AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE:
Determination of the occurrence of select pesticides and metabolic derivatives in finished wine, juice, nectar (bee and flower), and pollen

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SUMMARY:
A direct large volume injection high performance liquid chromatography – tandem mass spectrometry method was developed and validated for the quantitative determination of 16 systemic insecticides and their main plant metabolites. The assays were conducted on commercial samples of convenience from the United States and other countries. Using a 1:20 dilution and an injection volume of 800 µl, a limit of quantitation of 1 $\mu g\ L^{-1}$ for all compounds was achieved. Mass spectrometric detection was performed using multiple reaction monitoring and two specific transitions for each compound. Recoveries ranged between 71 and 120%. Precision, calculated as percent relative standard deviation (%RSD), ranged from 1 – 21%. Imidacloprid and methoxyfenozide were the most frequently detected parent insecticides in the wines reaching concentrations of 1 - 132 $\mu g\ L^{-1}$. The two important plant metabolites imidacloprid-olefin and the spirotetramat-enol were however found at higher concentrations. In five samples spirotetramat-enol was detected in the mg L^{-1} range. Most “organic” wines contained no detectable or low insecticide residues, except for one sample, which gave the highest Imidacloprid (14.7 $\mu g\ L^{-1}$) and Imidacloprid-olefin (331 $\mu g\ L^{-1}$) concentrations observed in this study. The reported concentrations indicate that organic grapes may have been treated with pesticide or came in contact with conventionally-treated grapes. Considering the maximum residue limit definition for the different insecticides, three “conventional” wine samples were non-compliant for Spirotetramat and its metabolite Spirotetramat-enol. This study highlights the importance to determine both parent and metabolite compounds of systemic insecticides in the finished wine product. Although not completed, we have made progress on our second objective, which was to apply the validated method in pesticide-treated grapes and blueberries as well as through the wine-making process.

OBJECTIVES:

The original grant application listed two objectives:

1] to expand and optimize an analytical method for quantifying select systemic and neonicotinoid pesticides and their bioactive forms in wines, juices, nectar (bee and flower), and pollen

2] to apply the validated method in a survey of finished wines made by various manufacturers and is several different years, grape juice, blueberry juice, nectar (bee and flower), and in pollen

PROCEDURES:

For **Objective 1**, a visiting senior scientist, Dr. Jean Daniel, developed and validated an analytical method to quantify Spirotetramat and its 'enol' biological metabolite along with 14 other pesticides and metabolites in wines. The list of pesticides and metabolites included Imidacloprid and its major metabolite Imidacloprid-olefin, a pesticide of concern for the health of bees. The analysis is accomplished by simply diluting wine samples and then directly injecting the mixture into a laboratory instrument (liquid chromatograph coupled with a tandem mass spectrometer detector) that was used to selectively identify and quantify pesticide residues. The accuracy, precision, and sensitivity of the analytical method for the pesticides and their major bioactive metabolites were determined, which informs confidence in the data (data quality). As a measure of accuracy, the recoveries of standard pesticides and metabolites spiked into wine ranged between 71 and 120%. Precision calculated as percent relative standard deviation (%RSD), ranged from 1 – 21%. Quality controls analyses were conducted including the analysis of blanks, wine samples in which the pesticides and their products do not occur. Now that the wine method is validated and 'turn key', the next phase of research will be to validate the approach for the analysis of berries/juices, nectar and pollen.

For **Objective 2**, several activities were conducted. First, the validated method developed for Objective 2 was applied to 61 wine samples, collected out of convenience and not for the purposes of conducting a market basket survey. The most frequently detected parent pesticides, Imidacloprid and methoxyfenozide, reached concentrations of 1 - 132 $\mu g\ L^{-1}$. However, the two metabolites imidacloprid-olefin and the spirotetramat-enol were found at higher concentrations. For example, in five samples spirotetramat-enol was detected in the mg L^{-1} range. Although the majority of organic wines did not have detectable pesticide residues, one organic wine actually gave the highest Imidacloprid (14.7 $\mu g\ L^{-1}$) and Imidacloprid-olefin (331 $\mu g\ L^{-1}$) concentrations. This finding indicates that not all wines labeled as organic can be characterized as having undetectable pesticide residues. A total of three non-organic wines gave Spirotetramat-enol, the metabolite of Spirotetramat, concentrations that were above maximum residue levels. Efforts to identify grower practices that may have led to exceedences are underway.

In a second activity for **Objective 2**, Dr. Walton applied pesticides at recommended application rates at recommended times to grapes and blueberries at the OSU Experimental Lewis Brown Farm. The grape plot that is located in OSU botany farm on Highway 34 was used for the wine residue study. Pinot noir and Pinot gris varieties were used for the wine experiment. Three treatments were applied including Control (water), Spirotetramat (Movento 240 SC, 240 g AI/L, Bayer CropScience), Imidacloprid (Admire Pro 550 g AI/L Bayer CropScience) as foliar application. Samples of foliage, fruit, and flower nectar were taken at various time intervals after pesticide application and are being frozen until analysis using the method developed and validated for Objective 1.

In a third activity for **Objective 2**, wine was produced in cooperation with Dr. James Osborne from the treated grapes and the concentrations of pesticides will be determined in the finished wines. Samples of the treated grapes and the finished wines were taken and are frozen until analysis.

SIGNIFICANT ACCOMPLISHMENTS:

- A visiting scientist, Dr. Jean Daniel Berset, created and validated a quantitative and precise analytical method for the determination of 16 systemic pesticides and their main plant metabolites in finished red and white wines.
- Dr. Berset trained an OSU graduate student, Serhan Mermer, to conduct the analyses. Mr. Mermer will conduct all subsequent analyses related to this project. OSU now possesses the capacity to analyze for 16 pesticides and their metabolites in wines and the method will be adapted to blueberries and cherries.
- A manuscript for publication in a peer-reviewed journal is in its final stage of co-author review. We anticipate submission of this manuscript for publication in February 2017.
- A total of 61 wines from California, Oregon, Washington, Spain, Italy, France, Australia, South Africa, Argentina, and Chile were analyzed for the 16 pesticides and key metabolites. Maximum residue levels were exceeded in three out of 61 commercial wines
- Outreach is being conducted by Dr. Vaughn Walton to determine which grower practices may have led to the high levels of residues in these wines.

BENEFITS & IMPACT:

- The analytical method is innovative and ‘green’ because it minimizes analysis time and laboratory solid and liquid (solvent) waste, while minimizing technician exposure to solvents.
- Publication of a peer-reviewed journal articles on the analytical method and its demonstration on a number of commercial wines will permit transfer of the technology to laboratories that conduct analyses of pesticide and metabolite residues in wine and other commodities.
- We identified that continued vigilance is required to ensure that organic wines are pesticide free (down to the limit of detection)

- Even with grower attention to pesticide applications, we identified that increased vigilance is required to identify and prevent conditions and procedures that lead to pesticide levels exceeding maximum residue levels in finished wines.
- We have engaged the College of Agriculture Communications team (Peg Herring) in order to release the information gathered to date on pesticide and pesticide metabolites in wine. Our team is cognizant of the potential importance of detecting pesticide and pesticide metabolite concentrations that exceed maximum residue levels.
- We are currently training a PhD student using funds from the below mentioned grants.
- The techniques and information from the student's work will enable growers to time the applications of chemicals in order to allow adequate breakdown of residues, thereby resulting in increased market access.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

Dr. Vaughn Walton received a 2-year grant from the Washington Cherry Board titled "Sustainable and rapid-response *Drosophila suzukii* management" that will utilize the analytical capabilities developed as part of this ARF project. Dr. Walton has since received funding from the Oregon Blueberry Commission and United States Department of Agriculture National Institute for Food and Agriculture award #2015-51181-24252, USDA OREI #2014-51300-22238.

FUTURE FUNDING POSSIBILITIES:

At this time, we are currently supporting the above-mentioned grants.