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VINICIUS HENRIQUE BELLO

DISPERSÃO DE *Bemisa tabaci* MEDITERRANEAN E VÍRUS ASSOCIADOS NO BRASIL E TRANSMISSÃO DE POLEROVIRUS POR AFÍDEOS E MOSCA-BRANCA EM ISRAEL

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Tese apresentada à Faculdade de Ciências Agronômicas da Unesp Câmpus de Botucatu, para obtenção do título de Doutor em Proteção de Plantas.

Orientadora: Professora Dra. Renate Krause Sakate

Coorientador: Professor Dr. Murad Ghanim

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AUTOR: VINICIUS HENRIQUE BELLO

ORIENTADORA: RENATE KRAUSE SAKATE

COORIENTADOR: MURAD GHANIM

Aprovado como parte das exigências para obtenção do Título de Doutor em AGRONOMIA (PROTEÇÃO DE PLANTAS), pela Comissão Examinadora:

Prof.ª Dr.ª RENATE KRAUSE SAKATE (Participaçao Virtual)
Proteção Vegetal / Faculdade de Ciências Agronômicas de Botucatu - UNESP

Prof. Dr. MARCELO AGENOR PAVAN (Participaçao Virtual)
(Docente Aposentado) / Faculdade de Ciências Agronômicas de Botucatu – UNESP

Dr. JULIO MASSAHARU MARUBAYASHI (Participaçao Virtual)
Pós-Doutorando Depto. Proteção Vegetal / FCA/ Unesp Botucatu

Prof. Dr. BRUNO ROSSITTO DE MARCHI (Participaçao Virtual)
Entomologia / University of Florida

P/

Prof. Dr. JORGE ALBERTO MARQUES REZENDE (Participaçao Virtual)
Fitopatologia / Universidade de São Paulo

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RESUMO

A mosca-branca, *Bemisia tabaci* (Hemiptera: Aleyrodidae) é uma das pragas agrícolas e vetora de vírus mais importantes mundialmente. No entanto, *Bemisia tabaci* é considerada um complexo de espécies crípticas composto por ao menos 44 espécies consideradas indistinguíveis morfologicamente. Dentre as espécies desse complexo, no Brasil as espécies Middle East-Asia Minor 1 (MEAM1, também conhecida como biótipo B) e Mediterranean (MED, também conhecida como biótipo Q) são as mais importantes. A espécie MEAM1 foi relatada no Brasil no início da década de 90 e desde então tem causado danos severos a diversas culturas, além de ser responsável pela emergência de begomovírus em tomateiro nunca observado previamente. A espécie MED foi relatada no Brasil em 2014 no extremo sul do país no estado do Rio Grande do Sul. Desde então, MED tem mudado o cenário da agricultura nos estados do Paraná e São Paulo associado aos danos severos causado pelo seu ataque e transmissão de vírus. Atualmente, MED pode ser encontrada no Rio Grande do Sul, Paraná (PR), Santa Catarina, São Paulo (SP) e Minas Gerais, associada a plantas hortícolas e ornamentais. Diante desse cenário, objetivou-se compreender melhor os impactos causados pela espécie MED como praga e vetora de vírus. O trabalho desenvolvido foi dividido em quatro capítulos.

No primeiro capítulo, levantamento populacionais demonstram que MED é a espécie predominante de mosca-branca em pimentão cultivado em estufa e a campo aberto no PR e SP. Além disso, o begomovírus tomato severe rugose (ToSRV) e o crinivírus tomato chlorosis virus (ToCV) foram encontrados associados a MED em plantas de pimentão e tomate, respectivamente. No segundo capítulo, foi identificado o ToCV infectando plantas de pepino pela primeira vez no Brasil, no município de Óleo (SP). Plantas infectadas apresentavam sintomas de mosqueado nas folhas jovens e se encontravam associadas a alta infestação de MED. No capítulo 3, apresentamos a primeira evidência de MED em campos de soja em SP e no PR. O carlavírus cowpea mild mottle virus (CPMMV) também foi encontrado associado a presença de MED e MEAM1. Além disso, estudos biológicos demonstraram que MED e MEAM1 são excelentes vetores do CPMMV, bem como plantas de soja infectadas pelo CPMMV são favoráveis para a reprodução de MED e MEAM1. No capítulo 4, realizado em Israel, foi demonstrado que os poleroviruses pepper vein yellows vírus - 2 (PeVYV-2) e o pepper whitefly-borne vein yellows virus (PeWBVYV) competem em plantas e também em seus respectivos vetores (pulgões e mosca-branca). No entanto, essa competição pode ser uma estratégia evolucionaria dos dois polerovirus para permitir a coexistência na mesma localização geográfica, evitando a exclusão competitiva.

Palavras-chave: mosca-branca; ToSRV; ToCV; CPMMV; *Polerovirus*.

ABSTRACT

The whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) is one of the most important agricultural pest and viral vector worldwide. However, *Bemisia tabaci* is considered a cryptic species complex composed of at least 44 species considered morphologically indistinguishable. Among these cryptic species, Middle East-Asia Minor 1 (MEAM1, also referred as B biotype) and Mediterranean (MED, also referred as Q biotype) are the main important in Brazil. The MEAM1 species was reported in Brazil in the early 90s and since then it has caused severe damage to several crops, beyond to be responsible the emergence of begomoviruses in tomatoes never previously observed. The MED species was reported in Brazil in 2014 in southernmost region of the country in Rio Grande do Sul State. Since then, MED has changed the scenario of Paraná and São Paulo States agriculture associated with the severe damage caused by its attack and transmission of virus. Currently, MED can be found in Rio Grande do Sul, Paraná (PR), Santa Catarina, São Paulo (SP) and Minas Gerais, associated to vegetable and/or ornamental plants. Due to this scenario, the goal was to better understand the impacts caused by the MED species as a pest and viral vector. The work developed was divided into four chapters.

In the first chapter, population surveys showed that MED is the predominant whitefly species in bell pepper cultivated in greenhouses and under field conditions in PR and SP. Besides, the begomovirus tomato severe rugose (ToSRV) and the crinivirus tomato chlorosis virus (ToCV) were found associated with MED in bell pepper and tomato plants, respectively. In the second chapter, was identified the ToCV infecting cucumber plants for the first time in Brazil, in the county of Óleo (SP). Infected plants showed mottling symptoms on young leaves and were associated with a high MED infestation. In the chapter 3, we present the first evidence of MED in soybean fields in SP and PR. The carlavirus cowpea mild mottle virus (CPMMV) was also found associated with the presence of MED and MEAM1. In addition, biological studies showed that MED and MEAM1 are good vectors of CPMMV, as well as CPMMV-infected plants are suitable for reproduction of MED and MEAM1. In the chaper 4 performed in Israel, was showed that the poleroviruses pepper vein yellows virus - 2 (PeVYV-2) and the pepper whitefly-borne vein yellows virus (PeWBVYV) compete in plants and their respective vectors (aphids and whitefly). However, this competition may be an evolutionary strategy for the two poleroviruses to allow coexistence in the same geographic location, avoiding competitive exclusion.

Keywords: Whitefly; ToSRV; ToCV; CPMMV; *Polerovirus*.

SUMMARY

GENERAL INTRODUCTION

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one the most important agricultural pest worldwide that causes serious economic damage to a wide variety of hosts, including field and greenhouse crops and ornamental plants. This pest causes damage by feeding on phloem, excreting honeydew and by transmitting plant viruses that belong to genera *Begomovirus*, *Crinivirus*, *Carlavirus*, *Torradovirus*, *Ipomovirus* and *Polerovirus* (NAVAS-CASTILLO; FIALLO-OLIVÉ; SÁNCHEZ-CAMPOS, 2011; FIALLO-OLIVÉ et al., 2020).

The diversity of *B. tabaci* species has been frequently studied and classifying as a cryptic species complex composed of at least 44 morphologically indistinguishably species (KANAKALA; GHANIM, 2019). Several biological characteristics can differ the species of this complex, such as their ability to transmit specific begomoviruses, plant host preference, tolerance to high temperature, different composition/infection frequencies of bacterial endosymbionts and resistance to insecticides (POLSTON et al., 2014; WATANABE et al., 2019; HOROWITZ et al., 2020). However, molecular biology analyzes of mitochondrial cytochrome oxidase I (mtCOI) gene are the main tools that have been used to differ and identify the different species of *B. tabaci* (DE BARRO et al., 2003, 2011).

Among these cryptic species, Middle East-Asia Minor 1 (MEAM1, also referred as B biotype) and Mediterranean (MED, also referred as Q biotype) are the most invasive and worldwide distributed *B. tabaci* species within this complex (GILBERTSON et al., 2015; KANAKALA; GHANIM, 2015, 2019). In Brazil, MEAM1 species was reported in the beginning of 1990s and after few years became widespread throughout the county (LOURENÇÃO; NAGAI, 1994; CHINTKUNTLA, 2015). Since then, tomato crop has been severely affected by the tomato severe rugose virus (ToSRV) and the tomato mottle leaf curl virus (ToMoLCV) begomovirus that emerged locally after the introduction of MEAM1 in Brazil (INOUE-NAGATA et al., 2016; MACEDO et al., 2018; QUADROS et al., 2019; REGO-MACHADO et al., 2019). The crinivirus tomato chlorosis virus (ToCV) was also reported in Brazil associated to MEAM1 (BARBOSA et al., 2008). Twenty nine years after MEAM1 introduction, this whitefly species is still predominant in Brazil and the most import vector of viruses (MORAES et al., 2018).

Two decades after introduction of MEAM1 in Brazil, MED was reported for the first time in the southernmost region of the country (BARBOSA et al., 2015). Nowadays, MED has been found in the states of Rio Grande do Sul (RS), Santa Catarina (SC), Paraná (PR), São Paulo (SP) and Minas Gerais (MG) associated to ornamental plants (SC, PR, SP and MG) and vegetable crops under greenhouse and field conditions (RS, SC, PR and SP) (MORAES et al., 2017, 2018).

It is also known that MED has several advantages over MEAM1. *Bemisia tabaci* MED is known to predominate over MEAM1 under greenhouse conditions with or without insecticides application (KONTSEDALOV et al., 2012). This can occur because MED is more resistant to high temperatures than MED (MAHADAV et al., 2009; XIAO et al., 2016). MED is also known to develop resistance to insecticides much faster than MEAM1 (HOROWITZ et al., 2005, 2014, 2020; KONTSEDALOV et al., 2012; SUN et al., 2013; YAO et al., 2017). In addition, studies of performance without insecticides from laboratory shows that MED has better performance on bell pepper and common bean than MEAM1, being able to displace MEAM1 in natural conditions (SUN et al., 2013; WATANABE et al., 2019).

In Brazil, the tomato severe rugose virus (ToSRV) is the main begomovirus that cause an important disease that affects tomato crops (INOUE-NAGATA et al., 2016). Symptoms caused by ToSRV and other begomoviruses on plants develop a yellow to light green mosaic and enhancing for a typical golden mosaic on younger leaves. ToSRV is transmitted by the whitefly *B. tabaci* MEAM1, MED and NW2 in a circulative persistent mode (DE MARCHI et al., 2017; BELLO et al., 2019). Under high begomovirus incidence, the losses caused by the begomovirus can reach 100%. Due to high incidence of begomovirus in processing tomatoes in Midwest region in 2003, a legislative control measure of tomato-free period of two months (December and January) was implemented in Goiás State (INOUE-NAGATA et al., 2016). However, a high incidence of ToSRV (60 to 100%) continues to occur in tomato crops (MACEDO et al., 2017a, 2019). Other management approach to control begomoviruses diseases in tomatoes depends on the use of resistant tomato hybrids. These resistant hybrids possess at least one of the resistance genes (e.g.,*Ty*-1, *Ty*2, *Ty*-3, *Ty*-4, *Ty*-5, *Ty*-6) that provides high level of resistance to tomato yellow leaf curl virus and a moderated level of resistance to bipartite begomoviruses (INOUE-NAGATA; LIMA; GILBERTSON, 2016). Thus, Brazilian tomato hybrids are moderately resistant and/or tolerant to infection with ToSRV.

Another current control is based mainly on frequent insecticides sprays (2 to 3 per week) to control the vector populations present in the field. However, insecticide sprays have failed in several tomato production areas in Brazil, once it is unable to reduce the primary spread, i.e., infection caused by the migration of viruliferous whiteflies coming from external sources of inoculum (GOUVÊA et al., 2017; MACEDO et al., 2019; FILHO et al., 2020). Generally, epidemiology of plant virus considers the reservoir as the main source of primary inoculum, responsible for introducing the pathogen into a previously pathogen-free area (HULL, 2014). Then, the secondary spread drives the epidemic, i.e., infection caused by viruliferous whiteflies from internal sources of inoculum. However, there are evidences that the incidence of ToSRV in weeds is low in Brazilian conditions and secondary spread is prevented by frequent and effective spraying of insecticides to control the whitefly MEAM1 (MACEDO et al., 2017a; FILHO et al., 2020).

Thus, the amplifier-host hypothesis can help to explain the occurrence of ToSRV epidemics in tomato fields in Brazilian conditions. Amplifier host occur in high density near to the target crop, support the growth of both virus and vector and acts as an intermediated host in off-season periods and provides a short-term source of infection (FILHO et al., 2020). Reports of the incidence of 2.9% and 3.3% of common bean and soybean plants, respectively, infected with ToSRV and asymptomatic in central Brazil (MACEDO et al., 2017b, 2017c). Further, one soybean field near of tomato crops recently transplanted showed an incidence > 10% of asymptomatic soybean plants infected with ToSRV in Sumaré/SP with an incidence of 57% to 70% of symptomatic tomato infected with begomovirus (FILHO et al., 2020).

In this way, challenges to the effectiveness of management measures include continuing studies that quantify the effect of vector control (*B. tabaci* species, virus-vector-plant interactions, chemical, etc.) and studies that quantify the effect of amplifier hosts such as common bean and soybeans in the ToSRV epidemics on tomato.

Beyond ToSRV, the crinivirus (ToCV) cause an important disease that affects several Solanaceas species in Brazil (INOUE-NAGATA et al., 2016). Symptoms caused by virus infection on plants mainly develop on older/lower leaves with interveinal yellowing and thickening of leaves. ToCV was first reported in Brazil infecting tomato plants in 2008 (BARBOSA et al., 2008). Currently, Solanaceas such as tomatoes, peppers and potatoes are the main ones infected by the virus, but also infect eggplants, scarlet eggplants and several weeds (FONSECA, 2016; MITUTI et al., 2019; SOUZA et al., 2020). ToCV is transmitted by the whiteflies MEAM1, MED, NW2 and *Trialeurodes vaporariorum* in a semi-persistent manner (DE MARCHI et al., 2017; WATANABE et al., 2018; BELLO et al., 2019).

In this kind of virus-vector relationship, insecticides are unable to kill insects before the virus is transmitted because the insect acquires and transmits the virus in a rather shorter period of time. Therefore, the effect of incidence on crops is not affected and the presence of few insects is enough to cause an epidemic in the field. In agreement with this knowledge, older tomato plants and weeds infected with ToCV play an important role in its epidemiology, representing an efficient inoculum source for younger tomato plants (FIALLO-OLIVÉ; NAVAS-CASTILLO, 2019). Thus, effective control measures for ToCV and whitefly hosts contribute to virus elimination, especially when combined with other methods.

In Brazil, the cowpea mild mottle virus (CPMMV) cause an important disease that inflict soybean and beans, causing symptoms of dwarfism/stunting, necrosis, chlorosis in both hosts and even plant death or asymptomatic (ZANARDO et al., 2014; ZANARDO; CARVALHO, 2017a). CPMMV belongs to *Carlavirus* genus, in which carlaviruses are transmitted exclusively by aphids. However, CPMMV is an exception of this genus transmitted by the whitefly *B. tabaci* MEAM1, MED and NW2 in a nonpersistent manner (MARUBAYASHI; YUKI; WUTKE, 2010; DE MARCHI et al., 2017; BELLO et al., 2019). Moreover, CPMMV is also transmitted by soybeans and beans seeds (ZANARDO; CARVALHO, 2017a; SILVA et al., 2020). However, CPMMV transmission by seeds may be dependent of several unclear factors (i.e., viral strain and host cultivar).

CPMMV was reported in Brazil infecting beans in 1983 and soybean in 2005 (ZANARDO; CARVALHO, 2017a). Since then, an intense increase in soybean and bean cultivations areas associated with the abusive use of non-selective insecticides has led to increasing occurrence of whitefly *B. tabaci* on soybean and bean fields (ARNEMANN et al., 2019). In addition, areas of soybean and bean are cultivated close to each other, which both plant species are the main host plants that act as virus and whitefly reservoir in the field. Thus, CPMMV has emerged as a problem in soybean and is re-emerging in beans (ZANARDO; CARVALHO, 2017a).

In this CPMMV-vector relationship, a single insect acquires and transmits the virus in a few minutes, however, the transmission rates increase proportionally with an increase of insect’s number and longer feeding periods (WHITFIELD; FALK;

ROTENBERG, 2015). Thus, vector control with insecticides cannot avoid virus transmission. Under these circumstances, CPMMV seems to present several challenges for its management. As vector control using insecticides cannot avoid virus transmission and also can be transmitted by seed prevenient of infected plants, the use of resistant/tolerant cultivars of soybeans and beans represents the best strategy (ZANARDO; CARVALHO, 2017a; SILVA et al., 2020).

Pepper vein yellows viruses (PeVYVs) are poleroviruses complex infecting pepper and causing interveinal yellowing of leaves, upward leaf curling and discoloration of mature fruits (LOTOS et al., 2017). Poleroviruses are members of the *Polerovirus* genus, exclusively transmitted by aphids in a circulative non-persistent manner (HOGENHOUT et al., 2008). Pepper vein yellows virus-2 (PeVYV-2) reported from Israel was one of the first described polerovirus species known to infect pepper and is transmitted exclusively by aphids (DOMBROVSKY et al., 2010). However, a disease outbreak in the pepper plantations in Israel with severe symptoms of interveinal yellowing and fruit discoloration as that caused by Poleroviruses was recently associated with a new recombinant polerovirus with PeVYV-2 being one parent of this new virus. This new recombinant virus was shown to be transmitted by the whitefly MEAM1 species and not aphids, and thus named as Pepper whitefly-borne vein yellows virus (PeWBVYV) (GHOSH et al., 2019a). PeWBVYV was the first polerovirus reported to be transmitted by whiteflies and not by aphids. Recently, another recombinant polerovirus infecting melons in Brazil has been reported to be transmitted by whitefly MEAM1 species and not aphids (COSTA et al., 2020).

This unusual poleroviruses transmission by whiteflies bring us new challenges in a management approach, since aphid and whitefly can occur simultaneously in the same cultivated area. However, the main control method is still based on insecticides use to the susceptibility of aphids and the whitefly MEAM1 species.

Since the first report of MED in Brazil, there has been a notable shift in the *B. tabaci* MED dynamics and its dispersion to other regions of our country. Previous works show that MED has several advantages under MEAM1. Among these, MED is known to increase some aspects that promotes benefits in the life cycle of MED and then MEAM1 (FANG et al., 2013), thus being able to suppress host plant defenses and promote subsequent whitefly infestations (SHI et al., 2014, 2018). In addition, the emergence of new viruses has been associated with MED, in which this species is known as an efficient vector of the ToSRV and ToCV to tomatoes, and CPMMV to beans, considered important viruses to several crops in Brazil (BELLO et al., 2019).

Thus, the goal of this work was evaluated the importance of *B. tabaci* MED in our country, which raises concerns as pest and vector of Brazilian plant viruses. Moreover, a study of relationships between whiteflies and Pepper yellows viruses was performed in Israel. Against, this work was divided into four chapters: 1) “Outbreaks of *Bemisia tabaci* Mediterranean species in vegetable crops in São Paulo and Paraná States, Brazil”, 2) “First report of Tomato Chlorosis Virus infecting cucumber in Brazil”, 3) “Detection of *Bemisia tabaci* Mediterranean cryptic species in Sao Paulo and Paraná Sates (Brazil) and interactions of cowpea mild mottle virus with whiteflies”, 4) “Competitive interactions between whitefly and aphid transmitted poleroviruses within the plant host and the insect vectors”**.**

FINAL CONSIDERATIONS

The data obtained here suggest that in São Paulo and Paraná States, Brazil, MED is now found on vegetable crops both in greenhouse and under field conditions, and ToCV-infected and ToSRV-infected plants were also associated to this this whitefly species. In addition, ToCV was identified infecting also cucumber plants for the first time in Brazil and associated with infestations of MED.

Our data also showed that MEAM1 is prevalent on soybean fields, but MED has started to spread and to colonize soybean under field conditions. Moreover, we found soybean plants infected with CPMMV associated with the presence of MED and MEAM1. In this work we show that in general MED is a better vector for CPMMV compared to MEAM1. Our performance assay also show that CPMMV-infected soybean plants seem to better hosts for the development and performance of MED and MEAM1 than healthy soybeans plants. The preference assays further show that viruliferous adults of both whitefly species preferred to settle more on CPMMV-infected soybean plants.

In the work performed in Israel, our results show that PeWBVYV was the weaker competitor inside the host plant and could only be transmitted efficiently when inoculated first and then challenged by PeVYV-2. Successive inoculations of plants with viruliferous whiteflies with PeWBVYV, followed by viruliferous aphids with PeVYV-2 led to co-infection rate of 60%. In contrast, PeVYV-2 was the weaker competitor inside the insect vector with reduced quantities of the acquired virus and reduced transmission rate by aphids when given prior acquisition on PeWBVYV. However, we also show that transmission efficiency of PeVYV-2 and PeWBVYV from co-infected plants by whiteflies and aphids remain comparable to that from singly-infected plants.

In summary, the results generated by the works developed here will have great practical applications in the way to correct manage whitefly in Brazil and avoid whitefly-transmitted viruses outbreaks. Finally, was possible to understanding a little more the pathosystem involving the poleroviruses PeVYV-2 and PeWBVYV, and aphids and whitefly in bell pepper crop in Israel.

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