Research Note

Sensory-Based Action Threshold for Multicolored Asian Lady Beetle-Related Taint in Winegrapes

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Abstract: Action thresholds were developed for the Frontenac winegrape to aid growers in the management of the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). Sensory thresholds were determined for the *H. axyridis*-related taint in winegrapes by artificial infestation of *H. axyridis*-free grapes with live adult beetles. In a series of three-alternative forced choice (3-AFC) tests, a tasting panel compared control wine prepared with *H. axyridis*-free grapes and wine produced with increasing insect levels. Logistic regression was used to relate the number of *H. axyridis* per kg of grapes to the proportion of correct 3-AFC tests. With the same logistic regression model, the probability of correct answers was estimated using the logit probability function. The same logistic model was also used to estimate the infestation level for a given probability of correct answers. The estimated threshold at which 10% of the population could detect the characteristic off-flavor of *H. axyridis* was 1.9 beetle/kg grape, or 0.27 beetle/grape cluster of Frontenac. The sensory thresholds presented here can be interpreted as action thresholds for *H. axyridis* in winegrapes (i.e., number of *H. axyridis* per kg of grapes). These new action thresholds add information in the management of the *H. axyridis* in grapes, which when combined with existing control methods and sampling plans, form the basis of integrated pest management for this insect in winegrapes.

Key words: *Harmonia axyridis*, tasting panel, winegrapes, contaminant pest

The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), has recently become an economically significant contaminant pest in the winemaking process. Adults tend to aggregate on clusters with injured berries just before-harvest and may eventually be incorporated with the grapes during wine processing (Pickering et al. 2004). Once disturbed or crushed, *H. axyridis*, like most Coccinellids, releases a yellow smelly fluid, which is called reflex bleeding and contains alkylmethoxypyrazines (Al Abassi et al. 1998). Alkylmethoxypyrazines could be partially responsible for the off-flavor produced by *H. axyridis* in wines (Pickering et al. 2005) and are well known for their contribution to vegetative, herbaceous, green bell pepper, and earthy character of wines such as Cabernet Sauvignon and Sauvignon blanc (Allen et al. 1994, Sala et al. 2002).

Known sensory thresholds of *H. axyridis* in wine are limited. For the white grape variety Riesling the threshold has been determined as ~1.76 *H. axyridis* adult/kg grape, or ~0.2/cluster, assuming an average weight of 0.1 kg/cluster (Pickering et al. 2006). Wine made from red grape varieties could be even more sensitive to taint and, therefore, could have an even lower threshold (Pickering et al. 2006).

Frontenac is a red grape variety that has become important for cold climates worldwide (Plocher and Parke 2001). It is a cold-hardy, vigorous, and disease-resistant variety that had been grown without the application of insecticides until the arrival of *H. axyridis*. Since then, control methods have ranged from insecticide applications to manual removal of this insect from grape clusters at harvest (Galvan et al. 2006). Regardless of the method used, growers of red grape varieties are making management decisions based on suggestions of empirical data since a sensory threshold for the *H. axyridis*-related taint does not exist.

Here the sensory threshold for *H. axyridis*-related taint in winegrapes was estimated using artificial infestation of *H. axyridis*-free grapes with live *H. axyridis* adults and a sensory panel. This sensory threshold can be interpreted as an action threshold for *H. axyridis* in winegrapes (i.e., number of *H. axyridis* per kg of grapes where action or

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management options are taken). Action thresholds and sampling plans (Galvan et al. 2007) provide the tools to estimate the level of insect population density at which control decisions should be taken.

Materials and Methods

Insects. *Harmonia axyridis* adults were collected during August 2005 in soybean fields at the Rosemount Research and Outreach Center, University of Minnesota, Rosemount. Following collection, adults were held in 1.96-L plastic dishes with ~100 beetles per dish, and maintained at 10 ± 1ºC and a photoperiod of 16:8 (L:D) hr. Three days before use, the dishes containing *H. axyridis* were warmed to 25 ± 1ºC with a photoperiod of 16:8 (L:D) hr. *Harmonia axyridis* adults were provided an *ad libitum* supply of live soybean aphids, *Aphis glycines* Matsumara, pea aphids, *Acyrthosiphon pisum* (Harris), and water in cotton balls placed in plastic Petri dishes (60 mm x 15 mm).

Grapes. In September 2005, 147 kg of Frontenac winegrapes were harvested from a commercial vineyard considered free from *H. axyridis* in Lake City, MN. The grapes were hand-picked and 7 kg of grapes were placed into a 22-L polycarbonate container and transported to the Enology Laboratory at the Horticultural Research Center, University of Minnesota, Chaska, where all clusters were carefully hand-sorted and any *H. axyridis* or other lady beetles were removed.

Infestation to determine reflex bleeding effects. Artificial infestations of the grapes with *H. axyridis* were prepared by adding 3.0 *H. axyridis*/kg grapes to 22-L polycarbonate containers containing 7 kg of grapes. Three container replicates of each treatment were prepared. The containers were closed, inverted, and rolled for 45 sec to mimic the disturbance that might be expected during grape harvest. Beetles were then removed from the containers and grapes were processed and winemaking was started using standard microvinification techniques. Wine was processed separately for each replicate. The effects of reflex bleeding in the wine taste were compared to the control (i.e., no beetles or reflex bleeding) using a chi-square test.

Infestation to determine sensory threshold. Artificial infestations of the grapes with *H. axyridis* were prepared by adding various densities of beetles to 22-L polycarbonate containers containing 7 kg of grapes (Table 1). Three container replicates of each treatment were prepared. The containers were closed, inverted, and rolled for 45 sec to mimic the disturbance that might be expected during harvest. The grapes with *H. axyridis* adults were then processed and winemaking was started using standard microvinification techniques. Wine was processed separately for each replicate. During the racking process *H. axyridis* were removed from the treatments.

Wine processing. The wine was prepared using microvinification procedures. There were 21 batches of wine; one for each replicate treatment combination (Table 1). Crushing and destemming was done mechanically in each 22-L polycarbonate container. Fermentation was conducted at 21°C until dryness in containers approximately 2/3 full. All wine batches were inoculated with Pasteur Red yeast (Red Star, Milwaukee, WI) at a rate of 265 mg/L. After 5 days, wine was racked to glass carboys to finish fermentation. Malolactic culture (CH 35; Chr. Hansen, Hørsholm, Denmark) was added toward the end of fermentation to reduce acidity. After malolactic fermentation completion, sulfur dioxide was added at 50 mg/L. Wine batches were racked to full containers and cold stabilized for two weeks. Each replicate was assessed for pH using an Accumet pH meter (Fisher Scientific, Pittsburgh, PA), sugar content using refractometer, and titratable acidity using standard NaOH titration. Finally, the wine was bottled in standard 350-mL splits with natural corks and stored at 21°C.

Table 1 Artificial infestation levels of *Harmonia axyridis* in Frontenac winegrapes and results of 3-AFC tests, 2005.

H. axyridis (/kg grape)	Correct 3-AFC[a]	*p* value (χ^2)[b]
0.0	-	-
0.3	0	0.99
0.5	1	0.72
1.0	2	0.35
3.0	5	<0.01
8.0	8	<0.01
3.0[c]	1	0.72

[a]As a result of correct identification of the same sample in two different randomized sets by the same subject (n = 11).

[b]Chi-square test comparing observed and expected number of correct 3-AFC tests ($p < 0.05$).

[c]*H. axyridis* were removed before crushing/destemming (i.e., beginning of wine processing).

Tasting panel. Panelists (n = 11) were recruited from the University of Minnesota, St. Paul. They evaluated the wines in individual booths, during four sessions, from 1900 to 2100 hr, at the Sensory Center, Department of Food Science and Nutrition. Panelists consumed red wine at least once per month. In a preliminary screening test 10 of the panelists correctly identified the odd sample in three of five triangle tests (i.e., comparing two very similar red wines), while one panelist correctly identified two of five triangle tests.

A series of three-alternative forced choice (3-AFC) tests (Meilgaard et al. 1999) were used for sensory threshold determination. Panelists participated in four sessions, two sets of two sessions one week apart. Samples were randomly assigned to either the first or second week. The serving order of the samples was randomized within each session. After use, each wine bottle had its headspace filled with nitrogen and was refrigerated between replicate days.

At each session panelists were first served "warm-up" samples (O'Mahony et al. 1988) consisting of 30 mL of the control wine and 30 mL of the wine from the treat-

ment with 8.0 *H. axyridis*/kg grape. The samples were labeled as "no taint" and "tainted," and panelists were asked to taste the samples back and forth five times or until they could perceive the difference.

After warm up, panelists received the 3-AFC tests. For each test, they were given two samples of the control and one of a tainted sample (15 mL of each sample in a wineglass). The instructions for each test were to evaluate each of the three samples in each set and identify the sample containing the most taint, taking into consideration all characteristics of the samples (smell, taste, texture, etc.). Panelists were asked to expectorate the samples and thoroughly rinse their mouths with water between all warm-up and the test samples. During the first session, panelists were given three 3-AFC tests, one each from the treatments that were infested with 0.3, 1.0, and 3.0 *H. axyridis*/kg grape. The latter treatment had beetles removed before crushing and destemming. The second session was a replicate of the first session. For the third session, the panelists were given three 3-AFC tests from the treatments that were infested with 0.5, 3.0, and 8.0 *H. axyridis*/ kg grape. (The 3.0 *H. axyridis*/kg grape tested in this session did not have the beetles removed before crushing and destemming.) The fourth session was a replicate of the third session.

Threshold determination. A correct response was recorded if the panelist correctly identified the tainted sample in both replicates. In a single 3-AFC test, the probability of correctly identifying the tainted sample would be 1/3 by chance alone and 1/9, or 11%, when the judge had to identify the correct sample in two different randomized sets (replicates).

Logistic regression (PROC LOGISTIC; SAS software) (SAS Institute, Cary, NY) was used to relate the proportion of correct 3-AFC tests to infestation level. In logistic regression, $\ln[p/1-p] = \alpha + \beta x$, p was the percentage of subjects who got the test correct, including those who got it correct by chance, x was the number of *H. axyridis* per kg of grapes processed in making the wine, α was the intercept, and β was the slope. The probability of correct answers, $P(x)$, for a given x, number of *H. axyridis* per kg of grapes, was estimated solving the logit probability function, $P(x) = e^{(\alpha+\beta x)}/(1+e^{(\alpha+\beta x)})$. The e in the equation is the base for natural logarithms (approximately equal to 2.71828). The 95% confidence intervals of the predicted correct answers were determined using:

$$P(x) \pm z_{1-a/2} * SE$$

where $P(x)$ is the estimated logit probability function for a given x (number of *H. axyridis* per kg of grapes); z is the value of probability for a normal distribution for a given a (significance level); and SE is the standard error, which is determined by the following equation:

$$SE = \left[s_\alpha^2 + x^2 * s_\beta^2 + 2*x*\mathrm{cov}_{\alpha,\beta}\right]^{1/2}$$

where s_α^2 is the estimated variance of α (i.e., intercept), s_β^2 is the estimated variance of β (i.e., slope), and $\mathrm{cov}_{\alpha,\beta}$ is the estimated covariance of α and β. Finally, we solve the logit probability functions, $P(x_1) = e^{(x1)}/(1+e^{(x1)})$ and $P(x_2) = e^{(x2)}/(1+e^{(x2)})$, where x_1 and x_2 are the lower and upper limits of the confidence interval, respectively (Agresti 2002).

The logistic regression equally was also used to estimate the infestation level (i.e., number of *H. axyridis* per kg of grapes) and 95% confidence intervals for a given probability of correct answers (Stokes et al. 2000). The predicted infestation levels were estimated by solving the logistic model for a given P, and its 95% confidence interval was estimated as:

$$x(P) \pm z_{1-a/2} * SE$$

where $x(P)$ is the estimated number of *H. axyridis* per kg of grapes for a given P (probability of correct answers); and SE is the standard error:

$$SE = x(P)^2 * \left[\frac{s_\alpha^2}{\alpha^2} + \frac{s_\beta^2}{\beta^2} - \frac{2*\mathrm{cov}_{\alpha,\beta}}{\alpha*\beta}\right]^{1/2}$$

Using this method, we estimated four infestation levels of *H. axyridis* by solving the logistic regression for $p = 0.12$, 0.20, 0.33, and 0.55. These are the values when 1, 10, 25, and 50% of the subjects got the test correct, without guessing, and the remaining panelists guessed and were correct 11% of the time.

Historically, thresholds are defined as "a stimulus intensity that will produce a response in half the population" (Bi and Ennis 1998). For sensory threshold determination, the 50% of correct answers is taken in addition to the percentage of correct decisions that occur by chance (Meilgaard 1991). By this definition, the sensory threshold would be calculated by solving the regression equation for concentration when p is set to 0.67 for a 3-AFC test with 33% of correct answers by chance (Meilgaard 1991). However, sensory threshold can be used to estimate thresholds of interest other than the ones determined by the 50% of correct answers above chance. For example, thresholds can be determined where individuals can detect off-flavor or taint 90% of the time or off-flavor and taint can be detected by 1% of a population (Meilgaard 1991, Bi and Ennis 1998).

Results

The presence of *H. axyridis* adults during crushing and fermentation did not affect alcohol content (12.2–12.97% v/v), pH (3.49–3.6), and tartaric acid (7.06–7.53 g/L) levels at bottling ($p > 0.05$). In the experiment that tested the effects of reflex bleeding in the wine, no significant differences were found between the control and the reflex bleeding-treated grapes ($p = 0.72$). Therefore, reflex bleeding from 3.0 *H. axyridis*/kg grape is not enough to taint the wine. However, panelists were able to identify the tainted samples of wine from treatments infested with

3 and 8 *H. axyridis*/kg grape according to a chi-square statistic at $p < 0.05$ (Table 1).

The logistic regression model, $\log[p/1-p] = -2.2116 + 0.44x$, fitted the probability of correct answers and the number of *H. axyridis* per kg of grapes very well according to the Deviance and Pearson's statistics ($p > 0.05$). The 95% confidence interval for the predicted probability of correct answers and the observed data are shown in Figure 1A. For example, for an infestation level of 1 *H. axyridis*/kg grape, the estimated probability of correct answers is 0.14 with the 95% confidence interval between 0.06 and 0.29. That is, there is a 95% chance that 6 to 29% of wine consumers will notice the taint in a wine with an infestation level of 1 *H. axyridis*/kg grape. Another characteristic of the logistic regression is the possibility to estimate the rate of increase in the probability of correct answers by an increase of one-unit in the infestation level. This rate of increase is determined by the odds ratio, which is 1.55 for the logistic model, meaning that the probability of correct answers will increase 1.55-fold for each adult *H. axyridis* added to a kg of grapes. For example, if the probability of a correct answer is 0.14 at 1 *H. axyridis*/kg grape, the probability will be 0.22 at 2 *H. axyridis*/kg grape.

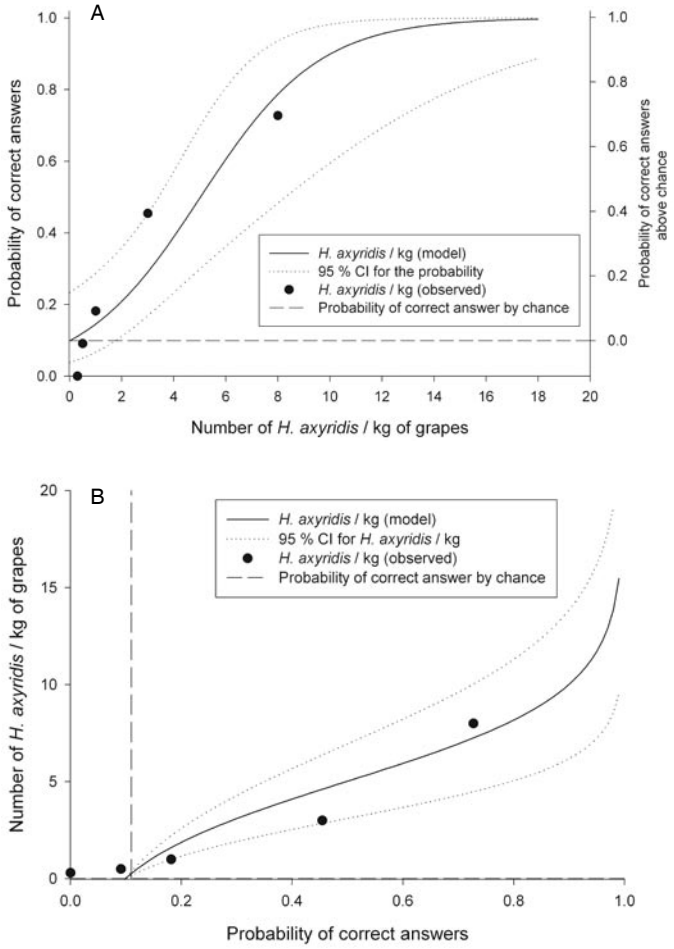

Figure 1 Logistic regression, $\log[p/1-p] = -2.2116 + 0.44x$, fitting the probability of correct answers (p) by the 3-alternative forced choice (3-AFC) test and the number of *H. axyridis* per kg of grapes (x). Deviance ($\chi^2 = 4.3361$, df = 3, $p = 0.2274$) and Pearson ($\chi^2 = 3.2479$, df = 3, $p = 0.3550$) statistics for the logistic regression model. Dotted lines represent the 95% confidence interval (CI) for the (**A**) predicted probability of correct answers and for the (**B**) predicted number of *H. axyridis* per kg of grapes.

The ability to estimate the proportion of wine consumers who will notice the *H. axyridis*-related taint is an important pest-management tool. For example, if a winemaker knows the *H. axyridis* infestation level and via the logistic model is able to estimate the probability of wine consumers that may notice the taint, then a "remediation" procedure can be used to decrease the taint. The remediation procedure could be to mix multiple wine batches with different infestation levels.

Alternatively, the ability to predict the number of *H. axyridis* per kg of grapes for a given probability of correct answers (Figure 1B) may be even more interesting from a pest-management perspective. Such estimations could help grapegrowers in making management decisions to prevent *H. axyridis* populations from reaching a predicted sensory threshold. In this case, the sensory threshold can be interpreted as the action threshold for *H. axyridis* management in the vineyard before harvest (i.e., number of *H. axyridis* per kg of grapes) since the grower is using the sensory threshold to determine when decisions should be made, thus preventing the population density from reaching undesirable levels and tainting wine. For example, the logistic regression model was used (Figure 1B) to estimate the sensory thresholds for *H. axyridis* per kg of grapes and the associated 95% confidence intervals when 1, 10, 25, and 50% of consumers would notice the taint (Table 2).

Discussion

In this study, sensory thresholds were estimated for *H. axyridis*-related taint for selected probabilities of correct responses. Such data analysis is more realistic for grapegrowers and winemakers than the traditional method of determining sensory thresholds. According to the traditional method, if 50% of a population can notice a change

Table 2 Predicted values of *Harmonia axyridis* in Frontenac winegrapes, 2006.

Correct answer above chance (%)	*(H. axyridis/* kg grape)[a]	95% CI[b]	*(H. axyridis/* cluster)[c]	95% CI[c]
1	0.5	0.3–0.7	0.07	0.04–0.1
10	1.87	1.16–2.59	0.27	0.16–0.37
25	3.42	2.11–4.72	0.49	0.3–0.67
50	5.48	3.39–7.58	0.78	0.48–1.08

[a]Predicted infestation levels estimated by solving the logistic model, $\log[p/1-p] = \alpha + \beta x$, for p is equal to 0.12, 0.20, 0.33, and 0.55. These values correspond to 1, 10, 25, and 50% of subjects answering the test correctly without guessing, respectively.

[b]95% confidence interval was estimated using the method described by Stokes et al. (2000).

[c]Assuming an average of 7 clusters/kg Frontenac grapes. Thus, we divided the number of *H. axyridis*/kg grape and its CI by 7.

in wine taste that results from *H. axyridis*-related taint, and if the taste change results in consumer rejection of that wine at similar proportions, then grapegrowers and winemakers could be losing 50% of their customers. The logistic model allows for the determination of sensory thresholds based on *H. axyridis* per kg of grapes when 1, 10, 25, and 50% (or any value between 1 and 99) of the population notice the taint, thus providing more flexibility and greater sensitivity.

Determination of sensory thresholds for *H. axyridis* in winegrapes is crucial for the implementation of pest-management tactics. To date, growers of red grape varieties in the Great Lakes region, where *H. axyridis* is a problem, base their management decisions on sensory thresholds suggested by grower associations and/or wineries, which have set the threshold at approximately one *H. axyridis* per cluster. Our results show that sensory thresholds could be much lower than one *H. axyridis* per cluster, depending on grape variety, wine style, and the grower's risk perception. Since each winegrape variety has unique physical and chemical characteristics, each will probably have its own sensory threshold. For example, the estimated threshold of *H. axyridis* in the white grape variety Riesling was ~0.2 *H. axyridis*/cluster (Pickering et al. 2006).

Discrimination tests, such as the 3-AFC, have been used for determination of sensory thresholds of added substances in food, beverages, cosmetics, paints, and solvents (Meilgaard et al. 1999). Logistic regression has also been used for the statistical analyses of results from 3-AFC tests, and it has been incorporated as a standard procedure for use with sensory thresholds (Meilgaard 1991). For example, the 3-AFC test and logistic regression have been used to determine the sensory threshold of different levels of diesel in fish (Davis et al. 1992) and diacetyl in beer (Kluba et al. 1993). The present study is the first to combine 3-AFC tests and logistic regression to estimate sensory thresholds for an insect pest.

Conclusions

We found that three live *H. axyridis* adults per kg of grapes were not enough to taint the wine if beetles were removed before crushing and fermentation. However, when the same number of beetles were crushed and incorporated in the wine process, trained panelists noticed the *H. axyridis*-related taint. In addition, we developed a logistic model based on the tasting panel results to estimate the level of *H. axyridis* infestation for a given percentage of people who would be able to notice the *H. axyridis*-related taint. These sensory thresholds can be interpreted as action thresholds for *H. axyridis* in winegrapes and can be used in conjunction with the appropriate sampling methods for Frontenac grapes. Finally, additional work should be done to develop similar models for other winegrape varieties commonly grown in the Great Lakes region.

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