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UNDERSTANDING THE DYNAMICS OF NEONICOTINOID INSECTICIDAL ACTIVITY AGAINST THE GLASSY-WINGED SHARPSHOOTER: DEVELOPMENT OF TARGET THRESHOLDS IN GRAPEVINES

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EXECUTIVE SUMMARY

Systemic neonicotinoid insecticides were effective against adult and immature stages of the glassy-winged sharpshooter (GWSS). One reason for the use of systemic treatments is that they exploit the xylophagous feeding behavior of the insect. In this study, we determined the concentrations of dinotefuran necessary to kill GWSS adults feeding from the xylem. Our data show that neonicotinoid treatments have an additional contact activity on emerging first instars before they begin feeding. Egg masses that were exposed to systemic treatments of dinotefuran were unaffected by the treatments and the first instars developed fully within the eggs. However, upon emergence, the insects became susceptible to the effects of insecticide residues that were present in the surrounding tissues. In a series of bioassays, we were able to show that there was a dose-response between insecticide residues and first instar mortality. From these data we were able to derive a target threshold for dinotefuran that would be effective against emerging first instar GWSS nymphs. This target value can be used as an indicator of the efficacy of treatments and the level of protection vines are receiving.

INTRODUCTION

Our research program focuses on the use of chemical insecticides for the management of glassy-winged sharpshooter (GWSS). We are dedicated to formulating safe and effective treatment programs for California growers, given the almost complete reliance by the grape industry on this method of control. We have conducted extensive trials in Coachella, Napa and Temecula valley vineyards to evaluate the uptake and persistence of three neonicotinoids – imidacloprid, thiamethoxam, and dinotefuran – under the diverse range of climatic, soil, and agronomic conditions associated with these regions. We have an understanding about how the different chemical properties, particularly water solubility, of these neonicotinoids can be exploited to achieve optimum uptake into vines, and we have developed sensitive techniques that allow us to monitor the levels of insecticide present within the vines. To exploit this knowledge further for the benefit of California grape production, we need to ensure that the concentrations of insecticide present within the vines are reaching levels that are effective at rapidly killing GWSS before they can infect vines with Pierce's disease (PD). We also need to understand whether there is a sub-lethal impact of these insecticides

on GWSS, since anti-feedant activity may not necessarily eliminate the threat that an infective sharpshooter poses to a vine. Our past and current research projects have established the threshold levels of imidacloprid needed to kill a GWSS at 10 ng/ml xylem fluid, and optimized treatment regimes for growers that will ensure these thresholds are attained following applications via different irrigation methods (drip, sprinkler). In 2007, a new systemic neonicotinoid, Venom (active ingredient dinotefuran), received full registration for use on grapes. Our work in this area has demonstrated the excellent uptake of these new insecticides following systemic application to vines (Toscano et al., 2007). This is good news for vineyard operators who have experienced problems with imidacloprid. Imidacloprid has been the predominant neonicotinoid in use in vineyards, but our research has shown that its uptake and persistence within vines varies dramatically between regions (Coachella Valley, Napa Valley, Temecula Valley). Despite its apparent poor uptake, growers continue to rely on imidacloprid in many areas. The perception is that the insecticide will work well in all areas given its successful implementation in Temecula vineyards (Byrne and Toscano, 2006). Dinotefuran offers a potential solution to overcoming the problems encountered with imidacloprid use – its rate of uptake is faster and it can reach higher concentrations at peak uptake than imidacloprid under the more challenging situations. It also exhibits favorable persistence. Having established that the uptake and persistence of dinotefuran is superior to imidacloprid in terms of insecticidal titers reached in the xylem, it is important to ensure that the levels attained in the xylem are active against sharpshooters. Comparative data on the efficacy of systemic dinotefuran against GWSS was not available, and this study addressed the gap in our knowledge of dinotefuran thresholds needed for effective control of adult and immature GWSS.

OBJECTIVE

The objective of this project was to establish the level of susceptibility of GWSS to the neonicotinoid insecticide dinotefuran. In our studies, we focused on two main areas: (1) evaluate the concentrations of dinotefuran present in xylem fluid that were necessary to kill a GWSS adult feeding on that xylem, and (2) determine the concentrations of dinotefuran distributed within leaves that were necessary to kill first instar GWSS as they emerged from the eggs.

MATERIALS AND METHODS

Insecticide Treatment of Plants

Venom 70 SG was used as the dinotefuran formulation. The product was dissolved in water to give the desired concentrations and then added directly to the soil in each pot. The potting media was pre-watered to ensure effective infiltration of the insecticide. The insecticide was applied in a volume of 50 ml, to avoid leaching from the bottom of the pot. 50-ml of water was used daily to water the plants.

Insects

Insects were collected on the day prior to bioassays from a citrus block at Agricultural Operations on the campus of UCR. They were allowed to acclimatize to the laboratory temperature prior to bioassay setup.

Bioassays

Two bioassay systems were utilized to determine the susceptibility of (1) adult GWSS feeding on plants and (2) 1st instar nymphs emerging from the egg mass.

Adult GWSS Feeding Bioassay

In vivo bioassays were conducted using cotton plants to determine the levels of toxicity of dinotefuran at different rates of application to adult GWSS. Cotton plants were used because large numbers could be grown to the desired size quite rapidly. Preliminary evaluations of different application rates were conducted in order to select a suitable range for the bioassays. This involved treating plants, extracting the xylem fluid at 5-days post-treatment, and quantifying the insecticide residues by ELISA. Plants used were of a consistent age and size in order to regulate the uptake between the different bioassays.

The concentrations of dinotefuran present within plants were controlled by varying the application rates. However, in bioassays, we were detecting mortality when insects fed on xylem that had levels of insecticide below the detection range of the ELISA. For this reason, we developed a simple model from which the concentrations in xylem from plants treated at the lowest rates (and which were undetectable by ELISA because they were out of range) could be determined by extrapolation. The method was very reliable. Figure 1 shows the concentrations of dinotefuran in the xylem fluid of extracts from cotton plants that were treated with different application rates on different occasions between 2008 and 2010. Note the consistency in residue levels at each application rate between the different experiments. The equation of this line was determined and used to estimate the concentrations of dinotefuran in plants treated with the lower rates of insecticide. As a check, all bioassays that were conducted included an application rate that could be evaluated by ELISA. The ELISA data was cross-checked with the model estimate for that particular application rate to ensure that the bioassay procedure was consistent.

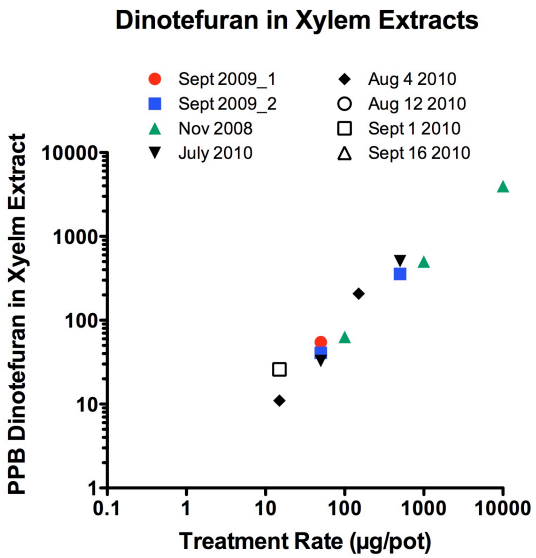

Figure 1. Relationship between dinotefuran concentrations in the xylem and the insecticide application rate. Data for 8 independent bioassays are plotted.

Cotton plants used for feeding bioassays were grown in 48 in^3 pots using the UCR #3 potting media. When plants had reached the required height of 2 ft, they were treated with Venom®. The formulated product was dissolved in water and then added to the soil as a drench application in a final volume of 50 mls. Plants were watered daily with 50 mls water to ensure that there was no leaching of insecticide from the base of the pots.

At 3 days after the plants were treated, GWSS (n=10 for each plant) were confined within ventilated cages to the cotton plants where they could feed on the main stem of the plants. Mortality was determined at 24 h and 48 h after initial confinement on the plants. At the completion of the bioassay (48 h), the xylem fluid was extracted from the plants for imidacloprid analysis. A minimum of 5 treatment rates was used to generate the dose response. Six plants (= replicate) were treated at each application rate, including 6 controls. The control plants were treated with water alone at the same time that the insecticide-treated plants were set up.

Following the completion of the bioassay, the xylem fluid was extracted from the plants and the dinotefuran concentrations measured using an ELISA method. Xylem fluid was extracted from plants using a pressure bomb. For xylem fluid extraction, the stems were cut as close as possible to the point on the stem where the GWSS adults were confined to feed. A section of cambium (1 inch long from the cut end of the stem) was removed and the plant was then inserted into the pressure chamber. The xylem fluid was extracted under a pressure of 30 bars and collected by pipette. Fluid samples were stored in 1.5 ml micro-centrifuge tubes at -20 ºC until insecticide residues were analyzed.

First Instar Bioassay

Adult GWSS were collected from the field source at Agricultural Operations, UCR and confined on cotton plants in insect cages. Cotton is an excellent oviposition host for *H. vitripennis* and for this reason was used as the host plant in this study. Leaves were checked daily for new egg masses to

ensure that no egg mass was older than 24 h when bioassays were set up. When eggs were detected, each leaf was excised from the plant and placed in a plastic leaf box, the design of which was based upon the original Blackman box used for aphid rearing (Blackman, 1971). To accommodate cotton leaves, the box size was increased to 12.5 cm x 8 cm x 2 cm. A plastic platform was inserted into the leaf box and glued into position at 1.5 cm from the base. A sponge strip was placed in the space beneath the platform. The leaf petiole was inserted through a hole in the platform into the sponge, and the box was then placed in a tray of water (to a depth of 1 cm) to saturate the sponge. This procedure ensured the survival of the cotton leaf for the duration of the bioassay.

The petiole was inserted into a vial containing 7 ml of a solution of freshly prepared dinotefuran. In order to ensure a range of insecticide concentrations in the leaves, the uptake vials were initially prepared at 0.1, 1.0, and 10 mg dinotefuran liter^{-1}. Once inserted into the uptake vial, each leaf was placed under fluorescent lights to allow uptake of the insecticide to proceed. After 24 h, the leaf was removed from the vial and returned to the leaf box. The concentration of dinotefuran within the leaf was determined from gravimetric measurements of the vial and its contents taken before and after the uptake period. The treated leaves (n=62) were maintained in the leaf boxes until emergence of the 1st instars was completed. At this time, the leaf area was measured in order to determine the distribution of dinotefuran per unit area of leaf. For controls (without insecticide), the cotton leaves were handled in the same manner, except that the uptake vials contained water instead of dinotefuran. Under the established experimental conditions, the mean developmental time of control eggs was 13 days (± 0.6 s.e.m.).

The egg masses were maintained in the leaf boxes until emergence of the insects. At this time, the total number of eggs in each egg mass was determined under a binocular microscope. To facilitate this, the leaf epidermis covering the egg mass was carefully folded back to expose the eggs. Eggs that had not developed were easily identified at this stage by the absence of the head cap, which normally appears when the developing embryo has reached 90-100 h (Al-Wahaibi, 2004). The microscope was also used to determine whether the mature sharpshooter embryo had broken through the egg chorion when insecticidal activity was assessed. Mortality was recorded at 48 h following the initial emergence of the 1st instar sharpshooter from the egg.

Data Analysis

POLO-PLUS software (LeOra Software, 1987) was used to perform a log dose-probit analysis in order to derive LC5, LC50, and LC95 data (that is the concentration of insecticide that will kill 5%, 50% and 95% of the test insects).

RESULTS

Adult Bioassays

There was an excellent dose-response in the adult GWSS bioassays with dinotefuran. Figure 2 shows the plot of percentage mortality vs the dose (on a logarithmic scale). The LD5, LD50 and LD95 values were calculated from bioassay data compiled from several independent experiments (Table 1).

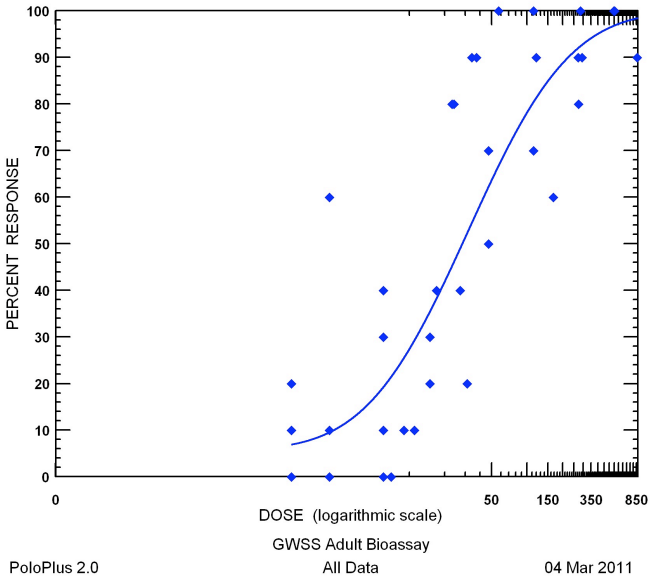

Figure 2. Log dose-response plot for GWSS adults in bioassays with dinotefuran. Mortality was determined at 12 hours after the insects were confined on treated plants. See Table 1 for LD values calculated at 5%, 50% and 95%

Table 1. Log dose-mortality data for GWSS adults bioassayed with dinotefuran. The LD is the Lethal Dose of dinotefuran required to kill a set number of insects (e.g. 50% at LD_{50}). See Figure 2 for plot of data.

Insect Stage	LD_5	LD_{50}	LD_{95}
GWSS Adults	2.4 ppb (0.9 – 5.0)	31 ppb (22 – 48)	411 ppb (203 – 1443)

First Instar Bioassays

We evaluated the effect of dinotefuran against the eggs of the GWSS. As with imidacloprid (Byrne and Toscano, 2007), the nymphs developed fully within the egg masses and only succumbed to the effects of contact with dinotefuran during emergence. This result indicates that the residues of dinotefuran lying in the vicinity of the egg act as a contact insecticide and the insecticidal activity does not require any feeding by the newly emerged nymph. In contrast to our previous data for imidacloprid, where we observed an LC_{50} of 39 ng/cm^2 leaf, results from this study showed that dinotefuran was slightly more toxic to the emerging first instars than imidacloprid (**Figure 3**). Also, the slope of the dose response curve was extremely steep, as was observed for imidacloprid, indicating that once threshold levels are reached within the leaves, the impact on emerging nymphs is highly efficient.

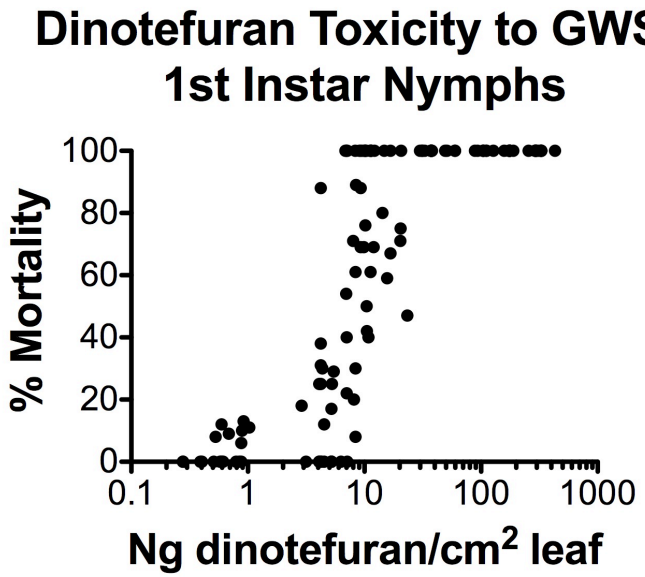

Figure 3. Toxicity of dinotefuran to emerging 1st instar GWSS. The petioles of leaves containing egg masses were placed in vials containing different concentrations of insecticide for 24 h systemic uptake. Leaves were then transferred to leaf boxes where the egg masses were allowed to continue their development. The survivorship of nymphs was determined for 5 days after the first indications of emergence.

Table 2. Log dose-mortality data for first instar GWSS nymphs emerging from egg masses on plants treated systemically with dinotefuran. Mortality units are expressed as ng dinotefuran per cm^2 of leaf tissue. See Figure 3 for plot of data.

Insect Stage	LD_5	LD_{50}	LD_{95}
1st Instar GWSS	1.4 ppb (0.9 – 2.0)	6.8 ppb (5.7 – 8)	33 ppb (25 – 48)

CONCLUSION

At current label recommendations, the rate of uptake of dinotefuran into grapevines is faster than imidacloprid and concentrations of dinotefuran at peak uptake are higher (Toscano et al., 2007). These two properties make dinotefuran a strong candidate for inclusion in a sharpshooter management strategy, provided that effective concentrations are reached within the xylem. The results of this study indicate that effective concentrations are being established in treated vines to kill adult GWSS. Additionally, dinotefuran is highly toxic to emerging first instars, and our data show that the insecticide is slightly more toxic than imidacloprid. As with imidacloprid, the toxic effect is not manifested until the nymphs emerge from the egg mass, suggesting that dinotefuran and imidacloprid act as contact insecticides.

The systemic neonicotinoids imidacloprid and dinotefuran are effective insecticides that growers can use for long-term management of GWSS populations. Because of the contrasting chemical properties of these insecticides, growers can now choose the most suitable product to meet their pest management needs. One of the interesting observations from this study has been that the concentrations of insecticide present within the xylem can be managed by choosing the appropriate application rate. This is a very powerful tool that could be used to optimize insecticide applications and manage insecticide use more effectively.

HOW THIS WORK AND ITS RESULTS WILL CONTRIBUTE TO SOLVING THE PD PROBLEM IN CALIFORNIA

Insecticides are used widely for the management of GWSS. Knowledge of how they work and how they persist within plants is important if we are to get the best use from them. The results of this study established threshold values for dinotefuran that can be used to evaluate the efficacy of an insecticide treatment, whether it be used in a vineyard, a citrus orchard, or a nursery. By using these thresholds, growers can determine the level of protection afforded their crop. It is important that GWSS adults and nymphs are prevented from feeding on vines in order to limit PD infections. Rapid kill is paramount to ensuring that vines do not become infected. The thresholds established in this study will contribute to better insecticide usage. Now that we know how much insecticide is needed to kill a sharpshooter, we can tailor treatment methods and application rates to ensure that those thresholds are achieved at the critical times during the season.

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