

EFFECTS OF SUBLETHAL DOSES OF IMIDACLOPRID ON VECTOR TRANSMISSION OF *XYLELLA FASTIDIOSA*

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Reporting Period: The results reported here are from work conducted from November 2003 to October 2004.

ABSTRACT

A computer-monitored flight mill was developed to study the effects on insect flight of sub-lethal dosages of soil-applied imidacloprid (Admire 2F, 21.4% AI) to glassy-winged sharpshooters (GWSS) in laboratory cages. Adult sharpshooters were glued to a 10 cm radius plastic arm that rotated on a pivot. The rotations per minute were recorded and tabulated by computer. The range of distances flown on flight mills by adult GWSS not exposed to insecticide treatment (negative controls) ranged from 8 m to 6,843 meters and averaged 3,853 m for males and 2,537 m for females. Over 90% of males and females flew at least 60 m ("fliers") during the 6-12 hour flight trials. More than 9 % of total distances flown by individual fliers occurred within 4 hours. Imidacloprid at sub-lethal dosages (9% mortality in 24 hours vs. 3% of untreated controls) that inhibit feeding did not reduce flight performance significantly, but dosages that killed 33% of the GWSS in 24 hours reduced flight in the surviving insects. Insects that had fed on insecticide-treated plants for 24 hours flew much less (fewer fliers), yet among those that did fly, the differences were not statistically significant. At 3.2 mg imidacloprid in 500 g soil, on average, one-third were killed after 24 hours, and less than 50 % of the survivors flew. However, there were occasional "outliers" that could fly just as well as, or sometimes more than, the control insects. Whether these individuals were resistant to imidacloprid or survived and flew as a result of uneven uptake of the insecticide by different replicate plants was not clear. There were no significant differences in flight distances of GWSS exposed to a dose of 0.1 mg in 500g soil.

INTRODUCTION

The systemic insecticide imidacloprid (Admire 2F, Bayer Co., Kansas City, MO) has been used to control glassy-winged sharpshooter (*Homalodisca coagulata*, GWSS) in citrus and grapes, mainly as a killing agent (Bethke et al. 2001). The main effect of insecticides in reducing the spread of Pierce's disease is to decrease the numbers of insects entering and remaining in vineyards. But beyond the numbers of GWSS, disease spread also depends on the level of infectivity of GWSS with *Xylella fastidiosa*, vector transmission efficiency to grape, and movements of the vector from plant to plant (Purcell 1981). GWSS movements from vine to vine should be especially important if this is the main mode by which GWSS establishes new infections of grape, as circumstantial evidence suggests (Perring et al. 2001; Purcell and Saunders 2001). Sub-lethal (low lethality) dosages may persist in treated crops longer than highly lethal dosages, as plant growth dilutes insecticide concentrations and the insecticide deteriorates to less toxic or non-toxic forms. Identifying the effects of sub-lethal dosages on the behavior of a plant disease vector is especially important because non-lethal doses of insecticide may repel some insects and increase plant-to-plant movements, leading to increased disease spread by surviving vectors. Our previous studies suggested that imidacloprid does not repel the GWSS or promote their small scale plant-to-plant movement.

Our objectives were to establish the effects of sub-lethal dosages of imidacloprid on GWSS transmission efficiency and movement. As we previously reported (Purcell 2003), systemic imidacloprid (soil applications) in grape reduced GWSS transmission of *X. fastidiosa* to grape, but the effects might have been mostly due to insect mortality rather than by affecting GWSS feeding behavior in such as way as to reduce vector transmission. Dosages that did not kill more than 10% of GWSS significantly reduced feeding by GWSS, but imidacloprid did not repel GWSS or blue-green sharpshooters in lab trials in which a documented repellent, Surround, did repel sharpshooters from plants (Purcell 2003).

We tested various dosages of imidacloprid that caused reduced GWSS feeding to determine the effects of the insecticide exposure on the flight performance of GWSS on flight mills. Computer-monitored flight mills have been used to study flight performance in other leafhoppers (Gorder 1990; Taylor et al. 1992), and we adopted a previously described flight mill design (Gorder 1990; Schumacher et al. 1997) to assess the flight performance of GWSS with or without exposure to imidacloprid treatments of grape. Flight mill performance usually requires about 30% of the power required for free flight (Riley et al. 1997), so flight mills underestimate free flight distances.

OBJECTIVES

1. Understand basic performance characteristics of GWSS flight.
2. Determine the effect of various doses of imidacloprid on the flight performance of GWSS in the context of Pierce's disease epidemiology.

RESULTS

Objective 1. Understand the Basic Characteristics of GWSS's Flight.

Flight mills were constructed as outlined by Schumacher et al. (1997), with slight modification. The rotating flight mill arm was a 20cm plastic drinking straw rotating on a jewel bearing fitted with a steel shaft. Custom computer software counted the number of revolutions in successive 60-second intervals and generated data on flight distance, duration, and velocity. For each trial, 3 replications of 4 to 10 GWSSs per cage were allowed to feed on grape for 24 hours. The prothorax of each insect was glued to a standard insect pin using water-soluble Styrofoam glue, and the insect pin (with the insect attached) was then inserted into the arm of the mill. Flight trials lasted for 12 hours, later reduced to 4 hours, during the day. GWSS were classified as “fliers” if they flew a total distance of 100 rotations (63 m) and “non-fliers” if they failed to complete 100 rotations. Table 1 summarizes the flight mill performance of GWSS from untreated plants. Males consistently flew longer and more frequently than females (Figure 1), so data for males only (Table 2) were summarized for comparisons of GWSS from treated and untreated grape. Figure 2 illustrates a typical flight profile for GWSS males from untreated (Figure 2A) and high dosage plants (Figure 2B).

Objective 2. Examine the Effect of Various Doses of Imidacloprid on the Flight Performance of GWSS in the Context of Pierce's Disease Epidemiology

To quantify the effects of sub-lethal dosages of Admire on GWSS flight performance, we measured the flight performance of insects exposed to both treated and untreated grape vines. Imidacloprid treatments were dilutions of a standard 3.2 mg in 500 g of soil. Dilutions used were 1/4, 1/8, 1/16, 1/32 of the standard dose; controls were untreated vines. The plants were allowed one week for pesticide uptake before caging the insects on them for 24 hours and then monitoring their flight mill performance. The 1/32nd dilution caused 9% mortality over a 24-hour period, compared to controls (3%) and did not significantly reduce total distance flown. A higher dose (1/4 of standard) did kill significantly more GWSS (33%) within 24 hours and reduced the numbers of surviving insects classified as “fliers”, but some individual GWSS from the 1/4th dosage plants flew as well as those from untreated plants (Table 2). This may have been because of physiological variation among individuals or the amount of imidacloprid taken up by plants on which the insects had fed. We collected and froze xylem saps to compare imidacloprid concentrations from each plant to the flight performance of the GWSS that fed on them before flight mill assays but have not yet analyzed these samples for imidacloprid content.

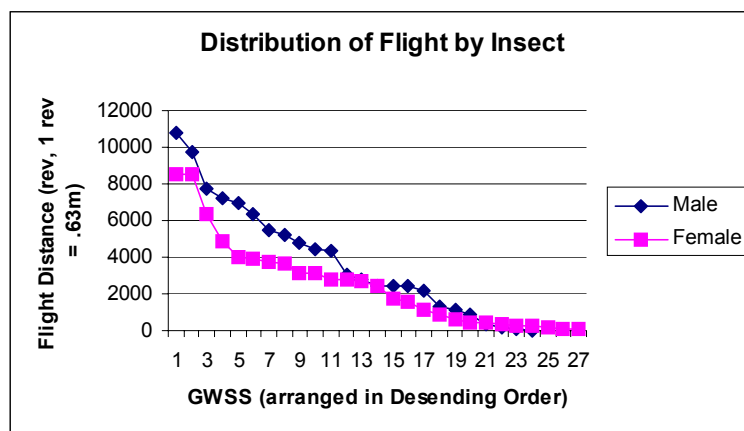


Figure 1. The flight distances of GWSS male (diamonds) and female (squares) from untreated plants.

Table 1. Flight mill performance of GWSS from untreated grape (control).

<u>Performance characteristics</u>		<u>Range</u>	<u>Average</u>	<u>Stand. dev.</u>
<u>Total Revolutions</u>	Males	12-10,826	3,853	3,085
	Females	72 - 8,557	2,537	2,410
<u>Total flight events</u>	Males	17-200	75	42
	Females	13-207	79	57
<u>Distance of longest flight event</u>	Males	6-1258 meters	358 m	359
	Females	6-495 m	149 m	140
<u>Average distance per flight event</u>	Males	6-178 m	70 m	46.9
	Females	6-151	37	40.8

Table 2. Mortality and flight performance of GWSS males after a 24-hour exposure to untreated grape or grape with imidacloprid applied at 1/4th or 1/32nd of a standard dose (3.2 mg/500 g soil) 10 days previously.

<u>Performance characteristic</u> (Males Only)	<u>Sample size</u>	<u>Range</u>	<u>Average</u> *	<u>Stand. dev.</u>	
<u>Mortality</u>	1/4 th dose	57	0- 100%	33% a	0.34
	1/32 nd dose	48	0 - 25%	9% b	0.09
	untreated	48	0 - 20%	3% b	0.08
<u>Percentage of surviving non-fliers</u>					
	1/4 th dose	38	0 - 100%	59% a	0.38
	1/32 nd dose	44	0 - 22%	7% b	0.09
	untreated	46	0 - 20%	3% b	0.08

*Numbers in a column followed by the same letter were not significantly different using chi-squared with Yates' correction and ANOVA.

The flight performance assays of GWSS exposed to 1/8th and 1/16th dilutions of the standard dosage of imidacloprid are still in progress. Preliminary indications are that the 1/8th dilution may reduce average flight activity but with some individuals flying as far as fliers from untreated plants.

Unreported Results that were Pending Last Year.

The effects of the insect-repellent kaolin clay (Surround) and Admire applied to potted grapevines were assessed in cages for possible repellency effects to GWSS and BGSS (Purcell 2003). In general Surround was repellent, whereas Admire was not. The test plants used in these behavioral experiments were saved for diagnosis for PD, as all sharpshooters used in the experiments had been exposed to plants infected with *X. fastidiosa*. Unfortunately, transmission rates in all treatments (including untreated controls) were too low (3% per plant for GWSS, 9-21% for BGSS) to be of value in assessing the effects of Admire or Surround applications on the vector transmission of *X. fastidiosa* where the insects had a choice of treated vs. untreated plants. This lower than normal transmission rate was probably due to low populations of *X. fastidiosa* in the PD-grapes used for acquisition feeding.

CONCLUSIONS

GWSS flew on flight mills for up to 4.2 miles (6.8 km), averaging over 1.5 miles in a 4 hr period. Soil-applied imidacloprid (Admire) dosages that caused 33% mortality during a 24-hr exposure to treated plants reduced average flight performance of surviving GWSS, but some of the insects that survived this exposure flew almost normally. Dosages that caused about 10% mortality and that have been shown to drastically reduce GWSS feeding did not significantly reduce flight on flight mills. Admire treatments probably reduce long distance movements of GWSS from treated crops having sap concentrations of imidacloprid that kill at least 30% of the GWSS within 24 hours.

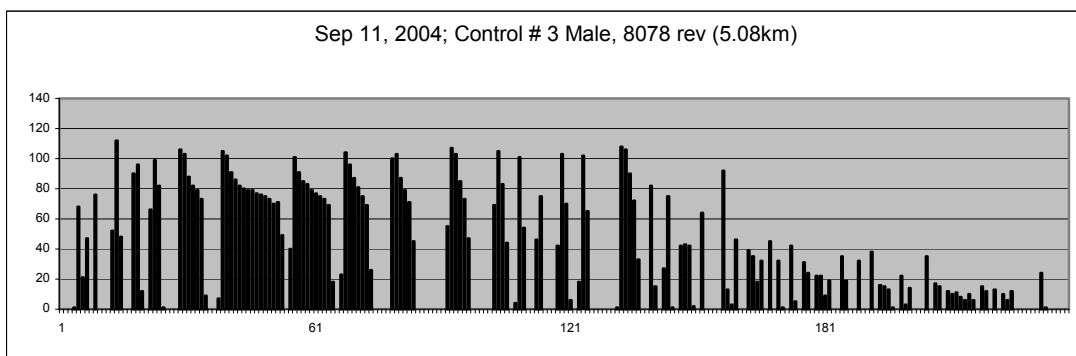


Figure 2A. Flight (flight mill rotations per minute) of a control GWSS (no insecticide); horizontal axis = minutes.

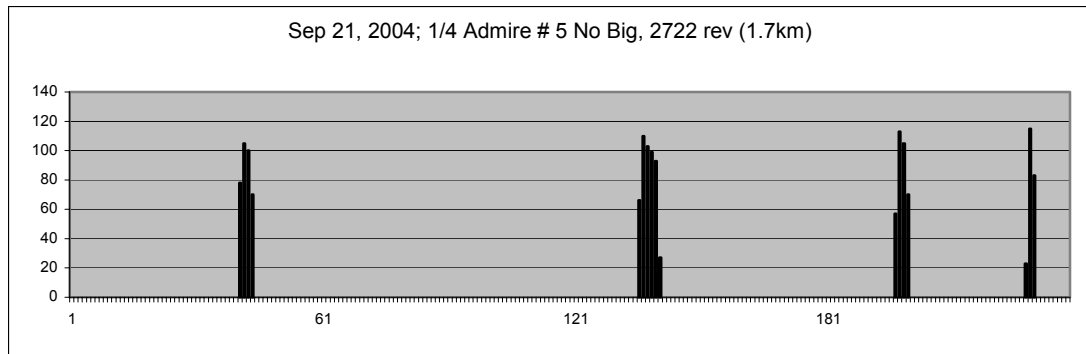


Figure 2B. Flight of a surviving GWSS fed on grape treated with 1/4 of standard dose. Note flights are fewer and shorter than untreated insects.

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FUNDING AGENCIES

Funding for this project was provided by the CDFR Pierce's Disease and Glassy-winged Sharpshooter Board and the University of California, Berkeley College of Natural Resources ARE Institute.