**IMPROVING WINTER AND SPRING VINE MEALYBUG CONTROLS: USING HPLC TO FOLLOW INSECTICIDE MOVEMENT IN THE VINE**

**Summary Report for CDFA Agreement 16-0556-SAFP**


**Principal Investigator:**
Kent Daane, Dept. ESPM, University of California, Berkeley, CA 94720-3114
kdaane@ucanr.edu

**Co-Principal Investigator:**
Sonet Van Zyl, California State University, Fresno, Dept. Viticulture and Enology, svanzyl@csufresno.edu

**Co-Principal Investigator:**
Monica Cooper, UC Cooperative Extension Napa Co., 1710 Soscol Ave, Suite 4, Napa, CA 94559
mlycooper@ucanr.edu

**Cooperator:**
Dr. Andreas Westphal, Dept. Nematology, University of California, Riverside, CA 92521
andreas.westphal@ucr.edu

**Post Doctorate Researcher:**
Dr. Valeria Hochman Adler, Dept. ESPM, University of California, Berkeley, CA 94720-3114 vhochman@ucanr.edu

**Staff Researchers:**
Geoffrey Dervishian, CSU Fresno,
Noemi Fonseca-Espinoza, UC Berkeley


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- The vine mealybug, *Planococcus ficus*, is one of the more important insect pests of California vineyards, and the pesticide Movento® (Bayer CropScience) is one of the better and most often used tools for vineyard mealybugs.

- We used an HPLC to obtain the concentration of the active ingredient of Movento® (Spirotetramat) and two of its primary metabolites, Spirotetramat -Enol (which is the metabolite that kills the mealybug) and Ketohydroxy (a breakdown metabolite that is not active against mealybugs as far as we know). To analyze the quantity of Spirotetramat, Enol and other metabolites in leaves, the extraction method "QuEChERS" (Quick Easy Cheap Effective Rugged Safe) was followed.

- Our first goal was to develop protocols for HPLC analysis of plant material for both Spirotetramat and its metabolic derivative Spirotetramat-Enol. Extraction and pre-HPLC processing of grape leaves were conducted using methodologies based on QuEChERS methodology. For bark tissues, we included additional steps to determine levels of Spirotetramat and its metabolites by placing bark tissue in a solution of 75% methanol and 25% water and then subjecting the solution to agitation in the dark for 3 hrs.

- The residues of Spirotetramat and it metabolites were estimated by HPLC fitted with an auto sampler Agilent 1100 HPLC composed of the following models: degasser G1379A, quaternary pump G1311A, autosampler G1313A, column compartment G1316A and DAD/UV-vis detector G1315A. The data were analyzed using ChemStation version B.04. The LC was fitted with a column Phenomenex Luna 3 µm C18(2), with accompanying guard cartridge of the same material. Column conditions were set to 40°C and peaks were observed at a wavelength of 215 nm after determining elution times using pure analytical standards provided by Bayer Crop Science Limited (USA).

- Leaf tissue analyses show that, in most vineyards, Spirotetramat was quickly (within 5 hr.) converted to the Enol and a portion of the Enol is also rapidly converted to Enol-Glucoside. We note that in some vineyards we found small amounts of Ketohydroxy 1 month after application. It is still unclear (from our studies) if the Spirotetramat found long after the application will eventually convert to Enol, or if this conversion process slows as the material moves from the leaf tissue.

- When looking closer at the amount of Spirotetramat and Spirotetramat -Enol in leaf tissue over the sampling period, it's clear that the amount Spirotetramat is reduced quickly, in one vineyard it dropped from about 100 ppm 5 hrs. after spray to about 40 ppb after 1-3 days, and <5 ppm after 1 month. What is needed now is a field bioassay that compares the amount of Spirotetramat-Enol in the plant to mealybug death (e.g., dose response) and information on how long the mealybug must feed to acquire this lethal dose.

- For many of the studied sites we sampled repeatedly over the year. In one of these vineyards, Spirotetramat

remained in the samples until 184 DAT but was gone 212 DAT from the brown senescing leaves and after a series of rain events. No Movento was applied in 2017 before the 320 DAT sample (9 April 2017), but small amounts of Spirotetramat-Enol were found. From this, we conclude that some amount of Spirotetramat-Enol was stored in the vine roots, trunk or cane and was in measurable levels the following season.

- In another trial, Movento was applied in May (label rate of 8 oz per acre) in a Crimson Seedless block and we recorded a complete conversion of Spirotetramat to the Enol in the leaves within 5 days. Interestingly, the amount of Enol varied among vines and this variation was still seen 72 days after application. There are many explanations for this; for example, we assume that the Spirotetramat and its metabolites move passively in the phloem and are therefore carried to new growing tissue. We do not know yet what dose is needed on different vine sections kill the mealybug and this, we hope will be determined next season. The variation then can be related to the leaves sampled, for example, an older leaf inside the canopy may have more or less Spirotetramat-Enol that a new leaf.

- In most sampled vineyards, we collected leaves from both exposed (sun) and protected (shade) areas. In one of the Thompson Seedless blocks, we show not a great deal of difference between sun and shade leaves for the amount of Spirotetramat, but at the 5 hr. collection there was significantly less Spirotetramat-Enol in the shaded leaves. We assume that there was either faster conversion of Spirotetramat to Spirotetramat-Enol in leaves in the sun (more actively photosynthesizing, or receiving more of the plant phloem), but as Spirotetramat-Enol was moved throughout the vine over time this difference dissolved.

- To determine how quickly the metabolites move to new or untreated leaves, we applied Movento but covered some of the leaves on each treated vine so that the insecticide spray did not contact the leaves directly, and there were a similar number of untreated vines. The bagged leaves were uncovered after the spray application and at 4 DAT leaves were sampled and showed that the amount of Spirotetramat was significantly different from the "bagged" and control treatments; however, the amount of Enol and Ketohydroxy was the same. This result shows that the metabolites are moving with the phloem/xylem from treated to untreated leaves rapidly and transforming from Spirotetramat to Spirotetramat-Enol rapidly as well.

- Movento can be applied up to 12 oz per acre per season but can only be applied up to 8 oz per acre on a single application. For this reason, growers have developed spray patterns from a single April-June 8 oz per acre application, to an early (e.g., April-May) 8 oz and a late (e.g., August) 4 oz rate, to an early and late split of 6 and 6 oz. From our samples, we found similar application rates (8-12 oz per acre per season) would have very different in season (June-September) amounts of Spirotetramat-Enol in the leaves, commonly ranging from 0.1 to 50 ppm. We believe that as yet unknow factors -such as vine physiology – may impact conversion of the active ingredient to the enol form – which is the main killing agent.

- We can report Enol and other 'downstream' metabolites such as the Spirotetramat, Spirotetramat-Enol, Enol-Glucoside and Ketohydroxy were found in the vine trunk, cordon and root tissues, although commonly at a lower amount than in the leaves. The most common metabolite found was the Spirotetramat-Enol. A common observation was that there was about a 10-fold reduction from the roots to the canes, and from the canes to the trunk and roots – for this reason there may be about 100x more Spirotetramat-Enol in the leaves than in the trunk 30-60 DAT.

- Overall, the tissue analyses show Movento metabolites are moving from the leaves to the trunk as expected. Some of the key findings that we will further study are the different rates of conversion from Spirotetramat to Enol in the leaves – in some vineyards nearly 100% conversion and in other vineyards closer to 50% conversion. Perhaps this results from vine leaf condition and age of the leaf, vineyard management practices, or even environmental conditions during application. This is important because it is the Enol that kills the mealybugs; however, the impact of this result is harder to interpret.