabernet Sauvignon wine; a variety known to have appreciable concentrations of methoxypyrazines (Boubee *et al.*, 2000). Exposure to light offers an interesting potential juice and wine treatment which has previously been investigated for application in reducing pesticide concentrations in wine (Stavropoulos *et al.*, 2001). Fluorescent light (Hashizume & Samuta, 1999) and sunlight (Allen & Lacey, 1998) have been shown to decrease methoxypyrazine concentrations in ripening grapes via photodecomposition, although treatment of wine with ultra-violet (UV) and other light wavelengths may also be deleterious to wine quality (Benitez *et al.*, 2003).

The objective of this study was to evaluate the efficacy of a range of commercially available fining agents and other interventions on IPMP concentration and the sensory characteristics of white and red wine affected by *HA*.

Materials and methods

Preparation of wine

Wines were initially prepared as 5:4 blends of the 1 and 10 beetle L^{-1} wines produced in the study done by Pickering *et al.* (2004). The blend provided a level of taint and IPMP concentration more typical of that observed in commercial *HA*-affected wines. *Harmonia axyridis* beetles were added directly into the juice and were present throughout the fermentation in order to produce the characteristic *HA* taint (Pickering *et al.*, 2004). After fermentation, wines were racked off their lees (which included removal of the beetles) and were stabilized following standard microvinification procedures. Finished wines were then stored in sealed carboys at 4 °C and blended immediately prior to use in these trials. The basic chemical composition of these wines were as follows; white wine: pH, 3.29; titratable acidity (g L^{-1}), 6.46; volatile acidity (mg L^{-1}), 402; free SO_2 (mg L^{-1}), 17.4; total SO_2 (mg L^{-1}), 167.1; red wine: pH, 3.39; titratable acidity (g L^{-1}), 6.86; volatile acidity (mg L^{-1}), 576; free SO_2 (mg L^{-1}), 12.0; total SO_2 (mg L^{-1}), 102.9.

A number of potential remedial treatments were bench-tested for their apparent effectiveness at reducing the *HA*-associated sensory characteristics, including bentonite, isinglass, combinations of bentonite and isinglass, activated charcoal, a range of oak chips including de-odourised oak, UV light, and other light sources. After bench-testing and investigating optimal dosage levels and processing conditions, the following treatments were applied to the blended *HA*-affected base wines described above:

(i) *Bentonite.* Bentonite (Vineco™, St Catharines, Ontario, Canada) 10 g was added to boiling water and stirred overnight. Forty millilitres of this solution was then added to 4 L of base wine to give a final bentonite concentration of 1 g L^{-1}. The wine was stored at 7 °C and racked after 3 and 7 days.

(ii) *Activated charcoal*. Activated charcoal (Sigma®, St Louis, MO, USA) 0.8 g was added directly into 4 L of base wine to give a final concentration of 0.2 g L^{-1}. The wine was stored at 7 °C and racked after 3 and 7 days.

(iii) *Oak chips*. French medium toast oak chips (Winemaster™, Vin Bon-Brew, St Catharines, Ontario, Canada) 16 g were seeped for three days in 4 L of base wine (final concentration of 4 g L^{-1}). The wine was stored at 7 °C and racked after 3 and 7 days.

(iv) *Deodourized oak*. Wines were processed as above except that the oak chips were deodorised prior to use. To deodourize, 60 g of oak chips were seeped in a 300 mL solution of 40% ethanol overnight. They were then washed three times with distilled water, boiled in water for 10 min, and dried in a 60 °C oven.

(v) *UV light (red wine)*. Wine was subject to a light source of the following specifications: UVG-43, wavelength = 254 nm, 18.3 W. In order to address the limited ability of UV light to penetrate far beneath the surface of wine, a transparent Perspex reactor was constructed which allowed a thin film of wine to be exposed under anaerobic conditions to the light source (Fig. 1). The reaction chamber had the approximate dimensions of 12 cm long × 4 cm wide × 0.5 cm deep, with the light source placed directly above at a distance of 30 cm. Four litres of wine was forced through the reactor using N_2 gas at a flow rate of 100 mL min^{-1} for 40 min.

(vi) *Visible light (white wine)*. The same reactor and conditions described above were used for white wine with the exception of the light source, which was white light provided by a halogen bulb of 120 W.

Determination of 2-Isopropyl-3-methoxypyrazine

The IPMP concentration of wines was assessed as follows: samples were concentrated in a C-18 SPE cartridge and eluted by ethyl acetate. The ethyl acetate extract was analysed by GC-MS using a DB5-MS column coupled with an Agilent 5973 MSD. IPMP was determined by selective ion monitoring of the target ion of m/e 152 and qualifying ions of m/e 137 and 124. Quantitative concentrations were determined by comparison to a standard solution of IPMP spiked into ethyl acetate. The limit of quantitation was approximately 4–5 ng L^{-1}. Full details of the method are given in Pickering *et al.* (2005).

Sensory evaluation

Panel recruitment and training

Wines were assessed using descriptive analysis. The panel was recruited from Brock University staff and students. A questionnaire was used to screen prospective panellists for anosmias or other conditions that might limit their suitability. Further selection was based on their interest and availability. The final panel consisted of seven females and one male aged between 21 and 53 years.
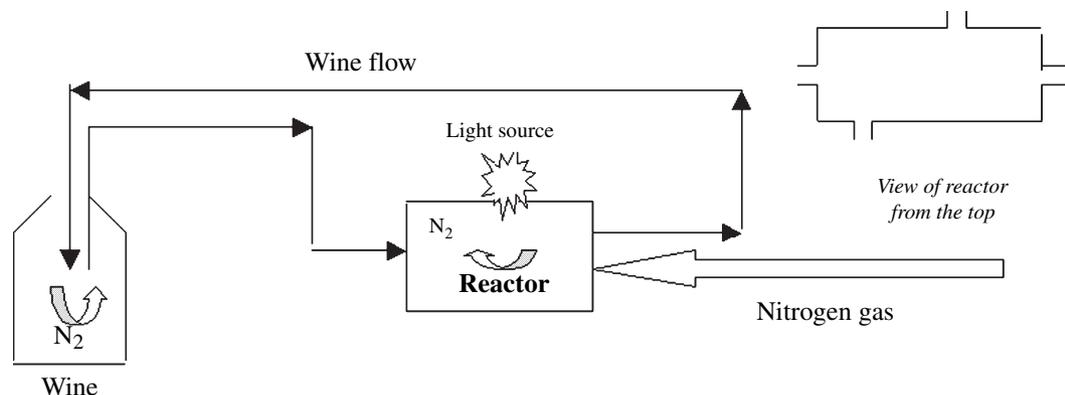

Figure 1 Reactor used for light-treatment of *Harmonia axyridis*-affected wines.

Seven training sessions of 1-h duration each were held over 4 weeks. Samples were presented blind, in coded ISO wine glasses, and were expectorated. In the first and second sessions, the panel was introduced to all white and red wine samples. The reference standards and descriptive terms used by Pickering *et al.* (2004) were provided to the panel to assist in the development of an appropriate lexicon. Panellists were also encouraged to generate new, appropriate descriptors for the aroma and flavour 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 & 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.

Tables 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.

Data collection
Formal assessment of the wines took place over six sessions. The evaluations were conducted in individual white booths with red lighting (130 V, 100 W Haskellite®, Robertson Electric, Rexdale, Ontario, Canada, red bulb covered with red cellophane) in the ventilated sensory lab at the Cool Climate Oenology and Viticulture Institute, Brock University. All wines were evaluated in duplicate for the aroma and flavour intensities of the predefined attributes using a randomized complete block design, with order of presentation of samples randomized within each group of wines (flight).

White and red wines were evaluated in separate sessions. In addition, panellists were asked to list

Table 1 White wine aroma and flavour descriptors with corresponding reference standards

Descriptor	Reference composition[a]
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' [#8947] + 10 mL 'Geranium leaf' [#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
SO$_2$	700 mg L^{-1} aqueous solution of potassium metabisulfite (Fisher Scientific) without base wine
Diesel	120 mg (148 μL) Isoamyl & 300 mg (375 μL) isobutyl alcohol in 300 mL base wine
Oak	0.3 L^{-1} French oak chips (Winemaster™, Vin Bon-Brew, St Catharines, Ontario, Canada) in base wine
Sweet	12.5 g L^{-1} sucrose in aqueous solution
Acid	1.5 g L^{-1} tartaric acid in aqueous solution
Bitter	12 mg L^{-1} quinine sulphate in aqueous solution

[a]All standards made up 1–2 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 flavour descriptors with corresponding reference standards

Descriptor	Reference composition[a]
Red berry	2–3 fresh whole blackberries heated in microwave oven for 20 s + 1/3 tsp strawberry jam
Cherry	10 mL cherry cocktail (DelMonte Quality[TM]) + 1/2 tsp canned cherry juice (E.D. Smith[TM])
Plum	2 tsp plum jam (S&F[TM])
Asparagus/bell pepper	1/2 tsp of canned asparagus juice (Equality[TM]) + one 5 × 10 mm strip of fresh bell pepper heated with naked flame for 20 s
Cheesy	1 g ripe Château Versailles[TM] 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.[TM]) in 60 mL base wine
Diacetyl	0.1 g L^{-1} Diacetyl (Sigma®) in base wine
Oak	0.3 L^{-1} French oak chips (Winemaster[TM]; Vin Bon-Brew, St Catharines, Ontario, Canada) in mL base wine per 60 mL + 0.5 μL 'Art toast (smoke) flavour,#8038–9138', Wine Awakening Inc.[TM]
SO_2	700 mg L^{-1} aqueous solution of potassium metabisulfite (Fisher Scientific) without base wine
Sweet	12.5 g L^{-1} sucrose in aqueous solution
Acid	1.5 g L^{-1} tartaric acid in aqueous solution
Bitter	12 mg L^{-1} quinine sulphate in aqueous solution

[a]All standards made up 1–2 h before tasting in control white base 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).

any additional descriptive terms they felt applicable. All data was collected using Compusense[TM] software (C5V4; Guelph, Ontario, Canada). Before each flight, panellists were instructed to re-familiarize 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 three-digit random numbers at ambient temperature (21 ± 1 °C).

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

Data analysis

Data for white and red wines were examined separately. For IPMP concentration, treatment means were compared with the untreated wine using one-tailed *t*-tests with alpha set at 0.05. For

the complete descriptive analysis data set, 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, the Bonferroni test was applied to separate means using the GLM procedure of SPSS (v. 11.0; SPSS Inc., Chicago, IL, USA). Independent sample *t*-tests (two-tailed) were also performed for each *HA*-associated attribute, with mean scores for each treatment compared with those of the untreated wine in order to further assess the impact of the remedial treatments. Levene's test for equality of variances was first applied, and the degrees of freedom modified appropriately if variances were not equal. Alpha was set at 0.10 for the *t*-tests because of the relatively low statistical power associated with 16 observations per treatment.

Results and discussion

White wine

The activated charcoal treatment reduced IPMP concentration ($t = 5.37$, $P = 0.017$) by approximately 34%, while all other treatments were

Table 3 2-Isopropyl-3-methoxy-pyrazine concentration of *Harmonia axyridis*-affected wines before and after treatment

		Treatment				
	Untreated wine	Bentonite	Activated charcoal	Oak chips	Deodourized oak	Light[b]
White wine						
Mean	10.9	11.0	7.2*	12.2	10.4	9.8
SD	0.73	0.92	0.57	0.92	0.99	0.80
Red wine						
Mean	10.8	11.8	9.6[a]	10.8	10.1*	10.4
SD	0.16	0.91	0.65	0.20	0.10	0.48

*Treatment mean is significantly different from untreated wine (one-tailed *t*-test, alpha = 0.05).
[a]$P(T < t) = 0.057$.
[b]For white wine, light source was visible spectrum; for red wine, UV was used.

unchanged from the untreated wine (Table 3). Activated charcoal possesses a large surface area ($500–1500 \ m^2 \ g^{-1}$) and its ability to adsorb and remove a wide range of molecules has previously been reported (Cabras *et al.*, 1997; Ying & Williams, 1999; Cabras & Angioni, 2000; Lopez *et al.*, 2001).

However, charcoal was not effective at reducing the sensory attributes directly associated with *HA* (Fig. 2), probably due to greater affinity for non-taint aroma-active wine compounds. Treatment with oak chips significantly reduced the intensity of asparagus flavour ($t = 2.747$, d.f. = 30, $P = 0.010$) and a trend of lower intensities for all taint
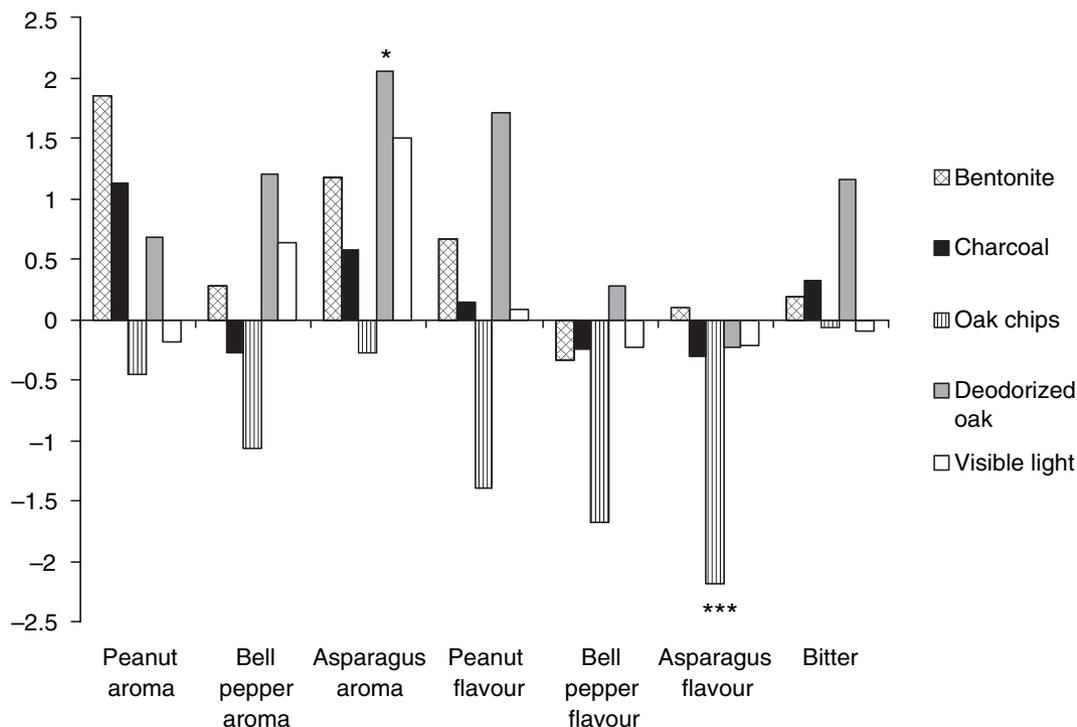

Figure 2 Change in mean intensity scores of taint attributes for *Harmonia axyridis*-affected white wine after various treatments ($n = 16$). Mean intensity of attribute significantly different from untreated wine (*t*-test) at alpha of *0.10 and **0.010.

attributes is observed for both aroma and flavour. As the deodorized oak treatment did not affect taint attributes, the oak chips very likely exerted their influence through masking of taint characteristics with oak-derived aromatic components, as corroborated by the higher oak intensity scores for aroma and flavour compared to all other wines ($P < 0.001$, Bonferroni). The ability of oak chips to mask other aspects of a wine's sensory profile is well acknowledged anecdotally and has been reported in the literature (Perez-Coello *et al.*, 2000). Other treatments did not affect the intensity of the taint attributes, although curiously, deodourized oak increased the intensity of asparagus aroma compared with the untreated wine ($t = 1.882$, d.f. $= 30$, $P = 0.070$).

With the exception of oak chips, treatments had relatively little impact on the overall flavour profiles of the wine (Fig. 3). There is a trend for all treatments of lower intensity of fruit and floral aroma descriptors compared with the untreated wine, although this is only significant for melon, where the light treatment has reduced intensity

($P = 0.044$, Bonferroni). In addition, sweetness was decreased in wines treated with deodorized oak when compared with bentonite-treated wines ($P = 0.023$, Bonferroni).

Red wine

Deodourized oak reduced the IPMP concentration of the untreated red wine ($t = 5.81$, $P = 0.014$) (Table 3). Other treatments had no effect, although the activated charcoal treatment was close to significance ($P = 0.057$, $t = 2.71$). With red wines there is a trend of *HA*-associated taint attributes being more responsive to treatment than with white wines, particularly for flavour, although only asparagus/bell pepper flavour was significantly reduced, with bentonite ($t = 1.905$, d.f. $= 30$, $P = 0.066$), charcoal ($t = 1.974$, d.f. $= 24.9$, $P = 0.060$), oak chips ($t = 1.992$, d.f. $= 30$, $P = 0.056$) and deodourized oak ($t = 1.793$, d.f. $= 26.9$, $P = 0.084$) all effective (Fig. 4). As reported with white wine, a trend of reduced intensity across the range of taint descriptors is observed with oak chip treatment.
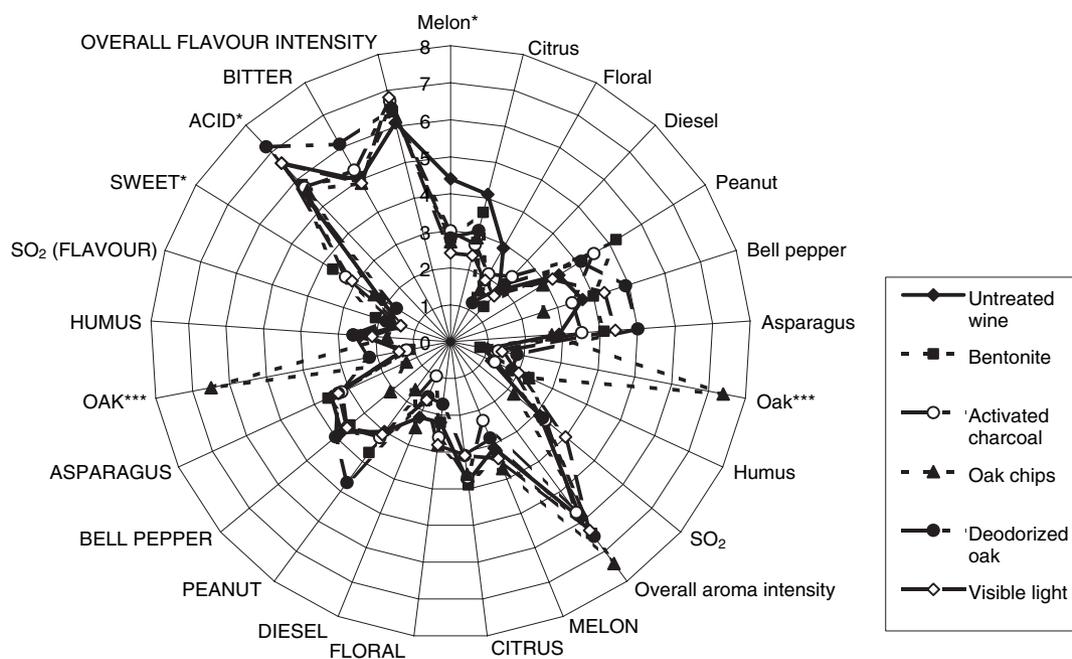

Figure 3 Mean aroma and FLAVOUR intensity scores for treated and untreated white wine affected by *Harmonia axyridis*. Treatment means are significantly different at alpha of **0.01 and ***0.001 [ANOVA].
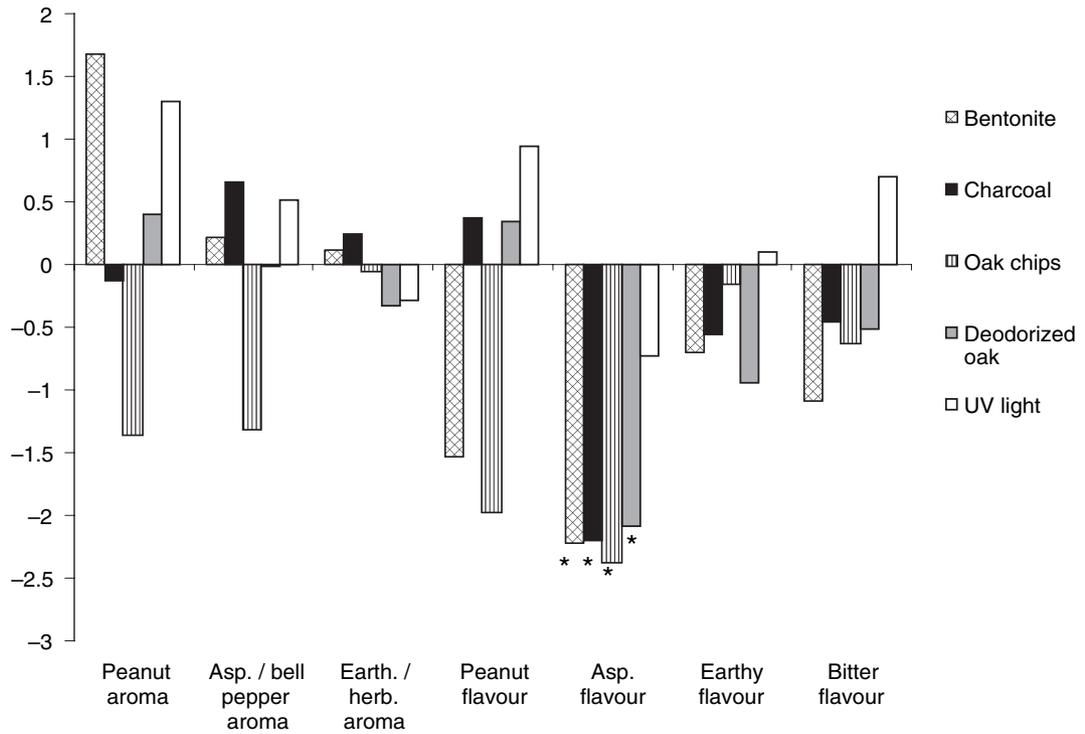
Figure 4 Change in mean intensity scores of taint attributes for *Harmonia axyridis*-affected red wine after various treatments ($n = 16$). Asp. = asparagus; Earth./herb and Earthy = Earthy/herbaceous; Asp. flavour = asparagus/bell pepper flavour; *mean intensity of attribute significantly different from untreated wine (*t*-test) at alpha of 0.10.
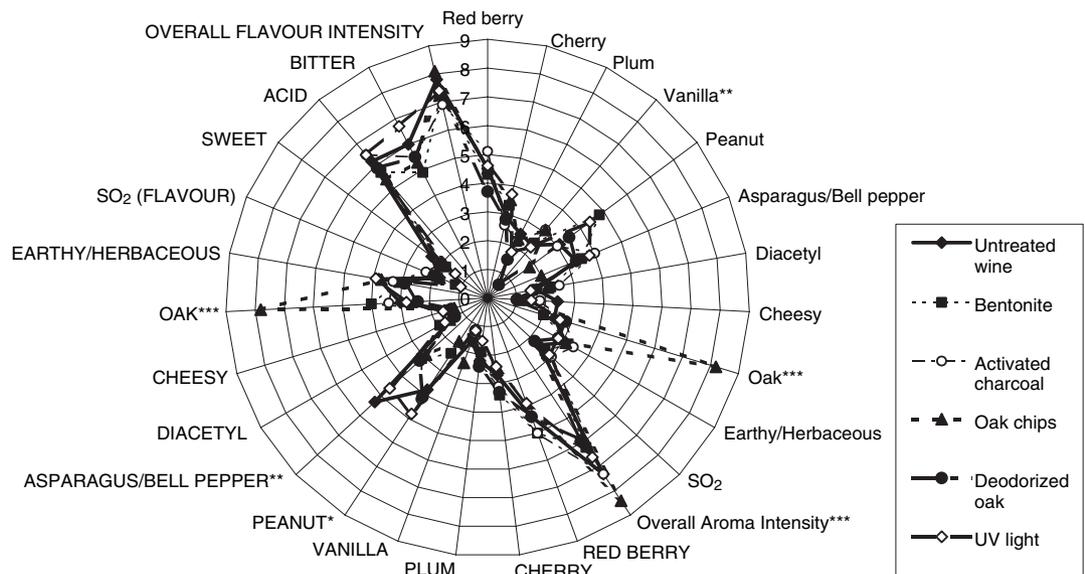

Figure 5 Mean aroma and FLAVOUR intensity scores for treated and untreated red wine affected by *Harmonia axyridis*. Treatment means are significantly different at alpha of **0.01 and ***0.001, respectively [ANOVA].

The oak chip-treated wine was rated more intense for oak aroma ($P < 0.000$, Bonferroni) and flavour ($P < 0.010$) compared with all other wines and more intense for vanilla aroma compared to the deodourized oak treatment ($P = 0.009$) (Fig. 5). Vanilla has previously been associated with the use of oak chips in wine (Afonso, 2002). In addition, the oak chip treatment was rated higher for overall aroma intensity than bentonite ($P = 0.002$, Bonferroni), deodourized oak ($P = 0.000$) and UV ($P = 0.022$) wines. Vanilla aroma was also significantly lower in the deodourized oak wine compared with the charcoal treatment ($P = 0.008$, Bonferroni), although vanilla is a relatively minor component of the overall flavour profile of these wines.

Further considerations

Taken overall, these results suggest that oak chips are effective in reducing *HA*-taint characteristics in wine. Oak chips possess a large surface area with polar hydroxyl groups and aromatic lignin structures that could potentially bind IPMP. However, Hartmann *et al.* (2002) concluded that oak does not have a strong affinity for alky-methoxypyrazines in model wine solutions. This result, and the limited performance of deodourized oak here, suggest that the oak chips are masking the *HA*-taint with oak-derived volatile components, such as vanillin (Perez-Coello *et al.*, 2000) and *cis* oak lactone (Sauvageot & Feuillat, 1999).

While treatment with oak chips has been successful for both white and red wines, it is not appropriate for all wine styles. Aromatic or delicate varieties, such as Muscat and Gewürztraminer, are not normally oaked, and other solutions are required for these styles.

Summary

Of the potential remedial treatments evaluated for wine affected by *HA*, activated charcoal was successful at reducing IPMP concentration in white wine and deodourized oak in red wine, although this did not generally translate into lower intensity of the sensory attributes associated with the taint. Oak chips were successful in reducing the intensity of *HA*-taint characteristics in both white and red wines, probably through a masking effect. Other processes investigated, including the non-traditional use of deodourized oak, UV and visible light, generally had no effect on white wine and limited effect on red wine.

While research is on going to investigate methods for preventing *HA* beetles from entering the juice and winemaking process, further work is also required to develop appropriate technologies to remove the taint from juice and wine. A limiting factor in these approaches may be legislation restricting permissible additions to these products. A successful technology would ideally target and selectively bind IPMP, with subsequent removal of the complex from the juice or wine.

References

Afonso, V.L.G. (2002). Sensory descriptive analysis between white wines fermented with oak chips and in barrels. *Journal of Food Science*, **67**, 2415–2419.

Aiken, J.W. & Noble, A.C. (1984). Comparison of the aromas of oak- and glass-aged wines. *American Journal of Enology and Viticulture*, **35**, 196–199.

Al Abassi, S., Birkett, M.A., Pettersson, J., Pickett, J.A. & Woodcock, C.M. (1998). Ladybird beetle odour identified and found to be responsible for attraction between adults. *Cellular and Molecular Life Sciences*, **54**, 876–879.

Allen, M.S. & Lacey, M.J. (1998). Methoxypyrazines of grapes and wines. In: *Chemistry of Wine Flavour* (edited by A.L. Waterhouse and S.E. Ebeler). Pp. 31–38. New York, USA: American Chemical Society.

Benitez, P., Castro, R. & Barroso, C.G. (2003). Changes in the polyphenolic and volatile content of 'Fino' sherry wine exposed to ultraviolet and visible radiation during storage. *Journal of Agricultural and Food Chemistry*, **51**, 6482–6487.

Boubee, D.R., Leeuwen, C.V. & Dubourdieu, D. (2000). Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines: effect of environmental conditions on concentrations in grapes during ripening. *Journal of Agricultural and Food Chemistry*, **48**, 830–4834.

Buttery, R.G., Seifert, R.M., Guadagni, D.G. & Ling, L.C. (1969). Characterization of some volatile constituents of bell peppers. *Journal of Agricultural and Food Chemistry,*, **17**, 1322–1327.

Cabras, P. & Angioni, A. (2000). Pesticide residues in grapes, wine, and their processing products. *Journal of Agricultural and Food Chemistry*, **48**, 967–973.

Cabras, P., Angioni, A., Garau, V.L. et al. (1997). Fate of some new fungicides (Cyprodinil, Fludioxonil, Pyramethanill, and Tebuconazole) from vine to wine. *Journal of Agricultural and Food Chemistry*, **45**, 2708–2710.

Hartmann, P.J., McNair, H.M. & Zoecklein, B.W. (2002). Measurement of 3-alkyl-2-methoxypyrazine by headspace solid-phase microextraction in spiked model wines. *American Journal of Enology and Viticulture*, **53**, 285–288.

Hashizume, K. & Samuta, T. (1999). Grape maturity and light exposure affect berry methoxypyrazine concentration. *American Journal of Enology and Viticulture*, **50**, 194–198.

Hoebeke, E.R. & Wheeler, A.G. (1996). Adventive lady beetles (Coleoptera: Coccinellidae) in the Canadian maritime provinces, with new eastern US records. *Entomological News*, **107**, 281–290.

Koch, R.L. (2003). The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. *Journal of Insect Science*, **3**, 32.

Laurent, P., Lebrun, B., Braekman, J., Daloze, D. & Pasteels, J.M. (2001). Biosynthetic studies on adaline and adalinine, two alkaloids from ladybird beetles (Coleoptera: Coccinellidae). *Tetrahedron*, **57**, 3403–3412.

Lawless, H.T. & Heymann, H. (1998). *Sensory Evaluation of Food: Principles and Practices*. New York, USA: Chapman and Hall.

Lopez, S., Castro, R., Garcia, E., Pazo, J.A.S. & Barroso, C.G. (2001). The use of activated charcoal in combination with other fining agent and its influence on the organoleptic properties of sherry wine. *European Food Research and Technology*, **212**:671–675.

Nalepa, C.A., Kidd, K.A. & Ahlstrom, K.R. (1996). Biology of *Harmonia axyridis* (Coleoptera: Coccinellidae) in winter aggregations. *Annals of the Entomological Society of America*, **89**, 2–6.

Perez-Coello, M.S., Sanchez, M.A., Garcia, E., Gonzalez-Vinas, Sanz, J. & Cabezudo, M.D. (2000). Fermentation of white wines in the presence of wood chips of American and French oak. *Journal of Agricultural and Food Chemistry*, **48**, 885–889.

Pickering, G.J., Lin, J.Y., Riesen, R., Reynolds, A., Brindle, I. & Soleas, G. (2004). Influence of *Harmonia axyridis* on the sensory properties of white and red wine. *American Journal of Enology and Viticulture*, **55**, 153–159.

Pickering, G.J., Lin, J.Y., Reynolds, A., Soleas, G., Riesen, R. & Brindle, I. (2005). The influence of *Harmonia axyridis* on wine composition and aging. *Journal of Food Science*, **70**, 5128–5135.

Ramirez, G.R., Lubbers, S., Charpentier, C. & Feuillat, M. (2001). Aroma compound sorption by oak wood in a model wine. *Journal of Agricultural and Food Chemistry*, **49**, 3893–3897.

Rothschild, M. & Moore, B. (1987). Pyrazines as alerting signals in toxic plants and insects. In: *Proceedings of the 6th International Symposium on Insect-Plant Relationships* (edited by V. Labeyrie, G. Fabres and D. Lachaise). Pp. 97–101. Pau: Kluwer Academic Publishers.

Sauvageot, F. & Feuillat, F. (1999). The influence of oak wood (*Quercus robur* L., *Q. petraea* Liebl.) on the flavor of burgundy pinot noir. An examination of variation among individual trees. *American Journal of Enology and Viticulture*, **50**, 447–455.

Seifert, R.M., Buttery, R.G., Guadagni, D.R., Black, D.R. & Harris, J.G. (1970). Synthesis of some 2-methoxy-3-alkylpyrazines with strong bell pepper-like odors. *Journal of Agricultural and Food Chemistry*, **18**, 246–249.

Stavropoulos, P., Athanasopoulos, P.E. & Kyriakidis, N.S. (2001). Degradation of pyrazophos and methidathion in fortified red and white wine under conditions of light and darkness. *Food Chemistry*, **72**, 473–477.

Ying, G.G. & Williams, B. (1999). Herbicide residues in grapes and wine. *Journal of Environmental Science and Health B*, **34**, 397–411.