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Project title

Laboratory and Field Evaluations of Neonicotinoid Insecticides Against the Glassy-Winged Sharpshooter

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Results of Research by Objective

Objective 1 - Determine regional differences in the uptake and persistence of imidacloprid and dinotefuran in grapevines in order to maximize protection of vineyards

Introduction

Admire® insecticide (Bayer CropSciences) is being widely used in southern California for the control of the glassy-winged sharpshooter (GWSS) in grapes and citrus. Admire is a soil-applied neonicotinoid insecticide that delivers the active ingredient imidacloprid, which has been shown to be very effective against GWSS and other sucking insects. In northern California, Admire has also been used against populations of blue-green sharpshooters, as well as other pests. However, the decision by growers in northern California to use Admire has largely been influenced by the success of this chemical at protecting southern California vineyards from PD. The value of this product to growers in the north needs further evaluation.

To date, most of the research on uptake and persistence of imidacloprid in grapevines has been done in southern California, where the climate is warm and the soils have relatively low clay content (Toscano and Byrne, 2005; Weber et al., 2004 and 2005). In the heavy soils and cooler climates common in North Coast vineyards, there are major questions about the effective use of soil applied neonicotinoid insecticides. In a 2004/5 study (Weber et al., 2004, 2005), we evaluated four treatment regimes for Admire in a North Coast chardonnay vineyard. Under the prevailing conditions of cool temperatures, loam soil (15% clay content) and limited irrigation, there was little uptake of imidacloprid and levels were never reached for effective control of sharpshooters. We concluded that limiting irrigation in order to manage vine growth and berry

size for premium wine production may prevent the effective use of Admire in such cooler growing regions. The solution to this problem may lie with Venom® (Valent USA Corporation), a newly registered product for use on grapevines with the active ingredient dinotefuran. It belongs to the same chemical class as imidacloprid, and has been shown in bioassays to be effective against GWSS. Like Admire, it is formulated for application through drip irrigation systems. Imidacloprid is less water soluble and has a higher Log P (1-octanol:water partition coefficient) than dinotefuran (Table 1). Water solubility is expected to increase the rate of uptake, while the Log P will determine how an insecticide will partition between water and soil organic matter.

Table 1. Chemical properties of neonicotinoids.

Neonicotinoid	Water solubility (mg/liter)	*Log P
Imidacloprid	514	0.57
Dinotefuran	39830	-0.64

*P = 1-octanol/water

The purpose of this objective was to compare inter-regional differences in the rate of uptake and persistence of imidacloprid and dinotefuran insecticides. This information will be especially critical in the event that GWSS becomes established in the Napa region where imidacloprid does not perform well, but will also have current value for use against blue-green sharpshooters.

Methods

Vineyard locations

Table 2 summarizes the vineyard details where the studies were conducted. All vineyards were commercially active enterprises, and our experimental plots were managed using normal agronomic practices appropriate to each location.

Table 2. Vineyard details for each trial location. Soil type analyses were conducted on ten 6" deep x 4" diameter soil cores for each site by the Division of Agricultural and Natural Resources Analytical Laboratory at University of California, Davis.

Vineyard Location	Variety	Age	Soil	% Organic Matter
			Sand,Silt,Clay (%)	
Coachella Valley	Perlette Seedless	15 years	83,10,7	0.8
Napa Valley	Chardonnay	12 years	42,38,20	1.1
Temecula Valley	Cabernet	8 years	70,18,12	1.8

Trials to evaluate the uptake of imidalcoprid and dinotefuran in vineyards located in three viticulture regions of California were conducted in Coachella, Napa and Temecula valley. Table 3 summarizes the rates used at each location. The insecticides were administered by the vineyard managers via chemigation using the drip irrigation systems established at each vineyard. In Coachella, we used the half label rate of Admire Pro (7 fl oz/acre) because this rate achieved the desired 10 ppb threshold concentration in the xylem necessary to kill a sharpshooter

(Byrne and Toscano, 2006). In Napa, we evaluated the same rates of Venom used in Coachella and Temecula, but used the full label rate of Admire Pro. In an earlier study, both the half and full label rates resulted in poor uptake (Weber et al., 2005), so we chose the maximum rate for this study.

Table 3. Neonicotinoid treatment rates used in Coachella, Napa and Temecula vineyards.

Vineyard Location	Imidacloprid	Dinotefuran
Coachella Valley	7 fl oz Admire Pro 4.6L	3 oz Venom 70SG/acre
		6 oz Venom 70SG /acre
Napa Valley	14 fl oz Admire Pro 4.6L	3 oz Venom 70SG /acre
		6 oz Venom 70SG /acre
Temecula Valley	7 fl oz Admire Pro 4.6L	3 oz Venom 70SG /acre
		6 oz Venom 70SG /acre

Imidacloprid and dinotefuran quantification

Xylem fluid was extracted using a plant water stress console (pressure chamber) (Scholander et al., 1965) from the terminal end of actively growing canes that were cut from vines on each sampling date (Byrne et al., 2005).

The concentrations of imidacloprid and dinotefuran within the xylem extracts were determined using a competitive ELISA technique, in which insecticide residues in unknown samples compete with an enzyme (horseradish peroxidase)-labeled insecticide for a limited number of antibody binding sites on the wells of a microplate. The levels of bound conjugate are determined colorimetrically, and are inversely proportional to the levels of insecticide present in the sample. ELISA kits are available commercially for both imidacloprid (QuantiPlate kit for imidacloprid, cat. # EP 006; EnviroLogix Inc., 500 Riverside Industrial Parkway, Portland, ME 04103, USA) and dinotefuran (SmartAssay Series Dinotefuran test kit, cat. # 9107001200USA; Horiba, 2 Miyanohigashi, Kisshoin, Kyoto, 601-8510 Japan) with reported lower sensitivities of 0.2 µg imidacloprid.litre^{-1} and 1.5 µg dinotefuran.liter^{-1}, respectively. The assays were calibrated before use to test for matrix effects associated with xylem fluid (Byrne *et al*., 2005). In this study, sample extracts were initially diluted 20-fold in distilled water before assays, and when readings measured above the detection limits of the ELISA, the samples were diluted further and the assays rerun.

Statistical analysis

The data from each study were analyzed by ANOVA, using a repeated measures model at a prob value of 0.05. The analyses were conducted using Prism 5 for Mac OS X (v5.0a) (GraphPad Software, Inc).

Results and Discussion

At each location, the differences in the temporal profiles of uptake and persistence for the three treatments were extremely significant (Coachella: $F_{2,869} = 300.63$; $P < 0.0001$; Napa: $F_{2,483} = 220.29$; $P < 0.0001$; Temecula: $F_{2,585} = 294.49$; $P < 0.0001$). The uptake of imidacloprid in the

Napa vineyard was especially poor, corroborating our earlier findings (Weber et al., 2005). Of the 144 samples taken during this study, only 1 vine recorded a value above the 10 ppb threshold, and most vines were below the detection limit of the ELISA (4 ppb). The uptake of imidacloprid in Coachella and Temecula was better, although levels were consistently better in Temecula. Levels in Coachella were marginally lower then the recommended threshold throughout most of the assessment. While we expected better concentrations, based on recent findings (Toscano and Byrne, 2005), it is likely that the irrigation regime at this vineyard was not conducive to providing the desired levels of uptake. In Temecula, imidacloprid moved into vines quickly. There was a period, however, when levels dropped below the required threshold. This dip in concentration occurred when irrigation water was withheld. During the period when weekly irrigation was practiced, the uptake rose well above the threshold levels and the vines were well protected from PD. For effective uptake of imidacloprid, it is vital that vines receive irrigation water.

Dinotefuran was detected in vines at each vineyard location, and the concentrations in the xylem were highest in vines treated at the full label rate. Even when it was applied at the lowest label rate (3 oz/acre), dinotefuran levels within the xylem surpassed those of imidacloprid. In Napa, dinotefuran levels rose quickly, suggesting that the uptake occurred at the time the treatments were made (the only time water was used in this vineyard). The rate of uptake in Coachella and Temecula was also rapid but more sustained, again suggesting the influence of the irrigation at these sites. With repeated irrigations, as part of a normal regular irrigation schedule, uptake of dinotefuran (and imidacloprid) is optimized. Breaks in the watering schedule could lead to lulls in insecticide concentrations within the xylem, putting the vines at risk from PD infection. In Temecula, the dip associated with imidacloprid uptake was evident at the lower rate of dinotefuran, but not at the high rate.

The effect of the greater solubility of dinotefuran, and its lower binding to organic matter, is reflected in the higher levels of dinotefuran at the three sites. But, this also results in a more rapid decline.

Dinotefuran proved better than imidacloprid in terms of uptake in the Napa vineyard. In this region, imidacloprid uptake is compromised by the heavy clay soils (which bind the insecticide tightly, making it unavailable for uptake through the vine roots) and the lack of irrigation (a consequence of the local climatic conditions). Although we observed rapid uptake at the full label rate of Venom 70SG, the concentrations began to decline after the first week. The spike in uptake occurred at the time of the applications and suggests that irrigation at the time of the applications will need to be carefully controlled if optimal delivery of the product is to be achieved.

It is clear from our study, that the uptake of dinotefuran is superior to that of imidacloprid at all sites. But the question remains whether the levels attained are actually high enough to provide effective pest management. This question is being addressed with our current CDFA-funded project, in which we are deriving an activity threshold for dinotefuran against the GWSS.

Objective 2 - Evaluate generic formulations of imidacloprid in grapevines

Introduction

In 2006, imidacloprid went off patent, and several generic formulations of imidacloprid were introduced to the market (in addition to Admire). To temper grower unease with switching to these newer products, we compared the efficacy of generic formulations of imidacloprid with the industry standard (Admire 2F). The purpose of this objective was to determine whether different formulations of imidacloprid behaved similarly when applied to grapevines.

Methods

We evaluated the uptake of imidacloprid applied as Admire 2F, Admire Pro and Nuprid 2F. The active ingredient in these products is imidacloprid, and the recommended label rates are the same. Nuprid 2F and Admire 2F are flowable formulations, while Admire Pro 4.6L is a liquid formulation. The insecticides were applied at equivalent rates, to deliver similar levels of imidacloprid to each vine. Standard agronomic practices were followed within the test plots to better represent normal conditions within a commercial vineyard.

Two studies were conducted at separate commercial vineyards in Coachella Valley. In the first, we compared the uptake and persistence of imidacloprid applied as either Admire Pro or Admire 2F to 2-year old table grapes (var. Superior). In the second study, we compared the uptake of imidacloprid applied as either Admire 2F or Nuprid 2F to 20 year old table grapevines (var. Perlettes).

A third study was conducted at a commercial vineyard in Temecula valley, where we compared the uptake and persistence of imidacloprid applied as either Admire Pro or Admire 2F to 6 year old wine grapevines (var. Cabernet).

At each location, the vines were pre-irrigated for at least 1 hour to ensure adequate soil moisture to permit better insecticide penetration to the root zone. Insecticides were applied using SeriPettors® which allowed for the administration of the required amounts of insecticide active ingredient to each vine. Irrigation was maintained for at least 1 hour after the treatments were completed. Throughout the trial, the standard irrigation schedule was adhered to, and was controlled by the vineyard managers at each study site.

Results and Discussion

The temporal profiles of imidacloprid uptake applied as different formulations are summarized in Figure 2.

Admire Pro v Admire 2F – Coachella Valley

The uptake and persistence profiles for both these products were not significantly different ($F_{1,60}$ = 0.03; P = 0.87). Peak levels within the xylem fluid were reached within 4 days and persisted within the vines at sharpshooter threshold levels of 10 ppb for approximately 30 days.

Admire 2F v Nuprid 2F – Coachella Valley

The uptake and persistence profiles for both products tracked each other well, and were not significantly different ($F_{1,70}$ = 1.63; P = 0.21). Although the target thresholds for sharpshooter

mortality were reached within 5 days, the peak uptake was not observed until about 12 days after the applications were made. The differences in uptake dynamics between the two sites are likely to reflect differences in vine age.

Admire Pro v Admire 2F – Temecula Valley

The profiles of imidacloprid uptake for both products tracked each other well and were not significantly different (($F_{1,480}$ = 51; P = 0.11). The initial rate of uptake was disappointing; however, the study was conducted in a commercial vineyard under normal operating practices. Water was minimal for several weeks following the initial application. The major peak in uptake coincided with increased water usage during a very hot period in the local weather. The data reinforce our earlier affirmation that irrigation is absolutely necessary to drive the imidacloprid into the vines, and withholding water could compromise efforts to prevent PD infection of vines.

General Comments

The peak levels of imidacloprid detected within vines at both Coachella study sites were very similar (30 – 45 ppb), indicating that the three products are suitable for use in this region. It is important that growers follow label instructions to ensure that each vine receives the correct dosage of active ingredient, and that subsequent water management does not compromise the uptake. Imidacloprid will bind to soil, especially soils heavy in clay and organic matter content. Imidacloprid has a slightly basic charge, making it attractive to the negative charge on clay particles. If water is not present to solubilize the insecticide, then the insecticide will bind to the clay, and will not be available for uptake. During irrigation, water will release some of the imidacloprid from binding sites in the soil, making it once again available for uptake. However, the amount of imidacloprid moving into the plant during irrigation will depend on the amount of imidacloprid released by the water, and if the binding is high, then the rate of release and subsequent uptake may not be sufficient to achieve target thresholds for pest management.

Objective 3 - Develop an ELISA for the detection and quantification of dinotefuran residues within plant tissues

The objective of this component of the study is to generate a hapten in which the nitroguanidine moiety of the dinotefuran molecule will be conjugated to a carrier protein for subsequent antibody production. The strategy is to generate antibodies to the 3-tetrahydrofuranmethly moiety, hopefully providing for a dinotefuran-specific antibody that will have low cross-reactivity with other neonicotinoids, particularly clothianidin. Currently, an antibody kit is available from a Japanese company, but it is expensive and not always immediately available for purchase when required for our studies.

Work on Objective 3 is still ongoing, since the synthesis chemist has encountered several problems related to the stability of intermediates in the synthesis. When the hapten is synthesized, Frank Byrne will conjugate it to the carrier protein and then send it to a commercial laboratory for Ab production.

Intellectual Property

None

References

1. Byrne, F.J., Castle, S.J., Bi, J.L., and N.C. Toscano. 2005. Application of competitive ELISA for the quantification of imidacloprid titers in xylem fluid extracted from grapevines. J. Econ. Entomol. 98: 182-187.
2. Byrne, F.J., Toscano, N.C., Urena, A.A., and Morse, J.G. 2005. Quantification of imidacloprid toxicity to avocado thrips, *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae), using a combined bioassay and ELISA approach. Pest Management Science 61: 754-758.
3. Byrne, F.J. and N.C. Toscano. 2006. Uptake and persistence of imidacloprid in grapevines treated by chemigation. Crop Protection 25: 831-834.
4. Scholander, P.F., Hammel, H.T., Bradstreer, E.D., and E.A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 148, 339–346
5. Toscano, N.C. and F.J. Byrne. 2005. Laboratory and field evaluations of neonicotinoid insecticides against the glassy-winged sharpshooter. *In* Proceedings of the Pierce's Disease Research Symposium, pp 380-383, The San Diego Marriott Hotel, San Diego, California, Dec 5-7, 2005.
6. Toscano, N.C. and F.J. Byrne. 2006. Laboratory and field evaluations of neonicotinoid insecticides against the glassy-winged sharpshooter. *In* Proceedings of the Pierce's Disease Research Symposium, pp 99-102, The Westin Horton Plaza, San Diego, California, Nov 27-29, 2006.
7. Toscano, N.C., Byrne, F.J. and E. Weber. 2007. Laboratory and field evaluations of neonicotinoid insecticides against the glassy-winged sharpshooter. *In* Proceedings of the Pierce's Disease Research Symposium, pp 98-100, The Westin Horton Plaza, San Diego, California, Dec 12-14, 2007.
8. Toscano, N.C. and F.J. Byrne. 2005. Laboratory and field evaluations of neonicotinoid insecticides against the glassy-winged sharpshooter. *In* Proceedings of the Pierce's Disease Research Symposium, pp 380-383, San Diego Marriott Hotel and Marina, San Diego, California, Dec 5-7, 2005.
9. Weber, E., Byrne, F.J., and N.C. Toscano. 2004. Optimization of Admire applications in north coast vineyards. *In* Proceedings of the Pierce's Disease Research Symposium, pp 388-389, Coronado Island Marriott Resort, Coronado, California, Dec 7-10, 2004.
10. Weber, E., Byrne, F.J., and N.C. Toscano. 2005. Optimization of Admire applications in north coast vineyards. *In* Proceedings of the Pierce's Disease Research Symposium, pp 395-398, San Diego Marriott Hotel and Marina, San Diego, California, Dec 5-7, 2005.

How this work and its results will contribute to solving the PD problem in California

Currently, insecticides are the only reliable means of controlling GWSS populations and PD transmission in vineyards. The neonicotinoid insecticides are the most reliable because of their systemic activity. Systemic activity occurs after the insecticide is applied to the soil. During irrigation, it is absorbed by the vine roots and then distributed throughout the plant in the xylem system. The xylem is the water-conducting system in plants and extends to all areas of the plant. The GWSS feeds from the xylem, and the neonicotinoids directly exploit their feeding habit. Since the xylem is present in all plant tissues, neonicotinoids have the potential to protect every part of the plant where the insect feeds. Our work determines how well the insecticides are working via this mode of action. We have shown that the uptake of the chemicals is not consistent between viticulture regions in California. Put simply, what works for one grower may not work for another because of their different locations. It is imperative that we understand how these insecticides are working under different agronomic conditions and climates, so that their use is optimized for the benefit of California viticulture. There are several neonicotinoid insecticides and each has its own unique properties. Their water solubility can vary greatly, and it is this property that we have addressed in this study in order to advise the industry on which neonicotinoid is more suited to a particular region. While imidacloprid activity in Coachella and Temecula vineyards can provide good control of GWSS, this is not the case in Napa where uptake was poor. However, the results of our work show that dinotefuran uptake in Napa vineyards is superior to imidacloprid. If the levels of uptake are sufficient to kill a GWSS, or other PD vectors more important within this region, then growers will have an effective material with which to protect their vines from PD.

It is important that when new formulations of insecticides are registered for use on grapes that they are carefully evaluated. This applies to new formulations of current insecticides (i.e. imidacloprid), as well as new active ingredients (dinotefuran). Our work has shown that the newer formulations of imidacloprid, introduced since the expiration of the imidacloprid patent in 2006, behave in a similar manner to Admire 2F, and that they are suitable for use in California vineyards where Admire 2F was originally shown to be effective.

Figure 1. Uptake of imidacloprid and dinotefuran in vines at three vineyard locations in California. Dinotefuran was applied as Venom 70SG at 3 oz/acre (half rate) and 6 oz/acre (full rate). Imidacloprid was applied as Admire Pro at 7 fl oz/acre in the Coachella and Temecula vineyards, and at 14 fl oz/acre at the Napa vineyard. Each point on the graphs represents the mean imidacloprid concentration in xylem fluid extracts, measured by ELISA, for at least 12 vines. The horizontal pink line at 10 ppb indicates the recommended concentration of imidacloprid required for the control of GWSS. Note that the scaling on the graphs is different. DAT = Days After Treatment.

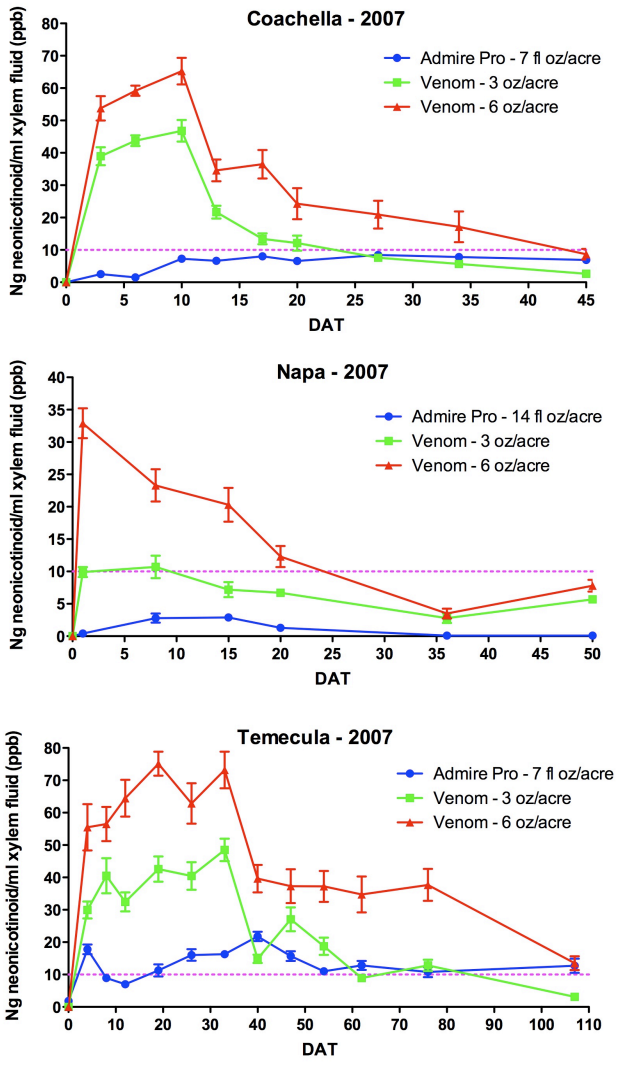

Figure 2. Uptake of imidacloprid applied as different formulations at two vineyard locations. Each point represents the mean concentration of imidacloprid in xylem fluid extracted from at least 16 vines. The horizontal pink line at 10 ppb indicates the recommended concentration of imidacloprid required for the control of GWSS. Note that the scaling on the graphs is different. DAT = Days After Treatment.