encouraged by minimizing the load of chemicals.

The above-mentioned recommendations, if adopted, for management of Red spider mite population of the Dooars and Terai tea plantations are expected to provide a solution to the growing menace, that is often causing heavy losses of tea production. The suggestion, if implemented in letters and spirit, would possible reduced the load of chemical acaricides on the crops and also the environment, all the same usher in a region wide practice of integrated management of the pest, the necessity of the hour.

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Research in Resistance Management

Preliminary Effects of Insecticidal Control of Asian Citrus Psyllid and Combinations of Nutrients and Systemic Acquired Resistance Elicitors on Incidence of Greening Disease in Citrus

ABSTRACT

A combination of systemic acquire resistance elicitors, with macro- and micro-nutrients have been used, with apparent success, by at least one Florida citrus grower to mitigate expression of symptoms related to Huanglongbing (HLB) or greening disease. A large scale field experiment to test the effects of this combination with and without insecticidal control of the vector, *Diaphorina citri* (Homoptera: Psyllidae) was initiated in a young block of citrus trees in southwest Florida. Visual symptoms of HLB were initially reduced, although differences compared to untreated trees became negligible four months after the first Special Foliar Treatment (SFT) in early summer application, and two months after the second application. Insecticide applications suppressed populations of *D. citri* adults and virtually no movement of the psyllid among plots inside the orange block was being observed. However, expression of HLB symptoms in the plants was not affected by insecticides.

INTRODUCTION

Huanglongbing (HLB) or citrus greening disease is considered by many as the most important disease of sweet orange, mandarin and grapefruits worldwide (Schwarz and Bove, 1975). HLB is found in over 40 citrus-producing countries in Asia, Africa, Oceania, and the Americas. The disease is associated with the presence of several genera of the bacterium *Candidatus* Liberibacter, of which only C. L. asiaticus is present in Florida where it is vectored by *Diaphorina citri* kuwayama (Bove, 2006).

Candidatus Liberibacter spp. are considered to be Gram-negative bacteria and restricted to the phloem of the plant (Garnier et al., 1984). The bacteria appears to reduce the distribution of nutrients through the phloem, and increase concentrations of starches in the leaves (Takushi et al., 2007). Foliar symptoms appear as micronutrient deficiencies and can sometimes be distinguished by a more characteristic blotchy mottling of older leaves that abscise early leading to dieback. Fruits are often misshapen, undersized, and green or remain green at the stylar end, green fruits, have aborted seeds and drop from the tree. Analysis of symptomatic leaves shows a higher potassium content and lower calcium, magnesium, and zinc (Da Graca, 1991). These low nutrient contents might be attributed to an obstruction of phloem function by the bacteria that would explain the resemblance between HLB and nutrient deficiency symptoms.

Systemic Acquired Resistance (SAR) is a plant response to the effect of pathogenic organisms or other stress factors induced by elicitors such as salicylic acid or jasmonic acid. Salicylic acid induces activation of pathogenesis-related genes (PR), which cascade into the production defensive metabolites or in the development of a hypersensitive reaction (Gaffney et al., 1993; Ryals et al., 1996). Salicylic acid is also known to induce antioxidants that could serve to attenuate pathogenesis directly (Huang et al., 2008).

At least one Florida citrus grower has been experimenting with nutrient/salicylic acid combinations to ameliorate symptoms of HLB with apparent success (Giles, 2009). Our objective was to evaluate separate effects of his formula and of insecticidal control of the vector on incidence and severity of the disease. The present report is intended to provide preliminary results of what will likely require more time and experiments under varying conditions to fully evaluate.

MATERIALS AND METHODS

A 12-acre parcel of young Valencia oranges located at 26° 29' N., 81° 23' W., in a large planting of oranges in Collier Co. in southwest Florida was selected. In this block HLB was first identified in 2005. The selected parcel showed a homogenous distribution of HLB symptoms and was divided into 16 plots organized in 4 replicates with 4 treatments set out in a randomized complete block design. The 4 treatments consisted of two factors, the SAR nutrient combination applied as a foliar spray, and insecticidal control, each at two levels (with and without).

Foliar treatment

A special foliar treatment (SFT) consisting of a combination of SAR inducer products including: Serenade Max ®WP (*Bacillus subtilis*) and Saver™ (salicylic acid source), macronutrients, and several micronutrients including zinc, manganese, molybdate, etc. (Giles, 2009). Treatments were sprayed using an airblast sprayer at 210 gallons per acre. Applications to the designed plots were conducted in 2008 on 19 March, 3 June, 8 September, and 16 October.

Insecticides

Foliar insecticide applications were made to the designated plots on 2 May (Danitol 2.4 EC (fenpropathrin) at 16 fl oz/acre [Valent USA, Walnut Creek, CA], and on 7 August Delegate WG (spinetoram) at 4 oz/acre [DOW AgroSciences, Indianapolis, IN]. In addition, two insecticides were used before the beginning of the experiments, Temik (aldicarb) [Bayer CropScience, Research Triangle Park, NC] applied to the soil at 20 lb/acre, and a dormant season spray of Danitol 2.4 EC (fenpropathrin) at 16 fl oz/acre during December 2007.

Sampling

Ten trees were randomly selected in the center row of each plot. To evaluate the population of adults of *D. citri* in the field, a white laminated field sheet divided into a 4/4 grid was held approximately one foot under a leafy branch. The branch was tapped three times and the number of *D. citri* adults that fell from the branch onto the sheet was counted. (Arevalo et al. 2009). This tapping process was repeated twice in each tree. This sampling system for psyllid adults has demonstrated to be efficient and reliable to estimate *D. citri* fluctuations in the field (Hall et al., 2007; Qureshi and Stansly, 2007).

Symptoms of HLB were evaluated on the same 10 trees used for the tap sample, and trees were designated as positive or negative based on the presence of leaves displaying blotchy mottle, dieback, shock bloom or other symptoms as mentioned above. The presence of one of more of these symptoms was enough to qualify a plant a positive.

RESULTS

Insecticide treatments significantly reduced the number of adult *D. citri* in the treated plots when compared with the untreated plots. Despite high populations of the psyllid in the treated plots, populations were very slow to re-infest treated plots even after several months following the insecticide applications (Fig. 3). Insecticides did not have an effect on the percentage of trees showing HLB symptoms (Fig 2).

Fig 1: Mean number of *D. citri* adults per tree (two tap samples) in plots treated or untreated with insecticides. Vertical lines represent the dates in which the insecticide treatments were sprayed

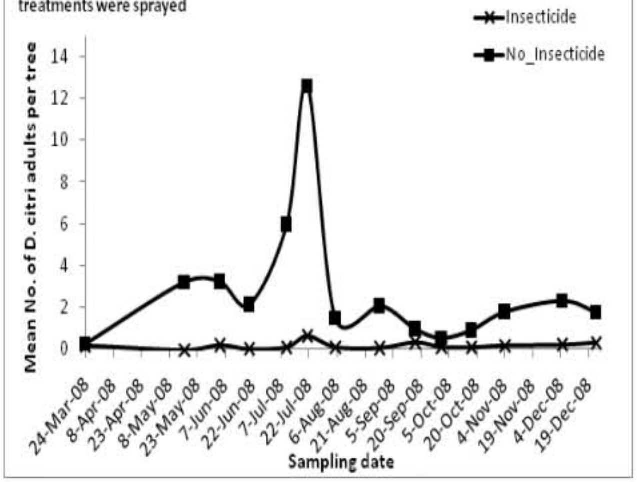
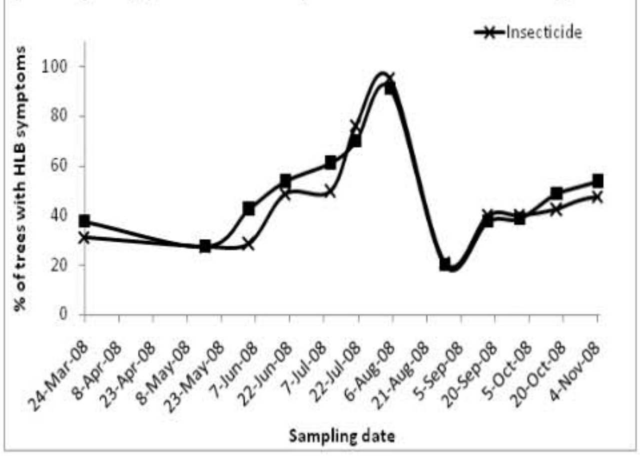


Fig 2. Effect of insecticide versus No insecticide applications on the percentage of trees presenting HLB symptoms. Vertical lines represent the dates for the Insecticide applications



A significant positive response was observed after the first (19 March, 2008) and second (3 June, 2008) applications of the SFT. Differences were observed from early May 2008 to late July 2008. Subsequently, the percentage of trees showing HLB symptoms did not differ between plots with STF and plots without the STF treatments (Fig. 3). Special foliar treatment did not have any effect on the mean number of adult psyllids observed (Fig. 4).

Fig 3. Percentage of trees observed with greening symptoms in plots with the special foliar treatment (SFT) versus the plots without SFT (No-SFT). Vertical lines represent the dates for the SFT application

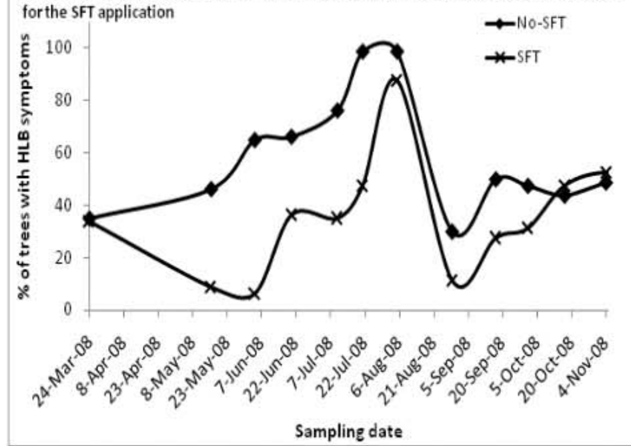
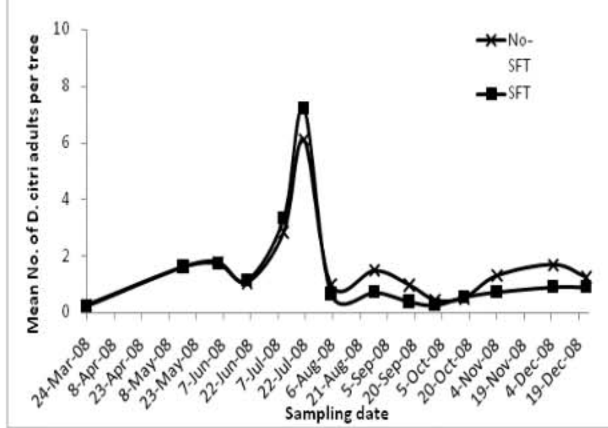


Fig 4: Mean number of *D. citri* adults per tree (two tap samples) in plots treated or untreated with SFT. Vertical lines represent the dates in which the treatments were sprayed



DISCUSSION

Plots that had insecticide applications presented a very slow re-infestation rate despite being adjacent to non-treated plots with high populations. Thus, the psyllid did not appear to be very vagile during the study period. Further studies of psyllid movement and dispersal are ongoing.

Results observed during the first year of the experiment indicated that the use of the SFT have a short effect on the presence or absence of HLB symptoms in the plants. This phenomenon could be explained by the temporary nature of the increase in gene expression of defense-related genes, as occurred in cucumber when *Trichoderma asperellum* is used to induce resistance these plants (Shoresh et al., 2005). Another explanation might include the high cost in fitness to the plant when producing SAR compounds (Cipollini et al., 2003). Hopefully, more light will be shed on these issues as this and other studies progress.

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Occurrence of resistance to the fungicide boscalid in isolates of Alternaria alternata pathogenic on pistachio

ABSTRACT

One hundred and twenty isolates of *Alternaria alternata* isolated during 2005 to 2007 from pistachio orchards with a history of Pristine® (pyraclostrobin + boscalid) applications and displaying high levels of resistance to boscalid fungicide (mean EC_{50} values > 100 µg / ml) were identified following mycelial growth tests. A cross-resistance study also revealed that the same isolates were resistant to carboxin, a known inhibitor of succinate dehydrogenase (SDH). To determine the genetic basis of boscalid resistance in *A. alternata* the entire iron sulfur gene (*AaSDHB*) was isolated from a fungicide-sensitive isolate. Comparison of AaSDHB full or partial sequences from sensitive and resistant isolates revealed that a highly conserved histidine residue (His) (codon CAC in sensitive isolates) was converted to either tyrosine (codon TAC, 53 mutants) or arginine (codon CGC, 27 mutants) at position 277. In forty other resistant isolates there was no mutation in the AaSDHB sequence, suggesting that resistance could be controlled by mutations at other loci. PCR-based assays were developed for the rapid identification of the mutants carrying the identified SDHB-mutations.

Key words: *Alternaria alternata*, succinate dehydrogenase, carboxamides, mechanisms of resistance