Mitigation of infestations of multi-coloured Asian lady beetle in Ontario vineyards

Final Report

Project Number: Activity 18b
Applicant Name: Wendy McFadden-Smith
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Program coordinator: Wendy McFadden-Smith

Executive Summary
Multicoloured Asian lady beetle (MALB) is a serious pest in wine and juice grapes in North America as beetles enter vineyards in the autumn and are harvested along with the grapes. When disturbed or crushed during grape processing, MALB release methoxypyrazines that taint the juice. If a single MALB is observed in a vineyard at harvest, many wineries require that it be treated with insecticides. Methods for controlling MALB in vineyards are largely limited to the use of insecticides. The goal of this research project is to evaluate alternative methods for removing MALB from vineyards and harvested material. Before harvest, behavior-modifying chemicals, such as repellents, can be used to discourage MALB from aggregating in vineyards. Alternatively, removal of MALB during sorting is a possibility and technologies exist that promise to do so, but these have not been thoroughly evaluated for MALB or their impact on methoxypyrazines.

3. Detailed Description of the Project

a) Objective and Project Input

Objective 1: Determine whether repellent treatments applied in the vineyard affect vinification or wine sensory profile.

Three repellent chemicals were tested to determine their potential effect on wine sensory profile. Each chemical was applied prior to harvest in October 2019, and wine was made from the treated grapes One row of grapes was donated by a grower. Research assistants applied sprays and hand harvested the grapes. Research assistants also de-stemmed and pressed the grapes and put the juice through the vinification process to create wine. A government issued vehicle was used for the transportation of grapes from the harvest site to the processing site, as well as from the processing site to the storage site.

Objective 2: Evaluate optical sorting to remove MALB from harvested grapes and reduce methoxypyrazines from juice.

In November 2019, an optical sorter was evaluated for efficacy in removing MALB from commercially harvested fruit infested with MALB. Material was collected from multiple points along the processing and sorting equipment. Collected material was examined for the presence of MALB and then juice was analyzed for methoxypyrazines.

This project involved in-kind participation of the owner and the operator of the sorter as well as in-kind donation of fruit. The operator ran the optical sorter and winery staff helped with the collection of material. Research assistants examined collected fruit for the presence of MALB and pressed the material. This project required MALB for infesting fruit. Research assistants were responsible for collecting MALB from the wild; as MALB populations levels were low in 2019 this task required a significant amount of time. Furthermore, research assistants also collected aphids from the wild to maintain captured MALB. An OMAFRA vehicle was used for all travel involved in the project (in-kind). Pressed juice samples were submitted to CCOV lab for testing for methoxypyrazines (fee service).

b) Project Activities and Outputs

Objective #1:

One row of Chardonnay was allotted for use and split into four plots for the three treatments plus the untreated control plot. Treatments included pine oil, basil oil and Buran (Table 1). A 1-panel buffer was left between treatments to minimize the effect of spray drift. The grapes were sprayed on 23 Oct. and harvested 24 and 48 hours after spraying to determine the residual effect on vinification and flavour profile of the wine. Each treatment was applied on 3 panels; 1.5 panels were harvested at 24 hours post-spray and the other 1.5 panels were harvested 48 hours post-spray. Treatments were applied at 800 L/ha using a backpack sprayer. The spray covered approximately 1 m width of the canopy, including the entire fruiting zone. No precipitation occurred between spraying and final harvest.

The grapes were processed separately by treatment and time before harvest. The grapes were put through a crusher-destemmer and then transferred into the press. Each load was pressed four times so that the grape skins were nearly free of juice. The crusher-destemmer and press were rinsed down with water between treatments to prevent the cross contamination of products. The juice was transferred directly into 19L plastic pales and sealed. Four days after being pressed, the juice was racked off the gross lees and inoculated with yeast. The vinification rate was monitored by testing for brix and temperature through the fermentation process (Figure 1). On 15 Nov primary fermentation was complete and the wine was racked into 1 gal glass carboys where they were stored to complete fermentation. On February 3, the wine was racked and bottled in 375mL glass bottles and corked with synthetic cork and stored for future tasting.

Table 1. Products and rate applied

Product	**Manufacturer**	**Formulation**	**Tested rate**
Buran	AEF Global	Suspension	29mL/400L
Pine oil	AEF Global	Suspension	1mL/1L
Basil oil	Sigma Aldrich	Suspension	0.56% v/v

None of the treatments had a significant effect on the rate of fermentation and all wines reached 0 Brix by Nov 15.

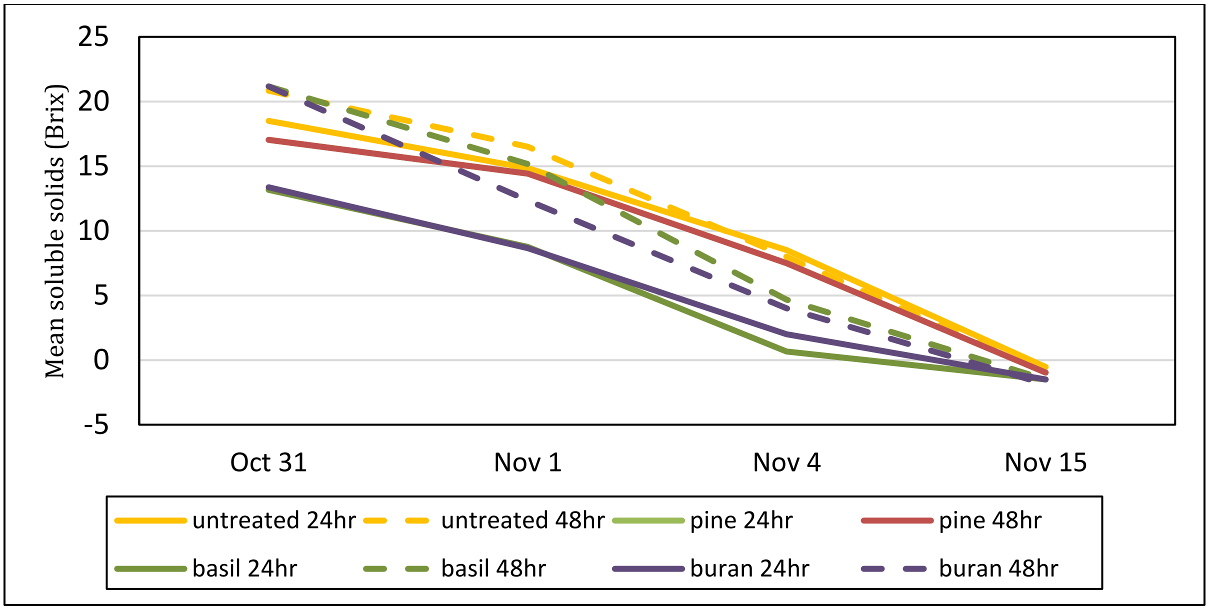

Figure 1. Vinification curves for repellent treatments and untreated harvested 24 and 48 hours post treatment (means of 3 replicates).

Objective #2:
MALB were collected from the Niagara region during the fall of 2019. Beetles were held in mesh cages outdoors and fed a mixture of aphids and grapes.

Cabernet Franc was machine harvested on 20 Nov, 2019. Multiple bins were delivered to the crush pad and one bin was randomly selected for experimentation. From this bin, harvested material was removed using a shovel and 93-94 kg was placed into four bins. Simultaneously, 6-7 kg of harvested material was placed into four buckets which were inoculated with 30 live MALB. Buckets were sealed and then vigorously shaken for 15-20 seconds to agitate MALB. Buckets were emptied into bins so the total weight of each bin was 100 ± 1 kg and the harvested material was thoroughly mixed together. Subsequently, a sample (1-2 L) was collected from each bin. Bins were covered with a lid and left on the crush pad for 30-40 mins before sorting. Immediately before sorting a sample (1-2 L) was collected from each bin.

Harvested material was processed at Vineland Estates using a Pellenc sorter, de-stemmer, and Selectiv' Process Vision 2. The equipment settings were set by the operator as per their normal operations (Figure 2). Bins were emptied into a semi-elevated hopper (Sthik). During the sorting process, material was collected in heavy duty plastic bags at multiple points (C - H, Figure 3). Collection bags were changed after each replicate. A replicate was considered to be fully processed when no more material passed through the optical sorter. The weight of material collected from each point, C to H, was measured using the crush pad scale.

Collected material was kept in a cool area and manually inspected for the presence of MALB. On 22 Nov, fruit from each station was pressed in the sample bags, except for station E (post mechanical sorter) which contained only rachises, and a 60 mL sample was collected and immediately frozen.

The number of MALB per collection point was determined and then the fruit was pressed with a small bladder press to 2 B. A 0.5 mL sample of juice was collected and submitted to CCOVI lab services for analysis of methoxypyrazines using headspace SPME GC-MS and a stable isotope dilution assay.

Figure 2. The operator set the parameter of the Pellenc sorter, de-stemmer, and Selectiv' Process Vision. These parameters were the same as normal operations.

Figure 3. Sampling points: A not shown; B = inoculated grapes in bin (pre-sorting); C = free run juice collected from hopper; D = waste from crusher; E = post mechanical sorter solids; F = post mechanical sorter liquids; G = post optical sorter, rejected; H = post optical sorter, accepted; G vs H = post optical sorter rejected (left) and accepted (right)

Prior to inoculation, no MALB were observed in the bin as a result of being collected during harvest. Before sorting, no MALB were observed on the bin walls or lids. The number of MALB recovered from collection points is shown in Table 1. The highest number of MALB were found in points B (inoculated grapes in bin) and G (post-optical sorter, waste). Overall, the number of MALB recovered after sorting was very low (19 of 120; Table 1). Of the material that reached the optical sorter, 95-96% of the material was accepted (Table 2). The material that was rejected represents a small amount of the initial weight (100 kg) but it contained a between 1-2 MALB, which represents a significant proportion of the recovered MALB.

Table 2. Number of MALB recovered from collection points in 2019. Each bin initially contained 100 kg of harvest material and 30 live MALB.

Collection point	Point description	Rep 1	Rep 2	Rep 3	Rep 4
A	Uninoculated grapes in bin	0	0	0	1
B	Inoculated grapes in bin (30-40 min)	0	4	0	2
C (HOPPER)	Free run after hopper	0	1	0	0
D	Entering crusher	0	0	0	0
E	Destemmer	0	0	0	0
F	Pre-optical sorter	0	1	1	0
G	Post-optical sorter, waste	1	1	2	2
H	Post-optical sorter, keep	1	0	2	0
Total		2	7	5	5

Table 3. Weight of material rejected and accepted by the optical sorter.

Rep	G point (rejected by optical sorter) (kg)	H point (accepted by optical sorter) (kg)	% of material accepted by optical sorter
1	2.5	59	96%
2	3.0	58	95%
3	2.5	51.5	95%
4	2.5	53.5	96%

Chemical analysis of pressed juice from collection point B showed that the level of IPMP was below the reliable quantitation limit of 4 ng/L. This result was disappointing as we anticipated that this collection point would have high levels of IPMP given the inoculation rate of 30 MALB per 100 kg fruit and the agitation that we did to the MALB in the buckets before inoculation. Given these results, chemical analysis was not performed on juice samples from the other collection points.

The primary challenge we experienced with this project was limited access to the sorting equipment. For example, due to scheduling challenges, it was not possible to do the trial on multiple varieties, including whites. It was also not possible to examine the equipment to look for unrecovered MALB. As previously mentioned, across all replicates only 16% (19 of 120) of MALB were recovered. This poor recovery makes it difficult to evaluate the effectiveness of the optical sorter. We suspect that the majority of MALB remained in the sorting equipment as we observed a significant amount of grape material stuck throughout the machine after each replicate was processed (Figure 3). The volume of each replicate (100 kg of fruit) is relatively small considering the size and processing capacity of the equipment.

Figure 4. Grape material left on the sorting line after the machine was turned off.

In total, the optical sorter removed 6 MALB (1-2 per rep) (point G) but 3 MALB, 1 in one rep and 2 in another, successfully passed through (point H). MALB that passed through the optical sorter may have appeared similar to the “desired” berry. Removing MALB from red grape varieties may be challenging given their similarities in shape, size and colour. We believe that the optical sorter would be more effective at removing MALB from white varieties when the colour contrast is greater; however, we were unable to test this hypothesis due scheduling challenges.

Based on published literature on MALB-wine taint, bins were inoculated with 30 MALB and this level should have resulted in measurable levels of IPMP in juice; however, analytical results failed to detect IPMP. We experienced the same problem in 2018 when bins were inoculated with 20 MALB/100 kg fruit. In this trial (2019), we increased both the number of MALB and the amount of agitation to get detectable levels of IPMP in the juice; however, this was not successful.

c) Reach and Communication

As the project objectives are to mitigate the economic impact of MALB on the grape and wine industry, the primary target audience is grape and wine producers. Additionally, those that support producers are a target audience, including crop advisers, government scientists, and academic researchers. Communication activities are described in the table below

Reach and Communication Table	
Activity	Communication details
Event: Ontario Fruit and Vegetable Conference (OFVC) Location: Niagara Date: February 19-20, 2020	A poster titled, "Evaluation of Compounds for Repellency of the Multicolored Asian Lady Beetle in Vineyards" was presented at OFVC. The poster presented the highlighted the discovery of repellent compounds for MALB and demonstrated their level of control in the field. In 2019 more than 3,000 people attended the OFVC The target audience for this poster was Ontario grape growers. OGWRI was acknowledged as providing financial support for the project in the acknowledgments section.
Event: scientific manuscript Journal: Pest Management Science Date: manuscript in preparation	A manuscript detailing the extensive laboratory and field trials has been prepared and is being submitted to the journal Pest Management Science. This is an international journal of research and development in crop protection and pest control. This journal is considered a premier forum for papers on the discovery, application, and impact on the environment of products and strategies designed for pest management. The target audience for this manuscript are those involved in pest management research. OGWRI will be acknowledged as providing financial support for the project.

4. Project Outcomes

a) Short-term

The expected short-term outcomes were to identify and evaluate alternative methods (i.e. non-pesticide) for controlling MALB in vineyards in order to minimize the potential for beetle-wine taint. From the outset of this project, we planned to test natural compounds for repellency to MALB and, secondly, to evaluate mechanical options for removing MALB from harvested fruit. After the completion of this study, we can conclude that the actual short-term outcomes were partially successful.

Research activities associated with identifying repellent compounds have exceeded the expected outcome as many of the products tested successfully repelled MALB in the laboratory. Many of these compounds were evaluated in the field in 2017 when high MALB populations permitted this testing.

However, low MALB populations in 2018 and 2019 prevented us from performing further field trials. As a result, some of the compounds identified as repellent in the laboratory were not tested in the field. From a scientific perspective, this was disappointing, but we recognized this potential limitation before the project started. Nevertheless, we have discovered many products that are repellent to MALB that could be used for their control.

Research activities focused on evaluating mechanical options for removing MALB from harvested fruit did not reach the expected outcome. In this project (2017-2019), we performed trials on optical sorting equipment and on harvesters that remove MALB during harvest. These trials aimed to measure the effect of sorting on the presence of MALB as well as IPMP in juice. In both cases, our evaluation of these mechanical options was not comprehensive as we received limited access to the equipment due to the busy schedules of operators. As a result, we are unable to make conclusions about the effectiveness of these technologies.

b) Long-term

The expected long-term objective of this study was to reduce the use of broad-spectrum pesticides to control MALB by providing grape growers with alternative control options. The long-term success of this project can be measured by a few key indicators; (1) products that we identified as repellent to MALB are registered for use on MALB in vineyards in the future; (2) grape growers in Ontario increasingly manage MALB infestation using integrated pest management, which can be determined in several ways, including grower surveys and pesticide usage data.

Currently, there is a strong reliance on broad-spectrum insecticide for controlling MALB just before harvest. The adoption of alternative control methods for MALB will likely reduce both environmental and health risks associated with broad-spectrum insecticides.

c) Doing things differently

Based on the optical sorter results from trials in 2018 and 2019, it would have been beneficial to increase the number of MALB added to bins to obtain detectable levels of IPM in juice. We used published literature on MALB-wine taint to determine inoculation levels for this trial; however, the number of MALB was evidently too low. The reason for this discrepancy from the published literature is not known.

As previously described, a challenge experienced with this project was getting permission to use optical sorting equipment. This technology is not common in the Niagara region so our opportunity to use it was limited. During the harvest season we maintained close contact with the winery and ensured that we were ready to perform our research trial at the winery's earliest convenience.

Field trials relied on natural MALB populations. Unfortunately, MALB populations were very low, not just in Ontario, but also in Quebec and Nova Scotia. There is no way to predict the population of MALB in advance of harvest. The only way to rectify this would be to extend the project to increase the likelihood of having a high MALB year.

5. Final Comments and Conclusion

From the onset of this research the project there were 3 activity objectives:

1. Evaluate the effectiveness of natural compounds for managing MALB infestations
2. Evaluate the efficacy of the optical sorter on harvesters for removing MALB in the vineyard
3. Development of best management practices for managing MALB in Ontario vineyards

Activities related to objective 1 were pursued in every year of this research project, 2017-2019. We believe that these activities fulfilled the original project plan. Furthermore, given the promising results, we decided to perform another trial to determine whether wine made from grapes sprayed with repellent compounds have an altered sensory profile. The wine tasting panel planned for 2020 will be used to determine this. This extra trial will provide valuable information on the effect of repellents on wine quality, which is critical information for developing best management practices.

Activities related to objective 2 were pursued in every of this project (2017: Opti-combine trials; 2018/2019: optical sorting equipment). These activities did not deviate from the project plan. Unfortunately, our investigation of these technologies does not allow us to provide recommendations on their effectiveness. We believe they have the ability to remove MALB and potentially reduce IPMP concentrations, but we are unable to demonstrate.

The completion of objective 3 is largely dependent on the successful completion of objectives 1 and 2. Objective 3 was expected to be completed in 2020. The completion of the wine tasting panel in 2020 should allow us to provide management practice recommendations for MALB using repellents.

This project did not experience any unforeseen budget or scheduling issues.