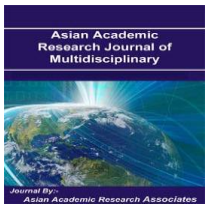
A Peer Reviewed International Journal of Asian Academic Research Associates

AARJMD

ASIAN ACADEMIC RESEARCH JOURNAL OF MULTIDISCIPLINARY



MACONELLICOCCUS HIRSUTUS (GREEN, 1908) (HEMIPTERA: PSEUDOCOCCIDAE): EXOTIC PEST INTRODUCED ON VINE IN THE SÃO FRANCISCO VALLEY

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Abstract

The pink hibiscus mealybug, *Maconellicoccus hirsutus* is a polyphagous pest that attacks more than 200 generous and about more than 74 botanical families of cultivated or uncultivated plants. Many of these plants are of economic importance to Brazil, including cotton, cocoa, coffee, coconut, citrus, cucumber, corn, beans, papaya, sweet potatoes, figs, grapes, guavas, peanuts, roses, hibiscus and ornamental palms. However, *M. hirsutus* was recently introduced in Brazil. This study reported the first time occurence this pest in São Francisco Valley, Northeastern Brazil. After the pest presence alert, samplings were taken inside and around of the plantation of vines. The mealybugs were found attacking all structures of grape plants (*Vitis vinifera*), native plants of caatinga, weeds, "windbreaks" and fruit trees. In grape plants, the mealybugs inside bunches caused the reduction of the fruits quality and the discard of them, and on the sprouts they cause the inhibition of branches development, compromising at least two harvests. Thus, by severity of the damage, *M. hirsutus* may be considered one of the most important pests of the grape culture in the region. The control of this new pest is a big challenge that will require a set of actions including chemical insecticides registration, the development of an effective monitoring plan as well as the search and use of natural enemies adapted to the region.

Keywords: Pink hibiscus mealybug, Vine pest, *Vitis vinifera*, Semiarid.

1. Introduction

Maconellicoccus hirsutus (Green, 1908) (Hemiptera: Pseudococcidae) known as pink hibiscus mealybug is a specie possibly native from the Southeastern Asia or Australia, having a large geographic distribution, being present in tropical and subtropical regions of the world (García et. al., 2016; OEPP/EPPO, 2005). The pink hibiscus mealybug is a polyphagous insect with hosts from 76 families which are included in more than 200 genus (García et al., 2016). They may cause severe damages in important crops like cotton, citrus, cocoa, coffee and grape (Tambasco et al., 2000).

M. hirsutus adult female have an elongated and ovoid shape; their body variates from brown to pink in color and are covered by a white wax. Adult male have a pair of wings and a pair of long wax tails (Chong, 2009; Miller, 1999). Ovipositing females produced an ovisac and in one week, deposits 260 to 300 eggs, depending on the host specie (Chong et al., 2008; Aristizábal et al., 2012). The eggs are orange, becoming pink in maturity. Nymphs are mobile and females ones develops through three instars while males ones through four instars (Chong et al., 2008; Miller, 1999).

When pink hibiscus mealybug feeds on the phloem sap, they inject into the plant a toxic saliva, resulting in malformed leaves, trunks, shoots, flowers and fruits (Kairo et al., 2010; Martínez, 2007; Rivero, 2007). High infestations results in distorted leaves and shoots, having a slow development. Blossom is affected and flowers drop. Fruits can be deformed and a black sooty mold can appears by developing in the honeydew (Rivero, 2007).

M. hirsutus first record in Brazil was in 2010 in the state of Roraima, in hibiscus seedlings (Marsaro Júnior et al., 2013) and two years later in state of Espírito Santo in okra (Culik et al., 2013). At 2013, it was recorded in cocoa in the states of Espírito Santo and Bahia (CEPLAC, 2014). Later, was also recorded in some fruits plants in Alagoas (Broglio et al., 2015), in hibiscus no Mato Grosso (Morais et. al., 2015) and more recently in the state of São Paulo also in hibiscus (Peronti et al., 2016; Moraes et al., 2015) being excluded of pests quarantine list.

In São Francisco Valley, which is responsible for 90% of Brazilian table grapes exportations, high infestations have been detected in grapevine. Mealybugs are founded feeding on all parts of the grape, causing severe lost in the production. According to the fast spread of *M. hirsutus* in Brazil, as well as is consider a potential pest due to its economic damages and environmental impacts (Culik et al., 2013), being a quarantine pest in some importers countries, there is a need to verified the presence of this mealybugs in grapevine. This work aims to relate the occurrence of pink hibiscus mealybug *M. hirsutus* in grapevine areas and in other plants inside or around the grape areas, at São Francisco Valley.

MATERIALS AND METHODS

The survey was taken in grapevine and others plants infested with mealybugs in 20 crop areas in São Francisco Valley, been 18 in the city of Petrolina-PE and 2 in the city of Casa Nova-BA (Figura 1). For each infested plant was collected 50 individuals mixing nymphs and/or adults. The material was conditioned in plastic tubes called eppendorf with

alcohol 96% and sent to the Laboratory of Bioinformatics and Evolutionary Biology, at the Department of Genetics, Federal University of Pernambuco, for molecular identification. The DNA extraction was made with Chelex®100 (BioRad, Berkeley, 111 California, USA) following the methodology of Walsh (1991). Each mealybug was put in plastic tubes of 1,5mL with 100 µL of 5% Chelex®100 (BioRad, 114 Berkeley, California, USA). Then, the samples were ret until become homogenized and rest in water bath at 54°C for one hour. The mixture was put in a plastic tube and maintained at 94°C for 30 minutes at thermocycler Multigene OptiMax TC 9610 (LABNETTM, Edison, New Jersey, USA). Then, the solution was centrifuged at 13.000 rpm for 6 minutes and the supernatant put in a plastic tube of 1,5mL and stored at -20°C.

To amplification the gene Citocromo-Oxidase I, associated to the DNA barcode (Hebert & Gregory 2005) were used LCO-M-2d-F (5'-123 ATAACTATACCTATYATTATTGGAAG-3') and LCO-M-2d-R (5'-124 AATAAATGTTGATATAAAATTGG-3') initiators described by (Malausa et al., 2011). In general, were used 12,5 µL of Mix Go Taq Colorless (Promega® Fitchburg, Wisconsin, 126 USA), 7,5µL of non-nuclease water, 1,5 µL of each initiator and 2 µL of DNA mold. The amplification reactions were realized in the thermocycler Multigene OptiMax TC 9610 (LABNETTM, Edison, New Jersey, USA), with the following cycle: initial denaturation at 95°C for 3 minutes; 35 cycles of denaturation at 95°C for 30 seconds, ringing at 48°C for 1 minute and extension at 72°C for 1 minute; and final extension at 72°C for 7 minutes. The amplification products were analyzed by electrophoresis agarose gel 1%. The electrophoresis gel was stained with SYBR® Safe DNA 134 Gel Stain (Invitrogen™, Carlsbad, California, USA) and visualized under ultraviolet light.

To purified the DNA amplification were used Wizard® SV Gel and PCR 137 Clean-Up System (Promega® Fitchburg, Wisconsin, EUA), following manufacturer's instructions. Later, sequencing was made at the automatic sequencer ABI 3500 (Applied Biosystems, Cleveland, 140 Ohio, EUA). Sequences were evaluate using Pregap4 v 1.5 and GAP4 programs, in the Staden package (Staden, 1996), using value Phred as 30. To find resemblance within the sequences in this study, the GenBank (National Center for 144 Biotechnology Information, Bethesda, EUA) were consulted, using Blastn (Altschul, 1990).

RESULTS AND DISCUTION

Individuals sequence fragments next to 400 pb were created from the DNA barcode through PCR. Taxonomic molecular results showed that the DNA obtained is from *Maconellicoccus hirsutus* with 99% of similarity when compared with other sequences in the GenBank.

Were observed the presence of *M. hirsutus* in all areas visited. This mealybug was founded in 13 plant species, 11 exotic ones and 2 native ones (Table 1). In the exotic group, five fruit tree, five weed and one used as windbreak, the pink hibiscus mealybug was founded. In the native group, the plants were *Mimosa tenuiflora*, *Spondias tuberosa*, founded next to the plantation and *Mimosa caesalpineafolia* used as windbreak (Table 1). In grapevine, *M. hirsutus* was founded in bunches (Figure 2A), trunk (Figure 2B) and in sprouts (Figure 2C). When founded in bunches, were observed a black sooty mold developing on the

honeydew, which one depreciate the fruit and cause lost. As *M. hirsutus* is a quarantine pest in many countries, the exportation of grapes may be impossible. In 2014 the producers observed a reduction in the crop harvest, being expected the same in 2015.

Because of the damages caused, this mealybug can be considered as one of the principals grape pest in the region. In India, *M. hirsutus* is an important grape pest, causing 50 to 100% lost production (Sahito et al., 2012). The economic impact in the entrance of this mealybug in São Francisco Valley is significantly and may be more as *M. hirsutus* were founded in others fruits plants in the region, as mango (Figure 2D).

The control in grapevine areas is a challenge. Currently, the control made is pruning and burning the structures of grapevine, weed or windbreak plants infested. However, others control methods are required. Chemical control is efficient and fast, but the products registered are for *Eurhizococcus brasiliensis* (AGROFIT, 2015). Is needed the register of chemical insecticides to control *M. hirsutus* in grapevine. Another fact is the presence of this mealybug in the native plant *Mimosa tenuiflora* (Figure 2E) which is observed in large quantities in all areas visited. It acts as a supply to spread out the mealybugs by the wind to the grapevine plants. So, even with the control of the mealybug in the grapevine, they will be infested again because of the infestation present in native plants next to it.

It is important to plan an efficient monitoring of this pest in areas infested, in other to help the decision in the control. To start, the monitoring needs to be done more frequently once the grapevine could be infested again any time. For monitoring, many authors (Marsaro Júnior et al., 2013; Culik et al., 2013) indicate the use of a pheromone, developed by Zhang et al (2004) which attracts the male adults of *M. hirsutus*. In Brazil, we don't have synthetic pheromone registered for mealybug (AGROFIT, 2015). In Mexico, studies are taken in order to validate pheromones to use for monitoring *M. hirsutus* (Gonzalez-Gaona et al. 2010) and also in Florida (Hall et al. 2008). This technique is secure because is specific and non-toxic, so its register needs to be encouraged. In USA, *Planococcus ficus* monitoring in grapevine are made using a commercialized pheromone (Walton et al,. 2006).

Also, biological control needs to be encouraged. Many biological control agents are related worldwide (Chong et al., 2015). In Brazil, in the state of Roraima, the parasitoid *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae) (Marsaro Júnior et al., 2013) was found associated with *M. hirsutus*. Many works report the potential and efficiency of this parasitoid as a control agent against pink hibiscus mealybug (Kairo et al., 2000; Roltsch et al., 2006; Chong, 2009; Reddy et al. 2009). Another natural enemy is the predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), consider one of the responsible for the success in the control of this mealybug at the countries that had imported it (Kairo et al., 2000; Roltsch et al., 2006; Kairo et al., 2013). Brazil was one of them that imported this predator, but the aim was controlling *Planococcus citri* Sanches & Carvalho, 2010). So, *A. kamali* and *C. montrouzieri* are good species for using as biological control of *M. hirsutus* in São Francisco Valley. However, more studies are needed in order to know the performance of them in semiarid region. There is an urgency in find new natural enemies, so the researcher group of Integrated Pest Management of Grapevine, of Embrapa Semiarid coordinated by Dr. José Eudes de Morais Oliveira, conduct weekly searches in infested areas. Were found recently, a specie of ladybug not identified already (Not published data). Biological control might have an important role in containing this new pest in the crops at the region.

CONCLUSION

This work presents the first record of *M. hirsutus* in grapevine areas and in 12 other species inside and around the grape areas, at São Francisco Valley. Causing severe damages to grape production, *M. hirsutus* could be consider one of the principal pests of grapevine in the region. Is needed a set of actions to control this new pest, including the register of synthetic insecticides, an efficient monitoring plan and the search and use of natural enemies adapted to the region.

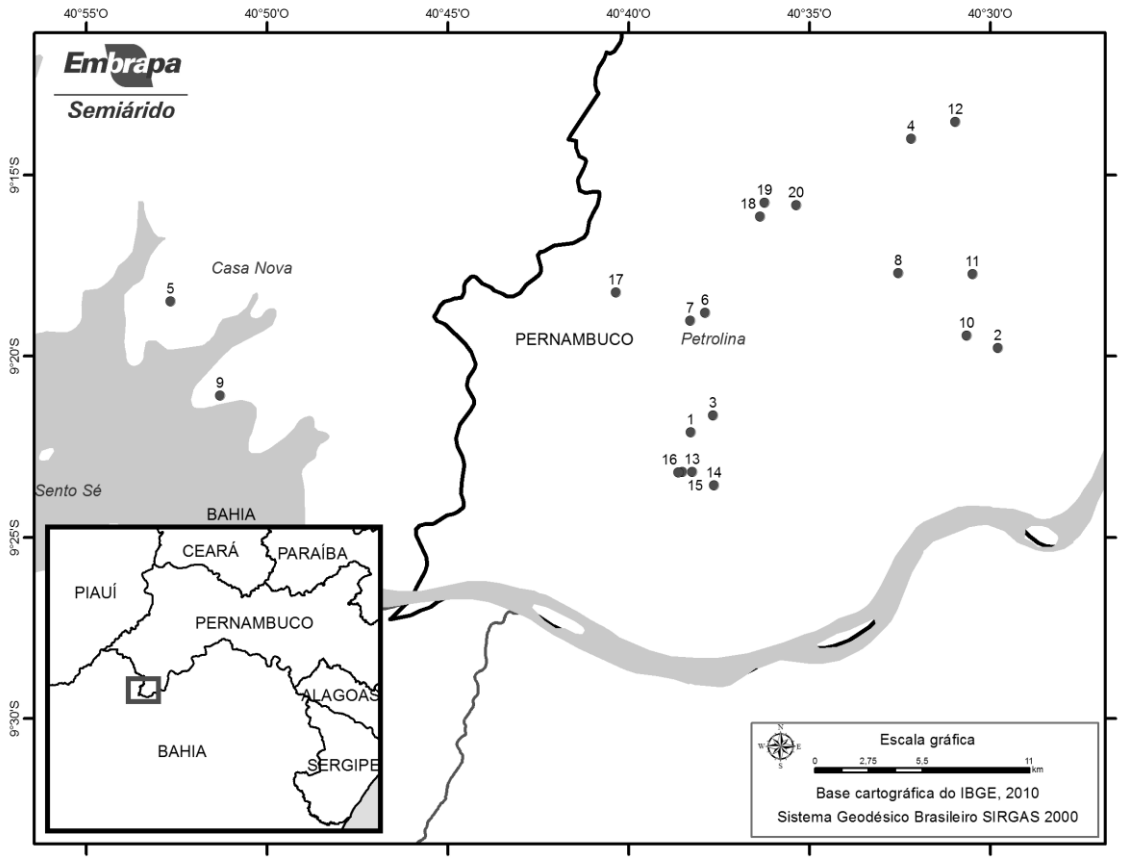

Figure 1: Map of the cities Petrolina-PE and Casa Nova-BA with the 20 grapevine areas which *Maconellicoccus hirsustus* were collected.

Table 1. Plant species infested with *Maconellicoccus hirsustus* registered in grapevine crop areas in São Francisco Valley.

Scientific name	Family	Locality *
Fruits		
Vitis vinífera L.	Vitaceae	8, 13, 14, 15, 16, 20
Mangifera indica L.	Anacardiaceae	12
Spondias tuberosa X *S. mombin*	Anacardiaceae	12, 19
Annona muricata L.	Annonaceae	12
Annona squamosa L.	Annonaceae	1
Weeds		
Commelina sp. L.	Commelinaceae	10, 13
Talinum paniculatum (Jacq.)	Portulacaceae	11
Sidastrum sp.	Malvaceae	8
Cucumis sp.	Cucurbitaceae	9
Momordica charantia L.	Cucurbitaceae	9
Windbreaks		
Grevillea robusta (Cunn.)	Proteaceae	3, 5, 9, 13
Native Plants		
Mimosa tenuiflora [Willd.] Poir.	Fabaceae	1, 4, 6, 7, 17, 18
Mimosa caesalpineafolia Benth.	Mimosacea	1, 2, 3, 6, 8, 11

*Grapevine areas presented in the map of Figure 1

Figure 2. *Maconellicoccus hirsustus* in bunches (A), trunk (B) and sprout (C) of grapevine plants (*Vitis vinifera*). Photos: José Eudes de Morais Oliveira.

ACKNOWLEDGMENT

To Embrapa Semiarid, CAPES, FACEPE and CNPq for the research financial and schorlarship and also to the grapevine producers in São Francisco Valley. Thanks to Dra Lúcia Helena Piedade Kiill for the plants identification.

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