Effect of Spirotetramat Concentration on *Anagyrus psuedococci* Mortality

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Abstract

Control of vine mealybug in vineyard systems is of critical importance due to economic injury potential including an ability to vector virus. Traditional management of this pest has relied heavily upon the use of systemic and contact insecticides. The systemic insecticide Movento®, registered for vine mealybug and active ingredient spirotetramat, has been shown to be compatible with biological control. However potential side effects of chemical controls on beneficial insect populations is known to affect non target arthropods. This study examined the effect of the systemic insecticide spirotetramat on populations of adult parasitic wasps, *Anagyrus psuedococci,* at two concentrations with wasps introduced onto treated leaves four hours and five days after application. Results of this study confirmed published reports spirotetramat did not cause a significant difference in wasp mortality concentration treatments or introduction interval treatments, and the interaction between concentration and interval was not found to be statistically significant. Lack of chemical ingestion combined with evolutionary characteristics of parasitic wasps may provide explanation to why a lipid synthesis inhibiting insecticides bear no adverse effect and warrants further investigation.

Introduction

Mealybugs are the most common soft scale insect affecting grapevines in California. Mealybugs are a family of homopteran insects that have piercing sucking mouthparts used to feed on the phloem of host plants. Four species of mealybugs are categorized as grapevine pests, the grape (*Pseudococcus maritimus*), vine (*Planococcus ficus*), longtail (*Psuedococcs longispinus*) and obscure (*Pseudococcus affinis*. Vine mealybug is present in almost all major grape growing regions worldwide (Daane et al. 2012). Invasive to California, vine mealybug (*P. ficus)* is the most abundant and problematic mealybug species globally (Mansour et al. 2011). The vine mealybug spends the majority of its juvenile stages underneath the bark of the grapevine roots and vine trunk, which makes control difficult. The emergence of female adults and winged adult males occur in the spring (Güleç et al. 2006). Upon the development of the vine canopy, mealybugs move from the roots and trunk to the shoots and fruit which if uncontrolled may result in economic injury. Damage from mealybugs is a result of feeding on fruit, roots, canes, trunks, clusters and leaves, which may result in delayed bud break and delayed sugar accumulation. Exuded from the pest is a honeydew, which acts as a substrate for the

development of black sooty mold (Daane et al. 2012). Presence of sooty mold on crop of both fresh market and wine grapes is of potential economic loss to the grower. Vine mealybug is one of the most problematic vineyard pests because not only of the economic injury caused from feeding but additionally an ability to vector virus. Vine mealybug has been shown to transmit a collection of single stranded RNA viruses, named grapevine leaf roll associated virus GLRaV, with the pathogen transmission rate in as little as 1 hour following feeding (Cooper et al. 2018). GLRaV causes a decline in vineyard productivity by inhibiting sugar accumulation, delaying bud break, and yield losses up to 40% (Daane et al. 2004).

Fundamental to Integrated Pest Management theory is the principal of utilizing selective chemicals in accordance with models for temperature related development, Economic Injury Level (EIL) and pest populations, in combination with cultural and biological controls. In addition to pheromone mating disruption, one of the most widely utilized alternative control measures for mealybugs in vineyard settings is the release of parasitic wasp *Anagyrus psuedococci* (University of Florida 2015**).** Current pesticides registered for control of mealybug populations include OMRI listed neem oil, insect growth regulators such as buprofezin and systemic insecticides including imidacloprid and spirotetramat. Unintended consequences of using certain pesticides has resulted in the negative impact on non-target populations of beneficial insects. Registered insecticides to control various species of mealybugs have been proven, with the exception of lipid synthase inhibitors (spirotetramat), to cause mortality of beneficial insect populations, with particular regard to *A. psuedococci* (Mansour et al. 2018).

Systemic insecticides are of preferred use in controlling phloem feeding insects as the likelihood of a contact insecticide reaching the target pest is far less than likely to occur due to shielding from leaves and the “hidden” nature of *P. ficus*. Spirotetramat is the active ingredient in the systemic insecticide Movento® (Bayer CropScience, Thane India) at concentration of 22.4 percent by volume. Proceeding foliar or drip application, spirotetramat is transformed into its active form in the xylem and phloem of the plant. Ingestion of spirotetramat by phloem feeding insects results in metabolic inhibition of acetyl coenzyme A, which prevents the synthesis of lipids resulting in both breakdown of existing and prevention of new cell wall formation effectively halting growth and ultimately leading to insect mortality (Nauen et al. 2007). Spirotetramat is most efficient on juvenile stages of *P. ficus*, and is of few systemic insecticides translocated within the plant through both the xylem and the phloem, making the control of

hidden pests (i.e. under bark) susceptible to effects of the chemical (Nauen et al. 2012). Spirotetramat has been classified as slightly harmful to beneficial arthropod populations of generalist predators such as spiders (*Lycosa spp*. and *Tetragnatha spp.*), earwigs (*Forficula auricularia)* and lacewings (*Chrysoperla spp*), and slightly toxic to specialist predatory mites (*Typhlodromus pyri*) (Brück et al. 2009).

The purpose of this study was to determine the impact of spirotetramat on populations of the beneficial insect *A. pseudococci* at two concentrations and two introduction intervals.

Materials and Methods

This study was conducted in October 2018 at Pacific Ag Research located in the Edna Valley of San Luis Obispo, California. This trial was performed on a lab bench in a climate controlled room maintained at 20°C, utilizing leaf discs sampled from a spirotetramat-treated *Vitis vinifera* L. cv. Chardonnay (clone three) vineyard planted on a 1.2 meter by three meter spacing and comprising approximately one hectare.

Experimental Design

This trial included three concentration treatments. Each treatment was replicated seven times. Concentration treatment one was distilled water as the control, concentration treatment 2 was spirotetramat at 365 ml/ha, and concentration treatment 3 was spirotetramat applied at 545 ml/ha. Introduction interval treatment one was at four hours post application and interval treatment two at five days post application. A section of 21 continuous vines apart from row ends and border rows was used for the all treatments. Within this area, a section of seven vines were used for the control treatment, seven vines were used for the 365 ml/ ha treatment and seven vines for the 545 ml/ ha treatment. Spray volume and rates were based on vine spacing and a 935 L/ hectare coverage rate, performed mid-day utilizing a calibrated Stihl SR450 backpack sprayer (Stihl, Waiblingen, Germany) with product applied until leaf wetness. Following a listed re-entry interval of four hours, 21 leaves were sampled, seven per treatment, for the four hour introduction interval. Leaf samples were collected from the middle five of the seven treated vines to create a buffer zone on either end of the treatment spray area. The sampling procedure consisted of collecting five fully expanded grapevine leaves at random from either side of the canopy within the fruiting zone. The same process was replicated for the five day introduction

interval treatment. Leaf samples were brought to the lab and from each a 3.8 cm diameter disc was cut and placed into sterile, labelled 100x15cm petri dish (Fisherbrand, Pittsburgh, PA) along with a cotton swab soaked in a 1% solution of organic honey and distilled water.

Parasitic wasp pupae were obtained from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands) and placed into a Quincy Labs model 12-140 incubation chamber (Quincy Labs, Chicago, IL) set at 27°C for a period of 24 hours or until 50% adult wasp emergence from mealybug mummies. Each was inoculated with five randomly selected *A. pseudococci* wasps through use of an insect aspirator. To better manipulate the wasps, they were placed into an ice chest for a period of 1-3 minutes. Petri dishes were immediately covered following inoculation, and left undisturbed for a 24 hour period in a 3x7 single layer grid upon the lab countertop. Treatments were laid on bench top and organized vertically by treatment with repetitions placed horizontally (Figure 2). Mortality rates of *A. pseudocci* were determined by visual observation and counting the number of wasps that had died compared to the five originally introduced 24 hours after wasp infestation. Mortality of wasp confirmed by agitating the petri dish and observing for movement.

Statistical Analysis

Analysis of data was conducted using ANOVA using JMP version 12.2 Software (SAS Institute, 2015).

Results

The ANOVA showed no significant effect of concentration treatment on adult wasp mortality and no significant effect of introduction interval treatment (Tables 1 and 2). Mortality for all treatments did not exceed 10% incidence for both the 0 day and five day introduction periods (Figure 1). The interaction of concentration treatment by introduction interval treatment was found to not be significant ($F=0.68$, $df = 2,36$ $p=0.51$).

Table 1. Comparison of percent mortality by concentration

	Control	**365 ml/ ha**	**545 ml/ ha**	**F**	**df**	**p**
Mortality (%)	5.71	8.57	7.14	0.159	2,36	0.853

Table 2. Comparison of average mortality rate by introduction interval.

	4 hour	**5 day**	**F**	**df**	**p**
Mortality (%)	9.52	4.76	1.31	1,36	0.25

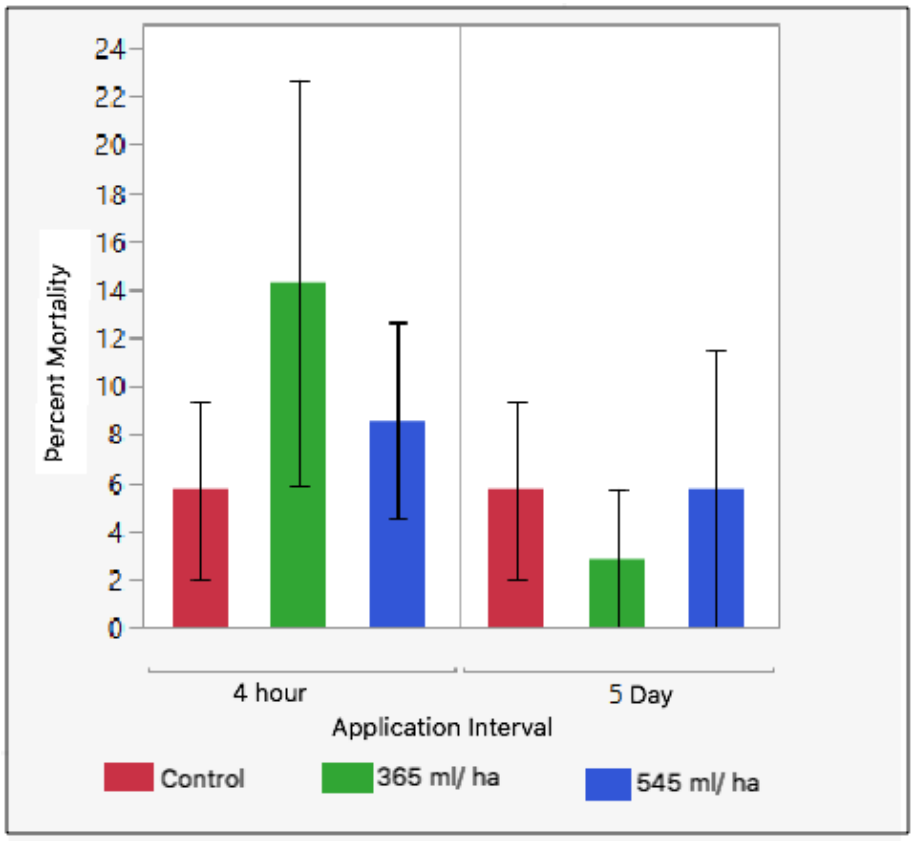

Figure 1. Comparison of average mortality rate by treatment with standard error bars and separated with by zero and five day introduction intervals.

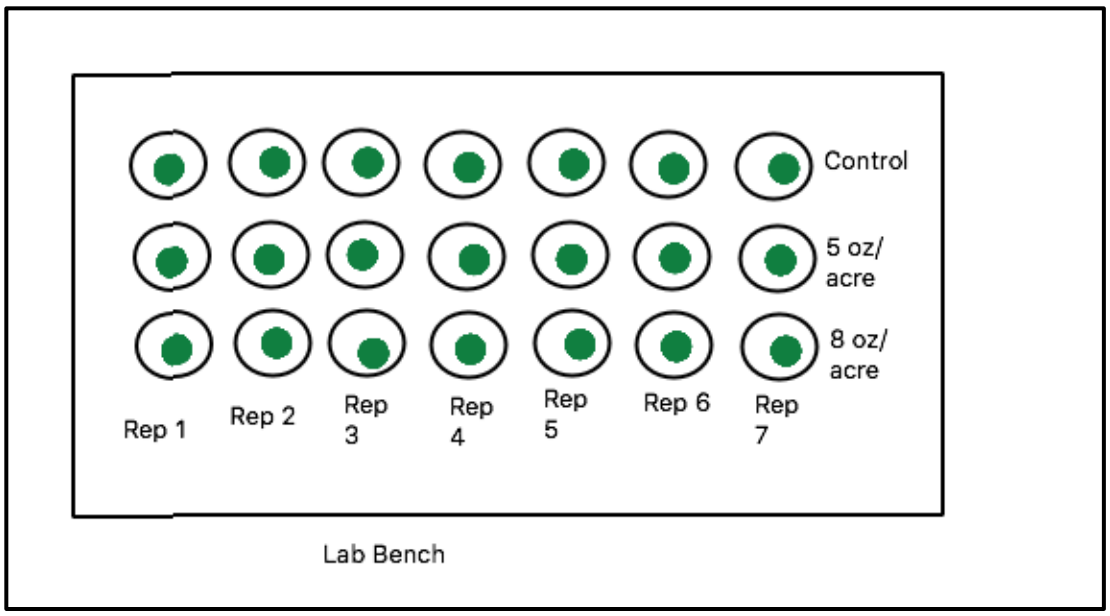

Figure 2. Experimental Design. Petri dishes with leaf discs on a 3 x 7 grid, organized horizontally by repetition and vertically by treatment used for both introduction intervals.

Discussion

This study confirms previous studies showing that the lipid biosynthesis inhibiting insecticide spirotetramat has no significant impact on mortality rates of *A. psudeococci* as measured by direct application of spirotetramat to pupae (Mansour et al. 2018) and additionally through indirect exposure in leaf dip assays when compared to controls. Results of the current study indicate no significant effect on mortality at the tested application rates and introduction intervals to adult wasps exposed to spirotetramat residues or breakdown products. Interestingly but not significant, mortality at five days was half that at four hours. The pattern of reduced mortality for the five day introduction interval shown for all concentrations is logical due to the degradation of spirotetramat. Non statistically significant differences of wasp mortality to control for both concentration and introduction treatments may be attributed to two factors. Parasitic wasp are not phloem feeders hence spirotetramat is not directly ingested from the plant. Management of *P.ficus* with fipronil, a systemic insecticide affecting the central nervous system of target pests, has been shown to cause mortality in *A. pseudococci* due to residual product on mealybug mummies ingested by emerging adult wasps (Mansour et al. 2011). However it has been shown that emergence rates of *A. pseudococci* from vine mealybug mummies treated with spirotetramat do not differ from that of their controls (Nauen et al. 2007), therefore direct

ingestion of spirotetramat is known to have no effect on mortality rates. Survival of *A. pseudococci* to concentrations of spirotetramat may be attributed to evolution of parasitic hymenopteran insects. Fatty acid synthesis, also known as "de novo lipogenesis" has been proven to be an evolutionary characteristic of parasitic wasps (Visser et. al 2010), meaning that hymenopteran insects acquire fatty acid building blocks, requirements for growth and development, without the presence of a specific lipogenesis metabolic pathway. Synthesis of fatty acids in parasitic wasps is analogous a parasitic plant lacking chlorophyll but obtaining nutrient from a host plant. Because spirotetramat causes death through the inhibition of this fatty acid pathway it stands to reason that this mode of action would not cause mortality in a species not possessing this trait. Literature regarding this phenomenon is scarce and warrants further investigation.

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