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Impact of pymetrozine on glassy-winged sharpshooter feeding behavior and rate of Xylella fastidiosa transmission

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Impact of pymetrozine on glassy-winged sharpshooter feeding behavior and rate of *Xylella fastidiosa* transmission

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Abstract

Pymetrozine is a compound that interferes with insect feeding and interrupts transmission of plant pathogens. The glassy-winged sharpshooter, *Homalodisca coagulata* Say (Hemiptera, Cicadellidae), is a vector of *Xylella fastidiosa*, the foregut-borne, propagative bacterium that causes Pierce's disease of grapevine. In this study, we recorded the behavioral response of *H. coagulata* to plants treated by soil drench with pymetrozine using time-lapse photography, quantified the reduction in liquid excreta produced by *H. coagulata* fed on pymetrozine-treated plants, and evaluated pymetrozine effectiveness in reducing transmission rate in grapevines. *H. coagulata* feeding on plants treated with 0.015 mg of pymetrozine was disrupted by decreasing the number of contacts made with the grapevine by more than 50% and by increasing movements away from the stem by more than 5-fold. Excreta production by *H. coagulata* was significantly reduced on plants treated with 0.015 or 0.0075 mg of pymetrozine. Contrary to the expected outcome, the mean number of *X. fastidiosa*-infected plants actually increased in the pymetrozine treatments relative to the controls.

This paper includes one video that can be accessed at http://insectscience.org/4.34

Introduction

Pymetrozine, a novel compound sold under the trade names Fulfill™, Endeavor™, and Chess 250 WP™, is a systemic antifeedant, belonging to the class of chemicals known as pyridine-azomethines (MSDS 2001). It has been approved for use on a variety of crops at rates varying from 0.09 to 3.12 lb/acre (Serafini 2001). Pymetrozine is mobile in the xylem tissues of plants (Wyss and Bolsinger 1997), so it is effective against xylem feeders when applied as a systemic chemical. In Hemiptera, pymetrozine affects the vector's feeding activities by paralyzing the cibarial muscle system (Harrewijn and Kayser 1997). Pymetrozine concentrations of 10^{-4} M in the hemolymph were sufficient to reduce probing and oviposition. The muscle contractions associated with the cibarial valves and the salivary pump decreased and became irregular after exposure to pymetrozine.

The glassy-winged sharpshooter, *Homalodisca coagulata* Say (Hemiptera, Cicadellidae), which feeds primarily from xylem, was introduced into California over 15 years ago. This agronomic and ornamental pest has greater dispersal capabilities and feeds on a wider variety of host plants than native leafhopper species (Perring *et al*. 2001; Blue and Morgan 2003; Blackmer *et al*. 2004) enhancing the spread of *Xylella fastidiosa,* the xylem-limited bacterium that causes Pierce's disease in grapes. While *X. fastidiosa* has been present in California for over a century, pathogen spread was limited because native sharpshooters remained close to riparian habitats and rarely ventured into the agricultural setting (Meadows 2001). To combat the threat of Pierce's disease, areawide management programs have depended on imidacloprid treatment on hosts near vineyards to suppress populations of *H. coagulata* infested with *Xylella* (Krewer *et al*. 2002). Population suppression techniques slows the spread of the pathogen by reducing the number of vector insects, but the pathogen will continue to spread even if low numbers of *H. coagulata* are present. Therefore, a direct approach would be to protect the grapevine by preventing feeding attempts. Use of an antifeedant could be beneficial if used in conjunction with an integrated pest management strategy (Slosser *et al*. 2000) if feeding were stopped before pathogen transmission occurs (Bedford *et al*. 1998).

Reduction of the ability of aphids to transmit cauliflower mosaic virus from infected plants within 1 hour after being treated with pymetrozine has been demonstrated (Bedford *et al*. 1998). It has been determined that *H. coagulata* can acquire *X. fastidiosa* from an infected plant in 2 hours of feeding and can spread the bacterium to a new plant within 3.9 hours (Purcell and Finlay 1979; Almeida and Purcell 2002, 2003). Therefore, we determined if systemic application of pymetrozine could disrupt *H. coagulata* feeding resulting in the prevention of pathogen transmission in laboratory studies.

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Materials and Methods

Collection and maintenance of H. coagulata

H. coagulata adults were collected from the lemon orchards at the University of California, Riverside campus and transported in a 30 × 30 × 30 cm collapsible, chiffon-screened collecting cage (Bioquip Products Inc., www.bioquip.com). *H. coagulata* were moved from the collecting cage and kept in four 60 × 30 × 60 cm rearing cages containing four *Chrysanthemum grandiflora* cv. 'White Diamond' plants as food and oviposition sources. The plants were watered daily and replaced weekly with four new White Diamond plants (12 L / 12 D, at 27° C and 70% humidity).

H. coagulata *feeding observation*

H. coagulata were caged individually on cowpea plants (*Vigna unguiculata* subsp. *unguiculata*) using a clip cage that allowed access to one side of the plant. Three clip cages were placed on each plant so that three replications were observed per plant. Three plants were treated with pymetrozine as follows: one water treated control plant, one plant treated with 0.0075 mg per plant, and one plant treated with 0.015 mg per plant. Amounts applied were calculated from the registration application rate suggested for other fruits (pome fruits and stone fruits) on a per acre basis and correlated to pot size. These amounts were then standardized for all experiments. Applications of pymetrozine were made 24 hours prior to the initial exposure of *H. coagulata* to plants and then in 24 hour intervals for 10 days. All three clip cages on all three plants were arranged so that they could be simultaneously recorded using a QuickCam Express, model number V-UH9 (Logitech, www.logitech.com). With Win TLV version 2.0 (http://www.c3sys.demon.co.uk), one frame was recorded every 30 seconds over a 24 hour period and played back at a rate of 5 frames per second resulting in a 9 minute 36 second video clip. This experiment was replicated on 3 separate dates with 9 *H. coagulata* /treatment so that the activities of 27 *H. coagulata* were observed per treatment. From the video surveillance, the mean number of *H. coagulata* that established contact with the plant within each 1 hour period was calculated. Contact establishment was determined when an insect landed on a plant for more than 10 minutes (4 seconds of time-lapse video). Observational data indicated that 100% of *H. coagulata* probed a host plant when they established contact with a plant for more than 5 minutes. Additionally, mean movements away from the stem after established contact was tabulated. Feeding observations on different dates were not significantly different, so data were pooled before the treatment effect was analyzed by ANOVA. When significant treatment effects were observed, means were separated by least significant difference tests (LSD) ($p = 0.001$).

H. coagulata *excreta study*

On two dates, sets of ten chrysanthemums (averaging 20 cm) were given one of three pymetrozine soil applied treatments daily for a two week period; (1) water treated controls (no pymetrozine), (2) pymetrozine at 0.0075 mg per plant, and (3) pymetrozine at 0.015 mg per plant. Leaves were removed from the proximal 10 cm of the plant so that only the bare stem was available for *H. coagulata* feeding. The removal of leaves also allowed liquid excreta to drip into the collection vial without being impeded by leaf material and did not affect feeding performance because the stem is a preferred feeding site. Beginning 24 hours after the initial treatment, 10 field-collected *H. coagulata* were caged in a vial on each plant so excreta could be collected (Anderson *et al*. 1992). Briefly, a 0.25 cm hole was bored in the center of a plastic vial lid and the plant was place through the hole. The 7 cm × 5 cm diameter plastic vial (Thornton Plastics Co., http://thorntonplastics.com/) containing 10 *H. coagulata* was fastened to the lid with the leaf-free stem terminal contained inside (Fig. 1). The plant was placed on its side (so the plant stem was horizontal) and the vial was slightly inverted so that any liquid excreta produced by the *H. coagulata* would drip to the bottom of the vial. Each day, 10 fresh *H. coagulata* were caged on each plant for 4 hours (10 am–2 pm). The weight (g) of excreta contained in each vial was determined. Mean fluid production was calculated for groups of *H. coagulata* fed on each treatment. Data were pooled over all dates within each replication. ANOVA followed by mean separation tests (LSD) was used to determine if differences ($p = 0.001$) in mean fluid production existed among treatments.

Transmission experiments

Four large grapevines (*Vitis vinifera* L. cv. 'Chardonnay') infected with *X. fastidiosa* were placed into a 60 × 30 × 60 cm rearing cage. Four hundred *H. coagulata* adults were allowed to feed on the infected grapevines for 5 days. Two holding cages (42 × 42 × 66 cm) were constructed using wooden frames with fine-mesh organdy covering all sides and top. The base, sitting on four legs, was designed to hold 20 square containers (2 × 2 × 12) with the bottoms of the containers coming through the base (Fig. 2). Plastic cups were then put under each of the containers so all liquid would stay in contact with plant soil and treatment would not be lost due to runoff.

Randomly, 20 grapevines (Sunridge Nursery, Bakersfield, CA) were assigned one of three treatments; water treated controls, 0.0075 mg pymetrozine per grapevine, and 0.015 mg pymetrozine per grapevine. The treatments were applied every 24 hours for 12 days starting 48 hours prior to *H. coagulata* exposure. Sixty *H. coagulata* that had been fed on *X. fastidiosa* were then transferred to each experimental rearing cage holding the pathogen-free grapevines. The plants were randomly distributed in the cage to provide *H. coagulata* choice tests. After 12 days, all surviving *H. coagulata* were removed from the cages. The grapevines were moved to pathogen-free greenhouse for 3 months under natural light conditions with daily watering from an automated irrigation system at 27° C. This experiment was replicated a total of 4 times on 2 dates.

Pathogen presence was detected by double antibody sandwich enzyme-linked immunosorbent assay (ELISA), specific for *X. fastidiosa,* using the extraction method included with the ELISA test system for *X. fastidiosa* kit (Agdia Inc., www.agida.com). Briefly, 100 µl of prepared sample was added to each sample well and the plate was incubated for 2 hours at 25º C in a humid box. Enzyme conjugate was then added to each well. After 10 minutes, wells were emptied and the plate was washed 6 times with 1X PBST. Then, enzyme conjugate was added at a rate of 100 µl of prepared enzyme conjugate per well and incubated in

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Figure 1. *Homalodisca coagulata* excreta study setup.

the humid box for 2 hours at 25° C. The plate was washed 6 times before adding 100 µl of o-Phenylenediamine solution per well and incubated for 15–30 minutes. The reaction was stopped by adding 50 µl of 3M sulfuric acid to each well. Results were evaluated by examining the wells using a Benchmark microplate reader (BioRad, www.bio-rad.com) and analyzed using the BioRad Microplate Manager V. 5.1 build 75 software. Wells in which color developed to greater than twice the OD^{490} of the negative control were considered positive. Test results were valid only if positive control wells tested positive and negative control buffer wells remained clear. Data were pooled between replications because no significant differences between dates were noted. Differences in rates of *X. fastidiosa* infections in grapevines receiving different doses were compared using ANOVA and LSD ($p = 0.001$).

Results

H. coagulata *feeding observation*

When *H. coagulata* were initially exposed to a control plant under laboratory conditions, they usually established contact within 10 min followed by probing. *H. coagulata* exposed to plants treated with either dose of pymetrozine were much more agitated than insects exposed to control plants. While 94% of *H. coagulata* exposed to control plants established contact with the test plant for at least 10 min per hour, 81% of *H. coagulata* exposed to test plants receiving 0.0075 mg of pymetrozine per plant and 38% of *H. coagulata* exposed to test plants receiving 0.015 mg of pymetrozine per plant established contact (Table 1) (see video). Percent contact in all groups of *H. coagulata* was statistically different among treatments ($p<0.001$). The percentage of *H. coagulata* establishing contact with the host plants varied little over a 24 h period; however, differences among treatment groups were evident (Fig. 3). More than 90% of the control insects established contact with the plant.

Figure 2. Transmission experiment plant arrangement. Note: a large rectangular cage was placed around the plants when insects were introduced.

However, contact was reduced by 20% for those exposed to plants given 0.0075 mg of pymetrozine, and by 60% for those exposed to plants given 0.015 mg of pymetrozine.

During the 24 h recording period, insects on plants treated with no pymetrozine moved away from the plant fewer than 6 times

per hour (3.56 ± 2.45) and these digressions were quickly followed by another landing on the plant where another long feeding bout ensued (n = 27) (Fig. 4). In comparison, insects caged on plants treated with 0.0075 mg of pymetrozine moved away from treated plants 13.07 ± 3.09 times per hour and insects caged on plants treated with 0.015 mg of pymetrozine moved away 19.65 ± 6.85 times per hour. The number of movements by all groups of *H. coagulata* were statistically different among treatments ($p < 0.001$) (Table 1).

H. coagulata placed on plants treated with pymetrozine were noticeably more agitated than insects placed on control plants (n = 27) in all replications. *H. coagulata* placed on treated plants landed on the plant, test probed, and moved away repeatedly. This irritation continued throughout a 24 hour period for insects exposed to plants treated with pymetrozine, while the insects exposed to control plants settled for long periods of time. Interestingly, when the insects exposed to control plants moved away from the stem they often stayed in one place for a relatively long period (<30 minutes); whereas, *H. coagulata* exposed to pymetrozine-treated plants were erratic in their movement, never staying in a position off the plant for more than 20 minutes.

H. coagulata *excreta study*

In both replications, *H. coagulata* fed on control plants produced significantly more fluid (1.32 g in replication one and 0.44 g in replication two) then those fed on plants receiving either 0.015 mg of pymetrozine (1.04 g in replication one and 0.27 g in replication two) or 0.0075 mg of pymetrozine (1.11 g in replication one and 0.29 g in replication two) ($p<0.001$) (Table 2). Mean fluid production between the two concentrations of pymetrozine were not different ($p<0.001$).

Transmission tests

Data from all replications on both dates were pooled. Incidence of *X. fastidiosa* in the experiments differed significantly between control plants and the pymetrozine treated plants ($p<0.001$) (Table 3). Forty-two percent (11 of 26) of control plants tested positive for the presence of *X. fastidiosa*, while 67.9% (19 of 28) of the plants that received 0.015 mg of pymetrozine, and 69.2% (18 of 26) of the plants that received 0.0075 mg of pymetrozine tested positive.

Discussion

Noticeable agitation of *H. coagulata* after probing the pymetrozine-treated plants was consistent with the effects of pymetrozine on other sap-feeding species including aphids (*Aphis fabae* [Harrewjin and Kayser 1997], *Myzus persicae* [Harrewjin and Kayser 1997, Sweeden and McLeod 1997; Wyss and Bolsinger 1997; Nauen *et al*. 1998], *Macrosiphum euphorbiae* [Harrewjin and Kayser 1997], and whiteflies; *Trialeurodes vaporariorum* [Bi *et al*. 2002] and *Bemisia tabaci* [Elbert and Nauen 2000]). The reaction of *H. coagulata* to pymetrozine was similar to the responses of these other pests where feeding was interrupted, but *H. coagulata* was not incapacitated to the point of any visible disorientation or bodily paralysis.

Application of pymetrozine through soil drench is an acceptable method of delivering the compound to sap feeding insects (Wyss and Bolsinger 1997). After application, pymetrozine can be found in most plant tissues especially in xylem and phloem (Wyss and Bolsinger 1997). This systemic activity and concentration in xylem and phloem vessels specifically targets sap-feeding insects.
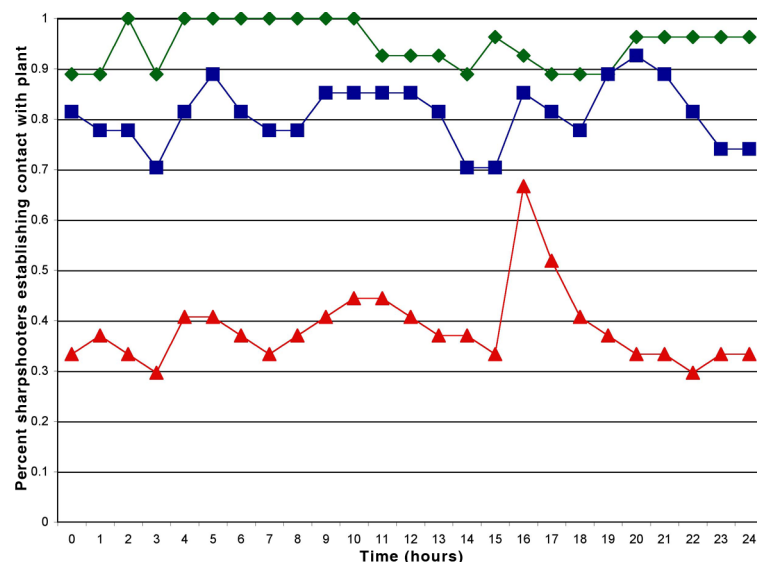

Figure 3. Proportion of *Homalodisca coagulata* establishing over 10 min contact time with a test plant observed through time-lapse video analysis. Top: 0 mg pymetrozine per plant; center: 0.0075 mg pymetrozine per plant; bottom: 0.015 mg pymetrozine per plant.
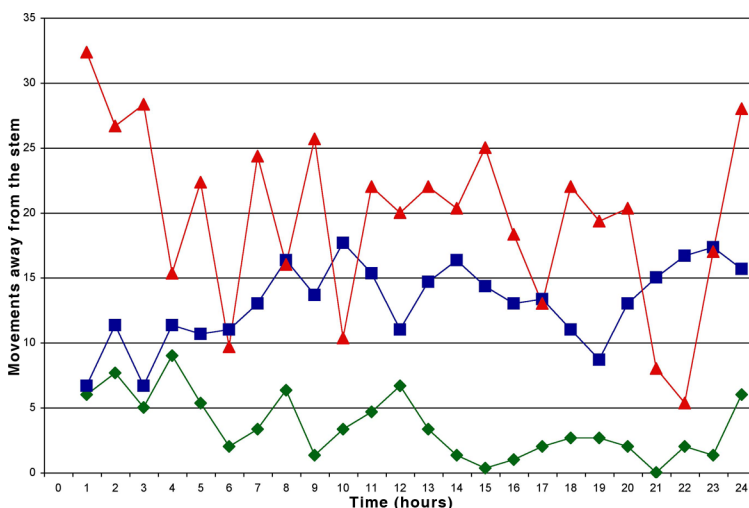

Figure 4. Number of movements away from a test plant by *Homalodisca coagulata* observed through time-lapse video analysis. Bottom: 0 mg pymetrozine per plant; center: 0.0075 mg pymetrozine per plant; top: 0.015 mg pymetrozine per plant.

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As a result, treatment with pymetrozine decreased transmission rates of cauliflower mosaic virus by *M. persicae* (Bedford *et al.* 1998). The amount of time and host contact necessary for cauliflower mosaic virus transmission to occur is similar to conditions required for *X. fastidiosa* transmission by sharpshooter vectors.

We hypothesized that the inactivation of the cibarial muscles after ingestion of pymetrozine (Harrewjin and Kayser 1997) would reduce the rate of *X. fastidiosa* transmission by *H. coagulata*. Unexpectedly, the rate of *X. fastidiosa* transmission to grapevines was not decreased when vines were treated with pymetrozine; in fact, a higher incidence of disease resulted in vines treated with pymetrozine compared to untreated control vines. Two possible explanations exist for these results. First, when *H. coagulata* initially test probed a plant treated with pymetrozine, they became agitated. This caused *H. coagulata* to remove their stylets, move to another feeding site, and test probe again when the effects of pymetrozine subsided (Table 1). Each time the insect test probed, an *X. fastidiosa* transmission event could occur. Because the bacterium is not always in the plant's xylem vessels, infection does not result from every transmission event. However, the greater number of transmission events due to the increased number of test probes could result in a higher the probability of infection. Second, if the cibarial pump muscles are inactivated by pymetrozine during the initial stages of feeding (Harrewjin and Kayser 1997), the negative pressure created by xylem fluid flow may cause a backflow of foregut contents into the xylem. A prolonged period of stylet insertion without ingestion to counter the flow of foregut contents into the plant may result in more *X. fastidiosa* moving into the plant along with foregut contents. With more *X. fastidiosa* being inoculated into the plant per feeding event, the probability of infection may increase. In both cases,

Table 1. Activity of *Homalodisca coagulata* observed through time-lapse video analysis.

	Mean Contact Establishment per hour	Movements away from stem per hour
Control	0.94 (+/-0.04)[a]	3.56 (+/-2.45)[a]
Half Rate[a]	0.81 (+/-0.06)[b]	13.07 (+/-3.09)[b]
Full Rate[b]	0.38 (+/-0.07)[c]	19.65 (+/-6.85)[c]

[a]0.0075 mg pymetrozine per grapevine.
[b]0.015 mg pymetrozine per grapevine.
[*]Means followed with the same letter within a column are not significantly different. (ANOVA, p>0.001)

Table 2. Mean liquid excreta from *Homalodisca coagulata* (±SD) collected over a 12 d period.

	Mean fluid collected (g)	
	Rep 1	Rep 2
Control	1.32 (+/-0.99)[a]	0.44 (+/-0.48)[a]
Half Rate[a]	1.11 (+/-0.84)[b]	0.29 (+/-0.33)[b]
Full Rate[b]	1.04 (+/-0.79)[b]	0.27 (+/-0.38)[b]

[a]0.0075 mg pymetrozine per grapevine.
[b]0.015 mg pymetrozine per grapevine.
[*]Means followed with the same letter within a column are not significantly different. (ANOVA, p>0.001)

Table 3. Mean number of infected plants by treatment.

Treatment	June, 2002		August, 2002		
	Rep 1	Rep 2	Rep 3	Rep 4	Total
Control	2/6	3/7	2/6	4/7	11/26 (.423)[a]
Half Rate[a]	5/7	4/6	4/7	5/6	18/26 (.692)[b]
Full Rate[b]	3/7	5/7	5/7	6/7	19/28 (.679)[b]

[a]0.0075 mg pymetrozine per grapevine.
[b]0.015 mg pymetrozine per grapevine.
[*]Means followed with the same letter within a column are not significantly different. (ANOVA, p>0.001)

effects of pymetrozine would increase the likelihood that a transmission event would occur. It is possible that the rates of pymetrozine were too low, causing ineffective doses to be administered to the insect allowing the repeated feedings. Plants treated in this study were in small pots and the amounts of pymetrozine used were translated from values prescribed in field studies. Therefore, the amount of pymetrozine taken up by the plants was probably substantial because the chemical was concentrated in the pot and not depleted in field soil.

Despite the inability of pymetrozine to stop the inoculation of *X. fastidiosa* to grapevines by *H. coagulata*, *X. fastidiosa* acquisition inhibition was not tested. While this would not protect naïve grapevines from *X. fastidiosa* infection, it could be used to limit sources of inoculum in the field. Because *H. coagulata* damage citrus through substantial removal of vitamins, minerals, and water (Perring *et al*. 2001), pymetrozine could deter this intense feeding. Additionally, predators were unaffected by pymetrozine when fed on exposed pest insects (Mori and Gotoh 2001) which suggests that this compound could be useful in an integrated pest management program when introducing a biological control component.

Acknowledgments

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