UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

FACULDADE DE CIÊNCIAS AGRONÔMICAS
UNESP | BOTUCATU

VINICIUS HENRIQUE BELLO

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

Botucatu

2020

VINICIUS HENRIQUE BELLO

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

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

Botucatu

2020

B446d

Bello, Vinicius Henrique
Dispersão de Bemisa tabaci Mediterranean e vírus associados no Brasil e transmissão de polerovirus por afídeos e mosca-branca em Israel / Vinicius Henrique Bello. -- Botucatu, 2020
117 p.

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agronômicas, Botucatu
Orientadora: Renate Krause-Sakate
Coorientador: Murad Ghanim

1. Whitefly. 2. Endosymbionts. 3. Virus. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agronômicas, Botucatu. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Botucatu

CERTIFICADO DE APROVAÇÃO

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

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:

RenateKSakate

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

Julio Massaharu Marubayashi

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

Bruno De Marchi

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

P/ RenateKSakate

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

Botucatu, 17 de dezembro de 2020

ACKNOWLEDGMENTS

First of all, I would like to thank my esteemed Major Advisor Dr. Renate Krause Sakate, professor of Faculdade de Ciências Agronômicas- Unesp, Botucatu, SP, Brazil, for her invaluable and inspiring guidance. I shall remain ever indebted to your care, sustainable encouragement, keen interest and precious time given me during this period of PhD course. I would also like to thank my Second Advisor Dr. Murad Ghanim, professor of Agricultural Research Organization (ARO), the Volcani Center, Israel, for your interest, confidence and precious time given me during this period of my PhD. I own my indebtedness and virtual veneration towards to this opportunity conceived me. It is my sovereign privilege to express my gratitude and moral obligation to my advisors.

I would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) (Finance Code 001) for my scholarship and to FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) grant number 2017/21588-7. I also extend my sincere acknowledgement to all my partners, professors and technician for their sincere attempts to increase my knowledge.

My appreciation also goes to my friends for making my time better. I would specially like to thank the friends who helped and supported me throughout: Luiz F.M. Watanabe, Bruno R. De Marchi, Felipe B. da Silva, Eduardo Vicentin, Eduardo S. Gorayeb, Marcos R.R. Junior, Giovana C.D. Cruciol, Lucas M. Fusco, Saptarshi Ghosh, Ola Jassar and Poulami Sarkar. I would also thank my fraternity friends where I spent great moments: Tiago Otani, Dennis Souza, Alberto Avilez, Lucas Guedes, Lucas Serrou, Fábio Araujo, Fábio Moreira, Vitoldo Neto, Murilo Marques, Antonio Higo, Rodrigo Barcellos, Diego Arcanjo, Laudelino Mota, Ricardo Gonçalves and Gabriel Baroni.

Finally, my sincerely gratitude is extended to my parents Varlei Bello and Ana Paula Gonçalves their encouragement and support in my decisions during this period.

I feel proud to be a part of UNESP, where I learned a lot and spent important moments of my life. I duly acknowledge to all those take care for me. Lastly, every name may not be mentioned, but none forgotten.

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 13

CHAPTER 1 - OUTBREAKS OF *Bemisia tabaci* MEDITERRANEAN SPECIES IN VEGETABLE CROPS IN SÃO PAULO AND PARANÁ STATES, BRAZIL 19

1.1 INTRODUCTION 20

1.2 MATERIAL AND METHODS 21

1.3 RESULTS 23

1.4 DISCUSSION 32

REFERENCES 36

CHAPTER 2 - FIRST REPORT OF *Tomato Chlorosis Virus* INFECTING CUCUMBER IN BRAZIL 45

DISEASE NOTE 45

REFERENCES 47

CHAPTER 3 - DETECTION OF *Bemisia tabaci* MEDITERRANEAN CRYPTIC SPECIES ON SOYBEAN IN SÃO PAULO AND PARANÁ STATES (BRAZIL) AND INTERACTION OF cowpea mild mottle VIRUS WITH WHITEFLIES 48

3.1 INTRODUCTION 49

3.2 MATERIAL AND METHODS 51

3.3 RESULTS 61

3.4 DISCUSSION 69

REFERENCES 74

CHAPTER 4 - COMPETITIVE INTERACTIONS BETWEEN WHITEFLY AND APHID TRANSMITTED POLEROVIRUSES WITHIN THE PLANT HOST AND THE INSECT VECTORS 85

4.1 INTRODUCTION 86

4.2 MATERIAL AND METHODS 87

4.3 RESULTS 94

4.4 DISCUSSION 102

REFERENCES 107

FINAL CONSIDERATIONS 112

REFERENCES 113

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”**.**

CHAPTER 1

OUTBREAKS OF *Bemisia tabaci* MEDITERRANEAN SPECIES IN VEGETABLE CROPS IN SÃO PAULO AND PARANÁ STATES, BRAZIL

Vinicius Henrique Bello[1], Luís Fernando Maranho Watanabe[1], Lucas Machado Fusco[1], Bruno Rossitto De Marchi[1], Felipe Barreto da Silva[1], Eduardo Silva Gorayeb[1], Mônika Fecury Moura[1], Isabela Morcilo de Souza[1], Cristiane Muller[2], Fernando Javier Sanhueza Salas[3], Valdir Atsushi Yuki[4], Regiane Cristina Oliveira de Freitas Bueno[1], Marcelo Agenor Pavan[1] and Renate Krause-Sakate[1]

[1]UNESP – Faculdade de Ciências Agronômicas, Botucatu, Brazil; [2]Corteva Agriscience™; Mogi Mirim; Brazil; [3]Instituto Biológico, São Paulo, Brazil; [d] Instituto Agronômico, Campinas, Brazil.

* Corresponding author; E-mail address: vhbello@hotmail.com

Cite this article: Bello VH et al (2020). Outbreaks of *Bemisia tabaci* Mediterranean species in vegetable crops in São Paulo and Paraná States, Brazil. Bulletin of Entomological Research 1–10.

Available at: https://doi.org/10.1017/S0007485319000841

Abstract

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of the most important agricultural pests and virus vectors worldwide. *Bemisia tabaci* is considered a complex of cryptic species with at least 44 species. Among them, the species Middle East-Asia Minor 1 (MEAM1, formerly B biotype) and Mediterranean (MED, formerly Q biotype) are the most important, and they have attained global status. In Brazil, MEAM1 was first reported in the 1990s and is currently the predominant species in the country, meanwhile, MED was recently reported in the South and Southeast regions and was found to be mainly associated with ornamental plants. Currently, an increasing problem in the management of whitefly infestations in greenhouses associated with bell pepper was observed in São Paulo State, Brazil. The whiteflies were collected and identified based on a microsatellite locus (primer pair BEM23F and BEM23R) and the mitochondrial cytochrome oxidase I gene followed by restriction fragment length polymorphism analysis and sequencing. We observed that MED was the predominant species collected on bell pepper, but it was also found on tomato, cucumber, eggplant, and weeds grown in greenhouses. In open field, we found MED on tomatoes, bell peppers, and eggplants. In addition, MED was identified in Goiás State in association with ornamental plants. The begomovirus Tomato severe rugose virus and the crinivirus Tomato chlorosis virus was detected on bell pepper and tomato, respectively. Only MED specimens were found associated with the virus-infected plants. Moreover, we also investigated the endosymbionts present in the MED whiteflies. The collected populations of *B. tabaci* MED harbored a diversity of secondary endosymbionts, with *Hamiltonella* (H) found predominantly in 89 specimens of the 129 tested. These results represent a new concern for Brazilian agriculture, especially for the management of the newly introduced whitefly MED species, which must be implemented to limit the spreading and establishment of this pest in different crops in this country.

Keywords: Bell pepper; cucumber, tomato; whitefly; Q biotype, endosymbionts

1.1 INTRODUCTION

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is an important global agricultural pest that is capable of damaging a wide range of hosts, including several greenhouse and field crops, ornamental plants and weeds, worldwide (Muñiz, 2000; Dinsdale *et al*., 2010). In addition, whiteflies are also considered to be a super vector, transmitting approximately 300 plant viruses, including viruses of the genera *Begomovirus, Carlavirus, Crinivirus, Ipomovirus,* and *Torradovirus*, highlighting the group of *Begomovirus*, which represents approximately 90% of the viruses transmitted by whitefly (Kanakala and Ghanim, 2015).

Bemisia tabaci is considered to be a complex of cryptic species, which includes at least 44 identified species (De Barro *et al*., 2011; Kanakala and Ghanim, 2019). Individual species of this complex are morphologically indistinguishable. However, they differ according to their molecular biology (Boykin and De Barro, 2014) and in several biological characteristics, such as their ability to transmit specific begomoviruses (Polston *et al*., 2014), ability to harbor different sets of bacterial endosymbionts

(Czosnek and Ghanim, 2016), adaptability to different host plants (Sun *et al*., 2013), insecticide tolerance (Horowitz and Ishaaya, 2014) and invasive capability (Liu *et al*., 2007).

Among these cryptic species, Middle East-Asia Minor 1 (MEAM1, formerly B biotype) and Mediterranean (MED, formerly Q biotype) are regarded as the most invasive and widespread (Kanakala and Ghanim, 2015). These cryptic species have attained the global status and are responsible for severe crop damage (Horowitz *et al*., 2003; Luo *et al*., 2010).

In Brazil, MEAM1 was first reported in the early 1990s (Lourenção and Nagai, 1994), and over the past 20 years, this species has become widespread throughout the country (Moraes *et al*., 2018). With MEAM1 introduction in Brazil, the emergence of begomoviruses also occurred in solanaceous crops (Ribeiro *et al*., 1998). More than 20 years later, MED was detected in 2014 in Rio Grande do Sul State (Barbosa *et al*., 2015), and a new invasion associated with ornamental plants was reported in São Paulo and Paraná States in 2015 (Moraes *et al*., 2017). Recently, in 2017, MED was also reported in Santa Catarina and Minas Gerais States in association with ornamental plants (Moraes *et al*., 2018), indicating the importance of ornamental plants for MED invasion in Brazil.

In this study, we report that MED now is also being found on vegetable crops, especially on bell peppers and cucumbers cultivated in greenhouses. However, the insect was also found infesting tomatoes, bell peppers, and eggplants cultivated in open fields. In addition, difficulties have been observed in insect management by spraying insecticides in bell pepper areas, and virus-infected plants were detected in these areas.

1.2 MATERIAL AND METHODS

Sampling of whiteflies

Bemisia tabaci samples were collected from several regions of São Paulo and Paraná States in 2017, 2018, and 2019. The samplings were performed in commercial greenhouses with *Capsicum annuum* Linnaeus (*Solanaceae*), *Solanum lycopersicum* Linnaeus (*Solanaceae*), *Cucumis sativus* (*Cucurbitaceae*), *Solanum melongena*

Linnaeus (*Solanaceae*) and weeds; and in open field containing tomatoes, bell peppers, and eggplants. The insects (adults) were collected with a hand-held aspirator, and the specimens were immediately transferred to a tube containing 100% ethanol and stored at −4°C until further analyses. For each population, analyses of ten adults were carried out for microsatellite and mitochondrial cytochrome oxidase I (mtCOI) regions. Table 1 summarizes the populations collected and analyzed in this study. Additionally, one *B. tabaci* sample was collected from ornamental plants in Formosa/Goiás.

MEAM1 and MED identification

The molecular analyses were carried out following the total DNA extraction from individual specimens following a modified Chelex method. Whitefly adults were macerated and homogenized in 50 µl of Chelex 5% solution in a 2.0 ml Eppendorf® tube. The tube was agitated for a few seconds and then incubated at 56°C for 15 min and at 99°C for 3 min. After centrifugation at 14,000 rpm for 5 min, the supernatant was then collected and used as a template for PCR amplification.

All individual DNA samples per population were first analyzed for an initial PCR using the BEM23 primer pair: Bem23F (5′-CGGAGCTTGCGCCTTAGTC-3′) and Bem23R (5′-CGGCTTTATCATAGCTCTCGT-3′) (De Barro *et al*., 2003). This primer pair differentiates the MED and MEAM1 based on the microsatellite locus of approximately 200 and 400 bp for each species, respectively (Kontsedalov *et al*., 2012; Škaljac *et al*., 2013). Furthermore, the whitefly species samples were confirmed by PCR and sequencing using the primer pair 2195Bt (5′-TGRTTTTTTGGTCATCCRGAAGT-3′) and C012/Bt-sh2 (5′-TTTACTGCACTTTCTGCC-3′), which amplifies a fragment from the mtCOI gene (Mugerwa *et al*., 2018). Subsequently, the mtCOI amplicons were submitted to a restriction fragment length polymorphism analysis (Bosco *et al*., 2006), and 6.5 µl of each PCR (800 bp) was digested with one unit of TaqI at 65°C for 2 h in a final volume of 16.5 µl. The restricted DNA was observed through electrophoresis in a 1.8% agarose gel stained with ethidium bromide.

The mtCOI amplicons of *B. tabaci* were purified (QIAquick Gel Extraction Kit, Qiagen, Hilden, Germany) and sequenced in both directions using the primers 2195Bt and C012/Bt-sh2. The curated dataset of mtCOI (Boykin and De Barro, 2014) was used to confirm the species. Nucleotide sequences of mtCOI were deposited in GenBank, and accession numbers are available in table 1.

Endosymbionts detection

The same DNA extracted from the individual MED whitefly specimens for identification was used for the screening of the six secondary endosymbionts *Hamiltonella, Rickettsia, Wolbachia, Arsenophonus, Cardinium*, and *Fristchea* using genus specific primers targeting the 16S and 23S rDNA genes (Marubayashi *et al*., 2014). Endosymbiont presence was confirmed by sequencing representative individuals. The nucleotide sequences were deposited in GenBank, and accession numbers are available in Supplementary table S1.

Virus identification

At least one plant sample for each locality was analyzed to confirm begomovirus and crinivirus presence. Begomoviruses were detected by DNA extraction (Dellaporta *et al*., 1983) and PCR using the degenerate primer pair PAL1v1978/PAR1c496 (Rojas *et al*., 1993). For crinivirus *Tomato chlorosis virus* (ToCV) detection, total RNA was extracted with a Total RNA Purification Kit® (Norgen, Canada), followed by RT-PCR with the primers HS-11/HS-12 and nested PCR specific for ToCV using the primer pair ToCV-5/ToCV-6 (Dovas *et al*., 2002). Representative PCR products amplified from samples were purified (QIAquick Gel Extraction Kit, Qiagen) and sequenced using the degenerate primers PAL1v1978/PAR1c496 for begomoviruses or the specific primers ToCV-5/ToCV-6 for ToCV. Respective sequences were analyzed and compared with other sequences in GenBank using BLAST tools (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Additionally, the same DNA extracted from individual whiteflies for identification was used to identify begomoviruses, and these identifications were confirmed by sequencing. All nucleotide sequences were deposited in GenBank, and accession numbers are available in table 1.

1.3 RESULTS

Samples collected under greenhouse conditions

A total of 360 individual whiteflies collected from bell peppers (*C. annuum*) were analyzed at 14 localities, and 100% were identified as MED. From cucumber crops (*C. sativus*), 110 individual whiteflies were tested, and only MED was detected. Among the tomato crops (*S. lycopersicum*), 110 individual whiteflies were analyzed, 107 were identified as MED, and three were identified as MEAM1.

MED was also detected infesting weeds associated with bell peppers in greenhouses (table 1, fig. 1) and was identified in Goiás state for the first time in association with ornamental plants collected from a flower shop.

TABLE 1. Information of whitefly *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) species collected in São Paulo and Paraná State, Brazil, between 2017 and 2019, and viruses identification. In bold samples collected in the field.

ID	**Location**	**Coordinates**	**Date**	**Host plant/environment**	**% species**		**Begomovirus**	**Crinivirus**
					MEAM1	**MED**		
1	Bandeirantes/PR	23° 06' 57" S 50°19' 55" W	2018	*Cucumis sativus (GH)*[1]		100	nt[3]	nt
2	Cambara/PR	23° 01' 22" S 50° 09' 04" W	2018	*Cucumis sativus (GH)*		100	nt	nt
3	São Pedro do Turvo/SP	22° 47' 10" S 49° 51' 17" W	2018	*Cucumis sativus (GH)*		100	nt	nt
4	São Pedro do Turvo/SP	22° 48' 14" S 49° 51' 55" W	2018	*Cucumis sativus (GH)*		100	nt	nt
5	Óleo/SP	22° 55' 51" S 49° 26' 5" W	2018	*Cucumis sativus (GH)*		100 (GenBank MK900721)	nt	nt
6	Óleo/SP	22° 55' 49" S 49° 26' 7" W	2018	*Cucumis sativus (GH)*		100 (GenBank MK900722)	nt	nt
7	Pirajuí/SP	22° 0' 39" S 49° 28' 48" W	2018	*Cucumis sativus (GH)*		100 (GenBank MK900723)	nt	nt
8	Bandeirantes/PR	23° 06' 57" S 50°19' 55" W	2019	*Cucumis sativus (GH)*		100	nt	nt
9	Bandeirantes/PR	23° 06' 56" S 50°19' 53" W	2019	*Cucumis sativus (GH)*		100	nt	nt
10	São Pedro do Turvo/SP	22° 47' 10" S 49° 51' 17" W	2019	*Cucumis sativus (GH)*		100	nt	nt
11	São Pedro do Turvo/SP	22° 47' 11" S 49° 51' 15" W	2019	*Cucumis sativus (GH)*		100	nt	nt
12	Óleo/SP	22° 55' 51" S 49° 26' 5" W	2019	*Cucumis sativus (GH)*		100	nt	nt

13	São Miguel Arcanjo/SP	23° 46' 31" S 48° 02' 07" W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/1)	Negative (0/1)
14	Bandeirantes/PR	23° 06' 57" S 50° 19' 55" W	2018	*Solanum lycopersicum (GH)*	10	90	Negative (0/1)	Negative (0/1)
15	Cambara/PR	23° 02' 55" S 50° 07' 57" W	2018	*Solanum lycopersicum (GH)*	20 (GenBank MK900724)	80 (GenBank MK900725)	Negative (0/2)	Negative (0/2)
16	Santa Cruz do Rio Pardo/SP	22° 52' 11" S 49° 35' 03" W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/1)	Negative (0/1)
17	São Pedro do Turvo/SP	22° 48' 13" S 49° 51' 54" W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/4)	ToCV^5 (4/4) (GenBank MK930366)
18	Óleo/SP	22° 55' 51" S 49° 26' 05" W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/2)	Negative (0/2)
19	Óleo/SP	22° 55' 51" S 49° 26' 05" W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/2)	Negative (0/2)
20	Óleo/SP	22° 55' 49" S 49° 26' 07" W	2018	*Solanum lycopersicum (GH)*		100 (GenBank MK900726)	Negative (0/1)	Negative (0/1)
21	Óleo/SP	22° 55' 5" S 49° 26' 09" W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/1)	Negative (0/1)
22	São Miguel Arcanjo/SP	23° 50' 30" S 47° 52' 45" W	2018	*Solanum lycopersicum (GH)*		100 (GenBank MK900727)	Negative (0/1)	Negative (0/1)
23	São Miguel Arcanjo/SP	23° 50' 29" S 47° 52' 42" W	2018	*Solanum lycopersicum (GH)*		100	Negative (0/1)	Negative (0/1)
24*	**Sumaré/SP**	**22° 53' 30" S 47° 15' 50" W**	**2018**	***Solanum lycopersicum (OF)***^{2}	**50**	**50** (GenBank MK900728)	**Negative (0/1)**	**Negative (0/1)**
25	**Monte Mor/SP**	**22° 54' 31" S 47° 21' 31" W**	**2018**	***Solanum lycopersicum (OF)***	**70**	**30**	**Negative (0/1)**	**Negative (0/1)**

26	**Sumaré/SP**	**22° 53' 45" S 47° 15' 41" W**	**2018**	***Solanum lycopersicum (OF)***	**40**	**60**	**Negative (0/1)**	**Negative (0/1)**
27	**Sumaré/SP**	**22° 53' 49" S 47° 15' 43" W**	**2018**	***Solanum lycopersicum (OF)***	**80**	**20**	**Negative (0/1)**	**Negative (0/1)**
28	São Miguel Arcanjo/SP	23° 50' 23" S 47° 53' 55" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
29	São Miguel Arcanjo/SP	23° 50' 21" S 47° 53' 54" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
30	Bragança Paulista/SP	23° 0' 54" S 46° 35' 31" W	2017	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
31	**Elias Fausto/SP**	**23° 04' 50" S 47° 22' 35" W**	**2017**	***Capsicum annuum (OF)***		**100** (GenBank MK900730)	**Negative (0/1)**	**Negative (0/1)**
32	Elias Fausto/SP	23° 06' 16" S 47° 25' 57" W	2017	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
33	Vitoriana/SP	22° 46' 42" S 48° 24' 19" W	2017	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
34	São Miguel Arcanjo/SP	23° 53' 40" S 48° 01' 13" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
35	Itápolis/SP	21° 35' 07" S 48° 47' 08" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
36	Itápolis/SP	21° 33' 30" S 48° 45' 34" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
37	Bauru/SP	22° 14' 40" S 49° 06' 20 "W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
38	São Miguel Arcanjo/SP	23° 50' 33" S 47° 52' 47" W	2018	*Capsicum annuum (GH)*		100 (GenBank MK900731)	Negative (0/1)	Negative (0/1)
39	São Miguel Arcanjo/SP	23° 50' 33" S 47° 52' 43" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)

40	Bandeirantes/PR	23° 06' 57" S 50° 19' 55" W	2018	*Capsicum annuum (GH)*	100 (GenBank MK900732)	Negative (0/1)	Negative (0/1)
41	Cambara/PR	23° 02' 55" S 50° 07' 56" W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
42	Cambara/PR	23° 02' 55" S 50° 07' 57" W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
43	Santa Cruz do Rio Pardo/SP	22° 52' 11" S 49° 35' 03" W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
44	São Pedro do Turvo/SP	22° 47' 10" S 49° 51' 17" W	2018	*Capsicum annuum (GH)*	100 (GenBank MK900733)	Negative (0/1)	Negative (0/1)
45	São Pedro do Turvo/SP	22° 48' 13" S 49° 51' 54" W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
46	São Pedro do Turvo/SP	22° 48' 13" S 49° 51' 54" W	2018	*Capsicum annuum (GH)*	100	ToSRV[4] (1/3) (GenBank MK930367)	Negative (0/1)
47	Bernardino do Campo/SP	22° 56' 32" S 49° 26' 12" W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
48	Bernardino do Campo/SP	22° 56' 32" S 49° 26' 14" W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
49	Óleo/SP	22° 55' 03" S 49° 24' 16" W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
50	Óleo/SP	22° 55' 05" S 49° 24' 19" W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
51	Óleo/SP	22° 55' 51" S 49° 26' 05" W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)
52	Óleo/SP	22° 55' 51" S 49° 26' 05" W	2018	*Capsicum annuum (GH)*	100	ToSRV (1/3) (GenBank MK930368)	Negative (0/1)
53	Óleo/SP	22° 55' 05" S 49° 26' 09" W	2018	*Capsicum annuum (GH)*	100	Negative (0/1)	Negative (0/1)

54	Óleo/SP	22° 55' 06" S 49° 26' 10" W	2018	*Capsicum annuum (GH)*		100	ToSRV (1/2) (GenBank MK930369)	Negative (0/1)
55	Pirajuí/SP	21° 55' 02" S 49° 23' 14" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
56	Pirajuí/SP	21° 55' 02" S 49° 23' 14" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
57	Pirajuí/SP	22° 0' 39" S 49° 28' 48" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
58	Pirajuí/SP	22° 0' 39" S 49° 28' 48" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
59	Pirajuí/SP	22° 0' 40" S 49° 29' 16" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
60	Pirajuí/SP	22° 0' 30" S 49° 29' 12" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
61	Pirajuí/SP	22° 0' 34" S 49° 17' 46" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
62	Pirajuí/SP	22° 0' 19" S 49° 17' 20" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
63	Pirajuí/SP	22° 0' 19" S 49° 17' 20" W	2018	*Capsicum annuum (GH)*		100	Negative (0/1)	Negative (0/1)
64	Pirajuí/SP	22° 0' 19" S 49° 17' 20" W	2018	*Capsicum annuum (GH)*		100 (GenBank MK900734)	Negative (0/1)	Negative (0/1)
65	**Itápolis/SP**	**21° 36' 10" S 48° 45' 16" W**	**2018**	***Solanum melongena (OF)***	**40**	**60**	**Negative (0/1)**	**Negative (0/1)**
66	**Elias Fausto/SP**	**23° 04' 50" S 47° 22' 35" W**	**2017**	***Solanum melongena (OF)***		**100** (GenBank MK900735)	**Negative (0/1)**	**Negative (0/1)**
67	**Pirajuí/SP**	**22° 0' 39" S 49° 28' 48" W**	**2018**	***Solanum melongena (OF)***		**100**	**Negative (0/1)**	**Negative (0/1)**

68	Pirajuí/SP	22° 0' 19" S 49° 17' 20" W	2018	*Amaranthus sp. (GH)*	100	nt	nt
69	Pirajuí/SP	22° 0' 34" S 49° 17' 46" W	2018	*Bidens pilosa (GH)*	100	nt	nt
70	Pirajuí/SP	21° 55' 02" S 49° 23' 14" W	2018	*Conyza* sp. *(GH)*	100	nt	nt
71	Óleo/SP	22° 55' 05" S 49° 24' 19" W	2018	*Artemisia absinthium (GH)*	100	nt	nt
72	Óleo/SP	22° 55' 05" S 49° 24' 19" W	2018	*Emilia fosbergii (GH)*	100	nt	nt
73	Óleo/SP	22° 55' 03" S 49° 24' 16" W	2018	*Chamaesyce* sp. *(GH)*	100	nt	nt
74	Mogi Mirim/SP	22° 25' 04" S 46° 53' 42" W	2017	Weed *(GH)*	100	nt	nt

---

[1] *GH* (Greenhouse) and [2]*OF* (Open Field); [3] not tested; [4]*Tomato severe rugose virus* (ToSRV); [5]*Tomato chlorosis virus* (ToCV);

*samples highlighted in bold belong to *OF* samples for a better visualization.

Figure 1. Sampling locations for *Bemisia tabaci* Mediterranean (MED) species in São Paulo and Paraná States, Brazil. These points represent places where MED was found in (a) bell pepper, (b) tomato, and (c) cucumber crops.

Samples collected under open field conditions

From 40 individuals collected on tomatoes, 16 were identified as MED, and 24 as MEAM1. On eggplants (*S. melongena*), 40 specimens were analyzed and 31 were identified as MED and nine as MEAM1. On bell peppers, ten specimens were analyzed and 100% were identified as MED.

Virus identification

The crinivirus ToCV was detected on tomatoes (sampling site 12), and the begomovirus *Tomato severe rugose virus* (ToSRV) was found infecting bell pepper (sampling sites 41, 47, and 49) (table 1). In addition, ToSRV was also detected by PCR in MED specimens. Both ToCV and ToSRV were found in areas where only MED was detected.

Endosymbionts detection

The endosymbiont analysis revealed that MED harbors *Hamiltonella, Rickettsia, Wolbachia*, and *Arsenophonus*, with a predominance of *Hamiltonella* in most of the individuals sampled (Supplementary table S1; fig. 2).

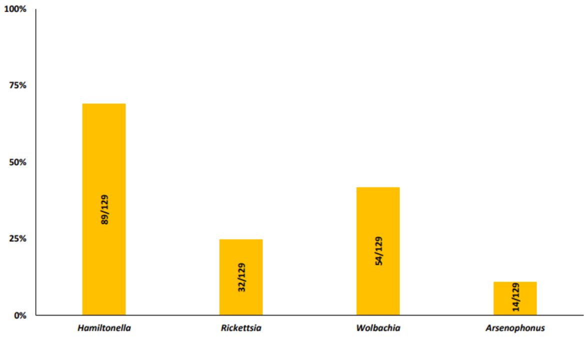


Figure 2. Individual infections by secondary endosymbionts found harbored *Bemisia tabaci* Mediterranean (MED) populations from São Paulo and Paraná States, Brazil. Numbers inside the columns represent the individual positive for the respective endosymbiont by total individuals analyzed. H: *Hamiltonella*, R: *Rickettsia*, W: *Wolbachia*, and A: *Arsenophonus*.

1.4 DISCUSSION

Our results showed that outbreaks of whiteflies in vegetable crops in two eastern states in Brazil (São Paulo and Paraná) associated with the MED species. High populations of MED infesting bell peppers, cucumbers, and eggplants not only under greenhouses, but also under open field conditions lead to the appearance of sooty mold growth (fig. 3). Difficult to manage the insects was also a common complaint on the visited areas. Moreover, ToCV-infected tomatoes and ToSRV-infected bell pepper plants were detected in greenhouses, where only MED was identified, indicating that MED may be contributing to virus transmission on these vegetables.

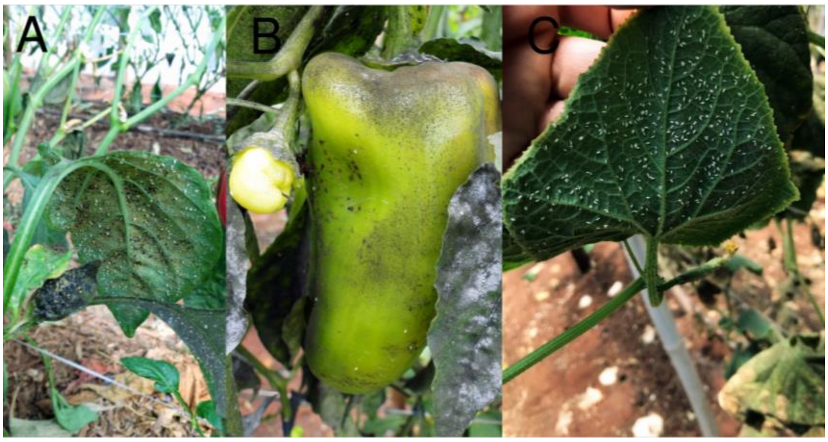


Figure 3. Outbreaks of *Bemisia tabaci* Mediterranean (MED) populations on bell pepper and cucumber crops. (a) Adults and nymphs of MED infesting bell pepper plants and causing the sooty mold (b). Adults of MED on cucumber leaves (c).

Bemisia tabaci MED was first reported in Brazil in Rio Grande do Sul State (Barbosa *et al*., 2015). After 1 year, a new MED introduction was reported in São Paulo and Paraná States (Moraes *et al*., 2017) and more recently in Minas Gerais and Santa Catarina States associated to ornamental plants (Moraes *et al*., 2018). In this study, we also detected MED on ornamental plants collected in the Midwest region (Goiás state) of Brazil, where the extensive agriculture is practiced, including vegetable and field crops.

The MED species is often found infesting greenhouses in two eastern states in Brazil. Studies have shown that MED is more adapted to greenhouse conditions than MEAM1. Kontsedalov *et al*. (2012) reported that MED predominated over MEAM1 under nethouse and greenhouse conditions, not only when insecticides spraying were used but also in the absence of insecticides application. It likely occurs because MED is more resistant to high temperatures than MEAM1. A study from Israel has shown that the mortality of MEAM1 is much higher at 37 and 40°C than that of MED (Mahadav *et al*., 2009). Another study from China also showed that MEAM1 has a higher mortality than MED under high-temperature conditions (Xiao *et al*., 2016). In general, the temperature between greenhouses and open field production is different, being higher and lower, respectively. Thus, as MED is more tolerant to high temperature, greenhouses can be a great environment to increase the population of this species. However, in this study, we have also found MED under open field conditions in mix infestation with MEAM1 on tomatoes and eggplants.

MED is also known to develop resistance much faster than other whitefly species, such as pyriproxyfen (Horowitz *et al*., 2003, 2005), acetamiprid (Horowitz and Kontsedalov, 2004; Horowitz and Ishaaya, 2014), imidacloprid (Karunker *et al*., 2008), thiamethoxam (Horowitz and Kontsedalov, 2004), and cyantraniliprole (Yao *et al*., 2017). In Israel, the dynamics of the whitefly populations on cotton were studied for several years in terms of management with insecticide and the predominant species. It was observed that in areas where MEAM1 was the predominant species, MED displaced MEAM1 with high insecticide use. However, after spraying cessation, MEAM1 returned as the predominant species, indicating that the insecticide directly influenced the prevalence of the species (Horowitz and Ishaaya, 2014). Similarly, it was verified in China that on eggplant and tomatoes on which MEAM1 was the predominant species, MED became the main species with high insecticide use (Sun *et al*., 2013). This lower susceptibility to several insecticides is often regarded as a main cause of the faster establishment and dominance of MED in several regions worldwide (Yao *et al*., 2017).

The introduction of an exotic pest into a country is always of great concern for its agriculture, mainly if it is a vector of viruses. The great concern started in the 1990s, when the exotic MEAM1 was first detected in Brazil. Ever since, several losses caused by this pest have been reported in practically all regions of Brazil (Lima, 2000), and outbreaks of begomoviruses infecting Solanaceae have occurred (Faria *et al*., 2000). Some years later, the crinivirus ToCV was first reported in Brazil associated with MEAM1 (Barbosa *et al*., 2008) and nowadays the begomovirus ToSRV and the crinivirus ToCV are the most important viruses transmitted by *B. tabaci* cryptic species infecting tomatoes in Brazil (Inoue-Nagata *et al*., 2016). We also verified in this work that ToCV-infected tomato plants and ToSRV-infected bell pepper plants were associated with the presence of MED specimens. The interactions between ToCV and MED in tomato plants are well known because MED fecundity and developmental time are increased by the presence of this virus, indicating that the virus is beneficial to MED (Orfanidou *et al*., 2016; Shi *et al*., 2018). The ability of *B. tabaci* MED to efficiently transmit ToSRV, a native Brazilian begomovirus and the main species infecting tomato in southwest Brazil, was recently demonstrated (Bello *et al*., 2019a). It is expected that changes in the epidemiology of whitefly-transmitted viruses may occur and this has been recently observed for cucumber plants found naturally infected with ToCV in association with MED specimens (Bello *et al*., 2019b). Cucumber plants have never been reported before as the host of ToCV, but what we could observe is that MED specimens are well adapted to cucumbers cultivated under greenhouse conditions in Brazil and ToCV in this crop can become an important problem to this host.

Bemisia tabaci MED has also been shown to be an excellent vector of the most important begomovirus in Europe and Asia: *Tomato yellow leaf curl virus* (TYLCV) (Ning *et al*., 2015; Czosnek *et al*., 2017), which has not yet been reported in Brazil but was detected in the neighboring country of Venezuela (Zambrano *et al*., 2007). In China, TYLCV became an emergent virus after MED introduction (Pan *et al.*, 2012; Ning *et al*., 2015). The transmission of TYLCV by MED is more efficient than that by

MEAM1 (Ning *et al*., 2015), and the fecundity of MED increases in the presence of this virus, while the developmental time decreases (Chen *et al.*, 2013; Fang *et al*., 2013). In this way, all results together point to the need for TYLCV monitoring in Brazil.

In Brazil, the variability in the secondary endosymbionts found in MED is quite large and may be explained by the recent and different introductions of this species into the country (Moraes *et al*., 2018). MED populations typically harbor *Hamiltonella, Rickettsia, Wolbachia, Cardinium*, and *Arsenophonus* (Gueguen *et al*., 2010; Czosnek and Ghanim, 2016). Our work has also shown that MED collected in this study harbors *Hamiltonella, Rickettsia, Wolbachia*, and *Arsenophonus* (fig. 2) and that *Hamiltonella* is the predominant secondary endosymbiont. The influence of endosymbionts on virus transmission is well described for *B. tabaci* MED, in which the absence or low frequency of *Hamiltonella* implies low transmission efficiency of TYLCV (Gottlieb *et al*., 2010). Additionally, individuals that harbor this bacterium show high benefits in reproduction factors that increase the populations of B. tabaci MEAM1 species (Kliot *et al*., 2019). In addition, populations of MED harboring high frequency of *Hamiltonella* proved to be better vectors of ToCV and ToSRV (Bello *et al*., 2019a).

In conclusion, our results suggest that in São Paulo and Paraná States, Brazil, MED is now found on vegetable crops both in greenhouses and under open field conditions, and ToCV-infected and ToSRV-infected plants were associated to this species. These results reinforce that MED is a real concern for Brazilian agriculture, because it has a great performance and can displace MEAM1 in some important crops (Watanabe *et al*., 2019), is a good vector of important viruses found in Brazil, and is less susceptible to insecticides than MEAM1 (Horowitz and Kontsedalov, 2004; Kontsedalov *et al*., 2012; Yao *et al*., 2017). Then, the management of *B. tabaci* should be performed according to which whitefly species is present in the area, in order to ensure a better control as well as to limit the spreading of MED species. Thus, the monitoring and identification of the *B. tabaci* cryptic species are essential for helping to choose the greatest management strategy, including not only the chemical control, but also other integrated pests, as biological control.

Acknowledgements. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001; FAPESP (processes number 2017/21588-7, 2017/50222, and 2018/18274-3) and

CNPq (405684/2018-5 and 303411/2018-0). RKS, RCOFB, and MAP received CNPq fellowships.

REFERENCES

Barbosa JC, Teixeira APM, Moreira AG, Camargo LEA, Bergamin Filho A, Kitajima EW and Rezende JAM (2008) First report of *Tomato chlorosis virus* infecting tomato crops in Brazil. *Plant Disease* **92**, 1. doi: https://doi.org/10.1094/PDIS-92-12-1709C

Barbosa LF, Yuki VA, Marubayashi JM, De Marchi BR, Perini FL, Pavan MA, Barros DR, Ghanim M, Moriones E, Navas-Castillo J and Krause-Sakate R (2015) First report of *Bemisia tabaci* Mediterranean (Q biotype) species in Brazil. *Pest Management Science* **71**, 501–504.

Bello VH, Watanabe LFM, Santos BR, Marubayashi JM, Yuki VA, De Marchi BR, Pavan MA and Krause-sakate R (2019a) Evidence for increased efficiency of virus transmission by populations of Mediterranean species of *Bemisia tabaci* with high *Hamiltonella* prevalence. *Phytoparasitica* **47**, 293–300. doi: https://doi.org/10.1007/s12600-019-00729-y

Bello VH, Gorayeb E, Watanabe LFM, De Marchi BR, Ribeiro-Junior M, Vicentin E, Barreto F and Krause-sakate R (2019b) First report of *Tomato Chlororis Virus* infecting cucumber in Brazil. *Plant Disease*. doi: https://doi.org/10.1094/PDIS-07-19-1490-PDN

Bosco D, Loria A, Sartor C and Cenis JL (2006) PCR-RFLP identification of *Bemisia tabaci* biotypes in the Mediterranean Basin. *Phytoparasitica* **34**, 243–251.

Boykin LM and De Barro PJ (2014) A practical guide to identifying members of the *Bemisia tabaci* species complex: and other morphologically identical species. *Frontiers in Ecology and Evolution* **2**, 45.

Chen G, Pan H, Xie W, Wang S, Wu Q, Fang Y, Shi X and Zhang Y (2013) Virus infection of a weed increases vector attraction to and vector fitness on the weed. *Scientific Reports* **3**, 2253.

Czosnek H and Ghanim M (2016) Management of Insect Pests to Agriculture: Lessons Learned from Deciphering their Genome, Transcriptome and Proteome. Switzerland: Springer International Publishing, pp. 1–290. https://doi.org/10.1007/978-3-319-24049-7

Czosnek H, Hariton-Shalev A, Sobol I, Gorovits R and Ghanim M (2017) The incredible journey of Begomoviruses in their whitefly vector. *Viruses* **9**, 273–292. doi: https://doi.org/10.3390/v9100273

De Barro PJ, Scott KD, Graham GC, Lange CL and Schutze MK (2003) Isolation and characterization of microsatellite loci in *Bemisia tabaci*. *Molecular Ecology Notes* **3**, 40–43.

De Barro PJ, Liu S-S, Boykin LM and Dinsdale AB (2011) Bemisia tabaci : a statement of species status. Annual Review of Entomology 56, 1–19. Dellaporta SL, Wood J and Hicks JB (1983) A plant DNA minipreparation: version II. *Plant Molecular Biology Reporter* **1**, 19–21.

Dinsdale A, Cook L, Riginos C, Buckley YM and De Barro P (2010) Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. *Annals of the Entomological Society of America* **103**, 196–208.

Dovas CI, Katis NI and Avgelis AD (2002) Multiplex detection of criniviruses associated with epidemics of a yellowing disease of tomato in Greece. *Plant Disease* **86**, 1345–1349.

Fang Y, Jiao X, Xie W, Wang S, Wu Q, Shi X, Chen G, Su Q, Yang X, Pan H and Zhang Y (2013) *Tomato yellow leaf curl virus* alters the host preferences of its vector Bemisia tabaci. *Scientific Reports* **3**, 1–5. doi: https://doi.org/10.1038/srep02876

Faria JC, Bezerra IC, Zerbini FM, Ribeiro SG and Lima MF (2000) Situação atual das geminiviroses no Brasil. Embrapa Arroz e Feijão-Artigo em periódico indexado (ALICE).

Gottlieb Y, Zchori-Fein E, Mozes-Daube N, Kontsedalov S, Skaljac M, Brumin M, Sobol I, Czosnek H, Vavre F, Fleury F and Ghanim M (2010) The transmission efficiency of *Tomato Yellow Leaf Curl Virus* by the whitefly *Bemisia tabaci* is correlated with the presence of a specific symbiotic bacterium species. *Journal of Virology* **84**, 9310–9317.

Gueguen G, Vavre F, Gnankine O, Peterschmitt M, Charif D, Chiel E, Gottlieb Y, Ghanim M, Zchori-Fein E and Fleury F (2010) Endosymbiont metacommunities, mtDNA diversity and the evolution of the *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex. *Molecular Ecology* **19**, 4365–4376.

Horowitz AR and Ishaaya I (2014) Dynamics of biotypes B and Q of the whitefly *Bemisia tabaci* and its impact on insecticide resistance. *Pest Management Science* **70**, 1568–1572.

Horowitz AR and Kontsedalov S (2004) Dynamics of resistance to the neonicotinoids acetamiprid and thiamethoxam in *Bemisia tabaci* (Homoptera : Aleyrodidae). *Journal of Economic Entomology* **97**, 2051–2056.

Horowitz AR, Denholm I, Gorman K, Cenis JL, Kontsedalov S and Ishaaya I (2003) Biotype Q of *Bemisia tabaci* identified in Israel. *Phytoparasitica* 31, 94–98.

Horowitz AR, Kontsedalov S, Khasdan V and Ishaaya I (2005) Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. Archives of Insect *Biochemistry and Physiology* **58**, 216–225.

Inoue-Nagata A, Lima MF and Gilbertison RL (2016) A review of geminivirus (begomovirus) diseases in vegetables and other crops in Brazil: current status and approaches for management. *Horticultura Brasileira* **34**, 8–18.

Kanakala S and Ghanim M (2015) Advances in the Genomics of the Whitefly *Bemisia tabaci*: An Insect Pest and a Virus Vector. Switzerland: Springer International Publishing, pp. 19–40. doi: https://doi.org/10.1007/978-3-319-24235-4_2

Kanakala S and Ghanim M (2019) Global genetic diversity and geographical distribution of *Bemisia tabaci* and its bacterial endosymbionts. *PLoS ONE* **14**, e0213946.

Karunker I, Benting J, Lueke B, Ponge T, Nauen R, Roditakis E, Vontas J, Gorman K, Denholm I and Morin S (2008) Over-expression of cytochrome P450 CYP6CM1 is associated with high resistance to imidacloprid in the B and Q biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Insect Biochemistry and Molecular Biology* **38**, 634–644.

Kliot A, Kontsealadov S, Lebedev G, Czonesk H and Ghanim M (2019) Combined infection with *Tomato Yellow Leaf Curl Virus* and *Rickettsia* influences fecundity, attraction to infected plants and expression of immunity-related genes in the whitefly *Bemisia tabaci*. *Journal of General Virology* **100**, 721–731. doi: https://doi.org/10.1099/jgv.0.001233

Kontsedalov S, Abu-Moch F, Lebedev G, Czosnek H, Horowitz AR and Ghanim M (2012) *Bemisia tabaci* biotype dynamics and resistance to insecticides in Israel during the years 2008–2010. *Journal of Integrative Agriculture* **11**, 312–320.

Lima LHC, Návia D, Inglis PW and Oliveira MRV (2000) Survey of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotypes in Brazil using RAPD markers. *Genetics and Molecular Biology* 23, 781–785. doi: https://doi.org/10.1590/S1415475720000004000012

Liu S-S, De Barro PJ, Xu J, Luan J-B, Zang L-S, Ruan Y-M and Wan F-H (2007) Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science* **318**, 1769–1772.

Lourenção AL and Nagai H (1994) Outbreaks of *Bemisia tabaci* in the São Paulo State, Brazil. *Bragantia* **53**, 53–59.

Luo C, Jones CM, Devine G, Zhang F, Denholm I and Gorman K (2010) Insecticide resistance in Bemisia tabaci biotype Q (Hemiptera: Aleyrodidae) from China. *Crop Protection* **29**, 429–434.

Mahadav A, Kontsedalov S, Czonesk H and Ghanim M (2009) Thermotolerance and gene expression following heat stress in the whitefly *Bemisia tabaci* B and Q biotypes. Insect *Biochemitry and Molecular Biology* **39**, 668–676. doi: https://doi.org/10.1016/j.ibmb.2009.08.002

Marubayashi JM, Kliot A, Yuki VA, Rezende JAM, Krause-Sakate R, Pavan MA and Ghanim M (2014) Diversity and localization of bacterial endosymbionts from whitefly species collected in Brazil. *PLoS ONE* **9**, e108363. doi: https://doi.org/10.1371/journal.pone.0108363

Moraes LA, Marubayashi JM, Yuki VA, Ghanim M, Bello VH, De Marchi BR, Barbosa LF, Boykin L, Krause-Sakate R and Agenor Pavan M (2017) New invasion of *Bemisia tabac*i Mediterranean species in Brazil associated to ornamental plants. *Phytoparasitica* **45**, 517–525. doi: https://doi.org/10.1007/s12600-017-0607-9

Moraes LA, Muller C, Bueno RCOF, Santos A, Bello VH, De Marchi BR, Watanabe LFM, Marubayashi JM, Santos BR, Yuki VA, Takada HM, de Barros DR, Neves CG, da Silva FN, Gonçalves MJ, Ghanim M, Boykin L, Pavan MA and Krause-Sakate R (2018) Distribution and phylogenetics of whiteflies and their endosymbiont relationships after the Mediterranean species invasion in Brazil. *Scientific Reports* **8**, 14589. doi: https://doi.org/10.1038/s41598-018-32913-1

Mugerwa H, Seal S, Wang H, Patel MV, Kabaalu R, Omongo CA, Alicai T, Tairo F, Ndunguru J, Sseruwagi P and Colvin J (2018) African ancestry of New World, Bemisia tabaci-whitefly species. *Scientific Reports* **8**, 1–11. doi: https://doi.org/10.1038/s41598-018-20956-3

Muñiz M (2000) Host suitability of two biotypes of *Bemisia tabaci* on some common weeds. *Entomologia Experimentalis et Applicata* **95**, 63–70.

Ning W, Shi X, Liu B, Pan H, Wei W, Zeng Y, Sun X, Xie W, Wang S, Wu Q, Cheng J, Peng Z and Zhang Y (2015) Transmission of *tomato yellow leaf curl virus* by *Bemisia tabaci* as affected by whitefly sex and biotype. *Scientific Reports* **5,** 10744.

Orfanidou CG, Pappi PG, Efthimiou KE, Katis NI and Maliogka VI (2016) Transmission of *Tomato Chlorosis Virus* (ToCV) by *Bemisia tabaci* biotype Q and evaluation of four weed species as viral sources. *Plant Disease* **100**, 2043–2049.

Pan H, Chu D, Yan W, Su Q, Liu B, Wang S and Wu Q (2012) Rapid spread of *Tomato Yellow Leaf Curl Virus* in China is aided differentially by two invasive whiteflies. *PLoS ONE* **7**, 1–9. https://doi.org/10.1371/journal.pone.0034817

Polston JE, De Barro P and Boykin LM (2014) Transmission specificities of plant viruses with the newly identified species of the *Bemisia tabaci* species complex. *Pest Management Science* **70**, 1547–1552.

Ribeiro SG, De Ávila AC, Bezerra IC, Fernandes JJ, Faria JC, Lima MF, Gilbertson RL, Maciel-Zambolim E and Zerbini FM (1998) Widespread occurrence of tomato geminiviruses in Brazil, associated with the new biotype of the whitefly vector. *Plant Disease* **82**, 830.

Rojas MR, Gilbertson RL, Russell DR and Maxwell DP (1993) Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. *Plant Disease* **77**, 340–347. doi: https://doi.org/10.1094/PD-77-0340

Shi X, Tang X, Zhang X, Zhang D, Li F, Yan F, Zhang Y, Zhou X and Liu Y (2018) Transmission efficiency, preference and behavior of *Bemisia tabaci* MEAM1 and MED under the influence of *Tomato Chlorosis Virus*. *Frontiers in Plant Science* **8**, 1–9.

Škaljac M, Anić K, Hrnčić S, Radonjić S, Perović T and Ghanim M (2013) Diversity and localization of bacterial symbionts in three whitefly species (Hemiptera: Aleyrodidae) from the east coast of the Adriatic Sea. *Bulletin of Entomological Research* **103**, 48–59.

Sun D-B, Liu Y-Q, Qin L, Xu J, Li F-F and Liu SS (2013) Competitive displacement between two invasive whiteflies: insecticide application and host plant effects. Bulletin of Entomological Research 103, 344–353.

Watanabe LFM, Bello VH, De Marchi BR, Silva F, Machado LF, Sartori MMP, Pavan MA and Krause-Sakate R (2019) Performance and competitive displacement of *Bemisia tabaci* MEAM1 and MED cryptic species on different host plants. *Crop Protection* **124**, 104860. doi: https://doi.org/10.1016/j.cropro.2019.104860

Xiao N, Pan LL, Zhang CR, Sham HW and Liu SS (2016) Differential tolerance capacity to unfavourable low and high temperatures between two invasive whitefly species. *Scientific Reports* **6**, 24306.

Yao FL, Zheng Y, Huang XY, Ding XL, Zhao JW, Desneux N, He YX and Weng QY (2017) Dynamics of *Bemisia tabaci* biotypes and insecticide resistance in Fujian province in China during 2005-2014. *Scientific Reports* **7**, 1–12.

Zambrano K, Carballo O, Geraud F, Chirinos D, Fernández C and Marys E (2007) First report of *Tomato Yellow Leaf Curl Virus* in Venezuela. *Plant Disease* **91**, 768.

Supplementary table 1. Endosymbionts composition of whitefly *Bemisia tabaci* Mediterranean (MED) species collected in São Paulo and Paraná State, Brazil, between 2017 and 2019. Middle East-Asia Minor 1 (MEAM1) species was not tested for endosymbionts.

ID	**Location**	**Host plant**	**% species**		**Endosymbionts**					
			MEAM1^a	**MED**	**H**	**R**	**W**	**C**	**F**	**A**
1	Bandeirantes/PR	*Cucumis sativus (GH)*		100	9/10 (GenBank MK967465)	0/10	0/10	0/10	0/10	0/10
2	Cambara/PR	*Cucumis sativus (GH)*		100	8/10	0/10	0/10	0/10	0/10	0/10
3	São Pedro do Turvo/SP	*Cucumis sativus (GH)*		100	7/10	2/10	10/10	0/10	0/10	0/10
4	São Pedro do Turvo/SP	*Cucumis sativus (GH)*		100	-*	-	-	-	-	-
5	Óleo/SP	*Cucumis sativus (GH)*		100	-	-	-	-	-	-
6	Óleo/SP	*Cucumis sativus (GH)*		100	-	-	-	-	-	-
7	Pirajuí/SP	*Cucumis sativus (GH)*		100	-	-	-	-	-	-
8	Bandeirantes/PR	*Cucumis sativus (GH)*		100	-	-	-	-	-	-
9	Bandeirantes/PR	*Cucumis sativus (GH)*		100	-	-	-	-	-	-
10	São Pedro do Turvo/SP	*Cucumis sativus (GH)*		100	-	-	-	-	-	-
11	São Pedro do Turvo/SP	*Cucumis sativus (GH)*		100	-	-	-	-	-	-
12	Óleo/SP	*Cucumis sativus (GH)*		100						
13	São Miguel Arcanjo/SP	*Solanum lycopersicum (GH)*		100	8/10	0/10	10/10	0/10	0/10	0/10
14	Bandeirantes/PR	*Solanum lycopersicum (GH)*	10	90	-	-	-	-	-	-
15	Cambara/PR	*Solanum lycopersicum (GH)*	20	80	8/8	8/8	0/8	0/8	0/8	0/8
16	Santa Cruz do Rio Pardo/SP	*Solanum lycopersicum (GH)*		100	-	-	-	-	-	-
17	São Pedro do Turvo/SP	*Solanum lycopersicum (GH)*	10	90	-	-	-	-	-	-
18	Óleo/SP	*Solanum lycopersicum (GH)*		100	-	-	-	-	-	-

19	Óleo/SP	*Solanum lycopersicum (GH)*		100	-	-	-	-	-	-
20	Óleo/SP	*Solanum lycopersicum (GH)*		100	10/10 (GenBank MK967466)	0/10	0/10	0/10	0/10	0/10
21	Óleo/SP	*Solanum lycopersicum (GH)*		100	-	-	-	-	-	-
22	São Miguel Arcanjo/SP	*Solanum lycopersicum (GH)*		100	-	-	-	-	-	-
23	São Miguel Arcanjo/SP	*Solanum lycopersicum (GH)*		100	-	-	-	-	-	-
24	Sumaré/SP	*Solanum lycopersicum (OF)*	50	50	0/5	5/5 (GenBank MK967462)	2/5	0/5	0/5	1/5 (GenBank MK967802)
25	Monte Mor/SP	*Solanum lycopersicum (OF)*	70	30	0/3	3/3	2/3	0/3	0/3	1/3
26	Sumaré/SP	*Solanum lycopersicum (OF)*	40	60	-	-	-	-	-	-
27	Sumaré/SP	*Solanum lycopersicum (OF)*	80	20	-	-	-	-	-	-
28	São Miguel Arcanjo/SP	*Capsicum annuum (GH)*		100	-	-	-	-	-	-
29	São Miguel Arcanjo/SP	*Capsicum annuum (GH)*		100	-	-	-	-	-	-
30	Bragança Paulista/SP	*Capsicum annuum (GH)*		*100*	-	-	-	-	-	-
31	Elias Fausto/SP	*Capsicum annuum (OF)*		100	2/10	8/10	10/10	0/10	0/10	1/10
32	Elias Fausto/SP	*Capsicum annuum (GH)*		100	-	-	-	-	-	-
33	Vitoriana/SP	*Capsicum annuum (GH)*		100	-	-	-	-	-	-
34	São Miguel Arcanjo/SP	*Capsicum annuum (GH)*		100	-	-	-	-	-	-
35	Itápolis/SP	*Capsicum annuum (GH)*		100	-	-	-	-	-	-
36	Itápolis/SP	*Capsicum annuum (GH)*		100	-	-	-	-	-	-
37	Bauru/SP	*Capsicum annuum (GH)*		100	-	-	-	-	-	-

38	São Miguel Arcanjo/SP	*Capsicum annuum (GH)*	100	8/10	3/10 (GenBank MK967463)	0/10	0/10	0/10	0/10
39	São Miguel Arcanjo/SP	*Capsicum annuum (GH)*	100						
40	Bandeirantes/PR	*Capsicum annuum (GH)*	100	8/10	0/10	0/10	0/10	0/10	0/10
41	Cambara/PR	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
42	Cambara/PR	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
43	Santa Cruz do Rio Pardo/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
44	São Pedro do Turvo/SP	*Capsicum annuum (GH)*	100	8/10 (GenBank MK967467)	0/10	10/10 (GenBank MK967968)	0/10	0/10	0/10
45	São Pedro do Turvo/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
46	São Pedro do Turvo/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
47	Bernardino do Campo/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
48	Bernardino do Campo/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
49	Óleo/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
50	Óleo/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
51	Óleo/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
52	Óleo/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
53	Óleo/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
54	Óleo/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
55	Pirajuí/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
56	Pirajuí/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
57	Pirajuí/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
58	Pirajuí/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
59	Pirajuí/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
60	Pirajuí/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
61	Pirajuí/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-
62	Pirajuí/SP	*Capsicum annuum (GH)*	100	-	-	-	-	-	-

63	Pirajuí/SP	*Capsicum annuum (GH)*		100	9/10 (GenBank MK967468)	0/10	0/10	0/10	0/10	0/10
64	Pirajuí/SP	*Capsicum annuum (GH)*		100	-	-	-	-	-	-
65	Itápolis/SP	*Solanum melongena (OF)*	40	60	-	-	-	-	-	-
66	Elias Fausto/SP	*Solanum melongena (OF)*		100	0/10	10/10 (GenBank MK967464)	10/10 (GenBank MK967969)	0/10	0/10	10/10
67	Pirajuí/SP	*Solanum melongena (OF)*		100	-	-	-	-	-	-
68	Pirajuí/SP	*Amaranthus sp. (GH)*		100	-	-	-	-	-	-
69	Pirajuí/SP	*Bidens pilosa (GH)*		100	-	-	-	-	-	-
70	Pirajuí/SP	*Conyza* sp. *(GH)*		100	-	-	-	-	-	-
71	Óleo/SP	*Artemisia absinthium (GH)*		100	-	-	-	-	-	-
72	Óleo/SP	*Emilia fosbergii (GH)*		100	-	-	-	-	-	-
73	Óleo/SP	*Chamaesyce* sp. *(GH)*		100	-	-	-	-	-	-
74	Mogi Mirim/SP	Wheed *(GH)*		100	-	-	-	-	-	-

*Populations of whitefly not tested for endosymbionts; [a] Insects of MEAM1 species were not tested for endosymbionts; (H) *Hamiltonella*, (R) *Rickettsia*, (W) *Wolbachia,* (C) *Cardinium,* (F) *Fristchea* and (A) *Arsenophonus*.

CHAPTER 2

FIRST REPORT OF *Tomato Chlorosis Virus* INFECTING CUCUMBER IN BRAZIL

V. H. Bello,† E. S. Gorayeb, L. F. M. Watanabe, B. R. De Marchi, M. R. Ribeiro-Junior, E. Vicentin, F. B. da Silva, and R. Krause-Sakate. Faculdade de Ciências Agronômicas, UNESP, Botucatu, SP, Brazil ˆ

†Corresponding author: V. H. Bello; E-mail Address: vhbello@hotmail.com;

Available at: https://doi.org/10.1094/PDIS-07-19-1490-PDN

Funding: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de N´ıvel Superior – Brasil (CAPES), Finance Code 001; Fundação de Amparo à Pesquisa do Estado de São Paulo ` (processes number 2017/21588-7 and 2018/18274-3); and Conselho Nacional de Desenvolvimento Científico e Tecnológico (405684/2018-5 ´ and 303411/2018-0). Plant Dis. 0:1, 2019; published online as https:// doi.org/10.1094/PDIS-07-19-1490-PDN. Accepted for publication 17 September 2019.

DISEASE NOTE

Cucumber plants (Cucumis sativus L.) exhibiting chlorosis and mottling symptoms on young leaves were found in a commercial greenhouse, in the county of Oleo, State of São Paulo, Brazil, in February 2019. The crop was highly infested by whiteflies. To identify the whitefly species in the greenhouse, total DNA was extracted from 10 whiteflies according to the Chelex protocol (Walsh et al. 1991). The DNA samples were subjected to initial PCR amplification of a mitochondrial cytochrome oxidase subunit 1 fragment using the primer pair 2196Bt and C012/Bt-sh2 (Mugerwa et al. 2018), followed by amplicon restriction fragment length polymorphism (RFLP) analysis of all samples and amplicon Sanger sequencing of one sample. The whiteflies were identified as *Bemisia tabaci* Mediterranean species (MED, also known as Q biotype) based on the RFLP analysis and BLATn search of the amplicon sequence (Gen Bank accession no. MN177614). To identify the agent that might have caused the disease, total RNA was extracted from five symptomatic leaves and 15 asymptomatic fruits using the PureLink Viral RNA/DNA Kit (Invitrogen). The RNA was pooled and used for construction of a cDNA library using the Complete ScriptSeq Kit (Epicenter, Illumina) and transcriptome sequencing with the Illumina HiSeq2500 platform (Roossinck et al. 2015). A total of 33,774,040 raw reads (average length 50 bp) were generated by highthroughput sequencing (HTS). The raw reads were trimmed and de novo assembled using CLC Genomics Workbench software version 9.0.3. The 1,441 obtained contigs (average length 350 bp) were subjected to a BLASTn using Geneious software version 9.1.5. The contigs then were blasted against the NCBI virus genome database. This analysis revealed the complete nucleotide sequence of two contigs of 8,573 bp (GenBank accession no. MN172419) and 8,223 bp (GenBank accession no. MN172420) in length, showing 99.8 and 99.6% sequence identity with tomato chlorosis virus (ToCV) RNA1 (JQ952600) and RNA2 (JQ952601), respectively. No other viruses were detected. Subsequently, individual RNA samples were tested for ToCV. Reverse transcription PCR (RT-PCR) was performed with a newly designed primer pair, p22-7537 (5’- ATAGGTACAATTTACCACCG-3’)/p22-8371-R (5’-GAATGTCAACAAAACACCTACT -3’), which was anticipated to result in an amplicon of 834 bp from RNA1 of ToCV. One leaf sample and three fruits tested positive by the RT-PCR assay. In order to validate the results, the amplicon from one sample was Sanger sequenced. The amplicon (GenBank accession no. MN164488) showed 100% nucleotide sequence identity with the HTSderived sequence and 99.6% with a tomato ToCV isolate (MF415521) from Brazil. To fulfill Koch’s postulates, adults of *B. tabaci* MED were transferred to ToCV-infected cucumber leaves for 72 h for virus acquisition, followed by inoculation access (50 *B. tabaci* per plant) to 10 tomato (cv. Santa Clara) and 10 cucumber (cv. Aodai) plants. Forty-five days postinoculation, ToCV was detected by RT-PCR in one tomato and three cucumber plants. The tomato plant infected with the ToCV-cucumber isolate developed typical ToCV symptoms, whereas the cucumber plants developed chlorosis and mottling in young leaves. ToCV is one of the most common whitefly-transmitted viruses of solanaceous plants in Brazil (Macedo et al. 2019), and its impact depends on vector prevalence and abundance (Fariña et al. 2019). These findings may raise a great concern for cucumber growers in Brazil, because *B. tabaci* MED can efficiently transmit ToCV (Bello et al. 2019) and is often found on cucumber crops grown in greenhouses (Moraes et al. 2018). Additionally, cucumbers may serve as inoculum sources of ToCV for other economically important solanaceous crops. To the best of our knowledge, this is the first report of ToCV infecting cucumber plants.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: ToCV, cucumber, MED, *Bemisia tabaci*

REFERENCES

Bello, V. H., et al. 2019. Phytoparasitica 47:293.
Fariña, A. E., et al. 2019. Plant Dis. 103:1132.
Macedo, M. A., et al. 2019. Plant Pathol. 68:72.
Moraes, L. A., et al. 2018. Sci. Rep. 8:14589.
Mugerwa, H., et al. 2018. Sci. Rep. 8:2734.
Roossinck, M. J., et al. 2015. Phytopathology 105:716.
Walsh, P. S., et al. 1991. Biotechniques 10:506

Figure S1. Symptoms of chlorosis and mottling on young leaves of *Cucumis sativus*.

CHAPTER 3

DETECTION OF *Bemisia tabaci* MEDITERRANEAN CRYPTIC SPECIES ON SOYBEAN IN SÃO PAULO AND PARANÁ STATES (BRAZIL) AND INTERACTION OF *cowpea mild mottle virus* WITH WHITEFLIES

Vinicius Henrique Bello[1,*], Felipe Barreto da Silva[1], Luís Fernando Maranho Watanabe[1], Eduardo Vicentin[1], Cristiane Muller[2], Regiane Cristina Oliveira de Freitas Bueno[1], Jessica Costa Santos[3], Bruno Rossitto de Marchi[4], Angélica Maria Nogueira[1], Valdir Atsushi Yuki[5], Julio Massaharu Marubayashi[1], Maria Márcia Pereira Sartori[1]; Marcelo Agenor Pavan[1], Murad Ghanim[6], Renate Krause-Sakate[1]

[1]UNESP – Faculdade de Ciências Agronômicas, Botucatu, Brazil; [2]Corteva Agriscience™, Mogi Mirim, Brazil; [3]Instituto Federal Catarinense – Campus Rio do Sul, Brazil; [4]Gulf Coast Research and Education Center, University of Florida, Wimauma, FL 33598, USA; [5]Instituto Agronômico, Campinas, Brazil; [6]The Volcani Center, Institute of Plant Protection, Bet Dagan, Israel
* Corresponding author; Vinicius Henrique Bello: vhbello@hotmail.com

Submitted to the “Plant Pathology”

Abstract

Since the detection of *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1) species in Brazil in the early 1990s, this whitefly has been the predominant species and the main viral vector in the country. In this work, whiteflies were collected from commercial soybean fields near and far from greenhouses where Mediterranean species had previously been detected infesting vegetable crops. Results indicated that MEAM1 was the predominant whitefly species in soybean in most sampling sites, while MED was found colonizing soybean plants in open field conditions alone and/or with MEAM1 in several places. Among the tested insects, MED species was mostly detected harbouring the facultative endosymbiont *Hamiltonella*. We also detected cowpea mild mottle virus (CPMMV; carlavirus) infecting soybean plants. Transmission assays demonstrated that MED species was more efficient transmitting CPMMV from beans to beans and from soybean to soybean plants than MEAM1, while MEAM1 was more efficient in transmitting the virus from soybean to bean than MED. Performance assays indicated that adult emergence of both species on CPMMV soybean infected plants was higher when compared with the emergence on healthy plants. Moreover, non-viruliferous MED and MEAM1 adults preferred to settle more often on healthy plants, while viruliferous adults settled more often on CPMMV-infected soybean plants. As MED has already been found in soybean open fields in São Paulo State and Paraná States, Brazil, and it is a good vector of CPMMV, we anticipate problems related to whitefly management and to increase in the incidence of the virus in soybean.

KEYWORDS
Whitefly; MED; MEAM1; endosymbiont; carlavirus

1.1 INTRODUCTION

Whiteflies are phloem-feeding insects that damage a wide range of agricultural crops, including soybean, vegetable, ornamental plants and weeds (Dinsdale *et al*., 2010). They can cause problems to plants in three ways: adults and nymphs cause damage by feeding directly on the phloem, weakening the plants. They also cause indirect damage via secretion of honeydew, which causes a sooty mould growth that interferes with photosynthesis, reducing crop productivity and fruit and fibre quality (Kanakala and Ghanim, 2015). However, the major impact caused by this insect in agriculture is the transmission of viruses (Navas-Castillo *et al*., 2011). *Bemisia tabaci* can transmit over 300 species of plant viruses, which include members of the genera *Begomoviru*s, *Carlavirus*, *Crinivirus*, *Ipomovirus*, *Torradovirus* and *Polerovirus* (Gilbertson *et al.*, 2015; Ghosh *et al.*, 2019; Costa *et al.*, 2020).

Bemisia tabaci is a cryptic species complex composed of ecological and genetically distinct species that can be differentiated based on molecular polymorphism markers, their ability to transmit viruses (Polston *et al*., 2014), endosymbiont composition (Gueguen *et al*., 2010), specific relationships with host plants (Sun *et al.*, 2013), ability to cause plant disorders (Sseruwagi *et al.*, 2005), resistance to insecticides (Kontsedalov *et al.*, 2012) and invasion ability (Liu *et al.*, 2007). Recently, the current definition based on molecular analyses of the mitochondrial cytochrome oxidase subunit I (mtCOI) gene classified this complex with at least 44 species (Kanakala and Ghanim, 2019). Among this cryptic species complex, Middle East-Asia Minor 1 (MEAM1, formerly known as the B biotype) and Mediterranean (MED, formerly known as the Q biotype) are considered the most invasive species worldwide and responsible for severe damage (Muñiz *et al.*, 2011; Alemandri *et al.*, 2012, 2015; McKenzie *et al.*, 2012; Barbosa *et al.*, 2014; Kanakala and Ghanim, 2015, 2019; Yao *et al.*, 2017).

Within the *B. tabaci* complex, the natives New World (formerly known as the A biotype) occur indigenously in Brazil (Marubayashi *et al.*, 2013; Barbosa *et al.*, 2014; Moraes *et al.*, 2018). However, soybean yield losses due to whitefly attack only became frequent and significant after the introduction of the Middle East-Asia Minor 1 (MEAM1, formerly known as the B biotype) in the early 1990s (Lourenção & Nagai, 1994) and is the predominant species in Brazil until nowadays (Moraes *et al*., 2018). Since then, an intense increase in soybean areas associated with the abusive use of non-selective insecticides has contributed to the occurrence of whitefly *B. tabaci* on soybean fields (Arnemann *et al.*, 2019; Pozebon *et al.*, 2020).

Bemisia tabaci MED was detected in the southernmost regions of Brazil in 2014 and since then, multiple detections of MED species were reported in the South, Southeast and Midwest regions of the country associated to ornamental plants and vegetable crops (Moraes *et al*., 2018; Bello *et al*., 2020). MED has been responsible for serious problems worldwide in the last two decades, mainly associated with resistance to insecticides, which caused its rapid invasion and dominance in new ecological niches and countries (Horowitz and Ishaaya, 2014; Horowitz *et al*., 2020). It is also known that resistance to insecticides has been reported affecting MEAM1 species (Gorman *et al.*, 2010; Dângelo *et al.*, 2018; Horowitz *et al.*, 2020), which is the most abundant whitefly in open-field agriculture (Kontsedalov *et al.*, 2012; Alemandri *et al.*, 2015; Kriticos *et al.*, 2020). The MED species might also suppress host plant defences and promote subsequent whitefly infestations (Chen *et al.*, 2013; Shi *et al.*, 2018a). Also, it is an efficient vector of viruses that are causing emerging diseases worldwide (Gilbertson *et al.*, 2015; Kanakala and Ghanim, 2019).

In Brazil, the cowpea mild mottle virus (CPMMV, carlavirus) can be transmitted efficiently by MED and MEAM1 (Bello *et al.*, 2019). CPMMV is a single-stranded positive-sense RNA virus (family *Betaflexiviridae*, genus *Carlavirus*) with a genome of 8,200 nucleotides and composed of six open reading frames (ORFs) as typical to other members of the genus (King *et al.*, 2011; Zanardo *et al.*, 2014). Carlaviruses are mostly aphid-transmitted, but several aphid species were tested as possible vector and none of these were able to transmit the CPMMV (Iwaki *et al.*, 1982; Thouvenel *et al.*, 1982; Muniyappa & Reddy, 1983; Almeida *et al.*, 2005). It was show that CPMMV is an exception for being transmitted by whiteflies in a non-persistent way. *Bemisia tabaci* can acquire and transmit the virus with only 10 minutes of acquisition and 5 minutes of inoculation period and without a latent period (Iwaki *et al.*, 1982; Marubayashi *et al.*, 2010).

CPMMV is considered an emerging virus in Brazil infecting soybean and beans in the South, Southeast, Midwest and Northeast production areas, causing an array of symptoms: dwarfism/stunting, necrosis, mosaic and chlorosis in soybeans and beans and even plant death. In some soybean genotypes, no symptoms are observed (Almeida *et al.*, 2005; Zanardo *et al.*, 2014; Barreto da Silva *et al.*, 2020). Although severe symptoms were described in soybean, mild mottle and mosaic were the most common symptoms observed associated with this virus in some cultivars in the last years (Zanardo *et al.*, 2014). However, even mild symptoms or no symptoms at all, yield losses varying from 174 to 638 kg ha^{-1} were observed depending on the cultivar (Barreto da Silva *et al.*, 2020). Thus, virus infection on soybeans can often be underestimated and the presence of *B. tabaci* can contribute to the virus spread and yield losses.

To better understand the dynamic of whiteflies in soybean fields from São Paulo and the Paraná States, insects were collected and identified based on the mtCOI analyses. The occurrence and frequency of endosymbionts were determined in MED populations. CPMMV surveys were conducted on different farmers. The goal of this study was also to reveal the importance of soybeans and beans as sources of inoculum of CPMMV, using MED and MEAM1 as vectors. Performance and preference assays of both species of whiteflis on CPMMV-infected and healthy soybean plants were conducted to better understand the effects of the virus on their reproduction and behaviour.

1.2 MATERIAL AND METHODS

Whitefly sampling

Whitefly sampling was carried out over two summer soybean seasons during January and February 2019 and 2020. Individual whiteflies were collected from 51 soybean fields distributed in 34 localities across São Paulo and Paraná States, Brazil, in which whitefly populations were defined by geographic region (Table 1). Adult whiteflies were collected from the leaves-underside selected at random across the soybean fields using a hand-held aspirator, transferred immediately to 100% ethanol and stored at -20 °C until further analyses. Up to fifty adults were collected per field. Soybean leaves of three sampling sites (samples 1, 13 and 14) with nymphs were also taken to the laboratory to prove whitefly colonization (Table 1). When fourth instars were present, these were also collected using an entomological needle and with the aid of a magnifying glass (Zeiss®), transferred to 100% ethanol and stored in the same conditions (-20 °C). Up to twenty nymphs were collected per selected sampling field.

Table 1. Information of whitefly species in São Paulo and Paraná States, Brazil, collected in January and February 2019/2020.

ID	Collection site	Host plant	Coordinates	Collection Date	Whitefly species	
					MED	**MEAM1**
1*	**Bandeirantes/PR**	***Glycine max (OF)***	**23° 7' 48" S 50° 23' 11" W**	**January 2019**	**100%** (GenBank MN318228)	
1A	**Bandeirantes/PR**	***Glycine max (OF)***	**23° 7' 48" S 50° 23' 11" W**	**January 2019**	**100%** (GenBank MN318229)	
2	Bandeirantes/PR	*Glycine max (OF)*	23° 04' 02" S 50° 23' 00" W	January 2019		100%
3	Bandeirantes/PR	*Glycine max (OF)*	23° 04' 02" S 50° 23' 05" W	January 2019		100%
4	Bandeirantes/PR	*Glycine max (OF)*	23° 6' 35" S 50° 21' 31" W	January 2019		100%
5	Londrina/PR	*Glycine max (OF)*	23° 25' 17" S 51° 16' 22" W	February 2019		100%
6	**Londrina/PR**	***Glycine max (OF)***	**23° 23' 47" S 51° 10' 50" W**	**February 2019**	**10%** (GenBank MN318231)	**90%**
7	Cambé/PR	*Glycine max (OF)*	23° 14' 16" S 51° 15' 03" W	February 2019		100%
8	Cambé/PR	*Glycine max (OF)*	23° 13' 19" S 51° 14' 24" W	February 2019		100%
9	Cambé/PR	*Glycine max (OF)*	23° 12' 13" S 51° 13' 35" W	February 2019		100%
10	Cambé/PR	*Glycine max (OF)*	23° 12' 27" S 51° 13' 45" W	February 2019		100%
11	Cambé/PR	*Glycine max (OF)*	23° 09' 59" S 51° 12' 14" W	February 2019		100%
12	Cambé/PR	*Glycine max (OF)*	23° 07' 43" S 51° 12' 13" W	February 2019		100%

13*	**Óleo/SP**	***Glycine max (OF)***	**22° 56' 33" S 49° 26' 14" W**	**February 2019**	**100%** (GenBank MN318230)	
13A	**Óleo/SP**	***Glycine max (OF)***	**22° 56' 33" S 49° 26' 14" W**	**February 2019**	**100%** (GenBank MN318231)	
14*	**Óleo/SP**	***Glycine max (OF)***	**22° 55' 35" S 49° 26' 57" W**	**February 2019**	**100%**	
14A	**Óleo/SP**	***Glycine max (OF)***	**22° 55' 35" S 49° 26' 57" W**	**February 2019**	**100%**	
15	Estiva Gerbi/SP	*Glycine max (OF)*	22° 12' 20" S 46° 57' 43" W	January 2019		100%
16	Estiva Gerbi/SP	*Glycine max (OF)*	22° 12' 12" S 46° 57' 37" W	January 2019		100%
17	**Canitar/SP**	***Glycine max (OF)***	**23° 00' 48" S 49° 46' 25" W**	**January 2020**	**10%**	**90%**
18	**Santa Cruz do Rio Pardo/SP**	***Glycine max (OF)***	**22° 56' 09" S 49° 32' 42" W**	**January 2020**	**40%**	**60%**
19	**Taciba/SP**	***Glycine max (OF)***	**22° 24' 38" S 51° 19' 30" W**	**January 2020**	**10%**	**90%**
20	**Rubiácea/SP**	***Glycine max (OF)***	**21° 25' 40" S 50° 49' 05" W**	**January 2020**	**70%**	**30%**
21	**Buritama/SP**	***Glycine max (OF)***	**21° 04' 08" S 50° 12' 08" W**	**January 2020**	**100%**	
22	**Pindamonhangaba/SP**	***Glycine max (OF)***	**22° 52' 05" S 45° 28' 18" W**	**February 2020**	**10%**	**90%**
23	Itaí/SP	*Glycine max (OF)*	23° 19' 07" S 49° 05' 17" W	January 2020		100%
24	Piraju/SP	*Glycine max (OF)*	23° 16' 13" S 49° 16' 30" W	January 2020		100%
25	Ipaussu/SP	*Glycine max (OF)*	23° 05' 11" S 49° 33' 03" W	January 2020		100%
26	Assis/SP	*Glycine max (OF)*	22° 40' 48" S 49° 46' 25" W	January 2020		100%

27	Assis/SP	*Glycine max (OF)*	22° 40' 01" S 50° 21' 59" W	January 2020	100%
28	Candido Mota/SP	*Glycine max (OF)*	22° 40' 02" S 50° 21' 26" W	January 2020	100%
29	Palmital/SP	*Glycine max (OF)*	22° 50' 08" S 50° 11' 30" W	January 2020	100%
30	Vargem Grande do Sul/SP	*Glycine max (OF)*	21° 50' 04" S 46° 55' 34" W	January 2020	100%
31	Porto Ferreira/SP	*Glycine max (OF)*	21° 48' 42" S 47° 25' 19" W	January 2020	100%
32	Pirassununga/SP	*Glycine max (OF)*	23° 03' 45" S 47° 30' 01" W	January 2020	100%
33	Paranapanema/SP	*Glycine max (OF)*	23° 28' 26" S 48° 45' 16" W	January 2020	100%
34	Mogi Mirim/SP	*Glycine max (OF)*	22° 27' 27" S 46° 56' 02" W	January 2020	100%
35	Mogi Mirim/SP	*Glycine max (OF)*	22° 28' 36" S 47° 03' 36" W	January 2020	100%
36	Casa Branca/SP	*Glycine max (OF)*	21° 55' 48" S 47° 04' 08" W	January 2020	100%
37	Luís Antônio/SP	*Glycine max (OF)*	21° 29' 59" S 47° 45' 45" W	January 2020	100%
38	Colina/SP	*Glycine max (OF)*	20° 46' 46" S 48° 30' 07" W	January 2020	100%
39	Miguelópolis/SP	*Glycine max (OF)*	20° 11' 43" S 48° 00' 54" W	January 2020	100%
40	Miguelópolis/SP	*Glycine max (OF)*	20° 06' 46" S 47° 57' 14" W	January 2020	100%
41	Guaíra/SP	*Glycine max (OF)*	20° 27' 41" S 48° 23' 33" W	January 2020	100%
42	Barretos/SP	*Glycine max (OF)*	20° 31' 29" S 48° 35' 09" W	January 2020	100%
43	Ituverava/SP	*Glycine max (OF)*	20° 18' 25" S 47° 48' 36" W	January 2020	100%

44	Jaboticabal/SP	*Glycine max (OF)*	21° 16' 46" S 48° 16' 42" W	January 2020	100%
45	Álvares Machado/SP	*Glycine max (OF)*	22° 01' 06" S 51° 24' 37" W	January 2020	100%
46	Anhumas/SP	*Glycine max (OF)*	22° 27' 03" S 51° 26' 01" W	January 2020	100%
47	Taquarituba/SP	*Glycine max (OF)*	22° 24' 38" S 51° 19' 30" W	January 2020	100%
48	Taquarituba/SP	*Glycine max (OF)*	23° 30' 54" S 49° 16' 14" W	January 2020	100%
49	Taquarituba/SP	*Glycine max (OF)*	23° 31' 27" S 49° 16' 15" W	January 2020	100%
50	Taquarituba/SP	*Glycine max (OF)*	23° 31' 09" S 49° 16' 52" W	January 2020	100%
51	Álvares Florence/SP	*Glycine max (OF)*	20° 17' 12" S 49° 55' 12" W	January 2020	100%

[*] Collected samples near of greenhouses where MED was identified previously by Bello et al. 2019; [1] OF: open field; and samples highlighted in red bold belong to MED samples for better visualization; Samples identified with “A” correspond to nymph whiteflies while unidentified samples correspond to adult whiteflies.

Whitefly identification

Molecular analyses were performed by extracting total nucleic acids from individual whiteflies, according to a modified Chelex protocol (Walsh *et al.*, 1991). For each population, 10 adults were analyzed and identified by amplification of microsatellite and mtCOI regions. In addition, 10 nymphs of some samples of soybean were collected directly from leaves and identified. Populations of adults and nymphs were assigned population IDs consisting of letters corresponding to the soybean plants from which they were collected (Table 1). Whitefly adults and nymphs were macerated and homogenized in 50 µl of Chelex 5% solution in a 1.5 ml Eppendorf® tube. The tube was mixed for few seconds and then incubated at 56 °C for 15 min and at 99 °C for 3 min. After centrifugation at 14,000 rpm for 5 min, the supernatant was collected and used as a template for PCR amplification.

All individual DNA samples from each population were first subjected to an initial round of PCR amplification using the following Bem23 primer pair: Bem23F (5′-CGGAGCTTGCGCCTTAGTC-3′) and Bem23R (5′-CGGCTTTATCATAGCTCTCGT-3′) (De Barro *et al*., 2003). The primer pair differentiates MED and MEAM1 species based in a microsatellite locus of about 200 bp and 400 bp for each species, respectively (Kontsedalov *et al*., 2012; Škaljac *et al*., 2013). Previously identified MED species (Samples 1, 6, 13, 14, 17, 18, 19, 20, 21 and 22; Table 1) were also confirmed by PCR using the prime pair: 2195Bt (5′-TGRTTTTTTGGTCATCCRGAAGT-3′) and C012/Bt-sh2 (5′-TTTACTGCACTTTCTGCC-3′) which targets a fragment of the mitochondrial cytochrome oxidase I (mtCOI) gene (Mugerwa *et al.*, 2018). Additionally, mtCOI PCR products were submitted to a restriction fragment length polymorphism (RFLP) analysis (Bosco *et al.*, 2006), in which 6.5 µl of each PCR (800 bp) and digested with one unit of *TaqI* at 65°C for 2h in a final volume of 16 µl. All restricted DNAs were analyzed by agarose gel electrophoresis (1.8%), stained with ethidium bromide and visualized by UV transillumination.

In addition, a representative number of PCR products from mtCOI amplicons of *B. tabaci* MED of soybean were purified (QIAquick Gel Extraction Kit Qiagen) and sequenced using the primer 2195Bt. Nucleotide sequences of mtCOI were deposited in GenBank, and their accession numbers are available in Table 1.

Endosymbionts

Due to the high variability of endosymbionts in MED, and the fac that they are well studied and stablished in MEAM1 populations in Brazil (Moraes *et al*., 2017; 2018; Bello *et al*., 2019; 2020), only MED specimens were screened for endosymbiont composition. Same DNA extracted from the individual MED whitefly specimens used for identification was used for screening the following whitefly endosymbionts: *Portiera aleyrodidarum, Hamiltonella*, *Rickettsia*, *Wolbachia*, *Arsenophonus*, *Cardinium* and *Fristchea*, using genus-specific primers targeting the 16S and 23S rDNA genes (Marubayashi et al., 2014). Here, *P. aleyrodidarum* was used as positive control for all other endosymbionts. Subsequently, PCR products from each positive endosymbiont were selected and sequenced using each forward primer to confirm the tests. The nucleotide sequences of endosymbionts were deposited in GenBank, and their accession numbers are available in Supplementary table 1.

Cowpea mild mottle identification

CPMMV detection was carried out in five soybean plants, collected at random across the soybean fields and associated with the presence of whiteflies for each sampled location. Total RNA was extracted with the Total RNA Purification Kit® (Norgen, Canada), followed by One Step RT-PCR using AMV reverse transcriptase (Promega, Brazil) with the specific primer pair: CPMMV1280-F (5′-GGCGTT CCAAAAGCTGCCGAT-3′) / CPMMV1696-R (5′-GGAGCCACCTTTCCAATCAA-3′), amplifying a region of the coat protein (De Marchi *et al.*, 2017). Additionally, representative samples of PCR amplicons were purified (QIAquick Gel Extraction Kit Qiagen) and sequenced using the primer CPMMV1280-F. Nucleotide sequences of viruses were deposited in GenBank, and their accession numbers are available in Supplementary table 2.

Biological studies

Transmission assay

To identify the importance of soybean and bean as an inoculum source of CPMMV, transmission assays were performed using two single-infected isolates of cowpea mild mottle virus (CPMMV). A CPMMV isolate (GenBank access number

MW264332) was collected from beans in Londrina county, Paraná, Brazil (April 2016) showing mild mottle, mosaic and stunting symptoms. The presence of the virus was confirmed by RT-PCR (as described in virus identification). Another CPMMV isolate (GenBank access number MT473963) was collected from soybean in Casa Branca county, São Paulo State, Brazil (February 2017). The soybean plant collected from field showed mild mottle symptoms and viral infection was confirmed by RT-PCR. After virus identification, the isolates were transmitted by whitefly (MEAM1) to common bean cv. Jalo and maintained in a whitefly-proof screened cage for 45 days until start the first transmission from and to beans and from beans to soybeans. Subsequently, infected soybeans were used for transmission from and to soybeans and back to beans. Before starting the assays and contact with whiteflies, all plants used as source of inoculum were tested for viral infection.

Virus acquisition was performed using 1,000 newly emerged adults (72h) of MEAM1 (GenBank access number MH186145) and 1,000 newly emerged adullts (72h) of MED (GenBank access number MH047295) species (maintained on cotton plants cv. IAC-20) for acquisition access periods (AAPs) of 24 h. Following virus acquisition, whiteflies (10 adults per plant) were transferred to cages containing healthy soybean plant cv. M6410 or beans cv. Jalo with true primary leaves, using a hand-held aspirator on which they remained for a 24 h inoculation access periods (IAP) under controlled conditions at 30 °C. Treatments were composed of CPMMV isolate from bean inoculated 1) from bean to bean, 2) from bean to soybean, 3) from soybean to soybean and 4) from soybean to bean. Its important to highlight that more than thirty plants were inoculated individually in separate cages; however, only 30 plants were used for each treatment in which all were alive after IAP (Table 2). The same experiment was performed using a CPMMV isolate from soybean (Table 2). Virus-free adult whiteflies collected from either rearing cage (MEAM1 and MED) were given inoculation access to 10 non-infected plants for 24 h at 30 °C as negative controls.

After inoculation, the plants were sprayed with a mixture of insecticides (Cartap® and Oberon®) to kill all the whitefly adults, nymphs, and eggs. Plants were grown in whitefly proof screened cages in greenhouses. Presence of the virus was analysed 30 days after inoculation (as described in virus identification).

Performance assay

To identify the importance of CPMMV-infected and healthy soybean plants for whitefly reproduction, performance assays were performed using the CPMMV isolate from soybean. Newly emerged adults (72h) of MEAM1 and MED raised on cotton were collected and transferred to clip-cages attached to healthy and CPMMV-infected soybean M6410 plants (third and fourth trifoliate of soybean) with 35 days after emergence and without symptoms but confirmed by RT-PCR. For each replicate (10 replicates per treatment and repeated twice), 10 whitefly couples were enclosed in one clip-cage per leaf and four per plant in the third and fourth trifoliate. Adults were maintained for 48 h under controlled conditions (26°C, 12L: 12D (Light 6:00–18:00). Subsequently, adults were removed from the leaves and the eggs were counted using a magnifying glass (Zeiss®) and maintained for further evaluations: eggs hatched, total of adults emerged, number of adults emerged per day, development time (from eggs to first adult emergence) and survival rate [(number of adults emerged/number of eggs laid)*100]. Daily evaluations were performed during all assays to determine the exact time between each evaluated phase of the whitefly cycle. Newly emerged adults were collected twice daily (9 am and 2 pm) using a hand-held aspirator and immediately counted. Assay was composed of four treatments: 1) MEAM1/healthy soybean plants, 2) MEAM1/CPMMV-infected soybean plants, 3) MED/healthy soybean plants, and 4) MED/CPMMV-infected soybean plants.

Preference assay

To understanding how CPMMV infection may change whitefly behaviour, preference assays were performed using the CPMMV isolate from soybean. Preference assays with free-choice were performed using MEAM1 and MED species on healthy and CPMMV-infected soybean M6410 plants with 35 days after emergence and without symptoms but confirmed by RT-PCR. For assays using viruliferous insects, each species (MEAM1 and MED) was placed separately in cages containing the infected plants (as described previously on transmission assays). Two plants, one healthy and one infected, were arranged in whitefly-proof screened-cages (45 x 45 x 55 cm; 2 plants per cage). Ten cages were used in this assay, totalizing 10 plants of each one (infected and healthy). A total of 100 whitefly couples were collected between 07:00 and 08:00 am and released near the centre-bottom of each cage, and cages were kept under controlled conditions (26°C, 12L: 12D (Light 6:00–18:00). Number of whiteflies that settled on each plant (healthy or CPMMV-infected) was determined in the following periods: 1, 3, 6, 12, 24, 48, 72 and 96 h. Whiteflies were counted with the aid of a mirror, positioned near the abaxial surface of leaves which allowed the visualization and counting of insects. There were 10 replicates for each kind of whitefly tested: 1) non-viruliferous MEAM1: healthy and CPMMV-infected soybean plants, 2) non-viruliferous MED: healthy and CPMMV-infected soybean plants, 3) viruliferous MEAM1: healthy and CPMMV-infected soybean plants, and 4) viruliferous MED: healthy and CPMMV-infected soybean plants.

Data analysis

Data on virus transmission efficiency by MED and MEAM1 were analyzed by chi-square test using a generalized linear model (GLM) with log link functions or a linear model only, using the software package R 3.1.0 (RDevelopment, 2018). For the number of eggs laid, number of eggs hatched, total of emerged adults, development time, survival rate and preference assays were transformed by log(x) before analysis to reduce the heteroscedasticity. Then, non-parametric statistical analyses were performed using the Kruskal-Wallis rank test ($P < 0.05$) employing the statistical software package Minitab 16 (Minitab, 2010).

1.3 RESULTS

Whitefly sampling

A total of 51 sites from São Paulo and Paraná States were sampled during January and February 2019 and 2020. Results indicated that the predominant whitefly species in soybean at most of the sampling sites was MEAM1. MED was found alone and/or co-occurring in soybean with MEAM1 in several sites (Figure 1, Table 1). MED was also found nearby (samples 1, 6, 13 and 14) and distant of greenhouses (samples 17, 18, 19, 20, 21 and 22).

In Paraná State, MED was found alone at sampling site 1, while MEAM1 and MED were sampled together at site 6; with predominance of MEAM1 over MED. In São Paulo State, MED was found alone at sites 13, 14 and 21, while MED and MEAM1 were also detected co-occurring in five sampling sites. MEAM1 predominating MED in four sites (17, 18, 19 and 22) and MED over MEAM1 at site 20 (Table1).

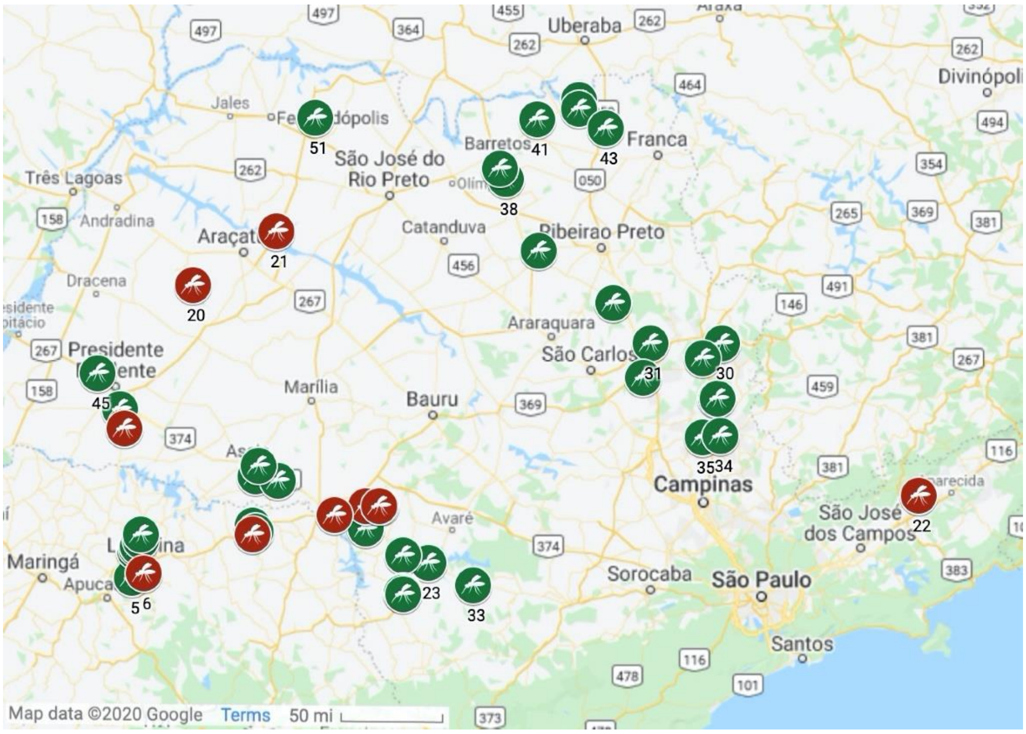


Figure 1. Sampling locations for *Bemisia tabaci* Mediterranean (MED) and Middle East Asia Minor 1 (MEAM1) species collected in soybean in São Paulo and Paraná States, Brazil. MED: red points; MEAM1: green points.

Endosymbiont analysis revealed that MED species in the sampled areas were infected by *Hamiltonella* with a high frequency (96.5 % - 82 positive individuals out of 85). *Rickettsia* was also found, but at a low frequency (2.55% - three positive individuals out of 85) (Supplementary fig. 1). All *Rickettsia* positive samples were found in mixed infection with *Hamiltonella. Portiera* was also identified in 100% of tested samples, confirming the quality of detection.

Virus identification

A total of 255 soybean plants were collected from 34 different collection sites. CPMMV was found infecting soybean plants at seven different collection sites in São Paulo and Paraná States. In four of these, only MED specimens were detected (samples 1, 13, 14 and 21). In two locations, the virus was found in a mixed MED and

MEAM1 population (samples 17 and 20), and only in one field with an MEAM1 population (sample 15) (Supplementary table 2). Although the number of plants tested was large, all collected samples in all sampled soybean fields were asymptomatic.

Virus transmission assays

Comparing the virus transmission only by MEAM1 between host combinations, CPMMV was better transmitted from soybean to bean and from bean to bean than from soybean or bean to soybean, for both isolates (*P* = 0.025162). Comparing the virus transmission only by MED between host combinations, MED was a better vector when CPMMV was transmitted from bean to bean, from bean to soybean or from soybean to soybean plants than from soybean to bean plants (*P* = 0.000016) for both isolates (Table 2).

Table 2. Efficiency of cowpea mild mottle virus transmission by *Bemisia tabaci* MEAM1 and MED species.

Virus	From/to[a]	MEAM1	MED
CPMMV – soybean isolate	Beans/Beans	28/30 [b] (93.3 [c])Aa	29/30 (96.7)Aa
	Beans/Soybean	24/30 (80.0)Bb	30/30 (100.0)Aa
	Soybean/Soybean	25/30 (83.33)Bb	28/30 (93.33)Aa
	Soybean/Beans	27/30 (90)Aa	22/30 (73.33)Bb
CPMMV – bean isolate	Beans/Beans	29/30 (96.7)Aa	30/30 (100)Aa
	Beans/Soybean	23/30 (76.6)Bb	30/30 (100.0)Aa
	Soybean/Soybean	22/30 (73.3)Bb	30/30 (100)Aa
	Soybean/Beans	28/30 (93.3)Aa	24/30 (80)Bb

[a] Hosts used for acquisition/transmission; [b] number of plants infected/tested; [c] percentage of infection; Means followed by different uppercase letters within columns for each isolate and lowercase letters within rows indicate significant difference (*P* < 0.05).

Comparing MED vs MEAM1, MED specimens were more efficient in transmitting the CPMMV- soybean isolate from beans to soybeans (100%) (*P* < 0.00001) and from soybean to soybean plants (93.7%) than MEAM1 (80% and 83.3%, respectively) (*P* = 0.0484) (Table 2). The same was also observed for the CPMMV-bean isolate when transmitted from beans to soybeans (100%) and from soybeans to soybean plants (100%) by MED than MEAM1 (76.6% and 73.3%, respectively) (*P* = 0. 00001) (Table 2). The MEAM1 was more effective in the transmission of CPMMV-soybean and CPMMV-bean isolates from soybeans to beans (90% and 93.3%, respectively) than MED (73.3% and 80%, respectively) (*P* = 0.0032; *P* = 0.025) (Table 2). No significant difference in the transmission of CPMMV from bean to bean plants could be observed between MED and MEAM1 (*P* = 0.5371) for both isolates (Table 2). CPMMV-infected beans showed mild mottle, mosaic and stunting, while infected soybeans were asymptomatic.

Performance assays

Number of eggs laid

Comparing MED vs MEAM1, performance assays showed that MEAM1 laid significantly more eggs than MED on CPMMV-infected soybean plants (*H* = 0.15, *P* = 0.049) (Table 3). The number of eggs laid by MEAM1 and MED on healthy soybean plants did not differ (*H* = 0.21, *P* = 0.65) (Table 3).

Comparing CPMMV-infected vs healthy soybean plants, more eggs were laid by MEAM1 on CPMMV-infected plant than on healthy soybean plants (*H* = 4.81, *P* = 0.028). The number of eggs laid by MED did not differ significantly between CPMMV-infected plants and healthy soybean plants (*H* = 0.28, *P* = 0.597) (Table 3).

Table 3. Performance evaluation of MEAM1 and MED on healthy and CPMMV-infected soybean plants

Whitefly species	**Eggs laid**		**Eggs hatched**		**Adults emerged**	
	Healthy	**CPMMV-infected**	**Healthy**	**CPMMV-infected**	**Healthy**	**CPMMV-infected**
B. tabaci MEAM1	40bA	87aA	38bA	85.5aA	32bA	72aA
B. tabaci MED	43aA	46aB	24aA	43aB	21bA	37aB

Medians followed by different uppercase letters within columns and lowercase lines indicate significant difference (*P* < 0.05) for each parameter evaluated.

Number of eggs hatched

There was no significant difference of the number of eggs hatched on healthy soybean between MEAM1 and MED (*H* = 0.07, *P* = 0.901), while on CPMMV-infected soybean, the eggs hatched was higher for MEAM1 compared to MED (*H* = 10.1, *P* = 0.01). (Table 3).

Comparing the CPMMV-infected vs healthy soybean plants, the number of eggs hatched for MEAM1 was higher on CPMMV-infected than on healthy soybeans (*H* = 4.17, *P* = 0.041). For MED, there was no significant difference on eggs hatched between CPMMV-infected and healthy soybean (*H* = 1.56, *P* = 0.212) (Table 3).

Number of emerged adults

The number of emerged adults of MEAM1 was significantly higher than that of MED on CPMMV-infected soybean plants (*H* = 9.61, *P* = 0.02). However, there was no difference in the median number of emerged adults on healthy soybean plants between MEAM1 and MED (*H* = 0.01, *P* = 0.91).

Comparing CPMMV-infected vs healthy soybean plants, the number of emerged adults was higher on CPMMV-infected soybean plants for both MEAM1 (*H* = 13.4, *P* = 0.04) and MED (*H* = 3.29, *P* = 0.07) (Table 3).

The daily evaluations showed that CPMMV-infected plants shortened the period of emergence of adults for both MEAM1 (9 days) and MED (9 days) compared to healthy plants (12 and 19 days, respectively) (Figure 2). In addition, it was observed that adults of MEAM1 had an earlier peak on CPMMV-infected soybean plants (the first to the fourth day) than those of MED. On healthy plants, MEAM1 had a peak on the fifth day and kept emerging for seven days, while MED had a peak on day 12 and kept emerging for seven days (Figure 2).

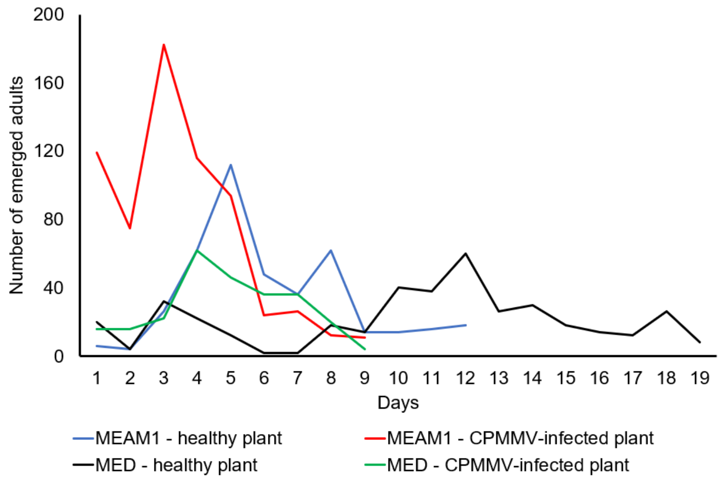


Figure 2. Number of emerged adults of *Bemisia tabaci* Mediterranean (MED) and Middle East-Asia Minor 1 (MEAM1) on healthy and CPMMV-infected soybean plants over 19 days. Medians followed by different lowercase letters within same day indicate significant difference ($P < 0.05$).

Development time and survival rate

No significant differences were observed for the development time from the egg laying to first adult emerged comparing CPMMV-infected plants and healthy plants ($H = 0.18$, $P = 0.5734$) and neither comparing MEAM1 and MED whiteflies ($H = 0.17$, $P = 0.5942$) (Figure 3A).

Survival rate between healthy plants and CPMMV-infected soybean plants did not differ for MEAM1 ($H= 0.52$, $P = 0.695$) (Figure 3B). However, survival rate of MED on CPMMV-infected plants was significantly higher than on healthy soybean plants ($H = 38.53$, $P < 0.0001$). While the survival rate of MED on CPMMV-infected soybean plants was lowest ($H = 40.16$, $P < 0.0001$) (Figure 3B).

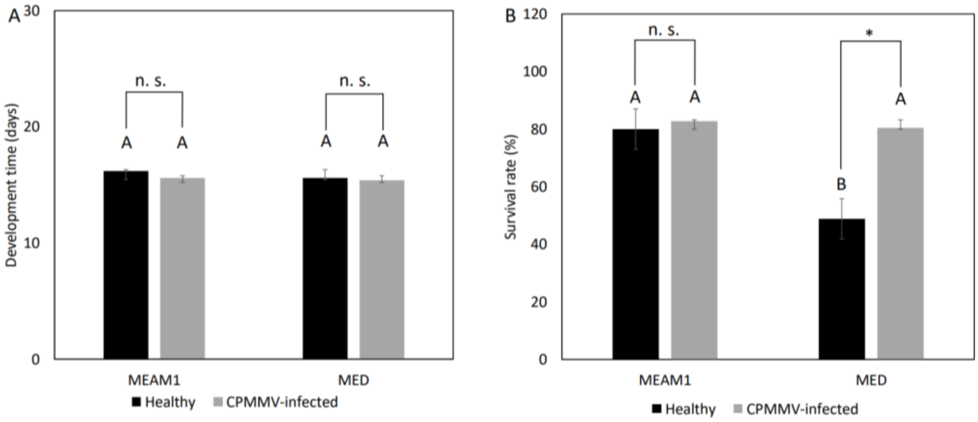


Figure 3. A) Mean development time and B) survival rate of *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) on healthy and CPMMV-infected soybean plants. In each panel, means followed by asterisks indicates a significant difference for each whitefly species on CPMMV-infected vs healthy soybean plants ($P < 0.05$) and n.s. indicates no significance ($P \geq 0.05$). While means followed by different uppercase letters are significantly different for all combinations between whiteflies (MEAM1 and MED) and hosts (CPMMV-infected and healthy soybean plants) ($P < 0.05$).

Preference assays

Non-viruliferous adults of both MEAM1 and MED species preferred to settle on healthy soybean plants over CPMMV-infected plants in all periods evaluated (from 1h to 96h) ($P < 0.05$) (Table 4) while viruliferous adults of MEAM1 and MED preferred to settle on CPMMV-infected soybeans plants in all periods evaluated (from 1h to 96h) ($P < 0.05$) (Table 5).

Table 4. Medians number of non-viruliferous *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) that settled on healthy and CPMMV-infected soybean plants in free-choice assays (preference assays) in various periods.

Non-viruliferous whiteflies	**Periods**							
	1H	**3H**	**6H**	**12H**	**24H**	**48H**	**72 H**	**96H**
MEAM1 - healthy plant	69.5a	80a	64a	81.5a	74a	71a	64.5a	63a
MEAM1 - CPMMV-infected plant	29.5b	17b	30.5b	48b	44b	37b	34b	29b
MED - healthy plant	107A	110.5 A	82.5A	84A	66A	57.5A	60A	66A
MED - CPMMV-infected plant	68.5B	76B	71.5B	66B	68.6A	56A	41.8 B	42B

Notes. Medians followed by the same columns (period) indicate same treatment (MEAM1 – healthy plants + MEAM1 – CPMMV-infected soybean plant or MED – healthy plant + MED – CPMMV-infected soybean plant); means followed by different uppercase or lowercase letters within columns of each whitefly species indicate significant difference (*P* < 0.05).

Table 5. Medians number of viruliferous *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) that settled on healthy and CPMMV-infected soybean plants in free-choice assays (preference assays) in various periods.

Viruliferous whiteflies	**Periods**							
	1H	**3H**	**6H**	**12H**	**24H**	**48H**	**72 H**	**96H**
MEAM1 - healthy plant	59b	71.5b	65b	55b	50.5b	50b	51b	50b
MEAM1 - CPMMV-infected plant	78a	91.5a	91.5a	81.5a	75.5a	62.5a	61.5a	62a
MED - healthy plant	63.5B	70B	73.5B	75B	60B	61B	65B	59B
MED - CPMMV-infected plant	78A	89.5A	91A	89A	92.5A	74A	74A	71A

Notes. Medians followed by the same columns (period) indicate same treatment (MEAM1 – healthy plants + MEAM1 – CPMMV-infected soybean plant or MED – healthy plant + MED – CPMMV-infected soybean plant); means followed by different uppercase or lowercase letters within columns of each whitefly species indicate significant difference (*P* < 0.05).

1.4 DISCUSSION

Previous studies in Brazil revealed that MED is found colonizing ornamental and vegetable crops in greenhouse conditions and that the dispersal of the insect was associated to ornamental plants (Moraes *et al*., 2017, 2018; Bello *et al*., 2020). Here our data show that MEAM1 is prevalent in soybean fields, but MED has also started to spread and colonize soybean under field conditions (Figure 4). In the 2018/19 whitefly survey, evidence reported recently by our group showed that MED might be disseminating to the field from greenhouse outbreaks (Bello *et al*., 2020). So the outbreaks near the greenhouses may have contributed to the dispersion of MED species and their occurrence in soybean fields, because the whitefly is able to disperse up to 7 km with the help of the wind (Byrne, 1999). However, in the 2019/2020 survey, we found MED in areas distant from greenhouses, suggesting that MED may be adapting to open field conditions and reaching different soybean producing areas of São Paulo and Paraná States.

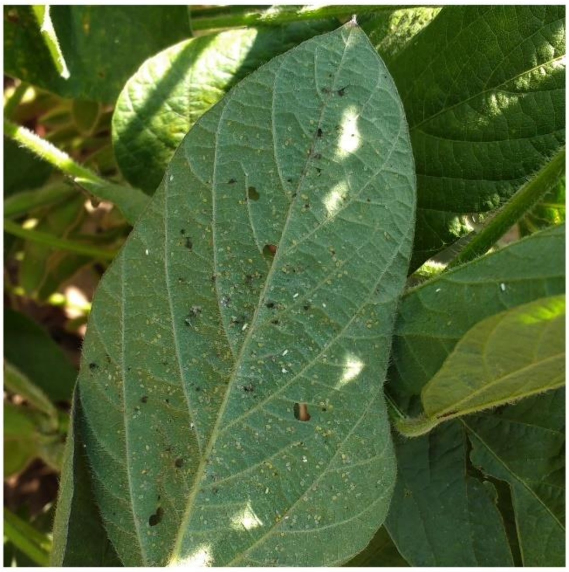


Figure 4. Adults and nymphs of *Bemisia tabaci* Mediterranean (MED) from a commercial soybean field in Oléo county, São Paulo State (Date: February 2019).

Although present in Brazil for at least seven years, MED had never been found on soybean plants in the country. However, here we have the first evidence of the adaptation of MED to open field conditions in São Paulo and Paraná States, which representa a new ecological niche and regions and raises many questions on the future of scenario of whitefly in Brazil.

Brazil is the largest soybean producer in the world, producing in the last soybean season (2019/20) 124.8 million tons of the grain from 36.949 million hectares cultivated in the "rainy season" from August to March (Conab, 2020; IBGE, 2020). This correspond to our summer season, where environmental conditions (temperature and humidity) are prone for insect multiplication. Whitefly outbreaks on soybeans were already frequent in Brazil (Pozebon *et al.*, 2020), as the presence of the whitefly transmitted virus CPMMV (Almeida *et al*., 2005; Zanardo and Carvalho, 2017).

Soybean is Brazil's highest-value agricultural product, its export generating at least US$ 35 billion per year (Cattelan and Dall'Agnol, 2018). However, the MED species occurrence on soybean fields, may negatively affect crop production. Several studies revealed that MED is more resistant to insecticides than MEAM1 which help to predominate over MEAM1 under high insecticide use (Horowitz *et al*., 2020; Horowitz *et al*., 2014; Sun *et al*., 2013). A study from Israel showed that MEAM1 can predominate over MED on cotton (Horowitz and Ishaaya, 2014). When chemical control was used, MED dominated over MEAM1 and MEAM1 predominated over MED again after chemical spray was ceased. In China, it was also observed that MED displaced MEAM1 after insecticides were spray under field conditions (Sun *et al.*, 2013). In laboratory studies without insecticide spray, MED can compete equally with MEAM1 on cotton and soybean plants and both species can colonize and co-habit plants together (Watanabe *et al*., 2019).

In Brazil, whitefly management strategies, not only for soybean, but also for common beans and cotton have often been based on the use of insecticides as the main control method (Pignati *et al.*, 2017). This control strategy might be an important factor favoring MED over MEAM1 on soybeans fields, since MED has the natural insecticide resistance advantage (Horowitz *et al.*, 2020). Tropical weather also contributes to the existence of whitefly populations throughout the year. Moreover, the cultivation system in Brazil with areas of soybean, common bean, cotton and vegetable crops cultivated close to each other heavily influences the occurrence of whiteflies under field conditions. Thus, a continuous monitoring of whitefly populations in Brazil is necessary to better understanding the dynamics of these insects in open field conditions.

In this study, we found asymptomatic CPMMV-infected soybean plants associated with both MEAM1 and MED whiteflies (Supplementary table 2). Although CPMMV infection can induce mild symptoms or asymptomatic in some cultivars, the damage caused by CPMMV is probably underestimated (Barreto *et al*., 2020). Soybeans and beans are known as the main hosts of CPMMV and also whiteflies in Brazil (Zanardo and Carvalho, 2017). Our transmission assays revealed that both MEAM1 and MED whiteflies can efficiently transmit CPMMV from beans to soybeans and vice versa. Previous studies also showed that MED and MEAM1 are efficient vectors of CPMMV for beans and soybeans (Marubayashi *et al.*, 2010; Bello *et al.*, 2019). Thus, we hypothesize that CPMMV occurrence in the soybean fields can be considered as a result of transferring virus by MEAM1 and MED whiteflies. Since CPMMV has been present in soybeans fields since early 2000’s (Almeida *et al.*, 2005), both MEAM1 and MED may be transmitting the virus to soybeans in São Paulo and Paraná States.

In this study, it was observed that CPMMV-infected soybean plants increased the performance of both MED and MEAM1 compared to healthy soybean plants. However, MEAM1 species seemed to have better performance than MED on infected plants. It has already known that infected plants by virus can influence the performance of herbivorous insects with effects that can be positive, negative, or neutral. A study from China involving MED whiteflies, tomato yellow leaf curl virus (TYLCV) and *Datura stramonium* showed that TYLCV-infected plants improved the overall performance, increasing their oviposition, longevity, fecundity, body length and survival rate (Chen *et al.*, 2013). In contrast, another study from the same country showed that TYLCV-infected tomato plants didn’t affect the performance of MED (Li *et al.*, 2011). Working with tomato chlorosis virus (ToCV) in Brazil, Watanabe *et al*. (2018) demonstrated that ToCV-infected tomato plants are harmful for MEAM1, and heavily affects its adult emergence and survival rate.

These results help to explain that the number of newly emerged *B. tabaci* adults that could feed on CPMMV-infected soybean plants would increase and, consequently, more adults would be able to transmit the virus to healthy plants in the field. Thus, CPMMV could contribute to both MEAM1 and MED species to increase their reproduction and, consequently, increase the whitefly population and promote their own dispersion.

Our preference assay showed that non-viruliferous adults of MED and MEAM1 chose to settle on healthy plants, while viruliferous adults of both whitefly species preferred to settle on soybean plants infected with CPMMV. These results are contradictory to those of (Shi *et al.*, 2018b), who observed a preference of MED whiteflies carrying ToCV preferred to settle on healthy plants over infected plants, contributing to the virus spread. Non-viruliferous MED whiteflies also showed to settle on TYLCV-infected plants rather than healthy plants (Chen *et al.*, 2013). In contrast, viruliferous MEAM1 whiteflies didn't chose to settle on healthy or ToCV-infected plants, while no-viruliferous whiteflies chose to settle on healthy plants rather than on ToCV-infected plants (Maluta *et al.*, 2017). These works help to support that to enhance virus spread, viruliferous vectors should prefer healthy plants while nonviruliferous should prefer infected plants (Mauck *et al.*, 2019). It is important to highlight that CPMMV is a non-persistent virus transmitted by whiteflies (Iwaki *et al.*, 1982; Muniyappa & Reddy, 1983). It seems that once the whitefly feeds on a CPMMV infected plant, it prefers to be continued on these plants that seems to increase their fitness, contributing for virus transmission.

The secondary endosymbiont associated with Brazilian MED populations is very diversified, possibly related to the recent and different introductions of the species in Brazil (Barbosa *et al*., 2015; Moraes *et al*., 2017; Moraes *et al*., 2018). MED populations are commonly found harbouring several facultative endosymbionts, such as *Hamiltonella*, *Rickettsia*, *Wolbachia*, *Arsenophonus* and *Cardinium* (Gueguen *et al*., 2010; Bing *et al*., 2013; Kanakala and Ghanim, 2019). High *Hamiltonella* frequency is very common in Brazilian MEAM1 populations and its often in sigle and mixed infections with other endosymbionts in MED as well (Moraes *et al.*, 2017; 2018; Bello *et al.*, 2020). Our results revealed that MED populations found on soybean harboured *Hamiltonella* at a high frequency and *Rickettsia* with lower frequencies. The endosymbiont was similar to that observed on MED specimens collected on *Solanaceae*, *Cucurbitaceae* and weeds (Bello *et al*., 2020). *Hamiltonella* was shown to be responsible for increasing transmission of the begomovirus TYLCV, as the GroEL protein produced by bacteria to safeguard the virus in the haemolymph (Gottlieb *et al.*, 2010; Ghanim, 2014). There is evidence that the Brazilian MED populations harbouring high frequencies of *Hamiltonella* are good vector of CPMMV (Bello *et al.*, 2019).

Presence of *Rickettsia* in MED also may be a factor to be considered, once *B. tabaci* populations that harbour this endosymbiont are known to efficiently transmit TYLCV (Kliot *et al.*, 2014). Altogether, here we present that MED may have a direct impact on the virus ecology and dissemination.

In summary, our data show that although MEAM1 is prevalent in soybean fields, MED has also started to spread and colonize soybean under field conditions in São Paulo and Paraná States. A high frequency of *Hamiltonella* was found in MED populations and CPMMV-infected soybean plants were also found together with MED and MEAM1 species. Moreover, CPMMV infected plants can contribute for an increase in whitefly infestation on soybean and, as a consequence, an increase in virus incidence, which might raise concerns for soybean production, associated potential damage and virus transmission by MED.

Despite all this, heavy dependence on insecticides remains the main strategy for managing whiteflies due to their ease of application and effectiveness in Brazil. However, using insecticides alone to control MED is inappropriate, because MED is resistant to several insecticides (Horowitz *et al.*, 2020), and their strong performance on soybeans can help to displace MEAM1 in the field (Watanabe *et al.*, 2019). Therefore, it is important to consider the unique ability of MED to develop insecticide resistance before strategizing the integrated pest management (IPM) approach to avoid displacement of MEAM1 by MED, otherwise this could lead to an increase in soybean yield losses and the incidence of CPMM.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001; Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (processes number 2017/21588-7) and Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (303411/2018-0). RKS, MAP, MMPS and RCOFB hold a CNPq fellowship.

Conflict of interest Statement

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

REFERENCES

Alemandri V, Barro P De, Bejerman N *et al.*, 2012. Species Within the Bemisia tabaci (Hemiptera: Aleyrodidae) Complex in Soybean and Bean Crops in Argentina. *Journal of Economic Entomology* **105**, 48–53.

Alemandri V, Medina CGV, Dum??n AD *et al.*, 2015. Three members of the Bemisia tabaci (Hemiptera: Aleyrodidae) cryptic species complex occur sympatrically in Argentine horticultural crops. *Journal of Economic Entomology* **108**, 405–413.

Almeida ÁMR, Piuga FF, Marin SRR *et al.*, 2005. Detection and partial characterization of a carlavirus causing stem necrosis of soybean in Brazil. *Fitopatologia Brasileira* **30**, 191–194.

Arnemann JA, Pozebon H, Marques RP *et al.*, 2019. Managing Whitefly on Soybean. *Journal of Agricultural Science* **11**, 41–51.

Barbosa L da F, Marubayashi JM, De Marchi BR *et al.*, 2014. Indigenous American species of the Bemisia tabaci complex are still widespread in the Americas. *Pest management science* **70**, 1440–1445.

Barbosa L da F, Yuki VA, Marubayashi JM *et al.*, 2015. First report of Bemisia tabaci Mediterranean (Q biotype) species in Brazil. *Pest Management Science* **71**, 501–504.

Barreto da Silva F, Muller C, Bello VH *et al.*, 2020. Effects of cowpea mild mottle virus on soybean cultivars in Brazil. *PeerJ* **8**, e9828.

Bello VH, Fernando L, Watanabe M *et al.*, 2019. Evidence for increased efficiency of virus transmission by populations of Mediterranean species of Bemisia tabaci with high Hamiltonella prevalence. *Phytoparasitica* **47**, 293–300.

Bello VH, Watanabe LFM, Fusco LM *et al.*, 2020. Outbreaks of Bemisia tabaci Mediterranean species in vegetable crops in São Paulo and Paraná States, Brazil. *Bulletin of Entomological Research* **110**.

Bing XL, Ruan YM, Rao Q, Wang XW, Liu SS, 2012. Diversity of secondary endosymbionts among different putative species of the whitefly Bemisia tabaci. *Insect Science* **20**, 194–206.

Bosco D, Loria A, Sartor C, Cenis JL, 2006. PCR-RFLP identification ofBemisia tabaci biotypes in the Mediterranean Basin. *Phytoparasitica* **34**, 243–251.

Byrne DN, 1999. Migration and dispersal by the sweet potato whitefly, Bemisia tabaci. *Agricultural and Forest Meteorology* **97**, 309–316.

Cattelan AJ, Dall’Agnol A, 2018. The rapid soybean growth in Brazil. *Ocl* **25**, D102.

Chen G, Pan H, Xie W *et al.*, 2013. Virus infection of a weed increases vector attraction to and vector fitness on the weed. , 3–8.

Costa TM, Inoue-Nagata AK, Vida AH, Ribeiro SG, Nagata T, 2020. The recombinant isolate of cucurbit aphid-borne yellows virus from Brazil is a polerovirus transmitted by whiteflies. *Plant Pathology* **69**, 1042–1050.

Conab 2020. Acompanhamento da safra brasileira: grãos, décimo segundo levantamento, Setembro 2020. Brasília: Companhia Nacional de Abastecimento, **7**, 1–33.

Dângelo RAC, Michereff-Filho M, Campos MR, da Silva PS, Guedes RNC, 2018. Insecticide resistance and control failure likelihood of the whitefly Bemisia tabaci (MEAM1; B biotype): a Neotropical scenario. *Annals of Applied Biology* **172**, 88–99.

De Barro PJ, Liu S-S, Boykin LM, Dinsdale AB, 2011. *Bemisia tabaci* : A Statement of Species Status. *Annual Review of Entomology* **56**, 1–19.

De Barro PJ, Scott KD, Graham GC, Lange CL, Schutze MK, 2003. Isolation and characterization of microsatellite loci in Bemisia tabaci. *Molecular Ecology Notes* **3**, 40–43.

De Marchi BR, Marubayashi JM, Favara GM *et al.*, 2017. Comparative transmission of five viruses by Bemisia tabaci NW2 and MEAM1. *Tropical Plant Pathology* **1**.

Dinsdale A, Cook L, Riginos C, Buckley YM, De Barro P, Barro P De, 2010. Refined Global Analysis of Bemisia tabaci (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) Mitochondrial Cytochrome Oxidase 1 to Identify Species Level Genetic Boundaries. *Annals of the Entomological Society of America* **103**, 196–208.

Ghanim M, 2014. A review of the mechanisms and components that determine the transmission efficiency of Tomato yellow leaf curl virus (Geminiviridae; Begomovirus) by its whitefly vector. *Virus Research* **186**, 47–54.

Ghosh S, Kanakala S, Lebedev G *et al.*, 2019. Transmission of a New Polerovirus Infecting Pepper by the Whitefly Bemisia tabaci (AE Simon, Ed,). *Journal of Virology* **93**, 1–14.

Gilbertson RL, Batuman O, Webster CG, Adkins S, 2015. Role of the Insect Supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the Emergence and Global Spread of Plant Viruses. *Annual Review of Virology* **2**, 67–93.

Gorman K, Slater R, Blande JD *et al.*, 2010. Cross-resistance relationships between neonicotinoids and pymetrozine in Bemisia tabaci (Hemiptera: Aleyrodidae). *Pest Management Science* **66**, 1186–1190.

Gottlieb Y, Zchori-Fein E, Mozes-Daube N *et al.*, 2010. The transmission efficiency of tomato yellow leaf curl virus by the whitefly Bemisia tabaci is correlated with the presence of a specific symbiotic bacterium species. *Journal of virology* **84**, 9310–7.

Gueguen G, Vavre F, Gnankine O *et al.*, 2010. Endosymbiont metacommunities, mtDNA diversity and the evolution of the Bemisia tabaci (Hemiptera: Aleyrodidae) species complex. *Molecular Ecology* **19**, 4365–4376.

Horowitz AR, Ghanim M, Roditakis E, Nauen R, Ishaaya I, 2020. Insecticide resistance and its management in Bemisia tabaci species. *Journal of Pest Science*.

Horowitz AR, Ishaaya I, 2014. Dynamics of biotypes B and Q of the whitefly Bemisia tabaci and its impact on insecticide resistance. *Pest Management Science* **70**, 1568–1572.

IBGE 2020. Levantamento Sistemático da Produção Agrícola. Availabe at: https://agenciadenoticias.ibge.gov.br/agencia-noticias/2012-agencia-de-noticias/noticias/29090-estimativa-preve-safra-recorde-de-252-milhoes-de-toneladas-em-2020 (acessed on November 18, 2020)

Iwaki M, Thongmeearkom P, Prommin M, … YH-P, 1982 U, 1982. Whitefly transmission and some properties of cowpea mild mottle virus on soybean in Thailand. *Plant Disease* **66**, 365–368.

Kanakala, S. and Ghanim M, 2019. Global genetic diversity and geographical distribution of Bemisia tabaci and its bacterial endosymbionts.

Kanakala S, Ghanim M, 2015. Advances in the Genomics of the Whitefly Bemisia tabaci: An Insect Pest and a Virus Vector. In: Springer International Publishing, 19–40.

King A, Lefkowitz E, Adams M, Carstens E, 2011. Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses.

Kliot A, Cilia M, Czosnek H, Ghanim M, 2014. Implication of the Bacterial Endosymbiont Rickettsia spp. in Interactions of the Whitefly Bemisia tabaci with Tomato yellow leaf curl virus. *Journal of Virology* **88**, 5652–5660.

Kontsedalov S, Abu-Moch F, Lebedev G, Czosnek H, Horowitz AR, Ghanim M, 2012. Bemisia tabaci Biotype Dynamics and Resistance to Insecticides in Israel During the Years 2008?2010. *Journal of Integrative Agriculture* **11**, 312–320.

Kriticos DJ, De Barro PJ, Yonow T, Ota N, Sutherst RW, 2020. The potential geographical distribution and phenology of Bemisia tabaci Middle East/Asia Minor 1, considering irrigation and glasshouse production. *Bulletin of Entomological Research*.

Li M, Liu J, Liu SS, 2011. Tomato yellow leaf curl virus infection of tomato does not affect the performance of the Q and ZHJ2 biotypes of the viral vector Bemisia tabaci. *Insect Science* **18**, 40–49.

Liu S-S, De Barro PJ, Xu J *et al.*, 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science* **318**, 1769–1772.

Lourenção AL, Nagai H, 1994. Outbreaks of Bemisia tabaci in the São Paulo State, Brazil. *Bragantia* **53**, 53–59.

Maluta NKP, Fereres A, Lopes JRS, 2017. Settling preferences of the whitefly vector

Bemisia tabaci on infected plants varies with virus family and transmission mode. *Entomologia Experimentalis et Applicata* **165**, 138–147.

Marubayashi JM, Kliot A, Yuki VA *et al.*, 2014. Diversity and localization of bacterial endosymbionts from whitefly species collected in Brazil. *PLoS ONE* **9**.

Marubayashi JM, Yuki VA, Rocha KCG *et al.*, 2013. At least two indigenous species of the Bemisia tabaci complex are present in Brazil. *Journal of Applied Entomology* **137**, 113–121.

Marubayashi JM, Yuki VA, Wutke EB, 2010. Transmissão do Cowpea mild mottle virus pela mosca branca Bemisia tabaci biótipo B para plantas de feijão e soja. *Summa Phytopathologica* **36**, 158–160.

Mauck KE, Kenney J, Chesnais Q, 2019. Progress and challenges in identifying molecular mechanisms underlying host and vector manipulation by plant viruses. *Current Opinion in Insect Science* **33**, 7–18.

McKenzie CL, Bethke JA, Byrne FJ *et al.*, 2012. Distribution of <I>Bemisia tabaci</I> (Hemiptera: Aleyrodidae) Biotypes in North America After the Q Invasion. *Journal of Economic Entomology* **105**, 753–766.

Moraes LA, Marubayashi JM, Yuki VA *et al.*, 2017. New invasion of Bemisia tabaci Mediterranean species in Brazil associated to ornamental plants. *Phytoparasitica* **45**.

Moraes LA, Muller C, Bueno RCO de F *et al.*, 2018. Distribution and phylogenetics of whiteflies and their endosymbiont relationships after the Mediterranean species invasion in Brazil. *Scientific Reports* **8**, 1–13.

Mugerwa H, Seal S, Wang H *et al.*, 2018. African ancestry of New World , Bemisia tabaci -whitefly species. *Scientific Reports*, 1–11.

Muniyappa V, Reddy DVR, 1983. Transmission of Cowpea Mild Mottle Virus by Bemisia tabaci in a Nonpersistent Manner. *Plant Disease* **67**, 391–393.

Muñiz Y, Granier M, Caruth C *et al.*, 2011. Extensive settlement of the invasive MEAM1 population of bemisia tabaci (Hemiptera: Aleyrodidae) in the caribbean and rare detection of indigenous populations. *Environmental Entomology* **40**, 989–998.

Navas-Castillo J, Fiallo-Olivé E, Sánchez-Campos S, 2011. *Emerging Virus Diseases Transmitted by Whiteflies*.

Pignati WA, Lima FAN de S e, Lara SS de *et al.*, 2017. Distribuição espacial do uso de agrotóxicos no Brasil: uma ferramenta para a Vigilância em Saúde. *Ciência & Saúde Coletiva* **22**, 3281–3293.

Polston JE, De Barro P, Boykin LM, 2014. Transmission specificities of plant viruses with the newly identified species of the *Bemisia tabaci* species complex. *Pest Management Science* **70**, 1547–1552.

Pozebon H, Marques RP, Padilha G *et al.*, 2020. Arthropod Invasions Versus Soybean Production in Brazil: A Review. *Journal of economic entomology* **113**, 1591–1608.

RDevelopment C, 2018. TEAM. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Shi X, Chen G, Pan H *et al.*, 2018a. Plants pre-infested with viruliferous MED/Q Cryptic species promotes subsequent Bemisia tabaci infestation. *Frontiers in Microbiology* **9**, 1–8.

Shi X, Tang X, Zhang X *et al.*, 2018b. Transmission Efficiency, Preference and Behavior of Bemisia tabaci MEAM1 and MED under the Influence of Tomato Chlorosis Virus. *Frontiers in Plant Science* **8**, 1–9.

Škaljac M, Anić K, Hrnčić S, Radonjić S, Perović T, Ghanim M, 2013. Diversity and localization of bacterial symbionts in three whitefly species (Hemiptera: Aleyrodidae) from the east coast of the Adriatic Sea. *Bulletin of Entomological Research* **103**, 48–59.

Souza TLPO, Faria JC, Aragão FJL *et al.*, 2018. Agronomic Performance and Yield Stability of the RNA Interference-Based Bean golden mosaic virus-Resistant Common Bean. *Crop Science* **58**, 579–591.

Sseruwagi P, Legg JP, Maruthi MN, Colvin J, Rey MEC, Brown JK, 2005. Genetic diversity of Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) populations and presence of the B biotype and a non-B biotype that can induce silver-leaf symptoms in squash, in Uganda. *Annals of Applied Biology* **147**, 253–265.

Sun D-B, Liu Y-Q, Qin L, Xu J, Li F-F, Liu S-S, 2013. Competitive displacement between two invasive whiteflies: insecticide application and host plant effects. *Bulletin of Entomological Research* **103**, 344–353.

Thouvenel J, Monsarrat A, Disease CF-P, 1982 U, 1982. Isolation of cowpea mild mottle virus from diseased soybeans in the Ivory Coast. *Plant Disease* **66**, 336–337.

Walsh PS, Metzger DA, Higuchi R, 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* **10**, 506–13.

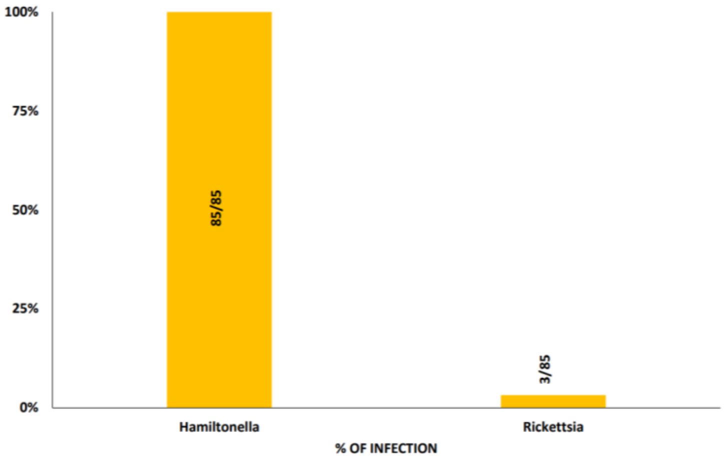
Watanabe LFM, Bello VH, De Marchi BR *et al.*, 2019. Performance and competitive displacement of Bemisia tabaci MEAM1 and MED cryptic species on different host plants. *Crop Protection* **124**.

Watanabe LFM, Bello VH, De Marchi BR, Sartori MMP, Pavan MA, Krause-Sakate R, 2018. Performance of Bemisia tabaci MEAM1 and Trialeurodes vaporariorum on Tomato chlorosis virus (ToCV) infected plants. *Journal of Applied Entomology* **142**.

Yao FL, Zheng Y, Huang XY *et al.*, 2017. Dynamics of Bemisia tabaci biotypes and insecticide resistance in Fujian province in China during 2005-2014. *Scientific Reports* **7**, 1–12.

Zanardo LG, Carvalho CM, 2017. Cowpea mild mottle virus (Carlavirus, Betaflexiviridae): a review. *Tropical Plant Pathology* **42**, 417–430.

Zanardo LG, Silva FN, Bicalho AAC *et al.*, 2014. Molecular and biological characterization of Cowpea mild mottle virus isolates infecting soybean in Brazil and evidence of recombination. *Plant Pathology* **53**, 456–465.



Supplementary figure 1. Secondary endosymbionts found harbouring *Bemisia tabaci* Mediterranean (MED) populations from São Paulo and Paraná States, Brazil. Numbers inside the columns represent the individual positive for the respective endosymbiont by total individuals analyzed.

Supplementary table 1. Endosymbiont composition of whitefly *Bemisia tabaci* Mediterranean (MED) species collected in São Paulo and Paraná State, Brazil, in January and February 2019/2020.

ID	**Collection site**	**Host plant**	**Whitefly species**		**Endosymbionts**					
			MED	**MEAM1**	**H**	**R**	**W**	**C**	**F**	**A**
1a	**Bandeirantes/PR**	***Glycine max (OF)***	**100%**		9/10	1/10 (GenBank 318319)	0/10	0/10	0/10	0/10
1b	**Bandeirantes/PR**	***Glycine max (OF)***	**100%**		10/10 (GenBank 318316)	0/10	0/10	0/10	0/10	0/10
2	Bandeirantes/PR	*Glycine max (OF)*		100%	-*	-	-	-	-	-
3	Bandeirantes/PR	*Glycine max (OF)*		100%	-	-	-	-	-	-
4	Bandeirantes/PR	*Glycine max (OF)*		100%	-	-	-	-	-	-
5	Londrina/PR	*Glycine max (OF)*		100%	-	-	-	-	-	-
6	**Londrina/PR**	***Glycine max (OF)***	**10%**	**90%**	1/1	0/1	0/1	0/1	0/1	0/1
7	Cambé/PR	*Glycine max (OF)*		100%	-	-	-	-	-	-
8	Cambé/PR	*Glycine max (OF)*		100%	-	-	-	-	-	-
9	Cambé/PR	*Glycine max (OF)*		100%	-	-	-	-	-	-
10	Cambé/PR	*Glycine max (OF)*		100%	-	-	-	-	-	-
11	Cambé/PR	*Glycine max (OF)*		100%	-	-	-	-	-	-
12	Cambé/PR	*Glycine max (OF)*		100%	-	-	-	-	-	-
13a	**Oleo/SP**	***Glycine max (OF)***	**100%**		10/10	0/10	0/10	0/10	0/10	0/10
13b	**Oleo/SP**	***Glycine max (OF)***	**100%**		10/10	0/10	0/10	0/10	0/10	0/10
14a	**Oleo/SP**	***Glycine max (OF)***	**100%**		10/10 (GenBank 318318)	0/10	0/10	0/10	0/10	0/10
14b	**Oleo/SP**	***Glycine max (OF)***	**100%**		10/10	0/10	0/10	0/10	0/10	0/10
15	Estiva Gerbi/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
16	Estiva Gerbi/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
17	**Canitar/SP**	***Glycine max (OF)***	**10%**	**90%**	9/9	0/9	0/9	0/9	0/9	0/9

18	**Santa Cruz do Rio Pardo/SP**	***Glycine max (OF)***	**40%**	**60%**	3/4	0/4	0/4	0/4	0/4	0/4
19	**Taciba/SP**	***Glycine max (OF)***	**10%**	**90%**	1/1	1/1	0/1	0/1	0/1	0/1
20	**Rubiácea/SP**	***Glycine max (OF)***	**70%**	**30%**	7/7	0/7	0/7	0/7	0/7	0/7
21	**Buritama/SP**	***Glycine max (OF)***	**100%**		9/10	0/10	0/10	0/10	0/10	0/10
22	**Pindamonhangaba/SP**	***Glycine max (OF)***	**10%**	**90%**	1/1	1/1	0/1	0/1	0/1	0/1
23	Itaí/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
24	Piraju/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
25	Ipaussu/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
26	Assis/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
27	Assis/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
28	Candido Mota/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
29	Palmital/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
30	Vargem Grande do Sul/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
31	Porto Ferreira/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
32	Pirassununga/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
33	Paranapanema/SP	*Glycine max (OF)*		100%						
34	Mogi Mirim/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
35	Mogi Mirim/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
36	Casa Branca/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
37	Luís Antonio/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
38	Colina/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
39	Miguelópolis/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
40	Miguelópolis/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
41	Guaíra/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
42	Barretos/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
43	Ituverava/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
44	Jaboticabal/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
45	Álavares Machado/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-
46	Anhumas/SP	*Glycine max (OF)*		100%	-	-	-	-	-	-

47	Taquarituba/SP	*Glycine max (OF)*	100%	-	-	-	-	-	-
48	Taquarituba/SP	*Glycine max (OF)*	100%	-	-	-	-	-	-
49	Taquarituba/SP	*Glycine max (OF)*	100%	-	-	-	-	-	-
50	Taquarituba/SP	*Glycine max (OF)*	100%	-	-	-	-	-	-
51	Álvares Florence/SP	*Glycine max (OF)*	100%	-	-	-	-	-	-

[*] Populations of whitefly not tested; H: *Hamintonella*, R: *Rickettsia*, W: *Wolbachia*, C: *Cardinium*, A: *Arsenophonus* and F: *Fristchea*.

Supplementary table 2. Detected virus on soybean plants associated to differentwhitefly species.

ID	Collection site	Host plant	Target virus on PCR	Plants tested	Detected virus	Whitefly species
1	Bandeirantes/PR	*Glycine max (OF)*	*Carlavirus*	5	CPMMV[1] (5/5[*])	MED
2	Bandeirantes/PR	*Glycine max (OF)*	*Carlavirus*	5	nd[2]	MEAM1
3	Bandeirantes/PR	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
4	Bandeirantes/PR	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
5	Londrina/PR	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
6	Londrina/PR	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1 + MED
7	Cambé/PR	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
8	Cambé/PR	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
9	Cambé/PR	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
10	Cambé/PR	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
11	Cambé/PR	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
12	Cambé/PR	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
13	Oleo/SP	*Glycine max (OF)*	*Carlavirus*	5	CPMMV (5/5) (GenBank MN318235)	MED

14	Oleo/SP	*Glycine max (OF)*	*Carlavirus*	5	CPMMV (5/5)	MED
15	Estiva Gerbi/SP	*Glycine max (OF)*	*Carlavirus*	5	CPMMV (2/5)	MEAM1
16	Estiva Gerbi/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
17	Canitar/SP	*Glycine max (OF)*	*Carlavirus*	5	CPMMV (4/5)	MEAM1 + MED
18	Santa Cruz do Rio Pardo/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1 + MED
19	Taciba/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1 + MED
20	Rubiácea/SP	*Glycine max (OF)*	*Carlavirus*	5	CPMMV (3/5)	MEAM1 + MED
21	Buritama/SP	*Glycine max (OF)*	*Carlavirus*	5	CPMMV (3/5)	MED
22	Pindamonhangaba/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1 + MED
23	Itaí/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
24	Piraju/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
25	Ipaussu/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
26	Assis/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
27	Assis/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
28	Candido Mota/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
29	Palmital/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
30	Vargem Grande do Sul/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
31	Porto Ferreira/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
32	Pirassununga/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1

33	Paranapanema/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
34	Mogi Mirim/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
35	Mogi Mirim/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
36	Casa Branca/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
37	Luís Antonio/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
38	Colina/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
39	Miguelópolis/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
40	Miguelópolis/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
41	Guaíra/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
42	Barretos/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
43	Ituverava/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
44	Jaboticabal/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
45	Álavares Machado/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
46	Anhumas/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
47	Taquarituba/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
48	Taquarituba/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
49	Taquarituba/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
50	Taquarituba/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1
51	Álvares Florence/SP	*Glycine max (OF)*	*Carlavirus*	5	nd	MEAM1

[1]CPMMV: cowpea mild mottle virus; [2]nd: not detected; [*]number of plants infected/tested

CHAPTER 4

COMPETITIVE INTERACTIONS BETWEEN WHITEFLY AND APHID TRANSMITTED POLEROVIRUSES WITHIN THE PLANT HOST AND THE INSECT VECTORS

Vinicius Henrique Bello[1,2],*, Saptarshi Ghosh[1],*, Renate Krause-Sakate[2] , Murad Ghanim[1]

[1]Department of Entomology, Volcani Center, Rishon LeZion, Israel; [2]UNESP – Faculdade de Ciências Agronômicas, Botucatu, Brazil
* These authors contributed equally to this work

Running title: Competition between two poleroviruses

Corresponding author: Murad Ghanim: ghanim@volcani.agri.gov.il
Available at: https://doi.org/10.1094/PHYTO-08-20-0369-R

Abstract

Pepper cultivation in Israel has recently been constrained by two sympatric poleroviruses, Pepper vein yellows virus-2 (PeVYV-2) and Pepper whitefly-borne vein yellows virus (PeWBVYV) which are transmitted specifically by aphids and whiteflies, respectively. The interaction between PeVYV-2 and PeWBVYV inside the host plant and the insect vectors were investigated in this study. Our results show that PeVYV-2 and PeWBVYV compete against each other inside the host plant and also inside aphids. PeWBVYV was the weaker competitor inside the host plant with diminished transmission rates when inoculated simultaneously or successively after PeVYV-2 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%, however with severely reduced titers of PeWBVYV in the co-infected plants compared to singly infected ones. 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. This is probably due to the reduced titers of PeWBVYV inside co-infected plants causing lesser impact on transmission of PeVYV-2 by aphids and the stronger competitiveness of PeWBVYV inside whitefly. Competitive interactions between PeVYV-2 and PeWBVYV inside the host plant and insect vector can thus be beneficial for their co-existence.

Keywords: Polerovirus, bell pepper, single infection, co-infection, whitefly, aphid, competition, co-existence.

1.1 INTRODUCTION

Most plant viruses depend on insect vectors for transmission to new hosts. However, the transmissibility by insects is highly specific and is maintained by strict conservation of genetic elements determining insect transmission (Power 2000). The viral determinants of insect transmission co-evolve with their insect vectors to maintain specific virus-vector interactions (Lefeuvre et al. 2019), greatly reducing the probability of emergence of new vectors of viruses (Power 2000; Lefeuvre et al. 2019). Transmission of closely related viruses by a single insect species increases the probability of plants to be co-infected with such viruses (Syller 2014). Co-occurrence of viruses within the same host plant or the insect vector may result in synergistic or antagonistic interactions, which influence the spread/transmission of the viruses from the mixed infected plant or insect host (Syller 2012; Moreno and López-Moya 2020). The nature of interaction between viruses co-infecting the same host plant depends upon the genetic similarity between the interacting viruses (Roossinck 2005); viruses with high genetic similarity compete whereas unrelated viruses interact synergistically. Mixed infections of host plants with closely related species of *Barley yellow dwarf virus* (Luteoviridae) are common examples of such virus-virus interactions within the host plants and their aphid vectors (Power 1996; Hall and Little 2013). Competitions inside the host plants can negatively influence the replication of the weaker competitor, while inside the insect vector, the competition may negatively influence its transmission (Power 1996).

Poleroviruses are members of the *Luteoviridae* family with a single stranded positive sense RNA genome (King et al. 2011). Poleroviruses, similar to other luteovirids, are phloem-limited and exclusively transmitted by aphids (Fereres and Raccah 2015). *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; Fiallo-Olivé et al. 2018; Kamran et al. 2018; Ghosh et al. 2019; Pantoja et al. 2019). *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, 2013). However, a disease outbreak in the pepper plantations in Israel with severe symptoms of interveinal yellowing and fruit discoloration as that caused by PeVYVs was recently associated with a new recombinant polerovirus with PeVYV-2 being one parent of this new virus (Ghosh et al. 2019). This new recombinant virus was shown to be transmitted by whiteflies, specifically the whitefly *Bemisia tabaci* (MEAM1), and not aphids, and thus named as Pepper whitefly-borne vein yellows virus (PeWBVYV). PeWBVYV was the first polerovirus reported to be transmitted not by aphids, but whiteflies, and recently, another recombinant polerovirus infecting melons in Brazil has been reported to be transmitted by whitefly (MEAM1) and not aphids (Costa et al. 2020). Pepper plants in Israel can be infected with both PeVYV-2 and PeWBVYV but only as single infections and no mixed infections were detected (Ghosh et al. 2019), albeit only a few samples were analyzed. During January 2019 to February 2020, we further screened 80 additional samples (with and without symptoms) randomly, for the presence of PeVYV-2 and PeWBVYV across Israel and report similar results of mutually exclusive presence of both viruses as before. Thus, in the current study we investigated the relationship between PeVYV-2 and PeWBVYV both inside the host plant and their respective insect vectors aiming to understand the absence of mixed infection with both viruses. The results presented here show that PeVYV-2 and PeWBVYV compete against each other inside the host plant and also inside the insect vector. PeVYV-2 dominates over PeWBVYV inside the host plant, whereas it was the weaker competitor inside the insect vector. The study also shows the conditions under which plants can be co-infected with both viruses. The antagonistic relationships inside both the host plant and insect vector might be an evolutionary strategy by the two poleroviruses to allow coexistence in the same geographical location avoiding competitive exclusion.

1.2 MATERIAL AND METHODS

Maintenance of aphid/whitefly populations and the PeVYV-2 and PeWBVYV isolates

PeVYV-2 and PeWBVYV infected plants naturally infected by their respective insect vectors were used as sources of inoculum. The identity of the infecting viruses and absence of other contaminating virus was ensured by Illumina sequencing of the maintained infected plants used as virus cultures. Additionally, no other whitefly or aphid-transmitted viruses are known to infect pepper in Israel and thus it is unlikely that other viruses transmitted by these two vectors exist in the source plants for inoculum.

Aphid (*Myzus persicae*) population were maintained on healthy and PeVYV-2/PeWBVYV infected bell pepper plants (cv. Cannon), respectively. Similarly, MEAM1 species of *Bemisia tabaci*, harboring the secondary symbiont *Hamiltonella* but free of *Rickettsia* were maintained on healthy and PeWBVYV/PeVYV-2 infected bell pepper plants (cv. Canon), respectively. *Rickettsia* remains undetected in MEAM1 populations in Israel in recent years but 100% of population of is infected with *Hamiltonella* (Brumin et al. 2020).

Competition assays between PeVYV-2 and PeWBVYV within the host plant

Simultaneous inoculation with viruliferous aphids and whiteflies

Viruliferous adults of *B. tabaci* (5-7 days of age) and nymphs of *M. persicae* were collected from the rearing boxes with PeWBVYV and PeVYV-2 infected bell pepper plants, respectively. Twenty individual seedlings of healthy bell pepper plants (cv. Cannon) enclosed inside transparent disposable milkshake cups with lids were inoculated with 20 whitefly adults and aphid nymphs each for seven days under controlled conditions (26 ±5 °C, 14L:10D). After seven days the plants were washed with water to remove all insects, treated with 0.5% (v/v) insecticide (acetamiprid) and stored inside insect proof cages under the above mentioned conditions. One more experiment with 20 additional plants was conducted, thus the total number of plants used in this experiment was 40.

Successive inoculation with viruliferous aphids followed by viruliferous whiteflies

Twenty individual seedlings of disease-free pepper plants were enclosed inside milkshake cups and each were inoculated with twenty viruliferous aphids previously reared on PeVYV-2 infected plants for seven days. The aphids were hence removed from the inoculated plants by brush and the leaves were thoroughly washed. Each seedling was re-inoculated with twenty viruliferous whiteflies previously reared on PeWBVYV plants for seven days. After seven days of inoculation, the plants were washed with water, treated with insecticide and stored in insect proof cages. The experiment was conducted under controlled conditions as described before. The experiment was replicated once with twenty additional seedlings to a total of 40 plants.

Successive inoculation with viruliferous whiteflies followed by viruliferous aphids

Twenty individual seedlings were inoculated with viruliferous whiteflies (PeWBVYV) for seven days followed by re-inoculation with viruliferous aphids (PeVYV-2) for the next seven days as described before. The experiment was replicated once to a total of 40 plants. Control inoculations with viruliferous aphids and whiteflies Twenty plants were inoculated individually with 20 viruliferous aphids and whiteflies separately for seven days using the milkshake cups as described before as control for single infections with PeVYV-2 and PeWBVYV, respectively. The control experiments were replicated once to a total of 40 plants each.

Virus detection and relative quantification of viruses

Total RNA was extracted from the top leaves of inoculated plants (30 days post inoculation, dpi) and screened for the presence of PeVYV-2 and PeWBVYV by two step multiplex RT-PCR detection (Ghosh et al. 2019). RNA samples from confirmed PeVYV-2 and PeWBVYV infected plants were mixed and used as positive control. Plant samples detected with single and double infections with PeVYV-2 and PeWBVYV by the above process were used for relative quantification of the viruses. PeWBVYV and PeVYV-2 were amplified using specific primers (Table 1) and normalized to the β-tubulin gene of the pepper plants. Relative quantities of the virus was calculated by deltadelta CT method. The PCR efficiency of the primers used for qPCR ranged between 97-106%. Significant differences between the mean relative quantities of the viruses were analyzed by Wilcoxon rank-sum test and pair-wise comparisons were made by Tukey's HSD test using the JMP software.

Table 1. Sequences of primers used for qPCR.

Name	Target	Sequence (5'→3')	Size (bp)	Reference
Common-F	PeVYV-2	ACAACTGGAATTCTGCTCTCA	270	(14)
PYLCV-R		TGATGTATGCTTGGCTAGTTATA		
PV-qF	PeWBVYV	GCGGCAACTGTAATAAGCCA	99	This study
PV-qR		GGGAGCCCAAGAGAGAGTTT		
Pep-tubulin F	β tubulin of pepper	GAGGGTGAGTGAGCAGTTC	167	(41)
Pep-tubulin R		CTTCATCGTCATCTGCTGTC		
MP_actin-F	β actin of *M. persicae*	TCGTCTTGGATTCTGGTGATG		(42)
MP_actin-R		GCAAGATCGAGACGAAGGATAG		
BT_actin-F	β actin of *B. tabaci*	TCTTCCAGCCATCCTTCTTG	130	(43)
BT_actin-R		CGGTGATTT CCTTCTGCATT		

Competition assays within the insect vector

Virus infected plant materials (30 dpi) with similar titers of PeVYV-2 and PeWBVYV (Fig. 1) were used for acquisition purposes for this assay. *M. persicae* nymphs reared on PeWBVYV infected bell pepper plants were given acquisition access to PeVYV-2 infected plants for 48 hours. Following this acquisition to PeVYV-2, the aphid nymphs were used for inoculating twenty virus free pepper seedlings (10 insect/plant) for 48 hours using small brushes. Aphids were allowed acquisition/inoculation access period of only 48 hours to study interaction between the viruses within the same generation of aphids. As a positive control, aphids that were given access to PeVYV-2 infected pepper plants for 48 hours, were used to inoculate twenty healthy pepper. Non-viruliferous aphids and aphids reared on PeWBVYV-infected bell pepper plants were used for inoculating healthy pepper plants as negative control.

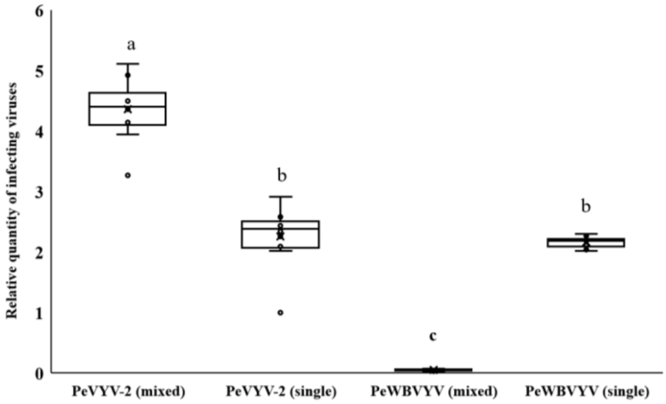


FIG 1 Means of relative quantities of PeVYV-2 and PeWBVYV in singly and mixed infected bell pepper plants. Different lowercase letters indicate significant difference of means (*P* < 0.05).

Newly emerged adults (1-7 days) of *B. tabaci* (MEAM1) reared on PeVYV-2 infected bell pepper plants were given acquisition access to PeWBVYV infected plants for 48 hours. After PeWBVYV acquisition, the whitefly adults were used for inoculating healthy pepper seedlings (10 insects/plant) for 48 hours. As a positive control, whitefly adults that have acquired only PeWBVYV for 48 hours were used for inoculating twenty healthy pepper plants. Non viruliferous whiteflies and whiteflies reared on PeVYV-2 were used for inoculating healthy pepper plants as negative control. Inoculated plants were sprayed with insecticide and stored in insect proof cages.

Acquisition of PeVYV-2 and PeWBVYV by aphids and whiteflies were confirmed by two step multiplex RT-PCR detection of viruses from insects after acquisition. Inoculated plants (30 dpi) were indexed for presence of viruses by multiplex RT-PCR.

Quantification of relative amounts of PeVYV-2 and PeWBVYV in aphids and whiteflies after 48 hours of acquisition access period

RNA (300 ng) extracted from a pool of twenty aphids or twenty whiteflies from the below mentioned experimental setups were used to synthesize first strand cDNA. The relative quantities of both viruses normalized to the β actin gene of the aphid and whitefly (Table 1), respectively were calculated by qPCR using the deltadelta CT method. The significance of difference of means of relative quantities of viruses were analyzed by Wilcoxon rank-sum test with post-hoc comparisons with Tukey's HSD test using the JMP software. The experimental setups are mentioned in Table (2).

Table 2. Experimental treatments used to quantify PeVYV-2/PeWBVYV in aphids and whiteflies after acquisition.

Treatments	Insect vector	Infection status of plants reared on	Acquisition source (48 hr)	Gut clearing on healthy plants (48 hr)
1	Aphids	PeWBVYV	PeVYV-2	-
2	Aphids	Healthy	PeVYV-2	✓
3	Aphids	Healthy	PeWBVYV	✓
4	Aphids	Healthy	PeVYV-2	-
5	Aphids	Healthy	PeWBVYV	-
6	Whitefly	PeVYV-2	PeWBVYV	-
7	Whitefly	Healthy	PeWBVYV	✓
8	Whitefly	Healthy	PeVYV-2	✓
9	Whitefly	Healthy	PeWBVYV	-
10	Whitefly	Healthy	PeVYV-2	-

Transmission efficiency of PeVYV-2 and PeWBVYV from co-infected plants

Virus infected plants obtained from the host plant competition experiments and maintained for six months without insects were used for this study. Two plants singly infected with PeVYV-2 and PeWBVYV and two co-infected each were used as source of acquisition and the relative 195 titers of the viruses were quantified using qPCR (S1). Young aphids with 48 hours of acquisition access to a leaflet of a bell pepper plant co-infected with PeVYV-2 and PeWBVYV were given inoculation access to healthy bell pepper plants (2 replicates of 15 plants each, 20 insects/plant) for five days. A control experiment was performed using aphids with 48 hours of acquisition access to PeVYV-2 infected plants followed by inoculation access to healthy bell pepper plants (2 replicates of 15 plants each, 20 insects/plant).

Similarly, young adult whiteflies were allowed 96 hours of acquisition access to another leaflet from the same bell pepper plant co-infected with PeVYV-2 and PeWBVYV and then given life-long inoculation access to healthy pepper plants (20 insects/plant). A control with whiteflies with 96 hours of acquisition access to PeWBVYV infected plants and then life-long access to healthy bell pepper plants (2 replicates of 15 plants each, 20 insects/plant) was also set up.

The inoculated plants were indexed for PeVYV-2 and PeWBVYV by two step multiplex RT-PCR. Relative amounts of PeVYV-2 and PeWBVYV acquired by aphids and whiteflies, respectively with 48 hours of acquisition access were compared between those with access to plants singly infected with each virus and plants co-infected with both viruses. RNA extracted from pools of 20 insects were converted to cDNA and used as template to quantify relative amounts of virus acquired by whiteflies and aphids from co-infected plants and singly infected plants by q-RT-PCR. Inferences of significant difference were drawn using the Wilcoxon rank sum test.

Screening for recombination events in co-infected plants

Recombination events within the two co-infected bell pepper plants (6 months old, Fig. S1) were screened by PCR-RFLP. Primers were designed (5’AAGCTCGAGTGGCGGCAAT3’ and 5’CTTTGGTGACGGGGAACTT3’) to PCR amplify a common amplicon (1780 bp) from both PeVYV-2 and PeWBVYV. The amplified PCR amplicon ranges from ORF2 (P2) to ORF3 (CP) which covers majority of known recombination breakpoints in the Luteoviridae family (Pagán and Holmes 2010). The PCR product was gel purified and cloned using pJET 1.2 cloning kit (ThermoFisher Scientific, USA). The colonies were screened for insert by colony PCR and 150 random colonies were analyzed by RFLP by *TaqI* restriction endonuclease enzyme as described in (Ghosh et al. 2020). The colonies were also indexed PeVYV-2 and PeWBVYV by multiplex RT-PCR, matched with the RFLP profiles and analyzed for recombination. Colonies with different RFLP profiles were Sanger sequenced and analyzed with RDP4 software for possible recombination events.

Comparison of host range of PeVYV-2 and PeWBVYV

Young aphid nymphs and newly emerged adult whiteflies were given 48 hours and 5 days of acquisition access to PeVYV-2 and PeWBVYV infected bell pepper plants, respectively. Viruliferous aphids and whiteflies were then allowed inoculation access to individual solanaceous plants (14 plants total) inside transparent disposable milkshake cups for seven days. Cultivated solanaceous plants such as bell pepper, hot chilli pepper, eggplant, tomato; and non-cultivated *Solanum nigrum*, *Datura stramonium* and *Physalis peruviana* were inoculated by viruliferous whiteflies and aphids in this study. After inoculation, the plants were sprayed with insecticide and kept in the same controlled conditions described above. The inoculated plants (30 dpi) were indexed for viruses by RT-PCR. Positive tested hosts for each virus (PeWBVYV and PeVYV-2) were used as inoculum source for transmission back to bell pepper plants.

1.3 RESULTS

Competition between PeWBVYV and PeVYV-2 within the host plant

Simultaneous inoculation of healthy pepper seedlings with viruliferous aphids with PeVYV-2 and whiteflies with PeWBVYV, for seven days significantly reduced transmission efficiency of PeVYV-2 by 15% (Fisher’s exact test, P= 0.026) compared to the aphid control which resulted in 100% infection, whereas PeWBVYV transmission was reduced by 80% (Fisher’s exact test, P< 0.0001) compared to that of the whitefly control experiment with 80% infection. Eighty five percent of the plants (34/40) became singly infected with PeVYV-2, but none were detected with PeWBVYV (Table 3).

Table 3. Transmission efficiency of PeVYV-2 and PeWBVYV when co-inoculated with viruliferous aphids and whiteflies at different time points.

Inoculation access by viruliferous vectors	Virus detected (infected plants/total inoculated plants)		
	PeVYV-2	PeWBVYV	Mixed infection
Simultaneous: Aphids and Whitefly	34/40 (85%)	0/40 (0%)	0/40 (0%)
Successive: Aphids succeeded by whitefly	40/40 (100%)	1/40 (2.5%)	1/40 (2.5%)
Successive: Whiteflies succeeded by aphids	32/40 (80%)	32/40 (80%)	22/40 (60%)
Aphids control	40/40 (100%)	-	-
Whitefly control	-	32/40 (80%)	-

Successive inoculation of healthy pepper seedlings with PeVYV-2-viruliferous aphids for seven days followed by PeWBVYV-viruliferous whiteflies for next seven days resulted in 100% (40/40) transmission of PeVYV-2 but only a single plant (2.5%) was detected with PeWBVYV as a double infection with PeVYV-2 (Table 3). PeWBVYV transmission efficiency was thus reduced by 77.5% (Fisher's exact test, $P < 0.0001$) compared to the whitefly control.

Successive inoculation using viruliferous whiteflies with PeWBVYV followed by aphids with PeVYV-2 significantly reduced the transmission of PeVYV-2 by 20% (Fisher's exact test, P = 259 0.005) compared to the aphid control, whereas PeWBVYV transmission was unaffected compared to the whitefly control (Table 3). PeVYV-2 and PeWBVYV were detected in 60% (24/40) of inoculated plants as co-infections (Table 3) while eight plants each were singly infected with PeVYV-2 (20%) and PeWBVYV (20%). The higher occurrence (Fisher's exact test, $P < 0.0001$) of co-infections by both viruses when inoculated first with whiteflies followed by aphids further reflects the weak competitiveness of PeWBVYV over PeVYV-2.

The relative quantities of PeVYV-2 and PeWBVYV in single and mixed infections were then compared using qPCR at 30 dpi. No significant difference could be observed between means of relative quantities of both viruses when the plants were singly infected (Fig. 1). However, relative quantities of PeVYV-2 in co-infected plants was 1.8 times higher than in plants with PeVYV-2 as single infections (Fig. 1). Most interestingly, PeWBVYV quantities in co-infected plants was forty fold lower compared to plants with PeWBVYV single infections and was at least 84 fold lesser than that of the PeVYV-2 in mixed infections with PeWBVYV (Fig. 1).

Competition between PeWBVYV and PeVYV-2 within the insect vectors

Transmission of PeVYV-2 was significantly reduced by 45% (Fisher's exact test, P< 0.0001) when aphids continuously reared on PeWBVYV-infected plants were used for transmitting PeVYV-2. In the inoculated plants, PeVYV-2 could only be detected in 55% (22/40) of the plants compared to the control aphids which were given acquisition access to only PeVYV-2 and then were used for transmission (100% infection) (Table 4).

Table 4. Transmission efficiency of PeVYV-2 and PeWBVYV using aphids and whiteflies with prior acquisition to PeWBVYV and PeVYV-2, respectively.

Acquisition access by insect vectors	Virus detected (infected plants/total inoculated plants)	
	PeVYV-2	PeWBVYV
Aphids control (PeVYV-2 only) 48 hours	40/40 (100%)	0/40 (0%)
Whitefly control (PeWBVYV only) 48 hours	0/40 (0%)	7/40 (17.5%)
Whiteflies on PeVYV-2 and then access to PeWBVYV for 48 hours	0/40 (0%)	5/40 (12.5%)
Aphids on PeWBVYV and then access to PeVYV-2 for 48 hours	22/40 (55%)	0/40 (0%)

Transmission of PeWBVYV however was not significantly affected (Fisher's exact test, P= 0.76) by the prior acquisition of PeVYV-2 compared to the control whiteflies (Table. 4). Whiteflies that acquired both viruses could transmit the virus to only 12.5% (5/40) of the plants, whereas the control whiteflies which acquired PeWBVYV had a slightly better transmission efficiency of 17.5% (7/40) (Table 4).

To further understand the competition between the viruses, we quantified their relative amounts after they have been acquired by the aphids and whiteflies used in the above experiment. Aphids continuously reared on plants infected with PeWBVYV and given subsequent acquisition access to PeVYV-2, contained similar amounts of PeWBVYV as that of aphids given only access to PeWBVYV for 48 hours and then transferred to healthy plants for 48 hours (Fig. 2A). Thus, PeWBVYV titers inside aphids were not affected by subsequent acquisition of PeVYV-2. Contrastingly, the presence of PeWBVYV inside the aphids significantly influenced the relative quantities of PeVYV-2, which was greater than 45 fold less compared to single acquisition of PeVYV-2 (Fig. 2B). Also, aphid controls with 48 hours of acquisition to PeVYV-2 and then transferred to healthy plants for 48 hours contained greater than 30 fold more PeVYV-2 (Fig. 2B) compared to aphids containing both viruses.

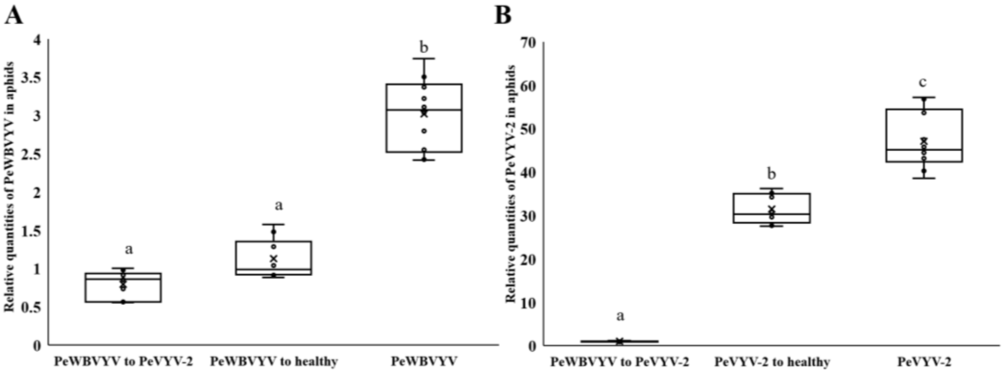


FIG 2 Means of relative quantities of PeWBVYV (A) and PeVYV-2 (B) acquired or contained in viruliferous aphids reared on PeWBVYV-infected plants and then given 48 hour access to PeVYV-2 (PeWBVYV to PeVYV-2), or control aphids reared on healthy plants after 48 hour acquisition from PeWBVYV/ PeVYV-2-infected plants and then transferred to virus free pepper plants for 48 hours (PeWBVYV/PeVYV-2 to healthy), or control aphids which acquired PeWBVYV/PeVYV-2 for 48 hours from infected plants. Different lowercase letters indicate significant difference of mean (*P* < 0.05).

Similar to the aphids, PeWBVYV was not negatively affected by the presence of PeVYV-2 inside whiteflies. Rather, relative quantities of PeWBVYV acquired by whiteflies which were previously reared on PeVYV-2 infected plants was slightly higher compared to the control whiteflies which acquired PeWBVYV alone (Fig. 3A). Surprisingly, PeVYV-2 titers inside whiteflies were reduced by more than seven fold following acquisition of PeWBVYV, compared to the controls in which aphids acquired PeVYV-2 for 48 hours and transferred to healthy plants for 48 hours (Fig. 3B).

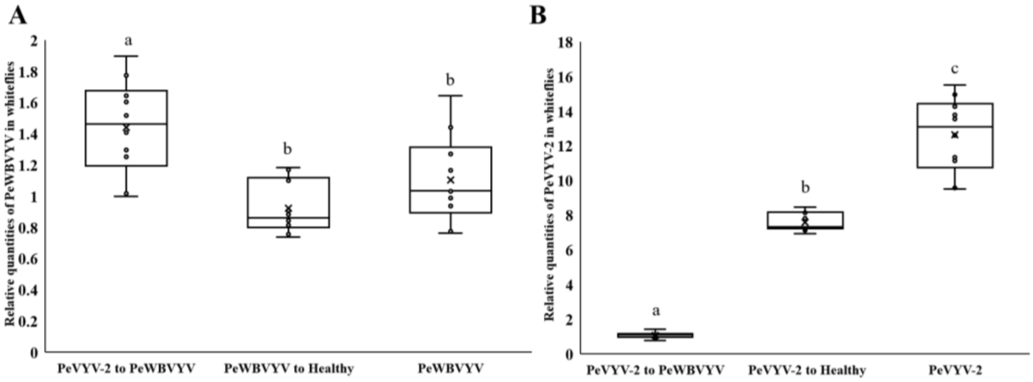


FIG 3 Means of relative quantities of PeWBVYV (A) and PeVYV-2 (B) acquired or contained by viruliferous whiteflies reared on PeVYV-2-infected plants and then given 48 hour access to PeWBVYV-infected plants (PeVYV-2 to PeWBVYV), or control whiteflies reared on healthy plants after 48 hour acquisition from PeWBVYV/ PeVYV-2-infected plants and then transferred to healthy pepper plants for 48 hours (PeWBVYV/PeVYV-2 to healthy), or control whiteflies which acquired PeWBVYV/PeVYV-2 for 48 hours from infected plants. Different lowercase letters indicate significant difference of mean (*P* < 0.05).

Nevertheless, comparing the relative amounts of the viruses acquired by aphids and whiteflies after 48 hours of acquisition revealed high specificity of aphids to PeVYV-2 acquisition, and whiteflies to PeWBVYV acquisition. Aphids acquired 160 fold more PeVYV-2 than PeWBVYV, while whiteflies acquired 40 fold more PeWBVYV than PeVYV-2 (Fig. 4). This implies that whiteflies acquire relatively higher quantities of PeWBVYV than PeVYV-2 and similarly aphids acquire PeVYV-2 in higher amounts than PeWBVYV.

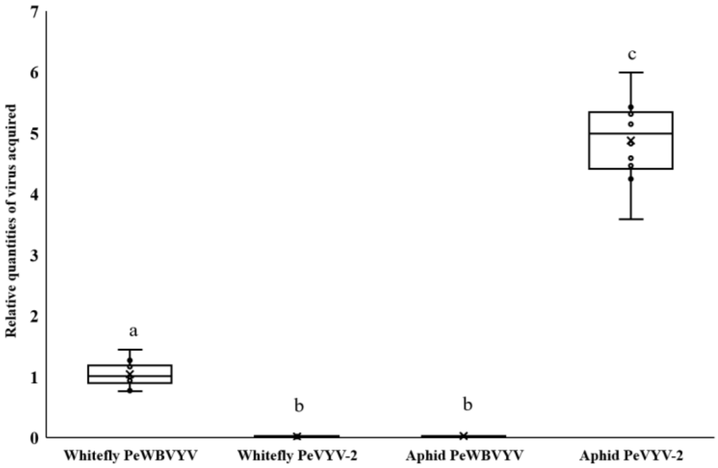


FIG 4 Means of relative quantities of PeVYV-2 and PeWBVYV acquired by non-viruliferous whiteflies or aphids after 48 hours acquisition access to PeVYV-2 or PeWBVYV infected plants. Different lowercase letters indicate significant difference of mean (*P* < 0.05).

Acquisition and transmission efficiency of PeVYV-2 and PeWBVYV from co-infected plants

The co-infected plants (6 months post inoculation) used as source of virus acquisition by aphids and whiteflies had two-fold higher relative quantities of PeVYV2 compared to single infections whereas, the PeWBVYV titer were almost half than that of singly infected plants (Fig. S1). Aphids acquired (48 hours AAP) greater than seven fold higher PeVYV-2 (P<0.01) when feeding on plant infected singly with PeVYV-2 compared to those feeding on co-infected plant (Fig. 5A). Acquisition of PeWBVYV by whiteflies feeding on co-infected plants was reduced by over 140 fold compared to those feeding on PeWBVYV singly infected plants (Fig. 5B). The efficiency of PeVYV-2 transmission after acquisition from singly infected pepper plant (48 hours AAP) was 100% (20/20) (S2A). Similarly, the transmission efficiency of PeVYV-2 was 100% (30/30 plants) using viruliferous aphids which acquired the virus from pepper plants co-infected with PeVYV-2 and PeWBVYV (48 hour AAP) (Fig. 6A). Interestingly, out of the thirty infected plants, one plant was also detected with PeWBVYV (Fig. 6A). Despite the low acquisition, whiteflies which were given access to co-infected plants transmitted PeWBVYV with 96% efficiency (29/30) which was comparable to the 90% (18/20) efficiency of the control whiteflies which were given access to singly infected plants (S2B). Also in this experiment, whiteflies unusually transmitted PeVYV-2 to two plants out of the thirty inoculated plants (Fig. 6B).

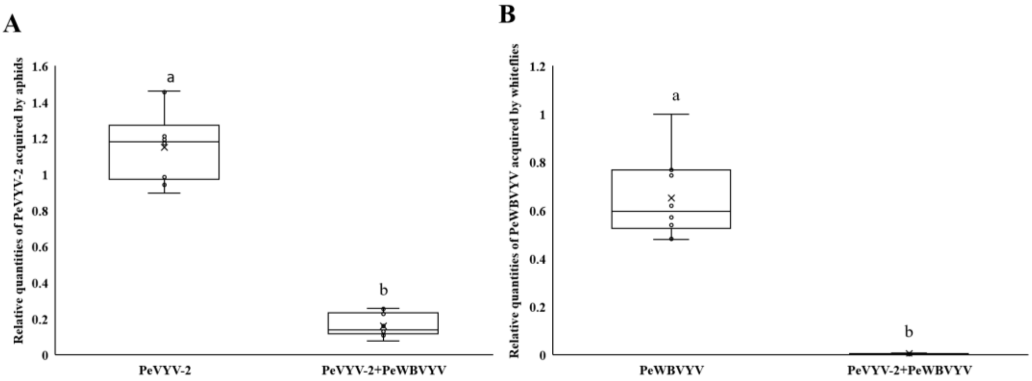


FIG 5 Relative quantities of PeVYV-2 acquired by aphids from single or co-infected plants with both viruses (A), and relative quantities of PeWBVYV acquired by whiteflies from single or co-infected plants (B), both after 48 hours of acquisition. Different lowercase letters indicate significant difference of mean (*P* < 0.05).

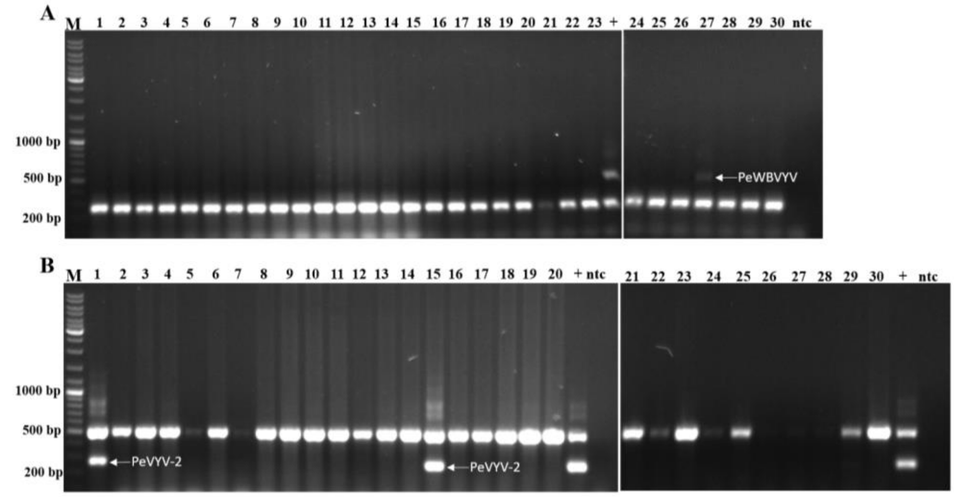


FIG 6 Transmission efficiency of PeVYV-2 by viruliferous aphids (A) and PeWBVYV by viruliferous whiteflies (B) when allowed acquisition access to co-infected plants with both viruses. The arrows indicate PeWBVYV transmitted by aphids (A) and PeVYV-2 (B) transmitted by whiteflies. + indicate positive control which is the plant used for acquisition. ntc is a no template control.

Recombination events inside co-infected plants

To test whether co-infections can serve as reservoirs for recombination events, we screened the co-infected plants (6 months post infection, Fig. S1) used for acquisition for recombinants by PCR-RFLP. A fragment (1780 bp) between ORF2 and ORF3 of both PeVYV-2 and PeWBVYV was PCR amplified from the co-infected plants by common primers and cloned. Screening of random colonies (N=150) by PCR-RFLP of the insert (1780 bp) identified few sequence variants of PeVYV-2 and PeWBVYV but no recombination event between the two viruses could be identified (data not shown). The screened colonies were also typed for the viruses by multiplex RT-PCR and the virus types of the screened colonies also matched with the respective RFLP profile. Thus, only PeVYV-2, PeWBVYV and few sequence variants, and no other recombinant could be detected from co-infected bell pepper plants.

Host plant range of PeVYV-2 and PeWBVYV

To investigate whether the transmission of PeWBVYV by whiteflies increases its host plant range, we compared the transmission of both PeWBVYV and PeVYV-2 from infected bell pepper plants to other commonly found solanaceous plants in Israel. The results showed no increase in host plant range of PeWBVYV, however PeVYV-2 was transmitted to more host plants (Table 5). Interestingly, both PeVYV-2 and PeWBVYV were found to be highly specific to pepper while infected weeds proved to be dead-end hosts of these viruses (Table 5).

Table 5. Host plant range of PeVYV-2 and PeWBVYV.

Inoculations	PeVYV-2 (virus infected/total infected)	PeWBVYV (virus infected/total infected)
Bell pepper to bell pepper	14/14 (100%)	12/14 (90%)
Bell pepper to *S. nigrum*	3/14 (21.4%)	11/14 (85.7%)
S. nigrum to bell pepper	0/14 (0%)	0/14 (0%)
Bell pepper to *P. floridana*	14/14	13/14 (93%)
P. floridana to bell pepper	0/14 (0%)	0/14 (0%)
Bell pepper to *D. stramonium*	14/14 (100%)	0/14 (0%)
D. stramonium to bell pepper	0/14 (0%)	-
Bell pepper to eggplant	0/14 (0%)	0/14 (0%)
Bell pepper to chilli pepper cv HP 1222	14/14 (100%)	1/14 (7.5%)
Chilli pepper to bell pepper	14/14 (100%)	1/14 (7.5%)
Bell pepper to tomato	0/14 (0%)	0/14 (0%)

1.4 DISCUSSION

Pepper vein yellows virus 2 (PeVYV-2) and the recently described Pepper whitefly-borne vein yellows virus (PeWBVYV) are poleroviruses infecting pepper crops in Israel, causing severe economic losses (Dombrovsky et al. 2010; Ghosh et al. 2019). PeVYV-2, like all poleroviruses, is exclusively transmitted by aphids; whereas PeWBVYV is a new recombinant virus with high similarity to PeVYV-2 on its 5' half, and is exclusively transmitted by the whitefly *B. tabaci* (MEAM1) and not by aphids. This presents a unique case wherein, two similar viruses infecting a common host plant but transmitted by different insect vectors and co-exist in the same geographical region. However, infected plants in the field have only been detected with single infections with either PeVYV-2 or PeWBVY and not as mixed infections. Interactions between these two closely related viruses inside their host plant, which were investigated in this study, revealed a competitive relationship between the two viruses with PeVYV-2 being the dominant virus.

Simultaneous inoculation of healthy plants by viruliferous aphids with PeVYV-2 and whiteflies with PeWBVYV, completely hindered transmission of PeWBVYV whereas, transmission of PeVYV-2 was reduced by 15% compared to the control experiment (inoculations by viruliferous aphids only) (Fig. 7A). Successive inoculation of plants with viruliferous aphids first followed by viruliferous whiteflies also resulted in very low transmission (2.5%) of PeWBVYV, but 100% transmission of PeVYV-2 (Fig. 7A). Interestingly, a single plant out of forty (2.5%) was co-infected with both viruses (Fig. 7A) suggesting that mixed infection with both viruses is possible but with very low incidence. Much to our surprise, successive inoculation of plants with viruliferous whiteflies followed by aphids led to sixty percent of the plants being co-infected with PeWBVYV and PeVYV-2 (Fig. 7A). The severely reduced transmission of PeWBVYV when inoculated simultaneously or successively after the PeVYV-2 and also the higher incidence of plants with co-infections when inoculated with PeWBVYV first, clearly demonstrates the dominant nature of PeVYV-2 inside the host plant. The weaker competitiveness of PeWBVYV was further reflected with its severely reduced titers inside the co-infected plants compared to plants with single infections (Fig. 7A). Such competitive interactions between two viruses inside the host plant leading to severely reduced titers of the weaker competitor has been well-documented (Chávez-Calvillo et al. 2016; Domingo-Calap et al. 2020; Gómez et al. 2009).

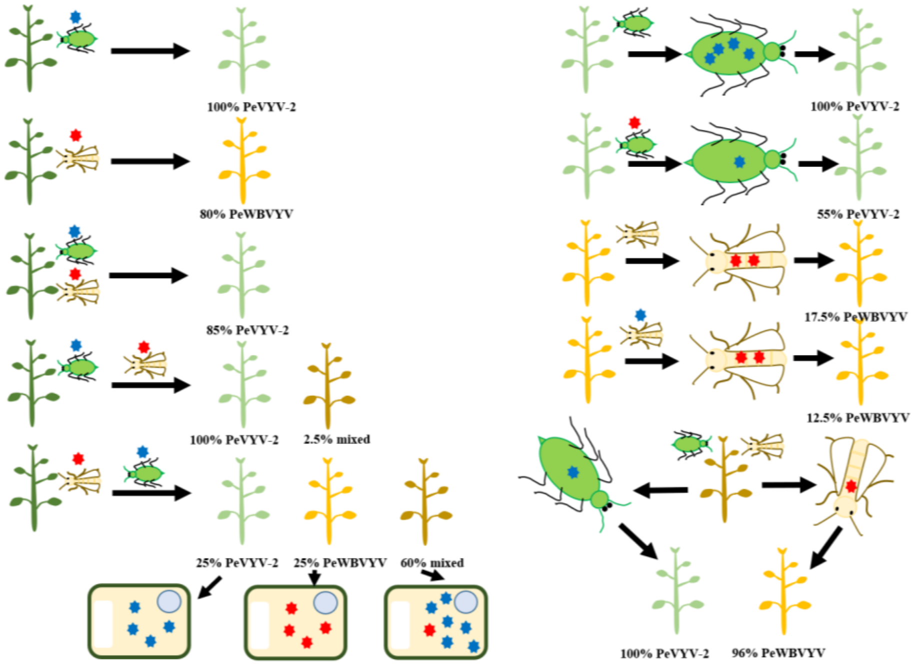


FIG 7. Summary of competitive interactions between PeVYV-2 (blue isometric particle) and PeWBVYV (red isometric particle) inside host plants (A) and the insect vectors (B). Healthy plants (dark green), plants infected with PeVYV-2 (light green), plants infected with PeWBVYV (light yellow), plants co-infected with PeVYV-2 and PeWBVYV (dark yellow) are presented. Virus particles inside insects indicate viruliferous insects. In A, the number of virus particles represent the titers of the respective virus inside the host plants (30 dpi) and insect vectors (48 hour acquisition).

Intra-host competitions between closely related viruses of the *Lutueoviridae* is well known, wherein mixed infections of grasses with different species of Barley yellow dwarf virus (BYDV) is a common event (Hall and Little 2013). Competitive interactions between two species of BYDV-PAV and BYDV-MAV isolates inside oat plants led to competitive exclusion of the latter virus between the years 1957-76 (Rochow 1979) with continued domination of PAV till the late 80’s (Miller et al. 1991). Similar to our results, PAV and MAV offer cross protection to each other of variable strength depending upon the time interval between inoculations of the two viruses (Jedlinski and Brown 1965; Aapola and Rochow 1971; Wen et al. 1991). Additionally, similar to PeWBVYV in this study, titers of MAV are severely reduced when co-occurring with PAV inside the plants (Wen et al. 1991), reducing its chances to be acquired by aphids (Gray et al. 1991). However, it is important to note that PAV is transmitted both by *Rhopalosiphum padi* (*R. padi*) and *Sitobian avenae* (*S. avenae*) while MAV is exclusively transmitted by *S. avenae*. The transmission of PAV by an additional aphid, *R. padi* not only gives it an advantage over MAV, but also reduces the probability of plants to be mixed infected with PAV and MAV which might be more detrimental for the survivability of MAV. Another such example of competitive interactions is that between PAV and PAS (an additional isolate of BYDV), both transmitted by the same aphid (*R. padi*), ensuring competitive exclusion of PAS (weaker competitor) when inoculated simultaneously or successively after PAV, but competes better when inoculated before PAV or under single infections (Hall and Little 2007, 2013). Similarly, if both PeVYV-2 and PeWBVYV were transmitted only by *Myzus persicae* (the most common aphid pest in pepper) then the competition between them would be fiercer and would have possibly led to the competitive exclusion of PeWBVYV. This is in accordance with the competition exclusion principle which states that two populations competing for a common resource cannot coexist without being displaced by the dominant population (Hardin 1960). Thus, the evolutionary choice of two different insect vectors by PeVYV-2 and PeWBVYV could be a strategy to avoid competitive exclusion and increase their chances to co-exist in a given geographical region.

The insect vector transmitting the viruses also plays an important role in the dynamics of the competing viruses. In this study we demonstrate reversal in interacting roles between PeVYV-2 and PeWBVYV inside their insect vector. Presence of PeWBVYV inside the aphids not only reduced the transmission of PeVYV-2 by 45% (Fig. 7B) but also reduced the amounts of PeVYV-2 acquired by the aphids by over forty five fold. Besides the aphid, PeVYV-2 titers also diminished by more than seven fold inside whiteflies after acquisition of PeWBVYV (Fig. 7B). In contrast, PeWBVYV transmission by whiteflies and also virus amounts acquired was unaffected by prior presence of PeVYV-2 inside the whiteflies (Fig. 7B). Although the transmission efficiency of PeWBVYV with 48 hours of acquisition and inoculation access was poor compared to when allowed higher periods of acquisition access periods. Similarly, PeWBVYV titers inside the aphids were also unaffected with the subsequent acquisition of PeVYV-2 by aphids. These results clearly indicate that PeWBVYV is the stronger competitor than PeVYV-2 inside the insect vectors and counterbalances the weak competitiveness of PeWBVYV inside the plant (Fig. 7B). The reasons for the selective advantage of PeWBVYV inside the insects remains unknown. Such reversal of interacting roles between the viruses inside the host plants and their insect vectors is also known with PAV and MAV (Gildow and Rochow 1980; Rochow, W.F.; Muller, I.; Gildow 1983). MAV, the weaker competitor inside the host plant is the stronger contender inside its aphid vector, *S. avenae* and its presence in the aphid inhibits transmission of PAV. Saturation of receptor sites by MAV inside the aphid vector has been proposed as the possible reason for the dominance of MAV over PAV inside the insect vector (Gildow and Rochow 1980). Similar dominance by competition for binding to common aphid receptors has also been suggested for non-circulative viruses such as zucchini yellow mosaic virus (ZYMV), which reduces the transmission of cucumber mosaic virus acquired sequentially after ZYMV (Fereres 2016). The coat proteins of PeVYV-2 and PeWBVYV have high sequence identity (Ghosh et al. 2019) and thus it is likely that they both bind to a common receptor inside midguts (Gildow 1993) of the insect vector which is crucial for their internalization and translocation across the midgut epithelial cells (Brault et al. 2007).

It is possible that PeWBVYV particles have stronger affinity to the midgut receptors and outcompetes binding of PeVYV-2 and thus resulting in lower titers inside the insect vectors. Interestingly, we also provide evidence of low cross-transmission of PeVYV-2 and PeWBVYV by whiteflies and aphids, respectively from co-infected plants. We suggest that this is probably due to heterologous encapsidation between the two viruses inside the co-infected plant host during the assembly of virions. Such swapping of insect vectors as a result of heterologous encapsidation between luteoviruses and poleroviruses inside co-infected plants are known to occur frequently (Rochow 1970; Creamer and Falk 1990; Wen and Lister 1991). However, this has to be experimentally determined.

Despite the dominance of PeVYV-2 over PeWBVYV inside co-infected plants, we show that the transmission efficiency of both these viruses remain unaffected from the co-infected plants by aphids and whiteflies although both vectors acquire reduced amounts of viruses from the co-infected plants. The reduced titers of PeWBVYV inside co-infected plants probably causes lesser hindrance to transmission of PeVYV-2 by aphids, while the stronger competitiveness of PeWBVYV over PeVYV-2 inside whiteflies ensures its transmission, despite low titers. If both PeVYV-2 and PeWBVYV were to be transmitted by aphids, transmission efficiency of PeWBVYV would be severely reduced as evidenced in other cases (Gray et al. 1991; Hall and Little 2013). Thus, the transmission of the two viruses by different vectors not only reduces competition by reducing co-infections, but also rescues them from competitive niches. We also show that the use of whitefly as a vector by PeWBVYV was not able to cause increased host plant range of PeWBVYV as thought in our previous work (Ghosh et al. 2019) and only infected bell pepper plants served as source of inoculum. We thus hypothesize that the use of two diverse vectors by PeVYV-2 and PeWBVYV is to avoid competitive exclusion and still coexist in the same geographical area. Continuous monitoring of the incidence of PeVYV-2 and PeWBVYV in the future will be crucial to understand the dynamics of the two viruses and whether they can co-exist without displacing either population.

Acknowledgements

This work was supported by grant number 20-02-0102 from the chief scientist of the Israeli 465 Ministry of Agriculture.

REFERENCES

Aapola, A. I. E., and Rochow, W. F. 1971. Relationships among three isolates of barley yellow dwarf virus. Virology. 46:127–141. 470

Brault, V., Herrbach, É., and Reinbold, C. 2007. Electron microscopy studies on luteovirid transmission by aphids. Micron. 38:302–312. 472

Brumin, M., Lebedev, G., Kontsedalov, S., and Ghanim, M. 2020. Levels of the endosymbiont *Rickettsia* in the whitefly *Bemisia tabaci* are influenced by the expression of vitellogenin. Insect Mol. Biol. 29:241–255. 475

Chávez-Calvillo, G., Contreras-Paredes, C. A., Mora-Macias, J., Noa-Carrazana, J. C., Serrano-Rubio, A. A., Dinkova, T. D., et al. 2016. Antagonism or synergism between papaya ringspot virus and papaya mosaic virus in Carica papaya is determined by their order of infection. Virology. 489:179–191. 479

Costa, T. M., Inoue-Nagata, A. K., Vidal, A. H., Ribeiro, S. G., and Nagata, T. 2020. The recombinant isolate of cucurbit aphid-borne yellows virus from Brazil is a polerovirus transmitted by whiteflies. Plant Pathol.

Creamer, R., and Falk, B. W. 1990. Direct detection of transcapsidated barley yellow dwarf luteoviruses in doubly infected plants. J. Gen. Virol.

Dombrovsky, A., Glanz, E., Lachman, O., Sela, N., Doron-Faigenboim, A., and Antignus, Y. 2013. The complete genomic sequence of Pepper yellow leaf curl virus (PYLCV) and its implications for our understanding of evolution dynamics in the genus Polerovirus. PLoS 487 One. 8:e70722. 488

Dombrovsky, A., Glanz, E., Pearlsman, M., Lachman, O., and Antignus, Y. 2010. Characterization of Pepper yellow leaf curl virus, a tentative new Polerovirus species causing a yellowing disease of pepper. Phytoparasitica. 38:477–486. 491

Domingo-Calap, M. L., Moreno, A. B., Díaz Pendón, J. A., Moreno, A., Fereres, A., and López-Moya, J. J. 2020. Assessing the impact on virus transmission and insect vector behavior of a viral mixed infection in melon. Phytopathology. 110:174–186.

Fereres, A. 2016. Aphid behavior and the transmission of noncirculative viruses. Vector mediated Transm. plant Pathog. APS Press. St. Paul. :31–45. 496

Fereres, A., and Raccah, B. 2015. Plant Virus Transmission by Insects. In eLS, Chichester, 497 UK: John Wiley & Sons, Ltd, p. 1–12. 498

Fiallo-Olivé, E., Navas-Hermosilla, E., Ferro, C. G., Zerbini, F. M., and Navas-Castillo, J. 499 2018. Evidence for a complex of emergent poleroviruses affecting pepper worldwide. Arch. 500 Virol. :1–8. 501

Ghosh, S., Kanakala, S., Lebedev, G., Kontsedalov, S., Silverman, D., Alon, T., et al. 2019. Transmission of a new polerovirus infecting pepper by the whitefly *Bemisia tabaci* ed. Anne E Simon. J. Virol. 93:e00488-19. 504

Ghosh, S., Sela, N., Kontsedalov, S., Lebedev, G., Haines, L. R., and Ghanim, M. 2020. An intranuclear sodalis-like symbiont and spiroplasma coinfect the carrot psyllid, bactericera trigonica (Hemiptera, psylloidea). Microorganisms. 8. 507

Gildow, F. E. 1993. Evidence for receptor-mediated endocytosis regulating Luteovirus acquisition by aphids. Phytopathology. 83:270. 509

Gildow, F. E., and Rochow, W. F. 1980. Transmission Interference Between Two Isolates of Barley Yellow Dwarf Virus in Macrosiphum avenae. Phytopathology. 70:122.

Gómez, P., Sempere, R. N., Elena, S. F., and Aranda, M. A. 2009. Mixed infections of Pepino mosaic virus strains modulate the evolutionary dynamics of this emergent virus. J. Virol. 83:12378–12387.

Gray, S. M., Power, A. G., Smith, D. M., Seaman, A. J., and Altman, N. S. 1991. Aphid transmission of barley yellow dwarf virus: Acquisition access periods and virus concentration requirements. Phytopathology. 81:539–545. 517

Hall, G. S., and Little, D. P. 2007. Relative quantitation of virus population size in mixed genotype infections using sequencing chromatograms. J. Virol. Methods. 146:22–28.

Hall, G. S., and Little, D. P. 2013. Within-host competition between barley yellow dwarf PAV and-PAS. Virus Res. 174:148–151.

Hardin, G. 1960. The competitive exclusion principle. Science (80). 131:1292–1297.

Jedlinski, H., and Brown, C. M. 1965. Cross protection and mutual exclusion by three strains of barley yellow dwarf virus in Avena sativa L. Virology. 26. 524

Kamran, A., Lotos, L., Amer, M. A., Al-Saleh, M. A., Alshahwan, I. M., Shakeel, M. T., et al. 2018. Characterization of Pepper leafroll chlorosis virus, a New Polerovirus Causing Yellowing Disease of Bell Pepper in Saudi Arabia. Plant Dis. 102:318–326.

King, A. M. Q., Lefkowitz, E., Adams, M. J., and Carstens, E. B. 2011. Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses. Elsevier. 529

Lefeuvre, P., Martin, D. P., Elena, S. F., Shepherd, D. N., Roumagnac, P., and Varsani, A. 2019. Evolution and ecology of plant viruses. Nat. Rev. Microbiol. 17:632–644.

Lotos, L., Olmos, A., Orfanidou, C., Efthimiou, K., Avgelis, A., Katis, N. I., et al. 2017. Insights into the etiology of polerovirus-induced pepper yellows disease. Phytopathology. 107:1567–1576.

Miller, N. R., Bergstrom, G. C., and Gray, S. M. 1991. Identity, prevalence and distribution of viral diseases of winter wheat in New York in 1988 and 1989. Plant Dis. 75:1105–1109.

Moreno, A. B., and López-Moya, J. J. 2020. When viruses play team sports: Mixed infections in plants. Phytopathology. 110:29–48. 538 Pagán, I., and Holmes, E. C. 2010.

Long-term evolution of the Luteoviridae: time scale and mode of virus speciation. J. Virol. 84:6177–6187.

Pantoja, K. F. da C., Rossitto De Marchi, B., Krause-Sakate, R., Rezende, J. A. M., Mituti, T., Ghanim, M., et al. 2019. First report of a putative new pepper vein yellow virus species associated with a vein yellows disease of bonnet pepper plants in Brazil. Plant Dis.

Power, A. G. 1996. Competition between Viruses in a Complex Plant--Pathogen System. Ecology. 77:1004–1010.

Power, A. G. 2000. Insect transmission of plant viruses: a constraint on virus variability. Curr. Opin. Plant Biol. 3:336–340.

Rochow, W.F.; Muller, I.; Gildow, F. E. 1983. Interference between two luteoviruses in an aphid: lack of reciprocal competition. Phytopathology. 73:919–922.

Rochow, W. F. 1970. Barley yellow dwarf virus: Phenotypic mixing and vector specificity. Science (80).

Rochow, W. F. 1979. Field variants of barley yellow dwarf virus: detection and fluctuation during twenty years.

Roossinck, M. J. 2005. Symbiosis versus competition in plant virus evolution. Nat. Rev. Microbiol. 3:917–924.

Syller, J. 2014. Biological and molecular events associated with simultaneous transmission of plant viruses by invertebrate and fungal vectors. Mol. Plant Pathol. 15:417–426.

Syller, J. 2012. Facilitative and antagonistic interactions between plant viruses in mixed infections. Mol. Plant Pathol. 13:204–216.

Wen, F., and Lister, R. M. 1991. Heterologous encapsidation in mixed infections among four isolates of barley yellow dwarf virus. J. Gen. Virol.

Wen, F., Lister, R. M., and Fattouh, F. A. 1991. Cross-protection among strains of barley yellow dwarf virus. J. Gen. Virol. 72:791–799.

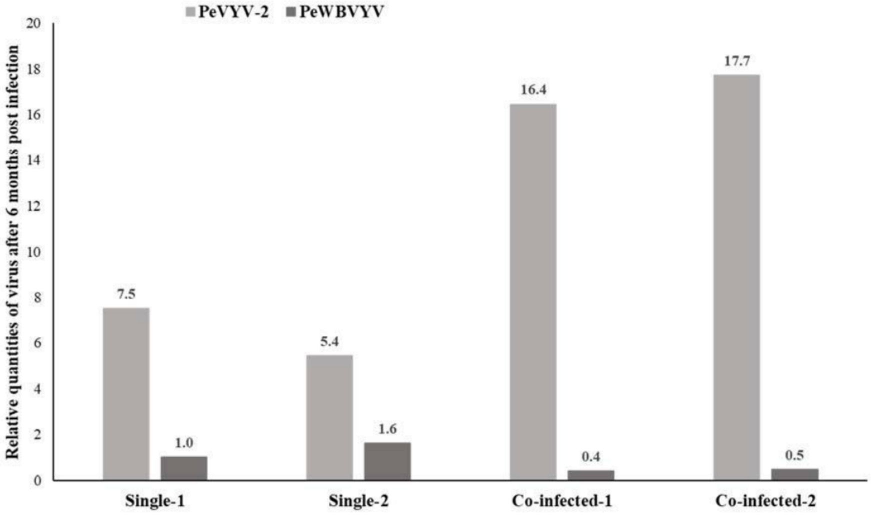


FIG S1 Relative quantities of PeVYV-2 and PeWBVYV in singly/co-infected plants (6 months post inoculation).

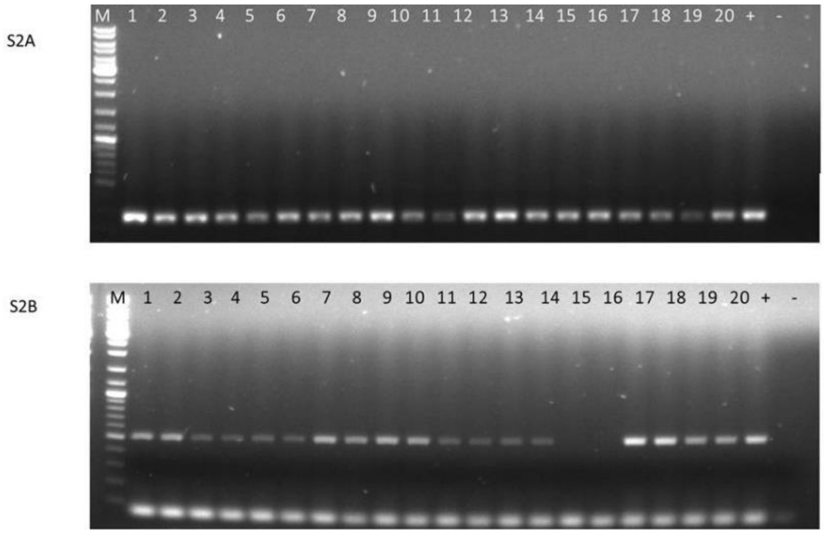


Fig. S2. Transmission efficiency of PeVYV-2 by control aphids (A) and PeWBVYV by control whiteflies (B) when allowed acquisition access to bell pepper plants singly infected with either PeVYV-2 or PeWBVYV, respectively.

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.

REFERENCES

ARNEMANN, J. A. et al. Managing Whitefly on Soybean. **Journal of Agricultural Science**, v. 11, n. 9, p. 41, 2019.

BARBOSA, J. C. et al. **First report of Tomato chlorosis virus infecting tomato crops in BrazilPlant Disease**, dez. 2008. .

BELLO, V. H. et al. Evidence for increased efficiency of virus transmission by populations of Mediterranean species of Bemisia tabaci with high Hamiltonella prevalence. **Phytoparasitica** v. 1, 2019.

CHINTKUNTLA. Survey of Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) biotypes in Brazil using RAPD markers. **Genetics and Molecular Biology**, v. 23, n. 4, p. 781–785, 2015.

DA FONSECA BARBOSA, L. et al. First report of Bemisia tabaci Mediterranean (Q biotype) species in Brazil. **Pest Management Science**, v. 71, n. 4, p. 501–504, 2015.

DE BARRO, P. J. et al. Isolation and characterization of microsatellite loci in Bemisia tabaci. **Molecular Ecology Notes**, v. 3, n. 1, p. 40–43, dez. 2003.

DE BARRO, P. J. et al. *Bemisia tabaci* : A Statement of Species Status. **Annual Review of Entomology**, v. 56, n. 1, p. 1–19, jan. 2011.

DE MARCHI, B. R. et al. Comparative transmission of five viruses by Bemisia tabaci NW2 and MEAM1. **Tropical Plant Pathology**, v. 42, n. 6, p. 495–499, 2017.

DE MORAES, L. A. et al. New invasion of Bemisia tabaci Mediterranean species in Brazil associated to ornamental plants. **Phytoparasitica**, v. 45, n. 4, 2017.

DE MORAES, L. A. et al. Distribution and phylogenetics of whiteflies and their endosymbiont relationships after the Mediterranean species invasion in Brazil. **Scientific Reports**, v. 8, n. 1, 2018.

DOMBROVSKY, A. et al. Characterization of Pepper yellow leaf curl virus, a tentative new Polerovirus species causing a yellowing disease of pepper. **Phytoparasitica**, v. 38, n. 5, p. 477–486, 2010.

FANG, Y. et al. Tomato yellow leaf curl virus alters the tabaci. **Scientific Reports** p. 1–5, 2013.

FIALLO-OLIVÉ, E. et al. Transmission of begomoviruses and other whitefly-borne viruses: Dependence on the vector species. **Phytopathology**, v. 110, n. 1, p. 10–17, 2020.

FIALLO-OLIVÉ, E.; NAVAS-CASTILLO, J. Tomato chlorosis virus, an emergent plant virus still expanding its geographical and host ranges. **Molecular Plant Pathology**, v. 20, n. 9, p. 1307–1320, 1 set. 2019.

FILHO, A. B. et al. Amplifier Hosts May Play an Essential Role in Tomato Begomovirus Epidemics in Brazil. **Frontiers in Plant Sicence** v. 11, n. April, p. 1–6, 2020.

FONSECA, M. E. N. First Report of Tomato chlorosis virus Infecting Eggplant and Scarlet Eggplant in Brazil. **Plant Disease** v. 100, n. 4, p. 11–12, 2016.

GHOSH, S. et al. Transmission of a New Polerovirus Infecting Pepper by the Whitefly Bemisia tabaci . **Journal of Virology**, v. 93, n. 15, p. 1–14, 2019a.

GHOSH, S. et al. Transmission of a New Polerovirus Infecting Pepper by the Whitefly Bemisia tabaci. **Journal of Virology**, v. 93, n. 15, p. 1–14, maio 2019b.

GILBERTSON, R. L. et al. Role of the Insect Supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the Emergence and Global Spread of Plant Viruses. **Annual Review of Virology**, v. 2, n. 1, p. 67–93, 2015. Disponível em: <http://www.annualreviews.org/doi/10.1146/annurev-virology-031413-085410>.

GOUVÊA, M. M. et al. Bioassay of insecticides on mortality of Bemisia tabaci biotype B and transmission of Tomato severe rugose virus (ToSRV) on tomatoes. **Phytoparasitica**, v. 45, n. 1, p. 95–101, 2017. Disponível em: <http://link.springer.com/10.1007/s12600-017-0562-5>.

HOGENHOUT, S. A. et al. Insect Vector Interactions with Persistently Transmitted Viruses. **Annual Review of Phytopathology**, v. 46, n. 1, p. 327–359, 2008. Disponível em: <http://www.annualreviews.org/doi/10.1146/annurev.phyto.022508.092135>.

HOROWITZ, A. R. et al. Biotypes B and Q of Bemisia tabaci and their relevance to neonicotinoid and pyriproxyfen resistance. **Archives of Insect Biochemistry and Physiology**, v. 58, n. 4, p. 216–225, 2005.

HOROWITZ, A. R. et al. Insecticide resistance and its management in Bemisia tabaci species. **Journal of Pest Science**, n. 0123456789, 2020. Disponível em: <https://doi.org/10.1007/s10340-020-01210-0>.

HOROWITZ, A. R.; ISHAAYA, I. Dynamics of biotypes B and Q of the whitefly Bemisia tabaci and its impact on insecticide resistance. **Pest Management Science**, v. 70, n. 10, p. 1568–1572, 2014.

HULL. **Roger Hull (Eds.)-Plant Virology-Academic Press (2014)**. 5th. ed. [s.l: s.n.]

INOUE-NAGATA et al. Vírus transmitidos por moscas-brancas no brasil: vetores, principais doenças e manejo. **Revisão anual de patologia de plantas**, v. 24, p. 7–29, 2016.

INOUE-NAGATA, A. K.; LIMA, M. F.; GILBERTSON, R. L. A review of geminivirus diseases in vegetables and other crops in Brazil: current status and approaches for management. **Horticultura Brasileira**, v. 34, n. 1, p. 8–18, 2016. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-05362016000100008&lng=en&nrm=iso&tlng=en>.

KANAKALA, S. AND GHANIM, M. Global genetic diversity and geographical distribution of Bemisia tabaci and its bacterial endosymbionts. **PlosONE** 2019.

KANAKALA, S.; GHANIM, M. Advances in the Genomics of the Whitefly Bemisia tabaci: An Insect Pest and a Virus Vector. In: [s.l.] Springer International Publishing, 2015. p. 19–40.

KONTSEDALOV, S. et al. Bemisia tabaci Biotype Dynamics and Resistance to Insecticides in Israel During the Years 2008?2010. **Journal of Integrative Agriculture**, v. 11, n. 2, p. 312–320, fev. 2012.

LOTOS, L. et al. Insights into the etiology of polerovirus-induced pepper yellows disease. **Phytopathology**, v. 107, n. 12, p. 1567–1576, 2017.

LOURENÇÃO, A. L.; NAGAI, H. Outbreaks of Bemisia tabaci in the São Paulo State, Brazil. **Bragantia**, v. 53, n. 1, p. 53–59, 1994.

MACEDO, M. A. et al. Temporal and spatial dynamics of begomovirus disease in tomatoes in central Brazil. **Plant Pathology**, v. 66, n. 4, p. 529–538, 2017a.

MACEDO, M. A. et al. First report of common beans as a non-symptomatic host of tomato severe rugose virus in Brazil. **Plant Disease**, v. 101, n. 1, p. 261, 1 jan. 2017b.

MACEDO, M. A. et al. First report of Tomato severe rugose virus, a tomato-infecting begomovirus, in soybean plants in Brazil. **Plant Disease**, v. 101, n. 11, p. 1959, 1 nov. 2017c.

MACEDO, M. A. et al. Characterization of tomato leaf curl purple vein virus, a new monopartite New World begomovirus infecting tomato in Northeast Brazil. **Archives of Virology**, v. 163, n. 3, p. 737–743, 2018. Disponível em: <https://doi.org/10.1007/s00705-017-3662-0>.

MACEDO, M. A. et al. Temporal and spatial progress of the diseases caused by the crinivirus tomato chlorosis virus and the begomovirus tomato severe rugose virus in tomatoes in Brazil. **Plant Pathology**, v. 68, n. 1, p. 72–84, 2019.

MAHADAV, A. et al. Thermotolerance and gene expression following heat stress in the whitefly Bemisia tabaci B and Q biotypes. **Insect biochemistry and molecular biology**, v. 39, n. 10, p. 668–676, 2009.

MARUBAYASHI, J. M.; YUKI, V. A.; WUTKE, E. B. Transmissão do Cowpea mild mottle virus pela mosca branca Bemisia tabaci biótipo B para plantas de feijão e soja. **Summa Phytopathologica**, v. 36, n. 2, p. 158–160, 2010. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-54052010000200009&lng=pt&nrm=iso&tlng=pt>.

MITUTI, T. et al. Survey of begomoviruses and the crinivirus, tomato chlorosis virus, in solanaceous in Southeast/Midwest of Brazil. **Tropical Plant Pathology**, v. 44, n. 5, p. 468–472, 2019.

NAVAS-CASTILLO, J.; FIALLO-OLIVÉ, E.; SÁNCHEZ-CAMPOS, S. **Emerging Virus Diseases Transmitted by Whiteflies**. [s.l: s.n.]v. 49

POLSTON, J. E.; DE BARRO, P.; BOYKIN, L. M. Transmission specificities of plant viruses with the newly identified species of the *Bemisia tabaci* species complex. **Pest Management Science**, v. 70, n. 10, p. 1547–1552, out. 2014.

QUADROS, A. F. F. et al. Two new begomoviruses infecting tomato and Hibiscus sp. in the Amazon region of Brazil. **Archives of Virology**, v. 164, n. 7, p. 1897–1901, 2019. Disponível em: <https://doi.org/10.1007/s00705-019-04245-6>.

REGO-MACHADO, C. M. et al. Complete genome sequence of a new bipartite begomovirus infecting tomato in Brazil. **Archives of Virology**, v. 164, n. 11, p. 2873–2875, 2019. Disponível em: <https://doi.org/10.1007/s00705-019-04380-0>.

SHI, X. et al. Three-Way Interactions Between the Tomato Plant, Tomato Yellow Leaf Curl Virus, and <I>Bemisia tabaci</I> (Hemiptera: Aleyrodidae) Facilitate Virus Spread. **Journal of Economic Entomology**, v. 107, n. 3, p. 920–926, 2014. Disponível em: <https://academic.oup.com/jee/article-lookup/doi/10.1603/EC13476>.

SHI, X. et al. Plants pre-infested with viruliferous MED/Q Cryptic species promotes subsequent Bemisia tabaci infestation. **Frontiers in Microbiology**, v. 9, n. JUN, p. 1–8, 2018.

SILVA, F. da et al. Effects of cowpea mild mottle virus on soybean cultivars in Brazil. **peerj.com**, 2020. Disponível em: <https://peerj.com/articles/9828/>. Acesso em: 12 set. 2020.

SOUZA, T. A. et al. Host range and natural infection of tomato chlorosis virus in weeds collected in Central Brazil. **Tropical Plant Pathology**, p. 84–90, 2020.

SUN, D.-B. et al. Competitive displacement between two invasive whiteflies: insecticide application and host plant effects. **Bulletin of Entomological Research**, v. 103, n. 03, p. 344–353, 2013. Disponível em: <http://www.journals.cambridge.org/abstract_S0007485312000788>.

T. M. COSTA, A. K. INOUE-NAGATA, A. H. VIDA, S. G. RIBEIRO, T. N. The recombinant isolate of cucurbit aphid-borne yellows virus from Brazil is a polerovirus transmitted by whiteflies. **Plant Pathology**, 2020.

WATANABE, L. F. M. et al. Performance of Bemisia tabaci MEAM1 and Trialeurodes vaporariorum on Tomato chlorosis virus (ToCV) infected plants. **Journal of Applied Entomology**, v. 142, n. 10, 2018.

WATANABE, L. F. M. et al. Performance and competitive displacement of Bemisia tabaci MEAM1 and MED cryptic species on different host plants. **Crop Protection**, v. 124, n. June, 2019.

WHITFIELD, A. E.; FALK, B. W.; ROTENBERG, D. Insect vector-mediated transmission of plant viruses. **Virology**, v. 479–480, n. August 2016, p. 278–289, 2015.

XIAO, N. et al. Differential tolerance capacity to unfavourable low and high temperatures between two invasive whiteflies. **Scientific Reports**, v. 6, n. October 2015, p. 1–10, 2016. Disponível em: <http://dx.doi.org/10.1038/srep24306>.

YAO, F. L. et al. Dynamics of Bemisia tabaci biotypes and insecticide resistance in Fujian province in China during 2005-2014. **Scientific Reports**, v. 7, n. December 2016, p. 1–12, 2017. Disponível em: <http://dx.doi.org/10.1038/srep40803>.

ZANARDO, L. G. et al. Molecular and biological characterization of Cowpea mild mottle virus isolates infecting soybean in Brazil and evidence of recombination. **Plant Pathology** p. 456–465, 2014.

ZANARDO, L. G.; CARVALHO, C. M. **Cowpea mild mottle virus (Carlavirus,**

Betaflexiviridae): a reviewTropical Plant Pathology Tropical Plant Pathology, , 2017a. .

ZANARDO, L. G.; CARVALHO, C. M. Cowpea mild mottle virus (Carlavirus, Betaflexiviridae): a review. **Tropical Plant Pathology**, v. 42, n. 6, p. 417–430, 2017b.