CHARACTERIZATION OF NEONICOTINOIDS AND THEIR PLANT METABOLITES IN CITRUS TREES AND GRAPEVINES, AND EVALUATION OF THEIR EFFICACY AGAINST THE GLASSY-WINGED SHARPSHOOTER AND THE EGG PARASITOID *GONATOCERUS ASHMEADI*

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ABSTRACT
The neonicotinoids are highly effective insecticides for the management of glassy-winged sharpshooters GWSS). The systemic activity of the insecticides exploits the feeding behavior of the sharpshooter at all life stages. Imidacloprid was also toxic to the egg stages. This toxicity was manifested at the time of emergence of the 1st instar from the egg mass, and not during the development of the embryo. Imidacloprid metabolites were found to be effective against adult and egg stages of the sharpshooter. In accordance with data from studies on aphids and whiteflies, the olefin and 5-hydroxy metabolites were most toxic, while the desnitro and diol derivatives lacked any toxic effect. Metabolites were detected in the xylem fluid extracted from citrus trees that had been treated with Admire. The source of these metabolites is not yet clear – they may originate from imidacloprid metabolism within the soil before uptake by the trees, or they may have been formed within the trees themselves. Nevertheless, our results confirm that sharpshooters feeding on citrus will encounter imidacloprid metabolites that will contribute to its toxicity. We are currently evaluating the metabolic fate of the other neonicotinoids.

INTRODUCTION
Without a cure for PD, the primary means of controlling its spread in California vineyards is through the elimination of its vector using insecticides. Systemic insecticides are currently being evaluated on both citrus and grapes. Of the various classes of insecticide under consideration, the neonicotinoids, especially imidacloprid, have proven to be the most effective at suppressing GWSS populations. Like all neonicotinoids, imidacloprid is a nicotinic acetylcholine receptor agonist that combines high potency with low mammalian toxicity and favorable persistence. As a systemic treatment, it has proved to be especially effective against the GWSS. The success of imidacloprid in controlling GWSS is due largely to its excellent systemic properties, which exploit the xylophagous feeding behavior of the insect, and thereby disrupt the transmission of PD and other *Xylella fastidiosa*-related diseases. The ability to deliver imidacloprid to the specific feeding zone of the GWSS is an extremely favorable attribute of imidacloprid, and one that has led to the widespread use of this chemical in area-wide management programs conducted in the Temecula Valley, southern Kern County (the General Beale Road Project), and the Coachella Valley.

We are currently evaluating the toxicity of several neonicotinoids insecticides against adult GWSS. These include acetamiprid, clothianidin, dinotefuran and thiamethoxam. Of particular interest to us are thiamethoxam and clothianidin, which are being evaluated for use against citrus and grape pests. Recently, it has been established that thiamethoxam is converted into clothianidin by insects and cotton plants (Nauen et al., 2003). This is an important finding, as it could have ramifications for the use of these products on grapes and citrus.

From a pest management perspective, there are legitimate reasons why it is important to study the neonicotinoid class of insecticides within the citrus and grapevine systems. Little consideration has been given to the impact of neonicotinoids on the eggs of the GWSS. It is important to establish this for two reasons. Firstly, egg mortality will contribute to the suppression of the population. And secondly, an impact on sharpshooter eggs could have a direct knock-on effect on parasitism levels. It remains to be determined whether or not the parasitoid can emerge successfully from a systemically treated plant after the parasitoid has reached the adult stage. This could be an important source of parasitoid mortality in treated citrus, where parasitism is reported to contribute, in some orchards, as much as 90% to overall mortality in the Summer generation. If the parasitoid can survive emergence from the egg, then this is good news for the integration of neonicotinoids and biological control. Parasitism by *G. ashmeadi*, for example, is especially effective against GWSS in Riverside orchards, at a time when insecticide titers arising from early applications of neonicotinoids are diminishing.

OBJECTIVES
1. Determine the metabolic fate of neonicotinoids within citrus trees and grapevines;
2. Determine the relative toxicities of neonicotinoids and their metabolites to the adult and egg stages of the GWSS;
3. Determine the impact of neonicotinoid metabolites on the egg parasitoid *Gonatocerus ashmeadi*.

RESULTS

Metabolic fate of neonicotinoids within citrus trees and grapevines

We are currently processing xylem fluid collected from treated citrus and grapes in order to quantify imidacloprid and its metabolites by HPLC. In Figure 1, the chromatogram of imidacloprid and two of its metabolites are shown. There are clear differences in retention time, enabling us to distinguish between metabolites and the parent compound. In Figure 2, chromatograms for 4 samples from Admire-treated citrus trees are shown. The main peak in each is parent imidacloprid, while there is evidence of metabolites in three of the samples (arrowed). As expected, the metabolites are more polar than the parent compound.

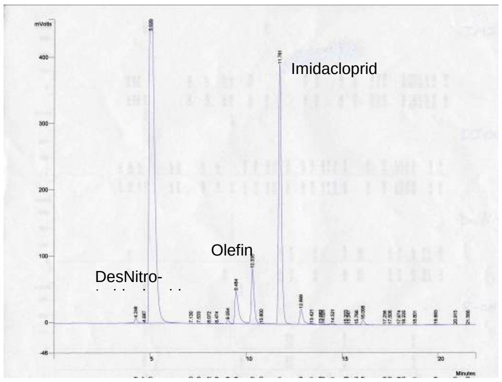

Figure 1. Chromatograms of imidacloprid and two of its metabolites - desnitro imidacloprid and imidacloprid olefin.

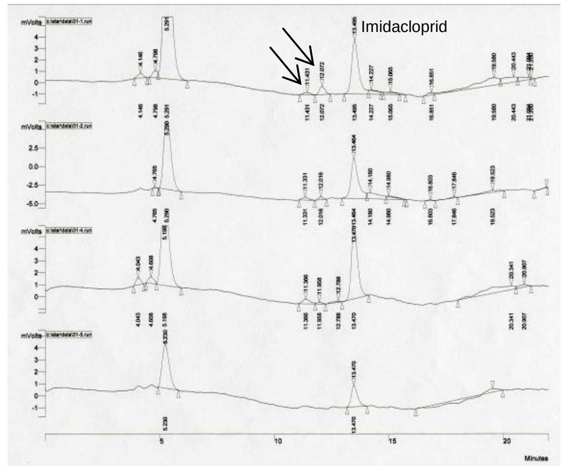

Figure 2. Chromatograms of 4 xylem fluid extracts from citrus trees treated with Admire. The arrows indicate polar metabolites that were detected in three of the extracts. Imidacloprid was the major peak in each chromatogram. The initial peak (at 5 min) is acetone. The presence of the olefin metabolite was also confirmed by TLC.

This is the first evidence that the GWSS feeding on xylem fluid from citrus will encounter metabolites. Furthermore, the presence of the metabolites in the xylem fluid indicates that they are likely to be deposited in the leaf tissue. It is at this location that their impact on the developing egg will be manifested.

Relative toxicities of neonicotinoids and their metabolites to the adult and egg stages of the GWSS

The results from our metabolic fate study vindicate the determination of toxicity profiles for the neonicotinoids and their metabolites. The GWSS are likely to encounter these chemicals during feeding, and so their toxicity is of interest. Furthermore, chemicals detected within the xylem system will be deposited within the leaves and there is, therefore, a strong likelihood that these chemicals could impact the egg stage of the sharpshooter.

The first phase of the study (presented in the 2004 PD/GWSS Report) focused on determining the general contact toxicity (topical application) of the neonicotinoids and some of the key metabolites. Data from that study showed that imidacloprid was highly toxic to GWSS adults. Two important plant metabolites of imidacloprid, the olefin and 5-OH derivatives, also showed high toxicity, while the diol and desnitro derivatives showed no toxicity. These data suggest that, if these metabolites are formed *in planta*, the efficacy of imidacloprid could extend beyond the lifetime of the parent material within the plant. Furthermore, because the metabolism of imidacloprid by microsomal oxidases within the insect is likely to produce the olefin and mono-hydroxy metabolites, this will also contribute to the lethal effects of imidacloprid.

We have completed a study of the effects of leaf residues of imidacloprid and its metabolites on GWSS eggs. Even at extremely high doses, the parent compound does not confer toxicity against the developing embryo. However, there is a lethal effect upon emergence of the immature from the egg that is dose-dependent (Figure 3).

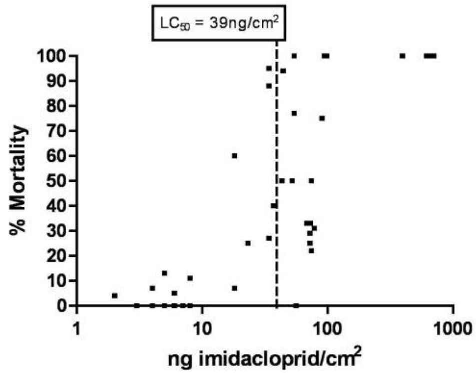

Figure 3. Dose response for GWSS eggs developing on imidacloprid-treated cotton leaves. The vertical line indicates the LC_{50} concentration.

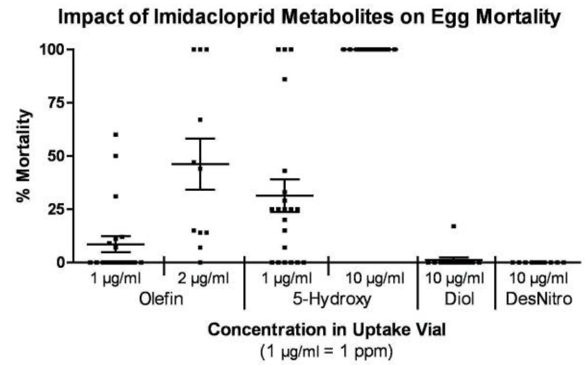

Figure 4. Impact of imidacloprid metabolites on GWSS egg survival. Horizontal bars indicate mean mortality at that dose. Vertical bars indicate the standard errors.

We have not developed full dose-response curves for the metabolites of imidacloprid. Instead, we have selected certain doses based on our results with imidacloprid. The toxicity profiles for the metabolites were the same for the emerging immature as for the adults (Figure 4). Both the olefin and 5-OH metabolites exhibited toxicity, while the diol and desnitro derivates had no effect. The significance of these results is two-fold. Firstly, they indicate that the GWSS egg receives no nourishment from the leaf during development. In Figure 5, the toxic effects of imidacloprid are evident in emerging immatures. And secondly, they raise the concern about the likely impact of these residues on a developing parasitoid. It would seem likely that the developing parasitoid would be protected inside the egg. However, during emergence from the egg, the parasitoid must pass through leaf tissue. This objective is currently under investigation.

Figure 5. Mortality of emerging immature GWSS on cotton leaf treated systemically with imidacloprid. Immatures develop fully within the egg mass, but succumb upon emergence.

CONCLUSIONS

The significance of our findings for the PD/GWSS problem is clearcut. Our data provides important information on the behavior of the neonicotinoids and their metabolites on the PD vector. The impact of this important chemical class extends to all stages of the GWSS, including the egg stage. In this report, we have provided the first evidence for a toxic effect of imidacloprid on the embryo as it emerges from the egg. Prior to emergence, the developing embryo remains protected within the confines of the egg. Our work now will focus on evaluating the potential toxic effect of imidacloprid against the developing parasitoid within the sharpshooter egg. The egg parasitoid adult may suffer the same fate as emerging nymphs as it passes from the egg through contaminated leaf tissue.

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