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Effects of Kaolin Particle Film and Imidacloprid on Glassy-Winged Sharpshooter (*Homalodisca vitripennis*) (Hemiptera: Cicadellidae) Populations and the Prevention of Spread of *Xylella fastidiosa* in Grape

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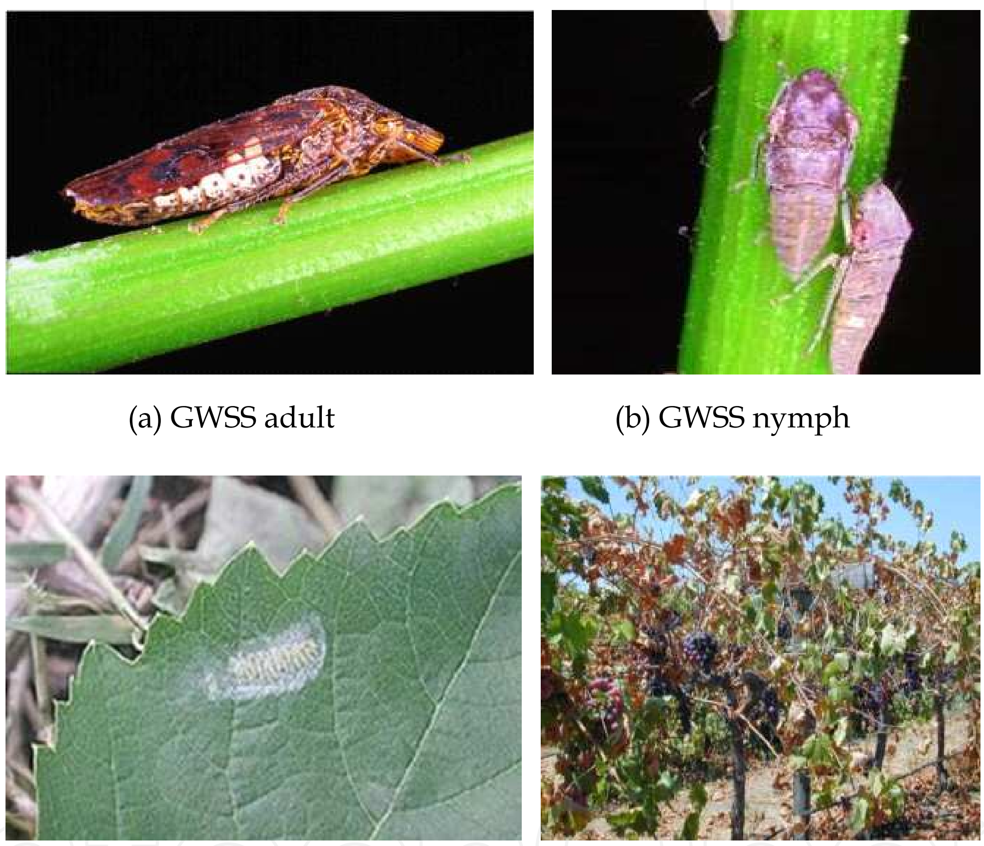
1. Introduction

The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar), (Hemiptera: Cicadellidae), is a major pest of important agronomic, horticultural, landscape, ornamental crops and native trees in California (Blua et al., 1999; Purcell et al., 1999; Purcell & Saunders, 1999). This insect is an invasive species in California that was first detected in the state in 1989 (Sorensen & Gill, 1996). This sharpshooter is a key vector of *Xylella fastidiosa* and has changed the epidemiology of *X. fastidiosa* (*Xf*) diseases affecting important agronomic and horticultural crops as well as landscape, ornamental and native trees in California based on the infection of these crops by the bacteria (Blua et al., 1999; Purcell et al., 1999; Purcell & Saunders, 1999) (Fig. 1). It is not clear if management strategies for Pierce's Disease (PD) developed in GWSS-free regions of California are suitable to manage the disease in vineyards where GWSS has become established.

Data from earlier studies suggest that GWSS has continued to increase in number (population density) and geographical range in California (Purcell & Saunders, 1999; Tubajika et al., 2004). GWSS populations are widely distributed over a large number of hosts including perennial agronomic crops, ornamental plantings, and weedy plant species (Hill & Purcell, 1995; Purcell et al., 1999; Raju et al., 1980). Previous studies by Blua et al. (1999) and Perring et al. (2001) showed that GWSS populations utilize citrus plants as their primary over-wintering host (other plants are available) when grapes, stone fruits, ornamental hosts, and weedy species are dormant during the winter. This vector may have a great impact on grape-growing areas in the Coachella, Temecula and the lower San Joaquin Valley due to numerous citrus orchards that support overwintering populations. Effective insect and disease management strategies are dependent upon knowledge of inoculum sources, and biology and the ecology of insect vectors and their natural enemies (Blua et al., 1999; Hill & Purcell, 1995; Tubajika et al., 2004). The GWSS transmits *Xf* to grape (Fig. 1), as well as to oleander (Costa et al., 2000) with the transmission efficiency greater than that of other vectors such as the green sharpshooter (GSS) [*Draeculacephala mineroa* Ball] and red-headed sharpshooter (RHSS) [*Carneocephala fulgida* Nottingham], RHSS) (Purcell & Saunders, 1999), but much less than that of the blue-green sharpshooter (BGSS) [*Graphocephala atropunctata* (Signoret] (Purcell & Saunders, 1999).



(a) GWSS adult (b) GWSS nymph

(c) GWSS egg (d) PD symptoms in grape

Fig. 1. Glassy-winged sharpshooter (GWSS) adult (a), nymph (b) (photos by B. Stone-Smith), and egg (c) found in grape in Bakersfield, CA. The GWSS transmits the bacterium *Xylella fastidiosa*, which causes Pierce's disease (PD) (d) in grape.

Imidacloprid, (Admire, Bayer Corp., Kansas City, MO, USA), is an neonicotinoid insecticide which interrupts the binding of nicotinergic acetylcholine in post-synaptic receptors of the insect (Hemingway & Ranson, 2005; Romoser & Stoffolano, 1998). In Georgia, treatment of grapevines with Admire slowed the rate of PD spread, but ultimately extended vineyard life by only a year when infestations were severe (Krewer et al., 2002). Imidacloprid is the most widely used neonicotinoid for the prevention of infestation of grapevines by GWSS in California. This product may be useful in reducing *Xf* transmission by the GWSS and in slowing the development of PD in grape. Contact insecticides offer short-term protection against GWSS infestations because of the continued movement of sharpshooter adults from citrus and non-treated hosts/areas which re-infest the grapevines. In a study on the transmission of *Xf* to grapevines by *H. vitripennis* (syn. *coagulata*), reported that *H. vitripenns* (syn. *coagulata*) transmitted *Xf* to grapevines in a persistent manner(Almeida & Purcell, 2003). This research also showed that the nymphal lost infectivity during molting, but there was no evidence of a latent period (delay after acquisition) of the pathogen by adult populations.

Likewise, kaolin (Surround®, NovaSource Tessenderlo Kerley, Inc., Phoenix, AZ.) is a potential alternative pest management product with improved safety to pesticide handlers and reduced environmental impact (Glenn et al., 1999)). It protects plants from insect feeding and oviposition by coating the plant surfaces with a protective mineral barrier (aka particle film) (Glenn et al., 1999; Puterka et al., 2000). Kaolin has been shown to suppress pear psylla, *Cacopsylla pyricola* (Forster); the spirea aphid, *Aphis spiraecola* Pagenstecher; the two-spotted spider mite, *Tetranychus urticae* Koch; and the potato leafhopper, *Empasca fabae* (Harris) in previous research (Glenn et al., 1999) (Fig. 2). The mechanism involved in reduction of pest density is reported to be reduced oviposition and feeding on treated plants (Glenn et al., 1999). Puterka et al. (2000) found Surround® reduced *C. pyricola* populations and controlled Fabraea leaf spot, fungal disease caused by *Fabraea maculata* Atk. Moreover, Glenn et al., (1999) reported that kaolin films contributed to control of fungal and bacterial plant pathogens by preventing the formation of a liquid film on the surface of pear leaves. In this chapter, the effects of kaolin particle film and imidacloprid on *H. vitripennis* (syn. *coagulata*) population levels and the prevention and spread of *Xf* in grape are described.

(a) Field study using kaolin (b) Close-up of kaolin-treated grape leaves

Fig. 2. Field study showing the application of kaolin (a) and a close-up of kaolin-treated grape used to control Glassy-winged sharpshooter in Kern County, Bakersfield in California.

2. Impacts of kaolin and imidacloprid on *Homalodisca vitripennis*

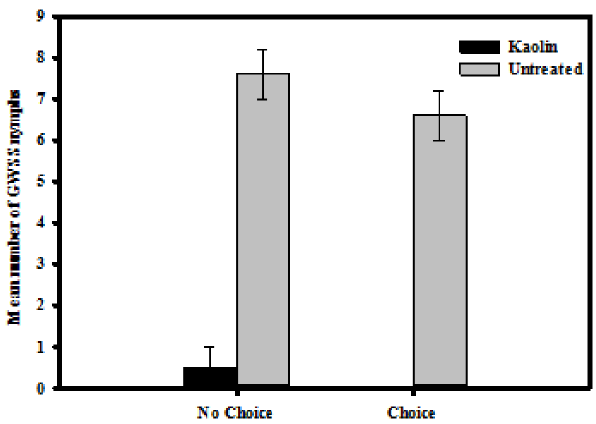
The use of insecticides is common for controlling pest populations (Hemingway & Ranson, 2005). However, the development of resistance and cross-resistance among widely used and newly applied systemic insecticides can affect the development of new strategies in integrated pest management. The GWSS has become a significant problem to California agriculture because it feeds readily on grape and, in doing so, transmits *Xf,* the causal agent of Pierce's Disease (PD) in grape (Fig.1d). Prior to the appearance of GWSS, California grape growers were able to manage PD in grape that is vectored by a number of other indigenous sharpshooters (Anon, 1992). Unfortunately, the GWSS more adapted to the citrus/grape agroecosystem than other sharpshooters, thus, making it a serious vector of PD that now threatens the grape industry in California (Purcell & Saunders, 1999). Although contact insecticides offer short-term protection against infestations of GWSS, the continued movement of GWSS adults from citrus and uninfested areas often re-infest grapes. Clearly, there is a need to investigate other approaches or technologies that could repel GWSS infestations or prevent them from feeding on grape vines and transmitting PD (Puterka et al., 2003).

In an effort to assess the efficacy of control using kaolin, experimental potted lemon trees in field cages at the Bakersfield location were used to test the efficacy kaolin on GWSS infestation and survival under no-choice conditions. 'Eureka' lemon trees were either treated with 6% kaolin (60 grams kaolin per liter of water) or left untreated as a control. Trees were sprayed with kaolin by a 4-liter hand-pump sprayer until all of the foliage was wetted and then allowed to dry. Either a treated or untreated tree was placed in screen-covered cages measuring 1 m^2 by 2 m high, and either 50 GWSS adults (Fig. 1a) or 10 instar nymphs (third to fourth stage) (Fig. 1b) were placed at the bases of the trees to disperse. Numbers of GWSS on trees and within the cages were recorded daily for four days to determine infestation rates and survival. GWSS numbers were converted to percentages and analyzed using analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

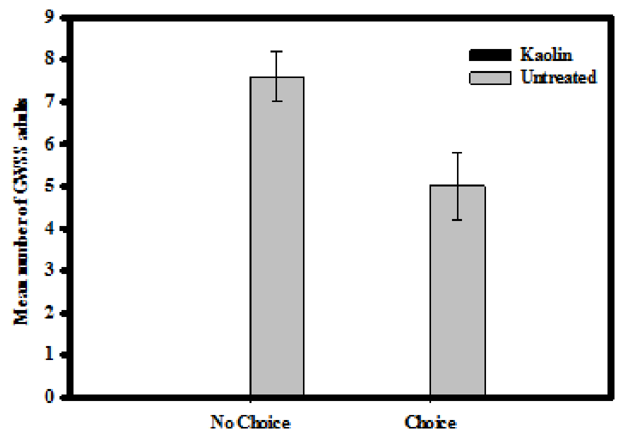
2.1 Survival effect of kaolin and imidacloprid on GWSS in citrus

Experimental results showed that treatment of grapes with kaolin significantly affected ($P < 0.01$) the number of nymph and adult populations. GWSS nymphs and adults were greatly repelled by kaolin application in treatments where choice and no-choice experiments were conducted (Fig.3ab). Very few nymphs or no adults were able to colonize lemon treated with kaolin in experiments where either GWSS nymphs or adults were given no choice for colonization, feeding, and oviposition on plant leaves treated with kaolin and the untreated citrus. There were no significant day or treatment-by-day interactions for nymphs ($P = 0.4$) or adults ($P = 0.19$) in the no-choice tests suggesting that GWSS did not vary over the 4-day period after they had found a suitable site on which to settle (Fig.3ab). Survival of nymphs under no-choice conditions was significantly reduced by kaolin treatments ($P < 0.001$) after being exposed for 4 days, and averaged 8.3 ± 4.2% on kaolin versus 75.0 ± 15.2% on untreated trees. There was no Adult GWSS survival on kaolin-treated lemons and 18.8 ± 4.4% on the untreated lemon 1 day after infestation (Fig.3ab). This relationship did not change over the 4-day period. Adult survival was extremely poor in caged tests because many individuals did not settle on lemon trees under caged conditions and clung to the side of cages until death.

Likewise, the choice tests produced very similar results as the no-choice tests. Neither GWSS nymphs nor adults settled on kaolin-treated lemon, which resulted in no colonization over a 4-day period (Fig.3ab). Treatment preferences of nymphs or adults did not change significantly over time. Survival of nymphs significantly declined over time, from 90.0 ± 4.5% at 1 day to 53.3 ± 6.4% at 4 days after infestation. We hypothesize that this decline in population levels was due to the nymphs falling from PF treated trees or from e movement between treated and untreated foliage and being unable to return to the trees. The number of surviving adults did not decline in untreated trees in the no-choice test indicating they had favorable conditions to colonize and thrive. Some GWSS nymphs that were able to find the few untreated spots on kaolin-treated foliage probably remained at those sites during the study period. In contrast to the above findings, adult survival on kaolin-treated foliage did not change over time in either the choice or no-choice tests. We observed that kaolin treated plants are undesirable hosts to both GWSS adults and nymphs in choice and no-choice environment.



(a) GWSS nymph counts



(b) GWSS adults counts

Fig. 3. Mean number of glassy-winged sharpshooter nymph (n=20) (a) and adults (n=50) (b) per tree 4 days after being released on trees that were treated or not treated with kaolin films in choice versus no-choice experiments.

However, in no-choice experiments, we observed that few nymphs managed to find an untreated site on which to settle when presented with the kaolin-treated plants. This finding implies that good coverage is important when using this material for insect control. When given a choice between kaolin-treated and untreated plants, no GWSS nymphs or adults infested kaolin treated plants.

The choice study may be more representative of what occurs under field conditions because GWSS can utilize more than 75 species of plants (Turner & Pollard, 1959). Therefore, GWSS could easily move to other plants or plant species if they encountered kaolin treated- plants. Results from other studies indicate that kaolin particle films protect plants from insect feeding, oviposition, and by repelling GWSS nymphs and adults by coating the plant surfaces with a mineral barrier (Puterka et al., 2003). This finding is supported by previous reports using kaolin particle film treatments (Glenn et al., 1999; Puterka et al., 2000; Sisterson et al., 2003). There are numerous examples where kaolin particle films repel insect infestations, thus prevent feeding and oviposition although there are at least six other possible mechanisms of action that depend on specific insect-plant relationships (Glenn and Puterka 2005, Puterka and Glenn 2008).

2.2 Mortality effect of kaolin and imidacloprid on GWSS on grape

The mortality effects of kaolin and imidacloprid on GWSS were assessed on grape artificially infested by *X. fastidiosa*. The GWSS was caged for 48 h on grape vines that was previously infested by *X. fastidiosa* (Tubajika et al., 2007). After acquisition, GWSS, in groups

of 10, were caged for 24 or 48 h in small sleeve cages (45 x 55 cm) containing plants either treated or not treated with kaolin and imidacloprid. After 24hr or 48 hr post-treatment, the number of affected GWSS was recorded. The criterion for mortality was insects without any movement (ataxic)[1]. The GWSS mortality was then expressed as percentage.
The GWSS mortality (%) was significantly ($P < 0.05$) impacted by particle film treatment (Tubajika et al., 2007). After 24 hr feeding time, GWSS mortality ranged from 0% (Negative and positive control plants) to 58% (kaolin-treated plants). For the 48 hr feeding time, the mortality ranged from 0% (negative control plants) to 100% (kaolin treated-plants) (Table 1).

Percent (±SE) Plant mortality[y]		
Treatment\Feeding time	24-hour	48-hour
Kaolin-treated plants	58 ± 14aB	100 ± 25aA
Imidacloprid-treated plants	34 ± 1bB	71 ± 21bA
Positive Control (Infested plants)[x]	0 ± 0cA	9 ± 1cA
Negative Control (Non infested plants)[x]	0 ± 0cA	0 ± 0cA

[x]Untreated plants control plants consisted of water-treated plants exposed or not exposed, respectively to infective GWSS.
[y]Calculated from the means for 5 replications. Within columns (small letters) and across rows (capital letters), means followed by the same letter did not differ significantly ($P < 0.05$) according to Fisher's least significant difference test.

Table 1. Effect of kaolin and imidacloprid treatments and length of glassy-winged sharpshooter (GWSS) feeding time on GWSS mortality in greenhouse experiments.

Overall, the GWSS mortality was greater on imidacloprid-, kaolin-, and the untreated plants when the GWSS fed for 48 h than when they were allowed to feed for 24 h.
However, mortality of the GWSS on untreated control plants and non-infested plants with *X. fastidiosa* was not impacted by the feeding time (negative controls) in these experiments (Table 1). The occurrence of insecticide resistance in GWSS depends on the insecticide used and duration of exposure (Tubajika et al., 2007). Therefore, effective insecticide management strategies and their implementation are necessary for the prevention of rapid resistance development. The mechanism of action of particle films do not rely on toxicity to insects thus resistance to particle films appears unlikely (Puterka & Glenn 2008).

2.3 Pierce's Disease (PD) incidence

Similarly, the impact of kaolin and Imidacloprid on incidence of PD was also assessed on the same grape vines. Each plant was visually assessed at 30-day intervals for PD symptom development which includes stunting, delayed growth, marginal leaf necrosis, and abscission between the petiole and leaf blade. On each assessment date, the total number of plants exhibiting PD symptoms was recorded. Ten leaf samples from each plant were bulked and assayed for the presence of *X. fastidiosa* using assays enzyme-linked immunosorbent assay (ELISA) and immunocapture polymerase chain reaction (IC-PCR)

[1] An inability to coordinate voluntary muscular movements that is symptomatic of some nervous disorders.

(Minsavage et al., 1997)). In determining the incidence of PD, the number of plants with symptoms of PD was expressed as percentage of total plant assessed.
The treatment of plants with kaolin and imidacloprid, exposure time of plants to the infectious GWSS, and the interaction of treatment by exposure time had an effect on PD incidence (Tubajika et al., 2007). PD incidence was 4%, 7%, and 32% in kaolin-, imidacloprid-treated plants, and in untreated controls, respectively (Table 2).

Percent (±SE) Pierce's Disease incidence[yz]		
Treatment\Feeding time	24-hour	48-hour
Kaolin-treated plants	8 ± 2bA	0 ± 0bB
Imidacloprid-treated plants	9 ± 2bA	4 ± 1bB
Positive Control (Infested plants)[x]	48 ± 12aA	19 ± 3aB
Negative Control (Non infested plants)[x]	0 ± 0bA	0 ± 0bA

[x]Untreated plants control plants consisted of water-treated plants exposed or not exposed, respectively to infective GWSS.
[y]Calculated from the means for 5 replications. Within columns (small letters) and across rows (capital letters), means followed by the same letter did not differ significantly ($P < 0.05$) according to Fisher's least significant difference test.
[z]Leaf samples from each plant were assayed for the presence *X. fastidiosa* using ELISA and IC-PCR.

Table 2. Effect of kaolin and imidacloprid treatment and length of glassy winged sharpshooter feeding time on Pierce's Disease incidence.

The incidence of PD did not differ among plants treated with imidacloprid and kaolin. *Xf* detection by ELISA assays was 8%, 11%, and 34% in kaolin-, imidacloprid-treated plants, and the untreated controls, respectively (data not shown). However, incidence of PD was greater when the GWSS were allowed to feed for 24 h versus 48 h. There was no difference in PD incidence on plants treated with imidacloprid and kaolin regardless of the length of the GWSS feeding time. In the untreated control plants, disease incidence was higher in plants exposed to infectious GWSS for 24 h than for 48 h (Table 2). The anti-feedant property of imidacloprid may be important in reducing the acquisition of PD bacterium, decreasing the transmission efficiency of infected GWSS, and subsequently, reducing the spread of PD bacterium (Tubajika et al., 2007). This finding is consistent with results obtained by Krewer et al., (2002), who showed that imidacloprid treatment slowed the development of PD in the field but was not effective in preventing the infection of PD in areas with prevalent sources of inoculum and high vector abundance.

3. Kaolin treatment as a barrier to GWSS movement into vineyard

Previous studies by Blua et al., (1999) and Perring et al., (2001) have shown that GWSS population utilize citrus as their primary reproductive host, and citrus is the predominant overwintering host, when grapes are dormant during winter. The application of insecticide to control insects can decrease spread and minimize yield loss in certain pathosystems (Purcell & Finley, 1979). Contact insecticides only offer short-term protection against GWSS infestations because of the continued immigration of sharpshooter adults from citrus which re-infest the grapes (Purcell & Finley, 1979).

The prevention of movement of GWSS from citrus to adjacent vineyards by kaolin particle film treatments as barriers was examined. The vineyard, which was comprised of an assortment of Thompson Seedless, Flames Seedless, and Chenin Blanc grape cultivars, was divided into six blocks 164.6 m wide by 365.7 m long (6.5 ha) and assigned treatments of kaolin or conventional insecticides (Puterka et al., 2003). Kaolin barrier treatments only extended 247.5 m into each block; the remaining 152.4 m was left untreated. The insecticide treatment block s had applications the entire 365.7 m distance of the block. Grapes received three bi-weekly applications of kaolin (11.36 kg kaolin, 378.5 per liter water) and six weekly applications of Dimethoate (Dimethoate 400, Platte Chemical Co., Greeley, CO, USA) applied at 4.67 l per ha, methomyl (Lannate LV, E. I. DuPont de Nemours & Co., Wilmington, DE, USA) applied at 2.33 l per ha, and Naled (Dibrom 8E, AMVAC Chemical Corp., Los Angeles, CA, USA) applied at 0.74 l per ha (Puterka et al., 2003). GWSS adults and nymphs were collected from the yellow sticky traps spaced every 30.5 m along the 365.7 m transects per block. Because of edge effect on movement of GWSS from citrus into grapes, plots were partitioned into four distances (0 to 4.3 m (interface); 4.4 to 123.7 m; 123.8 to 247.5 and 247.6 to 365.7 m) to better estimate the number of GWSS. GWSS adults and nymphs will be assessed by weekly monitoring of GWSS adults and nymphs caught per yellow sticky trap (Trece, Salinas, California placed 1 m high in the grape vines in vineyard bordering citrus (interface) and placed every 30.5 m into 365.7 m transects extending into each treatment block (transect) in Kern County, California. GWSS egg masses were sampled by inspecting 25 leaves per vine every 30.5 m along the sticky trap transects in each block.

3.1 GWSS adult counts from traps

Traps in kaolin-tread plants at the edge of vineyard (0-4.3 m) caught fewer GWSS than traps in the insecticide-treated plants based on one experiment from 9 March to 6 April, except on 16 March (Fig. 4a). After 6 April, there were no differences in Interface trap catches between treatments. Transect traps in kaolin barrier treatment resulted in fewer GWSS on 22 March and 6 April. Data from all other trap dates showed that treatment effects were not significantly different. Counts of GWSS adults were significantly different between sample dates, treatments, and blocks (Fig. 4ab).

Visual counts of in the grape-orange grove interface (0-4.3 meters) revealed higher number of GWSS adult in the insecticide treatment than in the kaolin treatment on week 1 (22 March) (kaolin = 0.06 ± 0.06, insecticides = 0.6 ± 0.6), week 2 (29 March) (kaolin = 0.14 ± 0.02, insecticide = 0.56 ± 0.10), and week 3 (6 April) (kaolin = 0.0 ± 0.0, insecticides = 0.9 ± 0.5). Overall, the GWSS adult counts were much lower in traps beyond the particle film barrier in grape (Fig. 4b). The treatment comparison of three applications of kaolin to six insecticide applications over a 2-month period showed that the kaolin barrier was equally effective or better than numerous insecticide applications in reducing GWSS movement into grapes (Puterka et al., 2003). This may appear to be contrary given the fact that the insecticide used was systemic; however, it appears that the kaolin was a sufficient barrier to prevent GWSS movement into the grape vineyards.

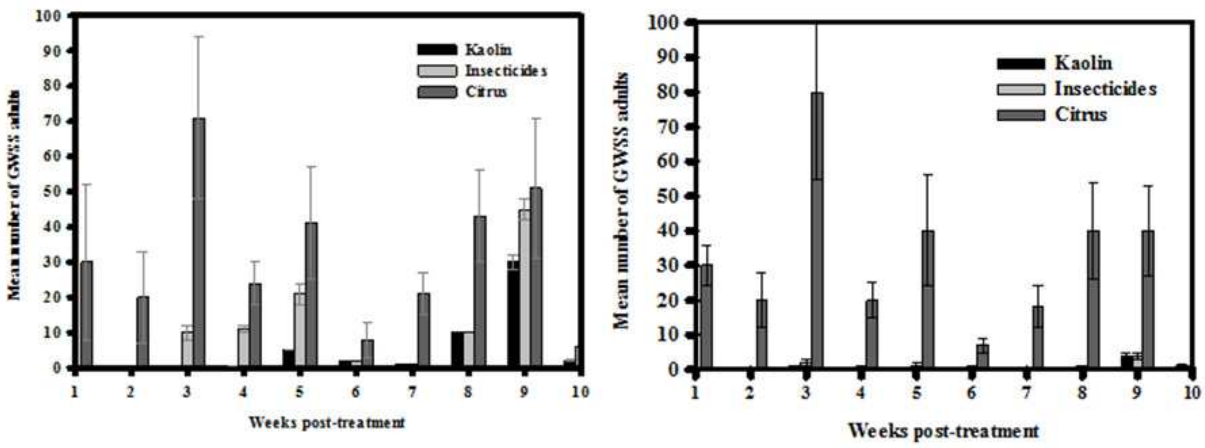


Fig. 4. Effects of kaolin and conventional insecticides on the mean number (±SE) of glassy-winged sharpshooter adults caught per yellow sticky trap (Trece, Salinas, California placed 1 m high in the grape vines in vineyard bordering citrus (interface) and placed every 30.5 m into 365.7 m transects extending into each treatment block (transect) in Kern County, California.

3.2 GWSS egg counts on grape

GWSS egg counts differed significantly (P = 0.0001) between the insecticide and barrier treatments in leaf samples taken by increasing distances from the grape-orange grove interface into the grape vineyard (P = 0.02). The insecticide treatment had significantly (P < 0.0001) larger numbers of GWSS eggs at the edge (0 meters) than at the distances away from the edge of grape leaves in vineyard bordering the citrus (Table 3). This suggest that oviposition on foliage can be affected by proximity to locales of insecticide application.

	GWSS egg counts (±SE)[y]	
Distance from edge of vineyard (m)[x]	Kaolin	Insecticides
0.0 - 4.3 (block 1)[y]	0.00 ± 0.02aA	14.1 ± 5.1aB
4.4 - 123.7 (block 2)	0.06 ± 0.02aA	5.3 ± 2bB
123.8 - 247.5 (block 3)	0.06 ± 0.02aA	1.6 ± 0.3cB
248.6 - 365.7 (block 4)	0.90 ± 0.03bA	1.9 ± 0.2cB

[x]Vineyard was partitioned to monitor the movement of glassy-winged sharpshooter into the grapes. Distance in meters from citrus.
[y]Values are means of 20 observations. Means followed by the same letter did not differ significantly (*P* < 0.05) according to Fisher's least significant difference test.
[z]Interface.

Table 3. Effects of kaolin barrier and conventional insecticides on the numbers ((±SE) of glassy-winged sharpshooter eggs sampled in Bakersfield vineyard bordering citrus from 0 – 365.7 meters from the edge. Yellow sticky traps were monitored from March 9 to May 9 on 25 grape leaves per vine.

In contrast, the oviposition averaged 0.0 to 0.9 GWSS eggs per block in the kaolin barrier treatment and did not differ (P = 0.50) from one another. Overall, the kaolin barrier resulted in undetectable levels of oviposition, whereas insecticides did not prevent oviposition (Puterka et al., 2003). The egg mass sampling has been shown as the best measure of how kaolin barrier treatments and insecticide treatments affected GWSS activity and host suitability (Puterka et al., 2003). This study suggests that unlike other vectors of Pierce's Disease, the GWSS disperse well into vineyard and is able to vector *Xf*, agent causal of PD in grape. This is consistent with the pattern of PD spread that we observed in Kern County, Bakersfield, CA (Tubajika et al., 2004).

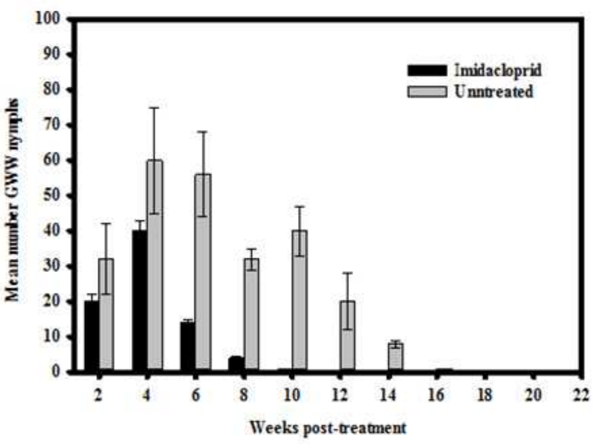
4. Impact of imidacloprid on GWSS in citrus

The epidemic of PD in the vineyards of Temecula in Riverside County, California brought into focus the urgent need to control GWSS populations in CA (Castle et al., 2005) around vineyards. Prior to the first applications of imidacloprid which was made in the spring of 2000 for control of GWSS infestations in Temecula, CA; there had been very limited experience with imidacloprid in citrus or against GWSS in any crop (Catle et al., 2005)). Experiments with imidacloprid treatment were carried out at the University of California's Agricultural Operations at Riverside, CA during 2001 and 2002. The experiment was conducted in a block of 10 rows (6.4 m centers) which were split equally between 30 year-old orange trees (var Frost Valencia grafted on Troyer citrange) and lemons (var Lupe grafted on Cook) situated in the center of a 12-ha orchard. The GWSS nymphs and adults were collected in each bag and counted to determine the effect of imidacloprid on GWSS infestations between treated and untreated citrus trees. A sample consisted of four to six rapid thrusts at five locations around each tree. The contents of the collecting jar were then emptied into pre-labeled ziplock bags before moving on to the next tree

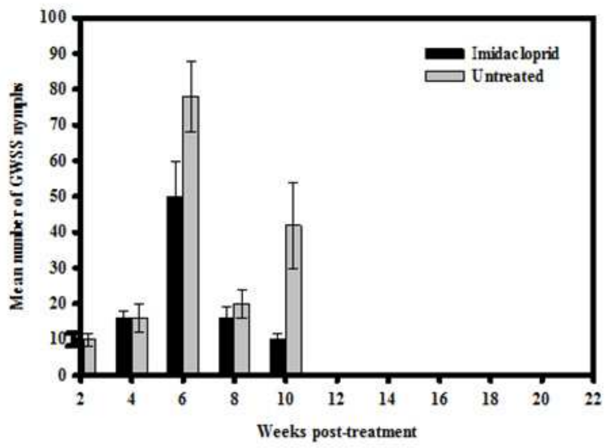
4.1 GWSS nymph counts in citrus

Based on the counts in the above experiments, sampling date proved to be a source of variation a two year study. In 2001, ifferences among the three sampling dates from April to June September. GWSS adults and nymphs were significantly ($P < 0.0001$) greater as observed among four dates in 2002 ($P < 0.0001$). Differences in GWSS counts between imidacloprid-treated and untreated trees were observed in 2001 (Fig. 5a) when populations were much larger than in 2002 (Fig. 5b). For the first four weeks following treatment, nymphal counts were high in both treated and untreated trees (Fig 4a). At week 6 post-treatment, a sharp decline in nymphal counts occurred in the imidacloprid-treated oranges coinciding with mean titers of imidacloprid surpassing 5 μg per liter (data not shown). Mean nymphal counts fell to 4.4 (±1.4) by week 8 compared with 30.49 (±4.4) in the untreated control.

The decline on number of nymphs at the beginning of week 9 may be due natural mortality, emigration and emergence to the adult stage, as observed in untreated control. However, mean number of nymphs remained between 30 and 40 through week 10 in the untreated trees while mean counts dropped below 2 at week 10 in the imidacloprid-treated orange trees (Fig 4a). Season-long differences between treated and untreated nymphal counts were significantly ($P < 0.0001$) high based on a repeated measures MANOVA. The decline in nymphal counts in 2001 corresponded nicely with the rise of imidacloprid titers in oranges, but this was not apparent in lemons or during the evaluation the following year when nymphal counts were so low (Catle et al., 2005). However the variability in the application through the irrigation system and/or the rate of uptake could also accounted for the significant variation in number of nymphs or adults among trees (Catle et al., 2005).



(a) GWSS nymph counts in 2001



(b) GWSS nymph counts in 2002

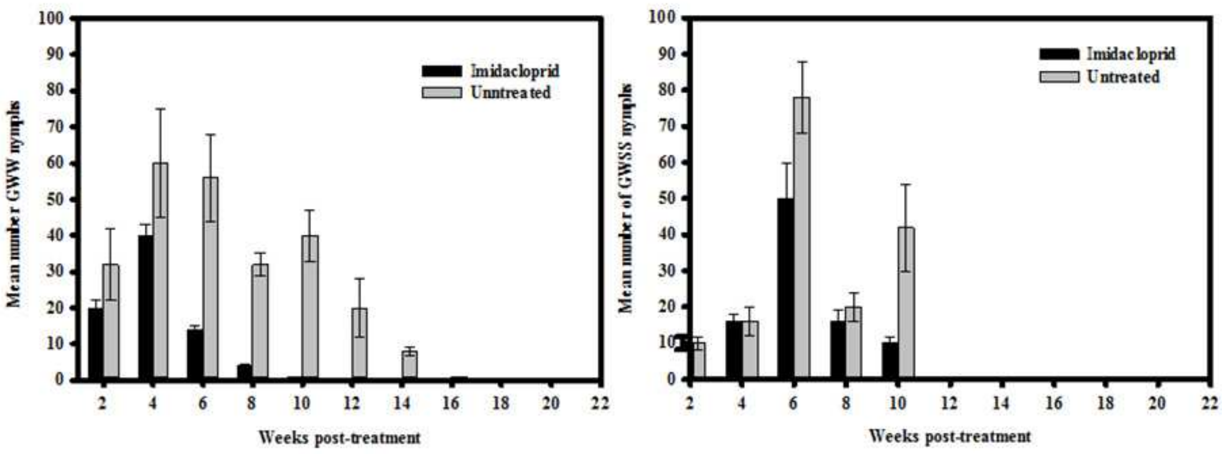
Fig. 5. Mean (±SE) number of (a) GWSS nymphs in 2001 and (b) in 2002 on oranges trees treated with imidacloprid compared with untreated trees. Sample size each week was n=12 trees using a bucker sampler thrusted a t five different locations per tree.

4.2 GWSS adult counts in citrus

GWSS Adults were not observed through the first eight weeks post treatment (Fig 4b). With maturation of the first nymphs and emergence to adults, numbers of adults rapidly increased in both treated and untreated oranges trees between weeks 9 and 12 (15 June–6 July). Contributing to the influx of young adults into the imidacloprid-treated oranges was the absence of any buffer zones between the treated and untreated trees (two rows of treated trees only). By week 14, however, a divergence in the mean number of adults caught in each treatment had begun, reaching its greatest difference in week 18 (17 August). Difference in adult densities was greater throughout week 25 (5 October) after which treated and untreated GWSS adult counts began to converge (Fig 4b). The mean number of GWSS adults between 15 June and 30 November 2001 was significant (P < 0.0001) between treated and untreated orange trees.

In contrast to the mean number of nymphs observed in 2001 in orange trees, the difference in number of GWSS nymphs in lemon trees in 2002 was inconsistent treated and untreated lemon trees (Fig 5a). Numbers of GWSS nymphs in untreated lemons were especially erratic, thus making it difficult to observe any clear treatment effect (P = 0.40). However, a very similar pattern to the orange trees was observed for GWSS adult counts in treated and untreated lemon trees (Fig 5b).

It is clear that protection by imidacloprid was not sporadic or spatially uneven, but rather was confronted by a phenomenon where mass emergence of adults coupled with heightened flight activity simply overwhelmed both treated and untreated trees in the orchard (Catle et al., 2005). After a few weeks, GWSS adult numbers began to decline and population remained consistently and significantly lower than the untreated orange and lemon trees. Similarly, a rapid increase in nymphal densities occurred in both treated and untreated orange trees in 2001, much as they were observed in Temecula in 2000 (Catle et al., 2005). The antifeedant effects of imidacloprid on other herbivores belonging to Hemiptera (Sternorrhyncha) are well established (Nauen et al., 1999).



(a) GWSS adult counts in 2001 (b) GWSS adult counts in 2002

Fig. 6. Mean (±SE) number of (a) GWSS adults in 2001 and (b) in 2002 on oranges trees treated with imidacloprid compared with untreated trees. Sample size each week was n=12 trees using a bucker sampler thrusted a t five different locations per tree.

Results from these experiments showed that a recruitment of adults from surrounding orchards during week increased densities in imidacloprid and untreated controls but at levels that were significantly lower for imidacloprid treated–trees. Moreover, Variability in the application through the irrigation system and/or the rate of uptake could have accounted for the significant variation observed among trees. Also, substantial reductions in GWSS nymphs and adults in imidacloprid treated-trees observed during 2001 production year were sustained for 4-5 months post treatment. Overall, GWSS infestations were reduced in imidacloprid-treated trees than in untreated-trees. Data on the number of GWSS adult and nymph counts from grapevines and citrus studies confirmed that citrus is the primary and preferred host for GWSS as observed in Temecula valley and when given other choices such as grape, almond, cherry, stone fruit, the insect feeds on these hosts as Kern County provides a perfect variety of hosts (Tubajika et al., 2007; Blua et al., 1999; Purcell & Saunder, 1999; Raju et al., 1980).

5. Pierce's Disease incidence

Our previous study on the analysis of the spatial patterns of PD incidence in the lower San Joaquin Valley, CA indicated that GWSS may not be infective but their movements within the vineyard after arrival are important in the spread of the infection, which resulted in the symptoms we recorded (Tubajika et al., 2004).

Experimental plots consisting of Thompson Seedless, Flame Seedless and Chenin Blanc cultivars which were approximately 15-years-old were assessed for Pierce's disease (PD) incidence using a visual evaluation of disease symptoms such as stunted shoot growth, leaf scorch, and persistent petioles, a condition that occurs when the leaf blades scald and abscise, leaving only petioles attached to the shoot (Anon, 1992; Purcell, 1974). Plants were assessed at 30-d intervals. Ten leaf samples from each plant were bulked and assayed for the

presence of *X. fastidiosa* using ELISA and IC-PCR and PD incidence was assessed as previously described.
When results were averaged across years, the incidence of PD was significantly (P < 0.05) lower (6%) in plots treated with kaolin than in plots treated with conventional insecticides (14%) (Tables 3, 4). There was no significant difference in the incidence of PD among grape cultivars in both years (P = 0.67). Also, chemical by cultivar interaction did not affect PD incidence in either year (Tables 3, 4).

Mean (± SE) percent PD incidence[xy]		
Cultivars	Kaolin	Conventional insecticides[z]
Thompson Seedless	8.4 ±1.1 aB	18.7 ± 2.4 aA
Flames Seedless	8.1 ± 1.1 aB	19.2 ± 2.1 aA
Chenin Blanc	7.8 ± 0.5 aB	17.5 ± 2.9 aA
Mean	8.1 ± 0.4	18. 5 ± 2.2

[x]Values are means of eight observations (Four replications x two years). Within rows (small letters) and across rows (capital letters), means followed by the same letter did not differ significantly (P< 0.05) according to Fisher's least significant difference test.
[y]Pathogen identity was confirmed by ELISA and IC-PCR assays. *Xylella fastidiosa* strain Temecula (ATCC 700964) collected from grape in Temecula, California was used as a reference control.
[z]dimethoate (Dimethoate 400) applied at 4.67 liter per ha, methomyl (Lannate LV) applied at 2.33 liter per ha and naled (Dibrom 8E) applied at 0.74 liter per ha.

Table 3. Effects of kaolin and conventional insecticides on Pierce's Disease incidence on Thompson Seedless, Flame Seedless, and Chenin Blanc cultivars during the 2001 production year at Bakersfield, CA.

Overall incidence of PD was 43% higher in 2001 than in 2002. All of the infected grape plants were removed from the plots. The inoculum sources for the 2002 season were subsequently reduced (Tubajika et al., 2007). In 2001, PD incidence was 18% on plants treated with insecticides and 4% in kaolin-treated plants (Table3). In 2002; the incidence of PD was 8% on plants treated with insecticides and 4% on kaolin-treated plants (Table 4).
Our previous study on the spatial patterns of incidence of PD in the lower San Joaquin Valley, CA indicates that GWSS may not be infective but their movements within the vineyard after arriving into the vineyard are important in the spread of the infection, and resulted in the symptoms we recorded (Tubajika et al., 2004). Field studies showed that plants treated with kaolin were less likely to become infected with *X. fastidiosa* and had a lower incidence of PD symptoms than untreated control plants (Tubajika et al., 2007). There are limited reports on application of particle film to control plant diseases caused by the vector population in the fields (Glenn et al., 1999; Blua et al., 1999; Puterka et al., 2000; Puterka et al., 2003). Also, data showed that the GWSS had a lower rate of survival following exposure to kaolin-treated plants for 48 h. This finding is similar to previous reports where kaolin completely protected plants from insect feeding (Blua et al., 1999; Puterka et al., 2000; Puterka et al., 2003). They suggested that the kaolin protects hosts against GWSS by camouflaging the plant with a white coating making them visually unperceivable, or by reflecting sunlight, which repels leafhoppers as well as aphids.

Mean (± SEM) percent PD incidence[xy]		
Cultivars	Kaolin	Conventional insecticides
Thompson Seedless	4.3 ±0.5 aB	8.8 ± 1.4 aA
Flames Seedless	4.2 ± 0.5 aB	8.1 ± 1.1 aA
Chenin Blanc	3.9 ± 0.2 aB	7.9 ± 0.9 aA
Mean	4.1 ± 0.3	8.3 ± 1.3

[x]Values are means of eight observations (Four replications x two years). Within rows (small letters) and across rows (capital letters), means followed by the same letter did not differ significantly ($P < 0.05$) according to Fisher's least significant difference test.
[y]Pathogen identity was confirmed by ELISA and IC-PCR assays. *Xylella fastidiosa* strain Temecula (ATCC 700964) collected from grape in Temecula, CA was used as a reference control culture.
[z]dimethoate (Dimethoate 400) applied at 4.67 liter per ha, methomyl (Lannate LV) applied at 2.33 liter per ha and naled (Dibrom 8E) applied at 0.74 liter per ha.

Table 4. Effects of kaolin and conventional insecticides on Pierce's Disease incidence on Thompson Seedless, Flame Seedless, and Chenin Blanc cultivars during the 2002 production year at Bakersfield, CA.

6. Conclusion

The GWSS was recently introduced into California and poses a serious threat to the grape industry because it is a very effective vector of the bacterium that causes Pierce's disease. This introduction of the GWSS has changed the epidemiology of *Xf* diseases affecting important agronomic and horticultural crops as well as landscape ornamental and native trees in CA by infesting these crops with the bacteria. The epidemic of PD in the vineyards brought into focus the urgent need to control GWSS populations, especially around vineyards. A new technology, called particle film and a systemic insecticide, imidacloprid were assessed both in greenhouse and field.

Results showed that kaolin protects plants from insect feeding, oviposition, and infestation by coating the plant surfaces with a protective mineral barrier. In caged field studies, we found that GWSS nymphs and adults were highly repelled by lemon trees treated with kaolin. In field studies that compared three biweekly kaolin treatments to six weekly contact insecticide treatments, kaolin performed as well as insecticides in reducing GWSS adult numbers and oviposition. A good coverage of plant leaves with kaolin is important when using this material for insect control. In greenhouse studies, GWSS adult counts were reduced greatly in kaolin-treated plants versus untreated control trees.

Based on data on GWSS nymph and adults counts in citrus, the persistence of imidacloprid in citrus varied as near-peak levels of imidacloprid were sustained for 6–10weeks before gradually declining as substantial reductions in GWSS nymphs and adults were observed in imidacloprid-treated trees during the 2001 trial and were sustained for 4–5months post-treatment. Imidacloprid effect on GWSS nymphs was not as well pronounced in the 2002 trial, when overall GWSS infestations were much reduced from the previous year. However, consistently lower adult infestations were observed in 2002 for imidacloprid compared with untreated trees.

The application of kaolin and imidaclorprid impacted GWSS mortality and PD incidence. Higher GWSS mortality rates were observed on kaolin-treated plants than on untreated-plants. The reduction in PD incidence observed in field studies suggest that either kaolin treatments as barriers to GWSS infestation or imidacloprid can be effective in reducing GWSS population and PD symptoms, and can be valuable tools for PD management. Both treatments strategies could also be used where insect resistance to imidacloprid would be a concern since insect resistance to particle films would not be a concern. Additionally, these treatments could be combined with other PD management approaches in integrated pest management.
The costs of chemicals and applications by either commercial applicators or growers were not determined in these studies. However, the choice of chemical to apply (alone or in combination) is important and the level of net returns will depend on this as well as other factors such as frequency of applications, disease intensity, and growth stage at which the chemical sprays are initiated. Additional work assessing these and other potential benefits are needed to fully determine the economic value of these treatments for grape production.

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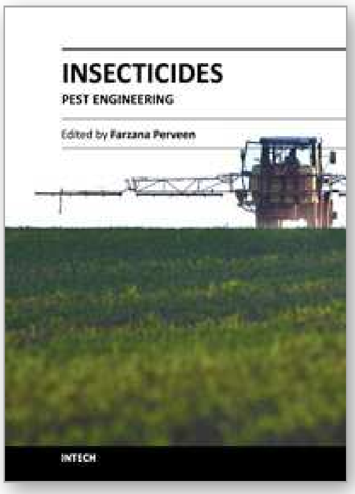

Insecticides - Pest Engineering
Edited by Dr. Farzana Perveen

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This book is compiled of 24 Chapters divided into 4 Sections. Section A focuses on toxicity of organic and inorganic insecticides, organophosphorus insecticides, toxicity of fenitrothion and permethrin, and dichlorodiphenyltrichloroethane (DDT). Section B is dedicated to vector control using insecticides, biological control of mosquito larvae by Bacillus thuringiensis, metabolism of pyrethroids by mosquito cytochrome P40 susceptibility status of Aedes aegypti, etc. Section C describes bioactive natural products from sapindacea, management of potato pests, flower thrips, mango mealy bug, pear psylla, grapes pests, small fruit production, boll weevil and tsetse fly using insecticides. Section D provides information on insecticide resistance in natural population of malaria vector, role of Anopheles gambiae P450 cytochrome, genetic toxicological profile of carbofuran and pirimicarp carbamic insecticides, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

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