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

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ABTRACTs

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. We show that in most vineyards, the April-June applications of Movento result in rapid conversion of the active ingredient Spirotetramat to the metabolite Spirotetramat-Enol (which is the primary killing agent). During most of the growing season the highest concentration of Spirotetramat-Enol is in the leaves, however some material is moved to the trunk and roots. There was an association of higher application rates with higher amounts of Spirotetramat, but not of Spirotetramat-Enol in the vine leaves and trunk. High concentrations of Spirotetramat-Enol in the vine appeared to vary more among vineyard sites than our application methods, suggesting an influence of vine physiology on the uptake and conversation of Spirotetramat to Spirotetramat-Enol in the leaves. In most studies, Spirotetramat-Enol remained in the vine, at least in trace amounts, for at least a year.

LAYPERSON SUMMARY

The vine mealybug has become one of the most important insect pests of California vineyards. Researchers, PCAs and farmers have developed relatively good controls that target exposed vine mealybugs – those on the leaves or canes. However, controlling the more protected mealybug population found under the bark of the trunk or on the roots has been more difficult. Our objectives are to improve pre- or post-harvest controls that target the winter-spring vine mealybug population and to better determine the spring emergence of vine mealybug crawlers to better time foliar applications. Currently, the pesticide Movento® (Bayer CropScience) is one of the better control tools for vineyard mealybugs. Our research sought to determine how to optimize this product, and if we could lower winter-spring mealybug populations by either increasing the dose response during the summer or increasing the amount of material in the vine's trunk during the winter-spring period. We show that in most vineyards, the April-June applications of Movento result in rapid conversion of the active ingredient Spirotetramat to the metabolite Spirotetramat-Enol (which is the primary killing agent). During most of the growing season the highest concentration of Spirotetramat-Enol is in the leaves, however some material is moved to the trunk and roots. There was an association of higher application rates with higher amounts of Spirotetramat, but not of Spirotetramat-Enol in the vine leaves and trunk. High concentrations of Spirotetramat-Enol in the vine appeared to vary more among vineyard sites than our application methods, suggesting an influence of vine physiology on the uptake and conversation of Spirotetramat to Spirotetramat-Enol in the leaves. In most studies, Spirotetramat-Enol remained in the vine, at least in trace amounts, for at least a year.

INTRODUCTION

The vine mealybug, *Planococcus ficus*, has become one of the most important insect pests of California vineyards, threatening economic production and sustainable practices in this multi-billion-dollar commodity. Insecticides are the primary control tool for vine mealybug (Prabhaker et al. 2012, Daane et al. 2013, Bentley et al. 2014), especially when leafroll diseases (GLDs) are a concern (Daane et al. 2013). Researchers, PCAs and farmers have developed relatively good controls that target exposed vine mealybugs – those on the leaves or canes. However, controlling the more protected mealybug population found under the bark of the trunk or on the roots has been more difficult. The vine mealybug population is primarily on the trunk and upper root zone near the soil line during the winter and early spring (Daane et al. 2013). This population has a refuge from natural enemies (Gutierrez et al. 2008) and can be the most difficult to control even with systemic insecticide applications (Daane, pers. obsrv.). Moreover, mealybugs can remain on even the remnant pieces of vine roots after vineyard removal, hosting pathogens and infesting new vines after replanting the vineyard (Bell et al. 2009).

Insecticides with systemic action are the best materials to control this protected population – but their proper use can vary among vineyards and regions. Moreover, vineyards with mealybug damage typically have large overwintering populations that are never fully regulated and can be the source for spring and summer generations that infest leaves and fruit or disperse to other vines and vineyards. Therefore, it is critical to develop better control programs for this overwintering population.

A delayed dormant (typically in February) application of chlorpyrifos (Lorsban®) was the standard post- harvest or pre-season control that targeted mealybugs on the trunk and cordon (Daane et al. 2006). The best in-season insecticide for vine mealybug that move from the trunk and cordon to the leaves, canes and fruit has been an application of Movento® (Bayer Crop Science), with the active ingredient Spirotetramat, which may also help control root feeding nematodes (Mike McKenry, pers. comm.). Still, the effectiveness of any systemic material will depend on application timing, soil moisture, vine condition, age and commodity (for example, post-harvest application timing). Our objectives were to improve controls that target the winter-spring vine mealybug population and to better determine the spring emergence of vine mealybug crawlers to better time foliar applications. Specifically, we followed the movement of Movento® in the vine using an "HPLC" to determine amounts of different metabolites associated with Movento®. For example, two of the questions we plan to address is whether Spirotetramat converts to the metabolite Spirotetramat-Enol (which is the primary toxicant) similarly under different vines condition, such as nutrient status or cultivar and where on the vine the metabolites move to and in what concentration are the metabolites found on different vine sections – such as the leaves versus the roots? We also used our protocols to help confirm the presence of Spirotetramat metabolites in the root system, in support of Dr. Andreas Westphal's work on the impact of Movento on nematodes.

OBJECTIVES

Our overall objective is to improve pre and post-harvest insecticide application to control vine mealybug overwintering population. Understanding the systemic uptake of the pesticide by the vine is vital to improve management decisions.

1) Improve the protocols to determine levels of Spirotetramat and its first metabolite, the Enol form, in vine tissue samples.
2) Investigate the dissipation and transformation mechanisms of the active ingredient of the pesticide Movento after application.

RESULTS AND DISCUSSION

Movento applied in different regions.

Applications of Spirotetramat (Movento, Bayer CropScience) were made in different vineyards, primarily in Fresno County. We sought to apply the pesticide and sample treated vines from vineyards that represented different commodities (table, raisin and wine), irrigation practices, age and cultivar in order to compare vine condition on the uptake and movement of Spirotetramat. We also sampled from numerous 'experimental' vineyard blocks at the Kearney Agricultural Research and Extension Center that represent wine and table grape blocks undergoing studies for nitrogen, irrigation, and wine grape cultivars. Together, the treated vineyards include several factors that could be affecting the pesticide efficiency, such as the age of vineyards, irrigation

type, commodity (table, raisin and wine grapes), the presence of a girdle, and geographical area. On each sample day, 10 g of leaf material, cane material, and upper or lower trunk material was collected (Photo 1), taken to the laboratory and stored at -20°C until it could be analyzed. After initial results showed the high costs per sample and the level of movement to the trunk, we modified the samples to include on leaf and lower trunk material.

Photo. 1: Sampling different vine sections (leaves and petioles, low and high trunk sections, and roots) taking leaf or bark chip samples for HPLC analyses.

To study how the pesticide Movento® moves through the vines, the pesticide uptake and movement of key metabolites in the plant was followed by means of high-pressure liquid chromatograph methodology (HPLC). To better understand our purpose, a description of how Movento® works to kill mealybugs is needed. Spirotetramat is sprayed onto the leaves where it has translaminar activity and gets absorbed. It is not the Spirotetramat that primarily kills mealybugs, but the first breakdown product or metabolite called "Enol" (Fig. 1, from Bayer CropScience). The Enol can change to other metabolites such as Enol-Glycoside and Ketohydroxy as some of the primary metabolites found, but it is the Enol metabolite that is most important for killing the mealybugs. The change from Spirotetramat to Enol appeared to be most effective in the leaf tissue, as described in Bayer-sponsored studies in apple, cotton and other crops. Whereas some translaminar pesticides remain in the leaves, Spirotetramat and its metabolites can be transported by the phloem (and to some extent the xylem) to other plant parts – and this is key in moving the product to where the mealybugs are.

We used the HPLC to obtain the concentration of the active ingredient of Movento® (Spirotetramat) and two of its primary metabolites, Spirotetramat -Enol and Ketohydroxy (the latest metabolite 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. This methodology allows the preparation and analyses of several samples at one time and provides extracts of several structurally different substances with good efficiencies. Afterwards the obtained results are compared to a standard curve created from a known amount of the pure product (Fig. 2A), and from this the presence and amount of each tested metabolite can be determined (Fig. 2B).

Our first goal was to develop protocols for HPLC analysis of plant material for both Spirotetramat and its metabolic derivatives Spirotetramat-Enol. We initially also looked at another 'breakdown' metabolite Ketohydroxy, however this was dropped because of costs and the relatively low levels of other metabolites found. The developed protocols were as follows:

Protocols for Leaf samples. Extraction and pre-HPLC processing of grape leaves were conducted using methodologies based on QuEChERS methodology (Mohapatra et al 2012). Leaf samples (10 g) were ground with dry ice using a mortar and pestle, for about 5 minute or until relatively powdered. After which, the ground leaf samples (10 g) were placed in 50 mL centrifuge tubes (Corning Inc) and 14 mL of gradient HPLC grade acetonitrile was then added and shaken vigorously for 1 min (vortex mixer). Then 4 g anhydrous magnesium sulphate and 1 g sodium chloride were added, and vortex mixed for 2 min and then centrifuged at 10,000 rpm for 10 min. An aliquot (4 mL) of the upper acetonitrile extract was then placed in a centrifuge tube containing 50 mg primary secondary amine (PSA) sorbent and 150 mg anhydrous magnesium sulphate. After which, 30 mg of graphitized carbon black (GCB) Norit (E-345-7 pn) was added and the tubes were shaken vigorously for 1 min and then centrifuged for 10 min at 10,000 rpm. About 2 mL of the supernatant acetonitrile phase was removed, passing through a Millipore 0.20µm filter, and placed in the HPLC vial for processing.

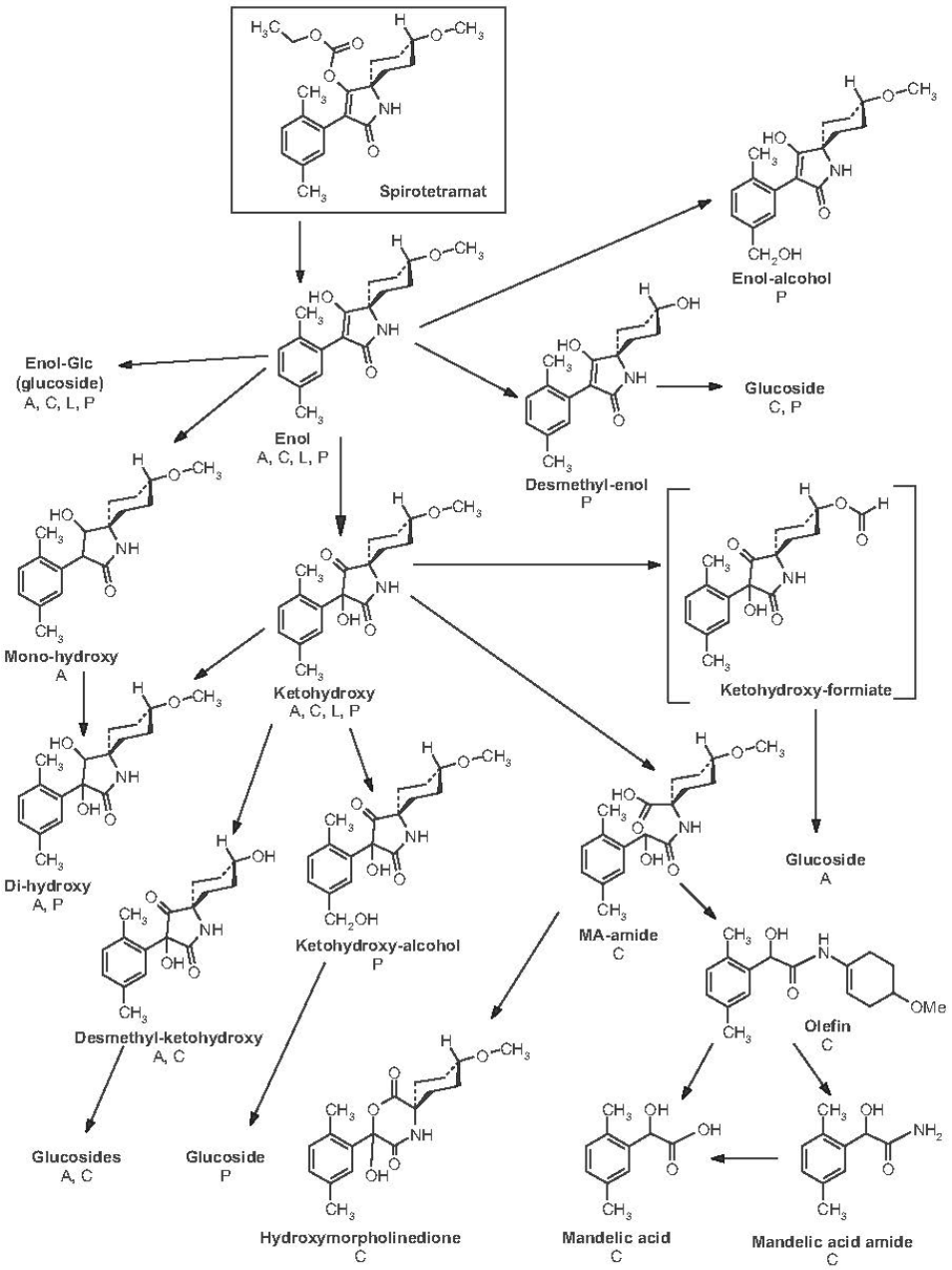

Figure 1. Proposed metabolic pathways of Spirotetramat in targeted crops, from Bayer CropScience Journal 61/2008).

Protocols for Bark samples.

Spirotetramat and its metabolites were extracted from a nominal 0.1g piece of bark tissue. Pieces were weighed using an analytical balance and the actual weight was recorded for downstream calculations. 1 ml of 75% methanol (Fisher Cat. No. A452-4), 25% water (Siemens Labostar Ultrapure Water System) was pipetted into the tube. Samples were extracted on a shaker in the dark at a speed of approximately 66 RPM for 2 hours. After shaking, 0.5 ml of the solution was transferred into a second 2 ml centrifuge tube and 1 ml of methylene chloride (Fisher Cat. No. D143-4) was added and the tube was put back onto the shaker for an additional hour in the dark at the same RPM. After this liquid-liquid extraction the infranatant was carefully aspirated and transferred to a 1.5 ml centrifuge tube and evaporated under vacuum using a Labconco centrivap (model 7810010) with a Labconco cold trap (model 7385020). After evaporation to dryness was achieved the extract was dissolved in 125 ul of HPLC grade methanol. Samples were then shaken vigorously (vortex mixer) and centrifuged at 17,000g for 5 minutes after which 100 µl was pipetted into an HPLC vial insert (Fisherbrand Catalog No. 13-622-207) which was placed into an Agilent HPLC vial (Part Number 5182-0716). Samples were stored at -20 °C for a period of 2-4 weeks before analysis.

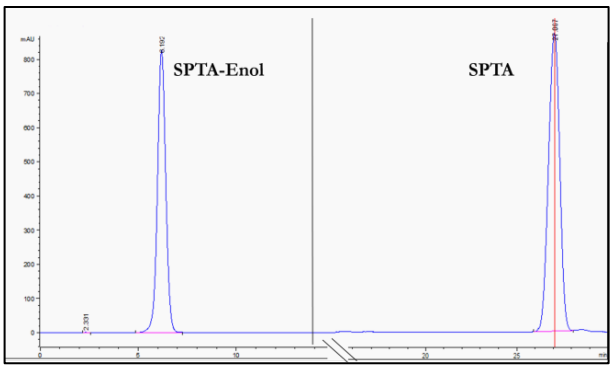

Fig. 2A: Example of known "standards" of Spirotetramat-Enol (SPTA-Enol) and Spirotetramat (SPTA) elution time. These compounds were eluted at 6.14 min at 27 minutes respectively and are compared with vine tissue samples.

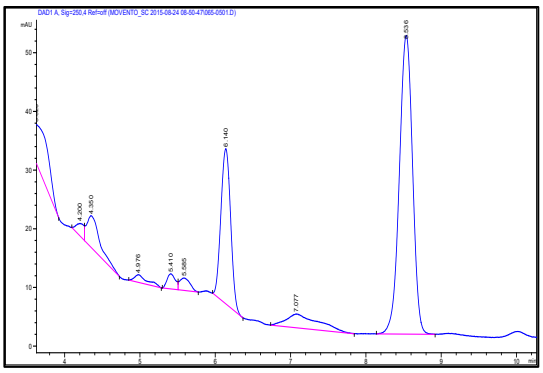

Fig. 2B: Example of a leaf sample, processed by HPLC, showing a peak that eluted at 6.14 min, matching the standard for SPTA-Enol and indicating its presence in the sampled leaf.

Protocols for HPLC Analysis.

The residues of Spirotetramat and metabolite, Spirotetramat-Enol 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), 100 Å, size 150x 4.6 mm, with accompanying guard cartridge of the same material. The injection volume taken was 20 µL, while chromatography conditions included an isocratic method wherein mobile phase of 60% deionized water (Siemens Labostar Ultrapure Water System) and 40% acetonitrile (Fisher Cat No. A955-4L Optima Grade) was held for 20 minutes with a flow rate of 1 mL min^{-1}. 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). (Spirotetramat purity 99.4% and Spirotetramat Metabolite BY108330-cis-Enol, purity 99.4%).

Sample collection and HPLC Analyses.

Concurrent with the development of the HPLC protocols, we collected leaf, cane and trunk tissues samples. In 2016, the fresh tissue sampling effort in 2016 resulted in approximately 6,000 samples, and another 6,000 samples in 2017. After six months of processing only about 500 samples, we then estimated the cost per sample, which was $9.36 per sample for materials (centrifuge tubes, pipette tips, syringe and nylon filter, HPLC vials, dry ice, acetonitrile, HPLC grade water, QueChERS Extract, etc.) and the labor ($10) per sample (grinding samples, extracting product, running HPLC). At approximately $20 per sample and with about 12,000 samples stored in the freezer, we were facing costs of $240,000 to process all of the samples. For this reason, we triaged the collection. First, we had collected 12 replicates for each vine tissue section (e.g., leaves) at each site on each sample date; we dropped the replication to 9 tissue samples per vine and tissue section. Second, we dropped vineyards located in different regions (e.g., Lodi, Napa) where we had only one sample date, deciding instead to first work study those Fresno County vineyards where we had multiple collections and could better control the application of Movento. Finally, we had initially taken six different tissue sections per vine (leaves, canes, cordon, upper trunk, lower trunk and roots) and we focused only on the leaves and the lower trunk (with the exception of some studies in collaboration with Dr. Westphal looking at control of root feeding nematodes.

How quickly does Spirotetramat convert to Spirotetramat-Enol?

To determine how quickly Spirotetramat is converted to Spirotetramat-Enol, a 22-yr. old Thompson seedless vineyard at the Kearney Agricultural Center was sprayed on 3 May 2016 at a rate of 12 oz Movento per acre (season-long limit and above label for a single spray). Leaf and trunk samples were collected at 5 hr. and 1, 3, 6, , 37, 110 and 184 days later (Fig. 3). Leaf tissue analyses show that, in this vineyard, Spirotetramat was quickly (within 5 hr.) converted to the Enol (remember that Enol is the metabolite responsible for killing the mealybugs), and a portion of the Enol is also rapidly converted to Enol-Glucoside (we do not show the Enol Glycoside metabolite (see Fig. 1) as we suspect the HPLC was also signaling on glucose in the vine). It has been reported that under the right circumstances the Enol-Glycoside can revert to Enol, although how common this occurs in vines is not known. Note that the Y-axis is using a log scale so there are great differences in the amounts of metabolites.

Also important was that some Spirotetramat and Enol was found in the leaf tissue up to 184 days after treatment and it is at this time that we first note Ketohydroxy metabolite (see Fig. 1), suggesting that the Spirotetramat-Enol is relatively stable in the vine. We note that in a different vineyard 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. In fact, the found Spirotetramat may be residue of the sprayed material on the sampled leaves that had not been washed off by winter rains. At this point, we assume that Enol found after 3-5 months is from either relative stable Enol remaining in the leaves, or Spirotetramat in the leaves that was later (in time) converted to Enol. Figure 3 also points out flaws in our processing of samples that we are still correcting; for example, there was no Spirotetramat-Enol recovered on 1 Day After Treatment (DAT) so either these samples were incorrect, or the previous 5 hr. sample was flawed.

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 (Fig. 3 is on a log scale), from about 100 ppm 5 hrs. after spray to about 40 ppb after 1-3 days, and <5 ppm after 1 month. There is not a corresponding increase in Spirotetramat-Enol, which is lower than Spirotetramat initially but shows a more stable presence during the five-month sampling period, around 20 ppb (Fig. 3). 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.

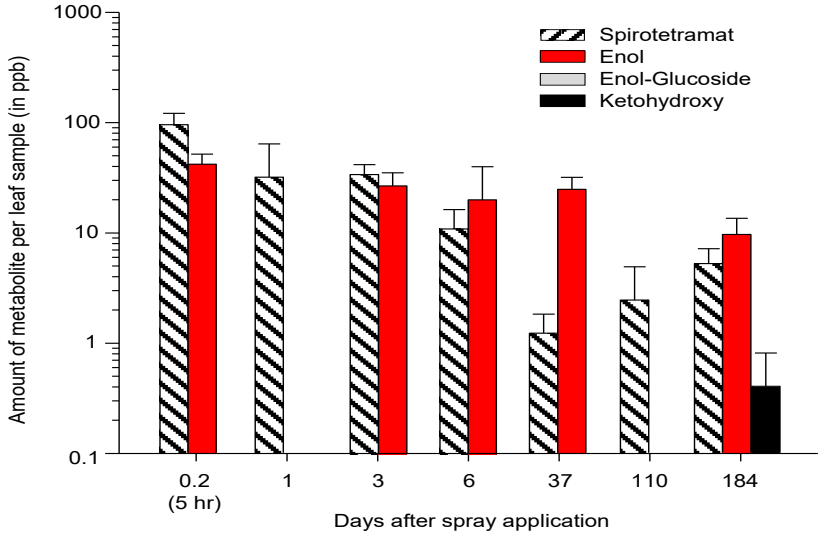

Fig. 3. Mean concentration of Spirotetramat and three of its metabolite in leaf samples from 5 hours after spray to 5 months after being treated with a label rate (8 oz per acre) of Movento® on 3 May 2016.

How long does Spirotetramat and Spirotetramat-Enol remain in the vine?

For many of the studied sites we sampled repeatedly over the year; however, in grower-managed sites we had less control of subsequent sprays. For this reason, we added a Movento application to a commercial 12 yr. old Crimson seedless table grape block on flood irrigation, where the vineyard manager had previously not included Movento as part of the insect management program. The application was an 8 oz per acre rate of Movento applied 24 May 2016. Figure 4 shows that Spirotetramat-Enol was found in the vine nearly a year after spray application (we have samples that go beyond this year that have still yet to be processed. In this vineyard, we did not find the rapid (5 hr.) conversion of Spirotetramat to Spirotetramat-Enol (Fig. 4). Three other interesting observations can be made. First, in most other trials the levels of Spirotetramat to Spirotetramat-Enol in the leaves range from 5-100 ppm, at this site there was much lower concentrations, ranging from 0.05 to 0.1 ppm over the year. Second, there was a very high concentration of Spirotetramat (Movento's a.i.) at 5 hr. after spray, and this was probably material both on the leaves and just moved into the plant. Spirotetramat remained in the samples until 184 DAT (24 November 2016) but was gone 212 DAT (22 December 2016) 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 can only conclude that some amount of Spirotetramat-Enol was stored in the vine roots, trunk or cane and was in measurable levels the following season. Note that

figures from these early trials are based on data sets with earlier transformation of the HPLC output, and how to best transform the HPLC graphic curves to ppm per sample is still being investigated and improved.

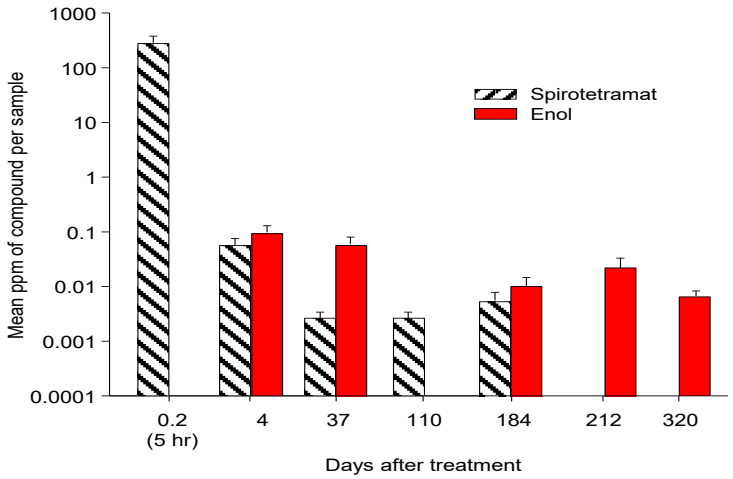

Fig. 4. Mean concentration of Spirotetramat and its metabolite Spirotetramat-Enol in leaf samples from 5 hours after spray to 1 year after being treated with a label rate (8 oz per acre) of Movento® in May 2016.

How evenly is the Spirotetramat-Enol Distributed?

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 (Fig. 5), which then is available to be transported via the phloem to other vine sections. What is interesting in this trial is that the amount of Enol varied among vines and this variation was still seen 72 days after application (Fig. 5). 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. Also, we expected that over time there would be a steady decrease in the amount (ppm or ppb) of Spirotetramat-Enol in the leaves, but at 5 and 72 DAT there was a similar amount – again this could be because of our leaf selection on these two sample days.

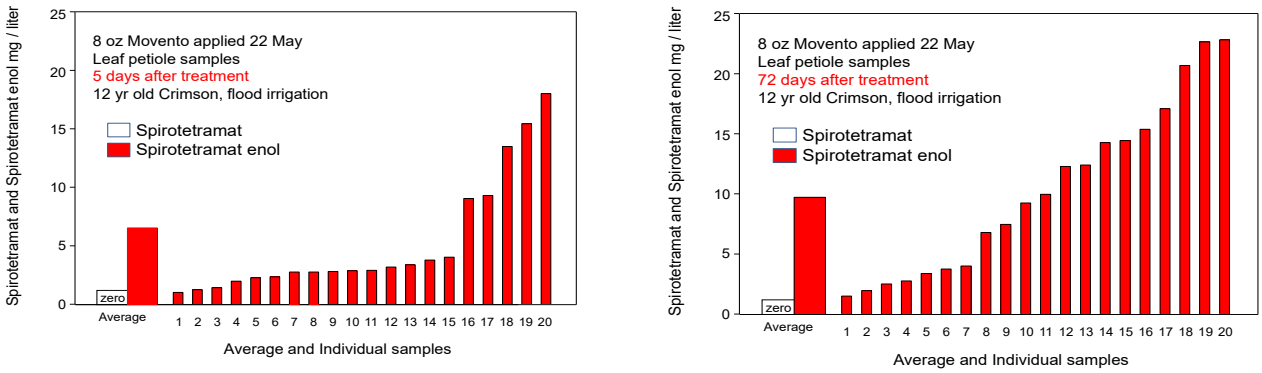

Fig. 5. Mean concentration of Spirotetramat and Spirotetramat-Enol in a Crimson Seedless vineyards with an 8 oz application of Movento at 5 and 72 DAT.

Leaves in the Sun vs Shade, Growing vs Senescent

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, although this was not apparent at 7, 30, and 78 DAT (Fig. 6). 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.

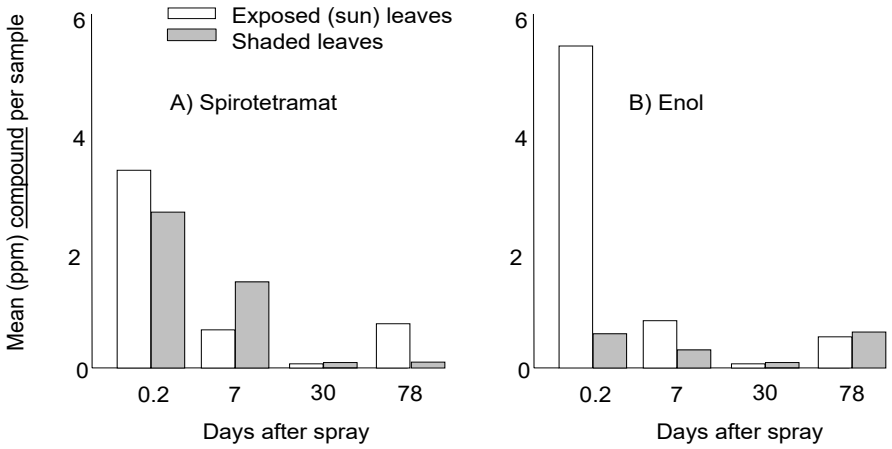

Fig. 6. Mean concentration of (A) Spirotetramat and (B) Spirotetramat-Enol in a Thompson Seedless vineyard with an 8 oz application of Movento at 5 hrs. and 7, 30 and 78 DAT for leaves collected in the sun compared with those collected in the shade.

Similar to sun vs shade, we also looked at spray application timing; in one trial this amounted to a post-harvest Movento application, which has been shown to be effective in trials conducted in Kern County (David Haviland, Pers. Comm.). In our trial, we applied an above label rate (24 oz per acre, subjected to crop destruct) very late in the season (13 October) in an older Thompson Seedless vineyard at the Kearney Agricultural Center. Our goal was to determine if the applied Spirotetramat and metabolized Spirotetramat-Enol might be moved to the trunk or root zone to be present in the vine on the trunk the following season, thereby killing the overwintered mealybugs on the trunk section. At the time of application most of the leaves were green but beginning to senesce. HPLC tissue analysis of leaves found very little conversion of Spirotetramat to Spirotetramat-Enol in the leaves (Fig. 7). We do not know if this material remained in the leaves and was lost to the vine after leaf drop, or if the Spirotetramat moved to the canes, trunk and roots and was later converted to Enol. Still, the results suggest that the leaves must be active for efficient conversion of Spirotetramat to Enol and for these late season applications to have a near-term impact.

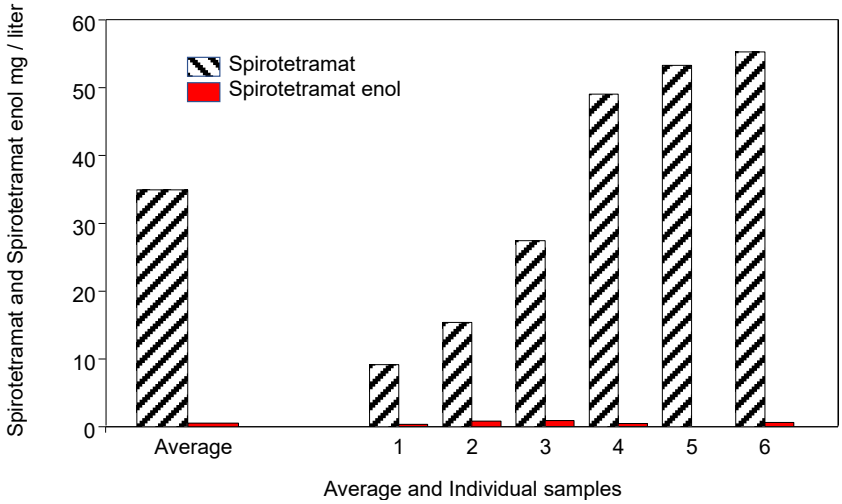

Fig. 7. Mean concentration of (A) Spirotetramat and (B) Spirotetramat-Enol at 7 DAT in a 22-yr. old Thompson Seedless vineyard with a 24 oz application of Movento (crop destruct) applied post-harvest on 13 October.

So clearly the results show Movento active ingredient and its metabolites move throughout the vine, but that there are conditions of leaf quality that may impact conversion to Spirotetramat-Enol. As an additional trial to determine how quickly the metabolites move to new or untreated leaves, in a May 2016 trial we applied Movento® in the Thompson Seedless vineyard block at Kearney at a rate of 12 oz per acre. Before being treated, 6 leaves per vine were covered 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 was somewhat dry (but no longer dripping) and 4 DAT leaves were sampled as usual. The analyses show that the amount of

Spirotetramat is significantly different from the "bagged" and control treatments ($F_{2,67}$=22.14, P<0.001); however, the amount of Enol and Ketohydroxy is no significantly different among treatments (Fig. 8). 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.

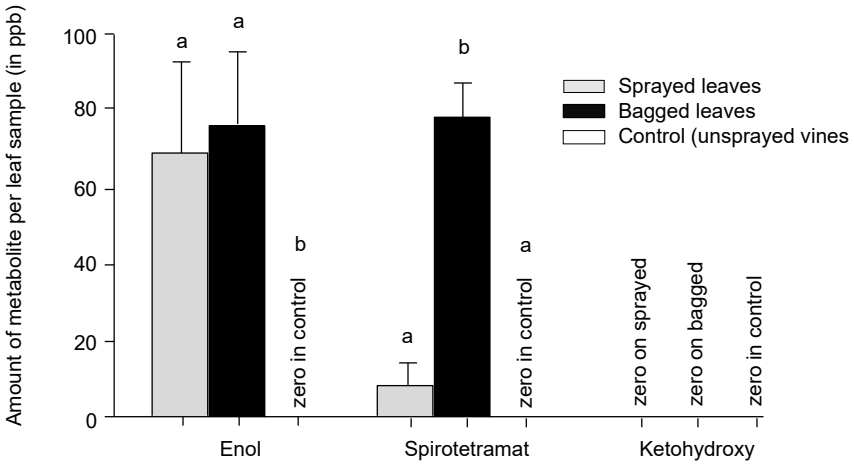

Figure 8. Spirotetramat and Enol content in covered and later uncovered leaves (in parts per billion) treated with a label rate (12 fl. oz per acre) of Movento® in May. Samples were collected four days after treated.

What rate of Movento should be applied?

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. However, from our samples of different commercial vineyards and experimental plots at Kearney Agricultural Center, 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. To determine if there was a dose response, in the experimental (e.g., crop destruct) Thompson Seedless vineyard at Kearney, we applied three rates (and a control) of 8, 12 and 24 oz per acre, applied in late May with the proper adjuvant (in most trials we used Dyne-Amic). Figure 8 shows that we did get the expected dose response of Spirotetramat, increasing in response to applied amounts and decreasing over time (and probably being metabolized to Spirotetramat-Enol (Fig. 9A). However, we did not get the same response in terms of the amount of Spirotetramat-Enol (Fig. 9B). 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. Moreover, we are still not sure what dose is needed to kill mealybugs.

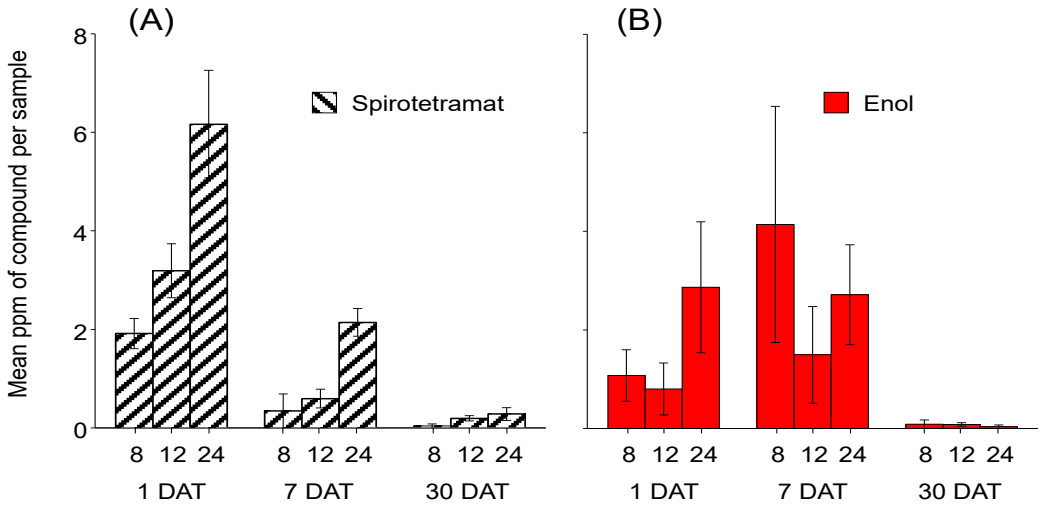

Fig. 9. Mean concentration of (A) Spirotetramat and (B) Spirotetramat-Enol at 1, 7 and 30 DAT in a 22-yr. old Thompson Seedless vineyard with 8, 12, and 24 oz rates of Movento (as in all trials, a no spray control was included and unless stated otherwise these data were 'zero' and so are not shown).

Can Different Surfactants be used?
Several factors might affect the rate of the pesticide uptake on the vine, and therefore, its efficiency to kill the pest. For instance, location and state of the pest population, soil moisture, application timing, vine age, cultivar and its condition and age, could affect how pesticides are absorbed by the plants. How the active ingredient is located inside plant tissues will differently affect the targeted pest spectrum, as pests have different feeding habits. Mealybug is a phloem feeding pest, thus we would like the killing material will stay in the phloem and not in other plant tissues or cell vacuoles.

Different surfactants are commonly used to applied Movento® (Bayer Crop Science) in vineyards to control mealybugs and other vineyard pests. Movento's active ingredient is Spirotetramat, but it is the breakdown metabolite Spirotetramat-Enol that is most important killing agent for mealybugs. Both the Spirotetramat and Spirotetramat-Enol will travel through the vines' the phloem and xylem from the leaves, to canes, to the spurs and trunk and eventually to the roots. Vintre® (Oro Agri) is a surfactant advertised as providing superior wetting and penetration. At low application rates, Vintre may be an excellent spreader that allows pesticides to reach deep within cracks and crevices and, when mixed at higher rates, may provide foliar penetration of inputs to the plants' phloem circulatory system. The goal of this trial was to compare Vintre to the surfactant Dyne-Amic® (Helena Chemical) in terms of the movement of Spirotetramat and Spirotetramat-Enol – again, the levels of Spirotetramat-Enol are most important. In fact, one of the yet-answered issues with the use of Movento is the complete conversion of Spirotetramat to Spirotetramat-Enol.

We compared Movento® applied at 12 fl. oz. (on 5 June 2017) with a Control (untreated vines), Movento and Dyne-Amic (mixed following manufacture recommendations), and Movento and Vintre (mixed following manufacture recommendations). The plot design was a randomized block design with 5 vines per block and 3 blocks; for this trial we used each vine as a replicated (n=15 vines per treatment). As with all of the trials at Kearney, a backpack sprayer was used, calibrated at 100 gallons of water per acre.

Our results show that 7 and 30 days after spraying, there was not significant difference between the two surfactants tested on the amount of Spirotetramat-Enol found (Fig. 10A, 1B, respectively). A larger study might have pulled more information out if there were subtle differences. For example, at 7 days there were statistically comparable amounts of both metabolites in the leaf samples. Whereas at 30 days, the Dyne-Amic treatment had almost exactly the same ratio of Spirotetramat to Spirotetramat-Enol but the Vintre treatment had nearly twice the Spirotetramat-Enol (compared with the same treatment at 7 days) and no Spirotetramat. Because of the large variance, this was not significant (it is not unusual for leaf samples to vary widely), however, if Vintre promoted better conversion of Spirotetramat to Spirotetramat-Enol after the spray date, this would be an important difference. We conclude that Vintre can be used as a surfactant with Movento. With this preliminary trial there is not enough information to determine if one surfactant might be better than another.

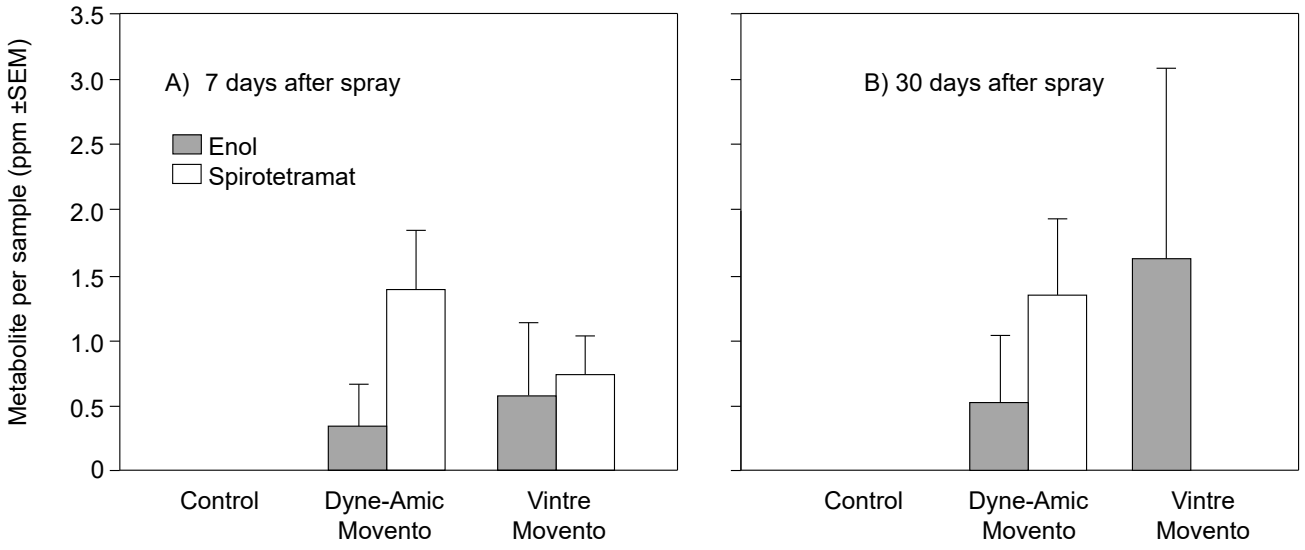

Figure 10. Mean Spirotetramat and Spirotetramat-Enol (ppm of leaf tissue sample ± SEM) found at (A) 7 and (B) 30 days after application of Movento.

How Much Spirotetramat-Enol goes to the Trunk and Roots/

While the project is officially over, we are still processing trunk and root samples.,. Still, 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. Again, we still don't know the chronic lethal dose for mealybugs, so it is not possible for us to know if the lowered amount still provides effective control.

An example of the reduction, and the metabolites found is from the experimental block at the Kearney Agricultural Center, where replicated vines were treated with 0, 8, 12, and 24 oz of Movento. Figure 9 shows the amount of Spirotetramat and Spirotetramat-Enol in leaves at 1, 7, and 30 DAT. Figure 11 shows the amount of these same compounds 7 and 30 DAT on samples taken from the upper and lower trunk sections (the metabolite Ketohydroxy was also found in trace amounts in the lower trunk 30 DAT sample, but these data are not shown). First, notice that for leaf samples (Fig. 9) the y-axis is in ppm (parts per million) whereas for the same vines the trunk samples (Fig. 11) has the y-axis in ppm (parts per million). Next, we found only one instance of Spirotetramat in the trunk sample (Fig. 11A - 12 oz per acre, 7 DAT, lower trunk), whereas we found Spirotetramat-Enol (Fig. 11B) in all but one treatment. Note also that, similar to the amount of Spirotetramat-Enol in the leaves there was not a clear association of application rate (8, 12, 24 oz per acre) with recovered amounts in the tissue samples. We believe this is due in part to sampling, conversion of the a.i. to Spirotetramat-Enol, and individual vine physiology. In the individual replicates what we note that is not apparent in the graphs is that trunk samples with Spirotetramat-Enol may have amounts nearly as high as leaf samples, however, there may be only 1 of 10 trunk samples with Spirotetramat-Enol. We suspect that this may be due to sampling – how much trunk sample was recovered with enough phloem tissue that would contain these metabolites, and possible to uneven transport of the metabolites from the leaves to the roots. One assumption that can be made is that those areas of the trunk with phloem tissue near the surface (where we sampled) would also be areas where the mealybugs would preferentially feed.

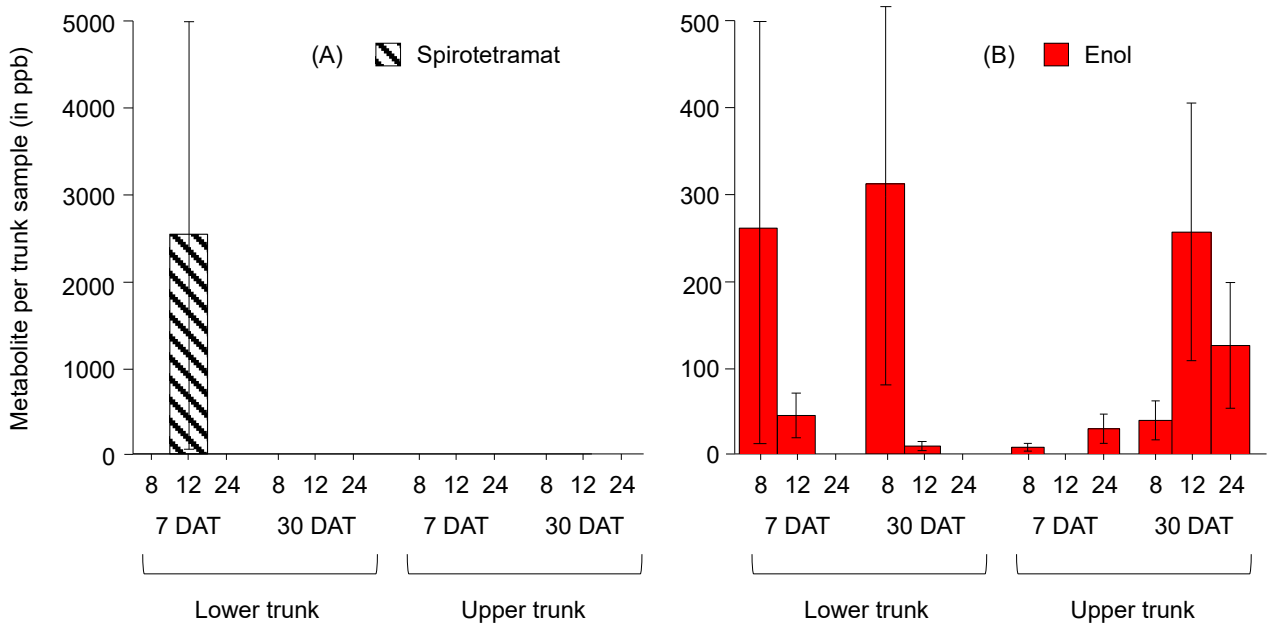

Fig. 11. Mean concentration of (A) Spirotetramat and (B) Spirotetramat-Enol at 7 and 30 DAT in a 22-yr. old Thompson Seedless vineyard with 8, 12, and 24 oz rates of Movento, separating tissue samples by lower (near the soil) and upper (near the arms or cordons) trunk regions.

Additionally, in a commercial Crimson Seedless block, with a grower standard application of 8 oz per acre of Movento, applied in May, we sampled upper and lower trunk tissue nearly a year after application and before the any future spray application. From these samples, we found Spirotetramat-Enol in the trunk samples at 212 DAT

and 320 DAT, suggesting that the Spirotetramat-Enol metabolite does remain fairly stable in the vine and can potential provide some reduction of mealybugs the following spring – depending on the amount of product that is carried over (Fig. 12). At this site, nearly a year after treatment we did not find the active ingredient Spirotetramat. This result shows that the metabolites are moving with the phloem from the leaves to other vine sections. There was no significant difference in the amount of Enol present 212 and 320 days after treatment. There was no difference between samples collected in upper and lower portions of the leaves.

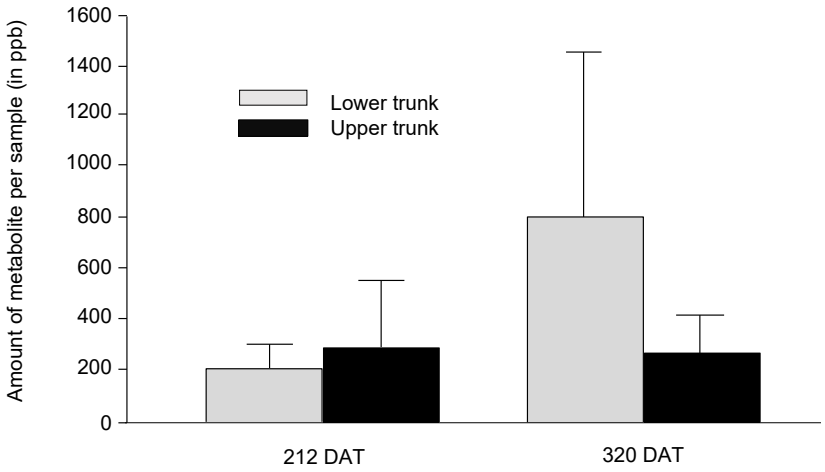

Fig. 12. Mean concentration (parts per billion) of Spirotetramat-Enol from lower and upper trunk section collected 212 and 320 DAT with Movento® applied to the leaves at label rate (8 oz per acre) in May.

Collaborative work with Dr. Westphal

As part of our efforts to look at movement of Spirotetramat and Spirotetramat-Enol to the root zone, we worked with Dr. Westphal on his study of the application of Movento and other products to control nematodes. Dr. Westphal was funded by the grape commodity boards, whereas our work with the HPLC was funded only through this CDFA Agreement 16-0556-SAFP and our collaboration was suggested to be a part of this project. Below is a summary of the pertinent parts of a report from Dr. Westphal to the grape commodities, which included our HPLC analyses of root tissue samples. The objectives of Dr. Westphal's proposal addressed post-plant nematicide applications to vineyards for suppression of key nematode pests of grape, specifically, to determine the nematocidal application rates of Movento in regard to watering regiment under controlled conditions. We provided evidence that the plants obtained from the nursery had probably been treated prior to the trial. Nevertheless, the treatments designed with Movento showed higher levels of Spirotetramat-Enol in the root tissue than the controls.

CONCLUSIONS

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. We assume that complete conversion to Enol is most desirable, but perhaps the movement of Spirotetramat through the phloem from leaves to trunk and roots will help kill mealybug later in the season if it is then converted to Enol. We also found a wide range of Enol 'dose' (parts per billion) in the leaves and other tissues. By itself, this does not provide any information on mealybug kill rate until we also determine the lethal dose in the vine (which also must include the feeding period). Finally, we have attempted different application timing of Movento® and found that the April-May period was associated with the most efficient conversion of Spirotetramat to Enol (as well as mealybug kill, data not shown here). We must still determine the fate of the unconverted Spirotetramat that is carried from the leaves to other tissue to understand if this material is eventually converted to Enol or if it metabolizes quickly from Enol to non-toxic downstream metabolites in the trunk and root tissue.

Finally, remember that Spirotetramat is a tetronic acid derivative and acts as a lipid biosynthesis inhibitor. Lipids

include fats, oils, waxes, certain vitamins, hormones and most of the non-protein membrane of cells. As such, lipids are vital to an animal's existence. For most insects, Movento ® will be most effective against juvenile stages by preventing molting but can also reduce fecundity and fertility of adult females. The juvenile mealybugs feeding on Enol should then be unable to molt – like a growth hormone – but also will have their energy transport system disrupted and should cease movement and feeding as well. For these reasons, there may be a delay from the time of field application to control of the population if there are large numbers of females already producing ovisacs, which Movento® will not effect. There may also be a time delay as Enol moves through the plant, although we found Spirotetramat or its metabolites in different tissue samples within days after spray application. As for questions of resistance, we sampled leaf tissue in a vineyard that had been treated with Movento but had damaging mealybug densities; however, HPLC analysis did not show clear peaks of Spirotetramat or Enol in any of the tissue samples, suggesting that a more plausible explanation would be that an active product was not delivered to the vine.

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RESEARCH RELEVANCE STATEMENT

The vine mealybug has become one of the more important insect pests of California vineyards, threatening economic production and sustainable practices in this multi-billion-dollar state industry. This work has begun to better understand and optimize registered insecticides used to control the vine mealybug in the winter and spring periods, when the mealybug bug population is located primarily under the bark on the trunk and cordons. In the initial we selected vineyards in three regions and have taken spring through fall samples. Our specific goal was to better understand the movement of Movento's active ingredient and metabolites in the vine to determine how best to optimize this product, which is one of the better control tools for vineyard mealybugs.

FUNDING AGENCIES

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