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

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ABSTRACT
The toxicities of established and new members of the neonicotinoid insecticide class were assessed against the glassy-winged sharpshooter in topical application bioassays. All compounds were highly toxic to the insect. Clothianidin elicited its toxic response more rapidly than thiamethoxam and was 3-fold more toxic overall at the LD50 level. Clothianidin has been proposed as an active derivative of thiamethoxam, so it is important to establish the fate of these chemicals within plant systems that are likely to be treated for GWSS control.

INTRODUCTION
The primary means of controlling the spread of Pierce’s disease (PD) in California vineyards is through the elimination of its vector using insecticides. The glassy-winged sharpshooter (GWSS) Homalodisca coagulata feeds directly from the plant xylem system and, therefore, 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. Imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine) is a nicotinic acetylcholine receptor agonist that combines high potency with low mammalian toxicity and favorable persistence. As a systemic, seed, soil or foliar treatment, it has proved to be especially effective against a wide range of homopterous insect pests, including the GWSS. The success of imidacloprid in controlling GWSS is due largely to its excellent systemic properties. Systemic applications exploit the xylophagous feeding behavior of the insect, and thereby disrupt the transmission of PD and other X. fastidiosa-related diseases.

This project is an extension of a one-year project that was funded by the UC Pierce’s Disease Research Grant Program. It will focus on the fate of imidacloprid and other neonicotinoid insecticides in citrus and grapes, and the impact of these chemicals on GWSS. In a previous study, imidacloprid and two of its derivatives were shown to be highly toxic to GWSS adults (Byrne and Toscano, 2003).

The aims of this study are to determine the extent to which metabolites of neonicotinoids are formed in citrus trees and grapevines, and to determine their toxicological significance towards GWSS. The presence of insecticidal metabolites in xylem sap could contribute to the excellent persistence of imidacloprid treatments against sharpshooters. As well as maintaining the toxic pressure of the initial application, the metabolism of neonicotinoids to yield equally or more toxic metabolites may also account for the stability of this chemical class to resistance.

Of particular interest to us are thiamethoxam and clothianidin, which are being evaluated for use against citrus and grape pests. During the past year, 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. When several products from the same class become available for pest management, it is important that their use be carefully monitored in order to circumvent potential resistance problems. The possibility that thiamethoxam is converted into clothianidin is, therefore, of concern when formulating management strategies based around the neonicotinoids. Receptor binding studies have suggested that thiamethoxam does not bind to the same receptor site as imidacloprid and so it has been proposed as a suitable product for alternation with imidacloprid because of the reduced resistance risk (Weisner and Kayser, 2000). Now that thiamethoxam has been shown to be a potential pro-insecticide, and clothianidin has been shown to bind to the same receptors as imidacloprid, new issues are raised about its suitability as a product for rotation with other neonicotinoids. This is an important reason for determining the fate of thiamethoxam in citrus and grapes.

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.

RESULTS
The toxicity of four neonicotinoid insecticides has been assessed for GWSS adults using a topical application bioassay (Table 1). Thiamethoxam, clothianidin and acetamiprid were all more toxic than imidacloprid. Clothianidin was
approximately 3-fold more toxic than thiamethoxam, and the dose-response was steeper as indicated by the higher slope. It was evident during these bioassays that the toxic effects of thiamethoxam were delayed compared with the other insecticides, suggesting that thiamethoxam may require activation to a toxic derivative within the GWSS.

Table 1. Toxicity of neonicotinoids to the GWSS in topical application bioassays.

<table>
<thead>
<tr>
<th>Compound</th>
<th>LD50 (ng a.i. per insect)</th>
<th>95% FL</th>
<th>Slope</th>
<th>No. of insects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid</td>
<td>4.8</td>
<td>2.8</td>
<td>1.5 ±0.4</td>
<td>100</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>2.6</td>
<td>2.0-3.3</td>
<td>1.4 ±0.3</td>
<td>200</td>
</tr>
<tr>
<td>Clothianidin</td>
<td>0.7</td>
<td>0.6-0.9</td>
<td>5.2±0.9</td>
<td>125</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>0.7</td>
<td>0.6-0.9</td>
<td>3.7±0.6</td>
<td>125</td>
</tr>
</tbody>
</table>

CONCLUSIONS
In this study, we tested four neonicotinoids against the GWSS. Although there were differences in LD50s, all compounds were highly toxic. These results confirm that the newer neonicotinoids could have a place in GWSS management programs. We are currently investigating the fate of these chemicals in both citrus trees and grapevines. Establishing the potential for conversion of thiamethoxam into clothianidin is of particular importance if these chemicals are to be incorporated into management strategies.

REFERENCES

FUNDING AGENCIES
Funding for this project was provided by the University of California Pierce’s Disease Grant Program.