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

FCA
FACULDADE DE CIÊNCIAS AGRONÔMICAS
UNESP | BOTUCATU

LUÍS FERNANDO MARANHO WATANABE

DINÂMICA COMPETITIVA DE MOSCAS BRANCAS EM DIFERENTES HOSPEDEIROS E OCORRÊNCIA DO CASSAVA COMMON MOSAIC VIRUS EM MANDIOCA

BOTUCATU

2020

LUÍS FERNANDO MARANHO WATANABE

DINÂMICA COMPETITIVA DE MOSCAS BRANCAS EM DIFERENTES HOSPEDEIROS E OCORRÊNCIA DO CASSAVA COMMON MOSAIC VIRUS EM MANDIOCA

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

Orientadora: Profª. Drª. Renate Krause Sakate

BOTUCATU

2020

W324d

Watanabe, Luís Fernando Maranho
Dinâmica competitiva de moscas brancas em diferentes hospedeiros e ocorrência do cassava common mosaic virus em mandioca / Luís Fernando Maranho Watanabe. -- Botucatu, 2020
89 p.

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

1. Mosca-branca. 2. Performance, competitividade, transmissão de vírus. 3. Mandioca: Levantamento e variabilidade de vírus. 4. Fitopatologia. 5. Proteção de Plantas. 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
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

FCA
FACULDADE DE CIÊNCIAS AGRONÔMICAS
UNESP | BOTUCATU

CERTIFICADO DE APROVAÇÃO

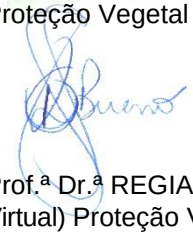
Título: DINÂMICA COMPETITIVA DE MOSCAS BRANCAS EM DIFERENTES HOSPEDEIROS E OCORRÊNCIA DO CASSAVA COMMON MOSAIC VÍRUS EM MANDIOCA.

AUTOR: LUÍS FERNANDO MARANHO WATANABE

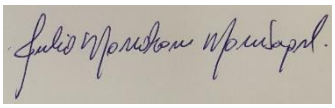
ORIENTADORA: RENATE KRAUSE SAKATE

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

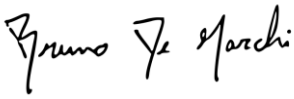
Prof.ª Dr.ª RENATE KRAUSE SAKATE (Participaçao Virtual)
Proteção Vegetal /Faculdade de Ciências Agronômicas de Botucatu - UNESP



Prof.ª Dr.ª REGIANE CRISTINA OLIVEIRA DE FREITAS BUENO (Participaçao Virtual) Proteção Vegetal / Faculdade de Ciências Agronômicas de Botucatu - UNESP



Dr. JULIO MASSAHARU MARUBAYASHI (Participaçao Virtual)



Prof. Dr. BRUNO ROSSITTO DE MARCHI (Participaçao Virtual) Research & Education Center / University of Florida Gulf Coast

Pesquisadora Dr.ª CRISTIANE MÜLLER (Participaçao Virtual) Entomologia / Corteva Agriscience

Botucatu, 16 de dezembro de 2020.

Aos meus queridos e amados pais,

Isabel e João,

Dedico

AGRADECIMENTOS

A Deus.

Aos meus pais, Isabel Maranho Watanabe e João Mikio Watanabe, e à minha irmã Ana Paula Maranho Watanabe, pelo amor e carinho incondicionais, apoio, incentivo, conselhos e por sempre estarem ao meu lado em todos os momentos.

À Professora Doutora Renate Krause Sakate, pela orientação, confiança, amizade, ensinamentos, apoio e incentivo que me forneceu durante todo o período da minha formação acadêmica.

Aos meus amigos de laboratório e de departamento Felipe Barreto da Silva, Vinicius Henrique Bello, Eduardo Silva Gorayeb, Marcos Roberto Ribeiro Junior, Daniele Nascimento, Juliana Uzan, Angélica Maria Nogueira, Julio Massaharu Marubayashi, Eduardo Vicentin, Leonardo Hipólito Dovigo, Flávio Nunes da Silva, Guilherme Augusto Gotardi e Daniel de Lima Alvarez por todos os momentos de ajuda, conversas, desabafos, momentos de descontração, pela amizade, que não serão esquecidos jamais.

À Júlia Carolina de Barros, minha namorada, pelo amor, amizade e companheirismo, por me apoiar e confiar sempre em mim, e me dar forças em todos os momentos.

To Dr. Francisco Ochoa-Corona for the support, confidence, friendship, patience, and teachings during my Ph.D. sandwich at Oklahoma State University (OSU), Stillwater.

To the researcher Andrés and to my friends from OSU Liz, Andrea, Patrick and Felipe for the friendship, support, lunches, and all moments spent together.

À família brasileira que tive durante o doutorado sanduíche em Stillwater, em especial ao Sr. Rolf e Sra. Bia, por me incluírem nesse grupo, e aos meus amigos Leonardo, João Arthur, Evandro, João Luís e Olívia pelos momentos de descontração, conversas, amizade e por me acolherem tão bem.

To my housemates Jun and Saad for the opportunity to share experiences in living together and for the conversations.

Aos Pesquisadores e Professores que me ajudaram muito durante todo meu ciclo acadêmico e que serão sempre lembrados com carinho: Bruno Rossitto de Marchi, Regiane C. F. Bueno, Cristiane Müller, Carlos Gilberto Raetano, Jorge Alberto Marques Rezende, Edson Luiz Lopes Baldin, Antônio Carlos Maringoni, Marcelo Agenor Pavan, Valdir Atsushi Yuki

A todos os funcionários da UNESP Botucatu e da Oklahoma State University.

À CAPES, pelo financiamento da minha bolsa de doutorado no Brasil e de doutorado sanduíche PDSE nos EUA.

RESUMO

As moscas-brancas causam danos diretos através da sucção de seiva do floema e danos indiretos, relacionados à secreção de "*honeydew*" e à transmissão de vírus. Entre as espécies presentes no Brasil, o complexo *Bemisia tabaci*, o qual inclui as espécies crípticas MEAM1, MED e do grupo NW, bem como a espécie *Trialeurodes vaporariorum* são as mais importantes. Atualmente, MEAM1 e MED possuem maior destaque no cenário brasileiro por serem altamente polífagas, possuírem alta capacidade adaptativa e de dispersão. Por isso, compreender o desempenho e a habilidade competitiva dessas duas espécies nas principais plantas cultivadas no Brasil é de fundamental valor. As moscas-brancas também são vetores de diversos vírus, incluindo o crinivirus tomato chlorosis virus (ToCV). As espécies MED, MEAM1, NW e *T. vaporariorum* são vetores desse vírus. Por *T. vaporariorum* ser mais comum em locais de temperaturas mais amenas e ser menos encontrada, poucos estudos acabam sendo realizados com essa mosca-branca envolvendo o ToCV, não havendo informação de como o vírus age sobre o inseto quanto ao desempenho. O potexvirus cassava common mosaic virus (CsCMV) é o principal vírus em mandioca reportado no Brasil. Até hoje, porém, não há informação sobre a incidência no Estado de São Paulo, sobre a variabilidade dos diferentes isolados presentes, nem sobre uma comparação dos genomas presentes no banco de dados. Sendo assim, esta tese traz, no Capítulo 1 dados sobre a performance e competitividade entre MEAM1 e MED em soja, algodão, feijão, tomate e pimentão, onde MEAM1 é mais adaptada ao tomateiro, MED é mais adaptada ao pimentão e ao feijoeiro e, no caso de soja e algodão, ambas aparentam se adaptar de forma semelhante; no Capítulo 2, dados sobre a performance de MEAM1 e *T. vaporariorum* em tomateiros sadios e infectados por ToCV, bem como a eficiência de transmissão de cada vetor para esse vírus, onde o vírus influencia negativamente ambas as espécies, porém mais gravemente MEAM1. E que MEAM1 é melhor vetor de ToCV; no Capítulo 3, um levantamento sobre a incidência do CsCMV no campo, assim como a variabilidade do genoma completo de um isolado brasileiro entre os isolados presentes no GenBank e; no Capítulo 4, descrição dos trabalhos realizados durante o intercâmbio na Oklahoma State University, Stillwater, nos Estados Unidos.

Palavras-chave: *Bemisia tabaci*. *Trialeurodes vaporariorum*. Soja. Algodão. Feijão. Tomate. Pimentão. RdRp. CP. Genoma completo

ABSTRACT

Whiteflies cause direct damage by sucking phloem sap and indirect damage, related to the secretion of “honeydew” and virus transmission. Among the species present in Brazil, the most important are the *Bemisia tabaci* complex, which includes the cryptic species MEAM1, MED and NW group as well as the species *Trialeurodes vaporariorum*. Currently MEAM1 and MED are more prominent in the Brazilian scenario because they are polyphagous and have an adaptive and dispersion capacity. Therefore, understanding the performance and competitive ability of these two species in the main crops grown in Brazil is of essential. Whiteflies are also vectors of several viruses, including the crinivirus tomato chlorosis virus (ToCV). The species MED, MEAM1, NW and *T. vaporariorum* are vectors of this virus. For being more common in places with milder temperatures and being less found, few studies have been performed with this relationship *T. vaporariorum* and ToCV, having a lack of information about the performance. The potexvirus cassava common mosaic virus (CsCMV) is the main cassava virus reported in Brazil. To date, however, there is no information on its incidence in São Paulo State, and the variability of the different isolates present. Therefore, this thesis brings, in Chapter 1, data of the performance and competitiveness between MEAM1 and MED in soybean, cotton, bean, tomato and pepper, where MEAM1 is more adapted to tomato, MED is more adapted to pepper and bean and, both seem to adapt similarly for soybean and cotton; in Chapter 2, data on the performance of MEAM1 and *T. vaporariorum* on healthy and ToCV-infected tomatoes, as well as the transmission efficiency of each vector for this virus, where MEAM1 is more negatively affected by ToCV than *T. vaporariorum*, but it is more efficient as a vector; in Chapter 3, high incidence of CsCMV in the field, as well as the variability of the complete genome of a Brazilian isolate among the isolates present on GenBank; and in Chapter 4, the description of the works performed during the PhD Sandwich at Oklahoma State University, Stillwater, USA.

Keywords: *Bemisia tabaci*. *Trialeurodes vaporariorum*. Soybean. Cotton. Common bean. Tomato. Sweet pepper. RdRp. CP. Complete genome

LISTA DE ILUSTRAÇÕES

CAPÍTULO 1

CAPÍTULO 2

CAPÍTULO 3

LISTA DE TABELAS

CAPÍTULO 1

CAPÍTULO 2

CAPÍTULO 3

LISTA DE ABREVIATURA E SIGLAS

CP	Coat protein
CsCMV	Cassava common mosaic virus
ICTV	International Committee on Taxonomy of Viruses
MEAM1	Middle East-Asia Minor 1
MED	Mediterranean
mtCOI	mitochondiral cytochrome oxidase I
NW	New World
RdRp	RNA dependent RNA polymerase
ToCV	Tomato chlorosis virus

SUMÁRIO

INTRODUÇÃO GERAL

O cultivo sucessivo de diversas culturas e condições de clima tropical no Brasil são favoráveis ao desenvolvimento de pragas e doenças, onde problemas fitossanitários foram se tornando cada vez mais frequentes, dos quais as moscas-brancas são de grande relevância. Dentre as moscas-brancas, o complexo *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) e a espécie *Trialeurodes vaporariorum* (Westwood) são as que possuem maior destaque. No complexo *B. tabaci* há espécies crípticas que apresentam alta polifagia, baixa suscetibilidade a inseticidas e eficiente dispersão no campo (GILBERTSON et al., 2015; KANAKALA; GHANIM; KANAKALA, S. AND GHANIM, 2019). A espécie *T. vaporariorum*, conhecida como a mosca-branca de casa de vegetação é comum em climas mais amenos e regiões de altitude (MANZANO; VAN LENTEREN, 2009).

Ambas podem ocasionar danos diretos, através da sucção de seiva do floema e indiretos através da secreção de "*honeydew*", uma substância açucarada que pode servir de substrato para crescimento de fungo do gênero *Capnodium* sobre as folhas e frutos, prejudicando a fotossíntese e, consequentemente, o desenvolvimento da planta (SUEKANE et al., 2013), e pela transmissão de vírus. Entretanto, os principais prejuízos causados pelas moscas-brancas consistem na transmissão de vírus. O complexo *B. tabaci* é capaz de transmitir vírus dos gêneros *Begomovirus*, *Crinivirus*, *Carlavirus*, *Ipomovirus*, *Torradovirus* e *Polerovirus*. (COSTA et al., 2020; GHOSH et al., 2019; GILBERTSON et al., 2015; NAVAS-CASTILLO; FIALLO-OLIVÉ; SÁNCHEZ-CAMPOS, 2011), enquanto *T. vaporariorum* é vetor de vírus dos gêneros *Crinivirus* e *Torradovirus* (NAVAS-CASTILLO; FIALLO-OLIVÉ; SÁNCHEZ-CAMPOS, 2011). No Brasil, já foram relatados vírus pertencentes aos gêneros *Begomovirus*, *Crinivirus*, *Carlavirus* e *Polerovirus* (BARBOSA et al., 2008; COSTA; GASPAR; VEGA, 1983; COSTA et al., 2020; INOUE-NAGATA et al., 2016; INOUE-NAGATA; LIMA; GILBERTSON, 2016), onde as principais plantas afetadas pertencem às famílias das Solanáceas e Fabáceas.

Insetos do complexo *B. tabaci* foram, inicialmente, classificados em biótipos considerando-se características como adaptação a diferentes hospedeiros, produção de toxinas como o prateamento das folhas de aboboreira, e a transmissão de vírus (BROWN; FROHLICH; ROSELL, 1995). Mais recentemente *B. tabaci* foi considerada um complexo de espécies crípticas morfologicamente indistinguíveis (DE BARRO et

al., 2011), porém diferenciados pela análise molecular da porção do gene mitocondrial denominado *mitochondrial cytochrome oxidase subunit I* (mtCOI). As principais espécies crípticas presentes no Brasil são Middle East-Asia Minor 1 (MEAM1 ou biótipo B), Mediterranean (MED ou biótipo Q) e New World (NW ou biótipo A). A origem geográfica de MEAM1 é provavelmente a região do oriente médio, enquanto MED a região do mediterrâneo e NW as américas (novo mundo) (DE BARRO et al., 2011). Atualmente, o complexo conta com ao menos 44 espécies crípticas (KANAKALA; GHANIM; KANAKALA, S. AND GHANIM, 2019).

No Brasil, *B. tabaci* foi primeiramente reportada no estado da Bahia na década de 1920 (BONDAR, 1928). Até os anos de 1970, com a expansão da cultura da soja e consequentes surtos populacionais de mosca-branca (COSTA, 1975), este inseto era considerado praga secundária, causando poucos prejuízos e danos. Acredita-se que, até início da década de 1990, os espécimes de *B. tabaci* presentes eram os nativos das Américas, denominados de biótipo A (BROWN; FROHLICH; ROSELL, 1995) e reclassificados, através da análise do gene mtCOI, como New World 1 (NW1) e New World 2 (NW2) utilizando-se o critério de 3,5% de divergência na sequência do mtCOI (MARUBAYASHI et al., 2013). Todavia, alguns trabalhos sugerem um critério com variação de 4% (LEE et al., 2013) e de 2,48 a 4,77% (VYSKOČILOVÁ et al., 2018) de divergência na sequência do mtCOI para diferenciação de espécies, de modo que, no caso de NW1 e NW2 ambas formariam um único grupo, denominado New World (NW).

Em meados da década de 1990, o cenário de mosca-branca no Brasil foi radicalmente alterado com a introdução da *B. tabaci* MEAM1, então conhecido por biótipo B, através do comércio de plantas ornamentais no estado de São Paulo (LOURENÇÃO; NAGAI, 1994). Desde então, essa espécie se dispersou rapidamente pelo território nacional sendo importante fator na disseminação e emergência de vírus. Vírus transmitidos por mosca-branca haviam sido relatados no Brasil desde 1960 em plantas daninhas (COSTA, 1955; COSTA; BENNETT, 1950; FLORES; SILBERSCHMIDT; KRAMER, 1960), em feijoeiro como o bean golden mosaic virus (BGMV) e o cowpea mild mottle virus (CPMMV) (COSTA, 1975; COSTA; GASPAR; VEGA, 1983) e o tomato golden mosaic virus em tomateiro (TGMV) (COSTA; OLIVEIRA; SILVA, 1975; MATYIS et al., 1975). Porém a introdução de MEAM1 permitiu a emergência de ao menos 18 novas espécies de begomovirus em tomateiro (ALBUQUERQUE et al., 2012; BARBOSA et al., 2011; CASTILLO-URQUIZA et al.,

2008; FERNANDES et al., 2006; INOUE-NAGATA et al., 2016; INOUE-NAGATA; LIMA; GILBERTSON, 2016; MACEDO et al., 2018; QUADROS et al., 2019; RIBEIRO et al., 2003; ROCHA et al., 2013), do crinivirus tomato chlorosis virus (ToCV) em tomateiro, pimentão e batateira (BARBOSA et al., 2008; BARBOSA; TEIXEIRA; REZENDE, 2010; FREITAS et al., 2012), além da transmissão do CPMMV para a soja (ALMEIDA et al., 2003) e BGMV em feijoeiro (INOUE-NAGATA et al., 2016).

Duas décadas mais tarde, a espécie críptica MED, também conhecida por biótipo Q, foi relatada pela primeira vez no Rio Grande do Sul, próxima à fronteira com o Uruguai (BARBOSA et al., 2015) e posteriormente, uma segunda introdução de MED foi reportada no estado de São Paulo, provavelmente associada ao comércio de plantas ornamentais (MORAES et al., 2017). A partir de então, MED se disseminou rapidamente ao longo da região sul e sudeste do país, tornando-se preocupante em várias áreas produtoras de hortaliças e plantas ornamentais (DE MORAES et al., 2018). No estado de São Paulo e no Paraná, algumas áreas comerciais de hortaliças, apresentaram altas infestações desta espécie, comprometendo a produção em estufas (BELLO et al., 2020). Além disso, também houve o primeiro relato de ToCV infectando pepino associado à MED (BELLO et al., 2019).

Atualmente, MEAM1 ainda é considerada a espécie predominante de mosca-branca presente no país (DE MORAES et al., 2018), porém, MED vem se destacando e se disseminando de forma acelerada, especialmente em hortaliças. Diante disso, tornou-se importante compreender o comportamento, com estudos de desempenho e habilidade competitiva de MED e MEAM1 em culturas de importância agrícola no país, como o feijoeiro, soja, algodão e hortaliças como pimentão e tomateiro.

Por outro lado, a espécie *T. vaporariorum* é encontrada no Brasil em regiões de elevadas altitudes e/ou em regiões de temperaturas mais amenas, como é o caso da região Sul e parte da região Sudeste do país (DE MORAES et al., 2018). Apesar de sua presença estar associada frequentemente a casas de vegetação, surtos populacionais de *T. vaporariorum* já ocorreram em campos no Brasil (LOURENÇÃO et al., 2008).

Pelo fato de ambos, o complexo *B. tabaci* e *T. vaporariorum* transmitirem o ToCV, esse vírus vem ganhando um destaque importante desde seu relato. O ToCV possui partículas alongadas e flexuosas, de 800 – 850 nm de comprimento, constituídas por duas moléculas de RNA de fita simples, senso positivo (LIU; WISLER;

DUFFUS, 2000). Seu primeiro relato ocorreu nos Estados Unidos (WISLER et al., 1998).

Nas últimas duas décadas, o ToCV se disseminou pela América do Norte, América do Sul, Europa, África, Oriente Médio e Ásia (ACCOTTO et al., 2001; ARRUABARRENA et al., 2014; FIALLO-OLIVÉ et al., 2011; HIROTA et al., 2010; SEGEV et al., 2004; WISLER et al., 1998). Atualmente, o ToCV é encontrado em 23 países (INOUE-NAGATA et al., 2016; NAVAS-CASTILLO; FIALLO-OLIVÉ; SÁNCHEZ-CAMPOS, 2011). No Brasil, o primeiro relato do ToCV ocorreu em tomateiro, no Estado de São Paulo (BARBOSA et al., 2008). Além do tomateiro, o ToCV também foi relatado infectando pimentão (NAVAS-CASTILLO et al., 2011), batateira (FORTES; NAVAS-CASTILLO, 2012; FREITAS et al., 2012), berinjela (*Solanum melongena*), jiló (*Solanum aethiopicum*) (FONSECA et al., 2016) e pepino (*Cucumis sativus*) (BELLO et al., 2020). Os sintomas causados pelo vírus são característicos de uma clorose internerval nas folhas do baixeiro da planta, podendo ocorrer bronzeamento, enrolamento das folhas voltadas para cima e engrossamento do limbo foliar (INOUE-NAGATA et al., 2016). Além disso, o ToCV pode causar danos expressivos na produção de tomateiro, chegando a 100% (LOZANO; MORIONES; NAVAS-CASTILLO, 2006; VELASCO et al., 2008). Em pimentão, o ToCV já foi associado a perdas na produção de até 75% de acordo com o genótipo (FORTES; MORIONES; NAVAS-CASTILLO, 2012). No Brasil, os danos decorrentes do ToCV são redução na produção de até 52% de acordo com o genótipo de tomateiro (MANSILLA-CÓRDOVA et al., 2018).

Como ambos *T. vaporariorum* e *B. tabaci* são vetores do crinivirus ToCV, alguns estudos sobre eficiência de transmissão já foram reportados, onde *B. tabaci* MEAM1 é um vetor mais eficiente do ToCV que *T. vaporariorum* (WINTERMANTEL; WISLER, 2006). Pelo fato de *T. vaporariorum* ser menos frequentemente encontrada que MEAM1, e até confundida com este inseto, esta tese teve como um dos objetivos estudar a interação entre esse inseto e o ToCV.

O terceiro tópico abordado nesta tese foi o vírus cassava common mosaic virus (CsCMV) em mandioca. A mandioca (*Manihot esculenta* Crantz), atrás do trigo, milho e arroz, é a quarta fonte de calorias mais importante no mundo, garantindo alimento para mais de 800 milhões de pessoas (LEGG et al., 2014, 2015). Na América do Sul, o Brasil se destaca como maior produtor da mandioca, chegando a 20 milhões de toneladas em 2018 (CONAB, 2018). De forma geral, a cultura da mandioca é cultivada

em áreas desfavoráveis à agricultura, sem manutenção adequada do solo e por pequenos produtores que não possuem grande recurso para investimento.

Referentes a problemas fitossanitários da mandioca, os vírus que se destacam especialmente na África, onde o cassava mosaic virus (CMV) e o cassava brown streak virus (CBSV) são os mais importantes da cultura (LEGG et al., 2014, 2015). No Brasil, até o momento foram relatados o cassava common mosaic virus (CsCMV) (COSTA, 1940; KITAJIMA et al., 1965; SILVA; KITAJIMA; OLIVEIRA, 1963), cassava vein mosaic virus (CsVMV) (COSTA, 1940; DE KOCHKO et al., 1998), cassava frogskin-associated virus (CsFSaV) (CALVERT et al., 2008), cassava symptomless virus (CsSLV) (KITAJIMA; COSTA, 1979) e cassava American latent virus (CsALV) (WALTER et al., 1989).

O CsCMV, pertencente ao gênero *Potexvirus*, é constituído de um RNA de fita simples (ssRNA) com uma partícula semi flexuosa com medidas de 15 nm × 495 nm (KITAJIMA et al., 1965). É o vírus mais relevante na cultura da mandioca no Brasil. Os danos causados pelo CsCMV podem chegar em torno de 30% na produtividade da parte aérea, da raiz e do amido (VENTURINI et al., 2016). Além de apresentar perdas consideráveis, o vírus está presente em boa parte das plantas no Estado do Paraná, de acordo com um levantamento que revelou que a incidência do vírus chegou a 90% no campo (SILVA et al., 2011). Essa alta incidência pode estar relacionada com o método de propagação utilizado para a cultura da mandioca e a transmissão mecânica do vírus, uma vez que até hoje nenhum vetor foi relatado para o CsCMV (LEGG et al., 2015). O modo de propagação da mandioca é vegetativo, realizado através do uso de manivas. Caso as manivas estejam infectadas com o vírus, esse tipo de propagação pode favorecer sua disseminação, uma vez que leva ao campo material contaminado.

Embora presente no Brasil há muitos anos, a variabilidade do CsCMV nunca foi estudada. De acordo com o Comitê Internacional de Taxonomia de Vírus (ICTV), a variabilidade dos potexvirus pode ser avaliada através dos genes RNA-dependente RNA-polimerase (RdRp) e capa proteica (CP), onde para serem consideradas espécies diferentes, a porcentagem de identidade deve ser inferior a 72% na comparação entre nucleotídeos ou 80% na comparação entre aminoácidos (https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/alphaflexiviridae/1330/genus-potexvirus). Na Argentina, ZANINI et al., 2018 concluíram que, com uma porcentagem de identidade dos nucleotídeos de parte do

gene RdRp entre 77,1 e 80,3%, os isolados podem ser classificados como estirpes distintas. No Brasil, até hoje não há dados relacionados à variabilidade do CsCMV e a presença de diferentes estirpes.

Quanto ao genoma completo, um isolado brasileiro sequenciado há mais de 20 anos por CALVERT et al., 1996 é o único do país. Após esse longo período, nenhum novo sequenciamento do CsCMV foi realizado no Brasil. Além desse genoma brasileiro, genomas da Argentina, Colômbia e outros da China já foram depositados no GenBank, porém não foram comparados entre si.

Além desses estudos, durante o doutorado sanduíche na Oklahoma State University, Stillwater, OK, EUA, foram realizadas análises moleculares, sequenciamento de nova geração (Next Generation Sequencing – NGS), e a construção de uma base de dados para vírus em videira na plataforma Mi-Fi, por E-probe Diagnostic Nucleic-acid Analysis (EDNA) (STOBBE et al., 2013).

Sendo assim, a partir dos assuntos abordados, a tese foi dividida em quatro capítulos: Capítulo 1, “Performance and competitive displacement of *Bemisia tabaci* MEAM1 and MED cryptic species on diferente host plants”, redigido em inglês e publicado nas normas da revista CROP PROTECTION; Capítulo 2, “Performance of *Bemisia tabaci* MEAM1 and *Trialeurodes vaporariorum* on *Tomato chlorosis virus* (ToCV) infected plants”, redigido em inglês e publicado em 2018 nas normas da revista JOURNAL OF APPLIED ENTOMOLOGY, o Capítulo 3, “título”, redigido em inglês, conforme as normas da revista PLANT PATHOLOGY; e o Capítulo 4 “Atividades desenvolvidas no período de intercâmbio na Oklahoma State University (Stillwater)”.

CAPÍTULO 1 – Performance and competitive displacement of *Bemisia tabaci* MEAM1 and MED cryptic species on different host plants

Luís Fernando Maranho Watanabe[a],*, Vinicius Henrique Bello[a], Bruno Rossitto De Marchi[a], Felipe Barreto da Silva[a], Lucas Machado Fusco[a], Maria Márcia Pereira Sartori[b], Marcelo Agenor Pavan[a], Renate Krause-Sakate[a]

[a]Department of Plant Protection, Faculdade de Ciências Agronômicas, São Paulo State University (UNESP), School of Agriculture, Botucatu, SP, 18610-034, Brazil
[b]Department of Production and Plant Breeding, Faculdade de Ciências Agronômicas, School of Agriculture, São Paulo State University (UNESP), Botucatu, SP, Brazil

*Corresponding author. Department of Plant Protection, Faculdade de Ciências Agronômicas, São Paulo State University (UNESP), Avenida Universitária, São Paulo, 3780, Brazil.
E-mail addresses: luiswatanabe92@hotmail.com, luis.watanabe@unesp.br (L.F.M. Watanabe).

https://doi.org/10.1016/j.cropro.2019.104860
Received 17 February 2019; Received in revised form 17 June 2019; Accepted 23 June 2019
Available online 25 June 2019

ABSTRACT

Bemisia tabaci comprises a complex of cryptic species, of which MEAM1 (known as B biotype) and MED (known as Q biotype) are the most invasive and widely distributed ones. In Brazil, MEAM1 was first reported in the 1990s, while MED was first reported in 2014. The goal of this study was to understand the behaviour of the newly introduced MED cryptic species in Brazil and to predict the susceptibility of soybean, cotton, common bean, tomato and sweet pepper to it in the absence of insecticides. We investigated the performance as well as the competitive displacement of these cryptic species in the selected hosts. The best performance of MED was observed on common bean, followed by sweet pepper, propitiating the emergence of a large number of adults and a higher survival rate (±60 and 50% for MED, respectively, while for MEAM1, 20% was reached). In addition, MED displaced MEAM1 in sweet pepper and common bean after four generations (after 120 days, all insects were 100% MED). By contrast, MEAM1 displaced MED only in tomato after four generations. The competitive displacement and the performance results were the same, indicating the most suitable host for each species. Both MED and MEAM1 are well adapted to cotton and soybean plants, and no advantage was observed for one particular species in the absence of insecticides. Common bean and sweet pepper are excellent hosts for *B. tabaci* MED cryptic species, requiring increased attention by the producer. The year-round common bean cultivation in Brazil could contribute to the adaptation of MED to open field production in Brazil and, consequently, to the migration to other crops such as soybean and cotton, to which MED is also well adapted. Further management studies with these cryptic species in Brazil, including insecticide experiments and biological control assays, are necessary.

Keywords: Common bean, Cotton, Soybean, Sweet pepper, Tomato, Whitefly

1.1 INTRODUCTION

The whitefly *Bemisia tabaci* (Gennadius) (Hemptera: Aleyrodidae) is one of the most damaging and invasive pests and is widespread around the world, causing losses to several crops (De Barro et al., 2011; Lapidot et al., 2014). It causes direct damage by feeding on the plant phloem and indirect damage by sooty mould growth; in addition, it is a vector of numerous viruses (Navas-Castillo et al., 2011). Previously, *B. tabaci* was stated as biotypes according to a range of biological characters, such as the capacity to induce silverleafing in squash and yellow vein in honeysuckle and nightshade, the host range, the capacity of begomovirus transmission, and the capacity to produce female offspring following interbiotype mating trials (Bedford et al., 1994; De Barro et al., 2011). Currently, *B. tabaci* classification has changed to a complex of cryptic species morphologically indistinguishable. However, the species can be differentiated via molecular analysis of the mitochondrial cytochrome oxidase I (mtCOI). This region is suitable for the identification of, to date, 44 cryptic species of *B. tabaci* (De Barro et al., 2011; Kanakala and Ghanim, 2019). Also, crossing experiments and observations on mating showed that the cryptic species reproduces isolated (Sun et al., 2011). The Middle East-Asia Minor 1 (MEAM1, known as B biotype) and the Mediterranean (MED, known as Q biotype) species are considered the most invasive cryptic species of the complex.

In the early 1990s, the MEAM1 (reported as B biotype) has first been reported in Brazil (Lourenção and Nagai, 1994) and is now widespread throughout the country (de Moraes et al., 2018; Marubayashi et al., 2013). Since then, it has been possible to verify MEAM1 whitefly infestations and virus outbreaks (Inoue-Nagata et al., 2016a).

Two decades later, MED species was first reported in Brazil in the state of Rio Grande do Sul, associated with sweet pepper plants (Barbosa et al., 2015), with subsequent findings in the states of Santa Catarina, Paraná, São Paulo, Minas Gerais (Moraes et al., 2018; Moraes et al., 2017), Goiás and Mato Grosso (data not published), associated with ornamental plants. More recently, MED was detected in greenhouse-grown vegetables crops (data not published). On a global level, MED and MEAM1 can co-exist in the same host/area, and significant switches in the species, in terms of predominance, are frequently being observed (Bertin et al., 2018). A previous study from China revealed that, in a condition without insecticide spraying, MED predominates over MEAM1 on sweet pepper (*Capsicum annuum* L.), while MEAM1 predominates over MED on cabbage (*Brassica oleracea* L.), cotton (*Gossypium*

hirsutum L.) and tomato (*Solanum lycopersicum* L.) (Sun et al., 2013). However, as MED is less susceptible to insecticides than MEAM1 (Horowitz et al., 2005; Horowitz and Ishaaya, 2014), it can have an advantage when the management is based on chemical control.

Brazil has a large territorial extension and a complex agricultural system, making it difficult to understand the behaviour of MEAM1 and MED in conditions where both species are present in the environment. The main concern about the characteristics of MED is that in Brazil pesticides are frequently used for whitefly control in numerous crops such as soybean (*Glycine max* L. Merr.), common bean (*Phaseolus vulgaris* L.), cotton, tomato and sweet pepper (Pignati et al., 2017).

However, although the behaviour of MED and MEAM1 needs to be investigated under conditions with insecticide spraying, it is also essential to understand the behaviour of these species on different host plants without insecticide spraying; such data would help to develop effective management strategies under conditions of low amounts of insecticides or where alternative control measures are used, such as biological control (Liu et al., 2016; Xu et al., 2018).

In this context, we investigated not only the performance, but also the competitive displacement of MEAM1 and MED in the most important Brazilian crops such as soybean, common bean, cotton, tomato and sweet pepper without insecticides, with the overall aim to understand the behaviour of the newly introduced MED cryptic species and to determine which crops are most susceptible to this pest.

1.2 MATERIALS & METHODS

1.2.1 Whitefly colonies, identification and host plants

The population of *B. tabaci* MEAM1 was collected from cabbage (*Brassica oleracea* L.) in Campinas, São Paulo, and maintained on cotton. The population of MED was collected from *Begonia* spp. in Mogi das Cruzes, São Paulo, and was taken to the Virology Laboratory of Faculdade de Ciências Agronômicas, UNESP, Botucatu, to start a colony on cotton plants. For whitefly identification, total DNA extraction was carried out following the Chelex protocol (Walsh et al., 1991). Subsequently, the DNA was used as a template for PCR analysis, using the primers Bem23-F and Bem23-R, amplifying a microsatellite locus of 200 and 400 bp for MEAM1 and MED (Konjevic, 2018; Skaljac et al., 2010), respectively, and primers C1-J-2195 and TL2-N-3014 of the mtCOI gene (Simon et al., 1994). The samples were purified (QIAquick Gel

Extraction Kit Qiagen), sequenced (Macrogen, South Korea) and confirmed as MEAM1 (GenBank access: MF624473) and MED (GenBank access: KX673609). The populations were maintained in insect-proof rearing cages in greenhouses, and colony purity was monitored every 6 months via molecular analysis. Whiteflies from each species used in these experiments were newly emerged (48 h after emergence).

As host plants, we used cotton, soybean cv. Campo Mourão, common bean cv. Pérola, tomato cv. Santa Clara and sweet pepper hybrid Magali-R. All plants used in the experiments had five to seven true leaves.

1.2.2 Whitefly performance on different host plants

All assays were conducted using two plants in insect-proof rearing cages (45 x 45 x 55 cm). Initially, 10 whitefly couples were transferred into a clip cage (5 x 5 x 5 cm), which was then attached to a leaf, resulting in a total of 10 leaves (10 replicates) (Chen et al., 2013; Jiao et al., 2012; Omondi et al., 2005; Watanabe et al., 2018; Xu et al., 2010; Zang et al., 2006). After 48 h, the clip cages and whitefly adults were removed, and only the eggs laid remained. The eggs were counted using a magnifying glass (Zeiss®) and maintained for further evaluation of the following parameters: hatchability [(eggs hatched/eggs laid)*100], number of adults emerged, survival rate [(number of adults emerged/number of eggs laid)*100] and development time (from eggs until adult emergence). Daily evaluations were performed during the entire assay to determine the exact time between each phase of the whitefly life cycle. Every day, newly emerged adults were collected with a manual insect aspirator and immediately counted. The temperature was maintained at 28 ± 2 °C.

1.2.3 Competitive displacement between MEAM1 and MED

The competitive displacement assays were conducted using two plants in each insect-proof rearing cage (45 x 45 x 55 cm), totalling four cages for each host (four replicates). The experiments were performed for sweet pepper, tomato, cotton, soybean and common bean, individually, under non-choice conditions. Per cage, 10 couples of each whitefly species (MEAM1 and MED) were transferred and let to reproduce (Sun et al., 2013). For each generation, whiteflies were sampled using a manual insect aspirator to further analysis. The time for each generation was determined based on the performance experiment (development time of whiteflies on each host). Additionally, two control cages were used on cotton plants, one for MEAM1

and another for MED (20 whitefly couples were introduced in each cage and let to reproduce). Subsequently, every 25 days (for cotton) and every 30 days (for soybean, common bean, tomato and sweet pepper), 100 insects of each cage were randomly sampled and analysed via PCR (as described above in section 2.1). During the experiments, in every sampling, the weaker plants in the cages were replaced by new plants to maintain optimal conditions.

The experiments were conducted either until one cryptic species displaced the other one or until they reached the field crop cycle time. The temperature was maintained at 28 ± 2 °C.

1.2.4 Data analysis

To analyse the performance experiments, non-parametric statistical analyses were performed using the Kruskal-Wallis rank test ($p < 0.05$), employing the statistical software package Minitab 16 (Minitab, 2010). We determined differences between the *B. tabaci* cryptic species MEAM1 and MED in each host plant as well as the differences among the host plants (cotton, common bean, sweet pepper, soybean and tomato) for each *B. tabaci* cryptic species.

1.3 RESULTS

1.3.1 Performance of MEAM1 and MED on different host plants

1.3.1.1 Number of eggs laid

For most of the host plants except sweet pepper, MEAM1 and MED were similar in terms of number of eggs laid. In sweet pepper, MED species laid considerably more eggs compared to MEAM1 species ($H = 14.29$, $P = 0.0002$). For the other host plants, there was no significant difference: cotton ($H = 0.01$, $P = 0.9097$), common bean ($H = 0.04$, $P = 0.85$), soybean ($H = 0.05$, $P = 0.8205$) and tomato ($H = 1.96$, $P = 0.1613$) (Table 1). Based on our results, common bean was the most suitable host plant for both MEAM1 ($H = 39.95$, $P < 0.0001$) (Table 2) and MED ($H = 24.74$, $P < 0.0001$) (Table 3), while sweet pepper was the least suitable host for MEAM1 (Table 2) and cotton was the least suitable host for MED (Table 3).

1.3.1.2 Hatchability

The hatchability of MED was significantly higher compared to that of MEAM1 on cotton ($H = 6.22$, $P = 0.0089$). The other host plants showed no differences in this

parameter between both species: common bean (H = 0.82, P = 0.3642), sweet pepper (H = 0.24, P = 0.6219), soybean (H = 0.21, P = 0.6501) and tomato (H = 3.02, P = 0.0757) (Table 1). In addition, the highest hatchability for MEAM1 was found for tomato (H = 11.95, P = 0.0165) (Table 2). For MED, there was no difference in hatchability (H = 7.07, P = 0.1284) (Table 3).

1.3.1.3 Number of adults emerged

The number of adults emerged differed significantly between MEAM1 and MED on common bean (H = 14, P = 0.0002), sweet pepper (H = 14.29, P = 0.0002) and tomato (H =11.57, P = 0.0007). For cotton (H = 0.82, P = 0.3642) and soybean (H = 0.46, P = 0.4955), there was no difference (Table 1). Additionally, higher numbers of adults were verified on tomato for MEAM1 (H = 35.97, P < 0.0001) (Table 2) and on common bean for MED (H = 17.83, P = 0.0013) (Table 3).

1.3.1.4 Survival rate

The survival rate (%) was higher for MEAM1 species compared to MED only on tomato (H = 13.72, P = 0.0002), while MED had higher survival rates on common bean (H = 14.29, P = 0.0002), cotton (H = 6.8, P = 0.0088) and sweet pepper (H = 8.69, P <0.0032) than MEAM1 (Table 1). Additionally, MEAM1 had the highest survival rate on tomato MEAM1 (H = 41.51, P < 0.0001) (Table 2), while MED survived better on cotton (H = 27.15, P < 0.0001) (Table 3).

1.3.1.5 Development time

The development time was similar for both species on most of the plant hosts assessed: cotton (H = 0.01, P = 0.9376), common bean (H = 0.14, P = 0.6147) and tomato (H = 0, P > 0.9999). However, MEAM1 had a shorter development time than MED on soybean (H = 14.29, P < 0.0001) and sweet pepper (H = 13.72, P < 0.0001) (Table 1). In addition, MEAM1 developed more rapidly on soybean, cotton and sweet pepper and more slowly on tomato and common bean (H = 39.34, P < 0.0001) (Table 2), while MED developed more rapidly on cotton and more slowly on common bean (H = 33.97, P < 0.0001) (Table 3).

Table 1 – Performance evaluation of MEAM1 vs MED on each host plants.

Host plant	Whitefly species	Eggs laid	Hatchability (%)	Adults emerged	Survival rate (%)	Development time (days)
Cotton	MEAM1	58a	83.1b	43.5a	73.7b	24a
	MED	48.5a	100a	45.5a	96.65a	24.5a
Common bean	MEAM1	177a	94.67a	30.5b	18.3b	29a
	MED	179a	89a	102a	60.45a	29a
Sweet pepper	MEAM1	27.5b	89.76a	4.5b	18.8b	25a
	MED	125.5a	91.85a	44.5a	40.45a	28b
Soybean	MEAM1	83.5a	91.73a	47a	60.35a	23.5a
	MED	79a	86.95a	52.5a	63.25a	27b
Tomato	MEAM1	104.5a	99.13a	97a	90.1a	28a
	MED	89a	96.63a	52b	60.65b	28a

Medians followed by different letters within columns indicate significant difference ($p < 0.05$) between MEAM1 and MED on each different host plant for each parameter evaluated.

Table 2 – Performance evaluation of host plants for MEAM1.

	Parameters	Cotton	Common bean	Sweet pepper	Soybean	Tomato
MEAM1	Eggs laid	58cd	177a	27.5d	83.5bc	104.5ab
	Hatchability (%)	83.1c	94.67ab	89.76bc	91.73bc	99.13a
	Adults emerged	43.5b	30.5b	4.5c	47b	97a
	Survival rate (%)	73.7ab	18.3c	18.8c	60.35b	90.1a
	Development time (days)	24a	29b	25a	23.5a	28b

Medians followed by different letters within rows indicate significant difference ($p < 0.05$) among the different host plants.

Table 3 – Performance evaluation of host plants for MED.

	Parameters	Cotton	Common bean	Sweet pepper	Soybean	Tomato
MED	Eggs laid	48.5d	179a	125.5ab	79cd	89bc
	Hatchability (%)	100a	89a	91.85a	86.95a	96.63a
	Adults emerged	45.5b	102a	44.5b	52.5b	52b
	Survival rate (%)	96.65a	60.45bc	40.45c	63.25b	60.65bc
	Development time (days)	24.5a	29c	28bc	27b	28bc

Medians followed by different letters within rows indicate significant difference ($p < 0.05$) among the different host plants.

1.3.1.6 Emergence of adults per day

The emergence of adults throughout the entire emergence period is shown in Fig. 1. The sum of the adults per day indicates the total of adults emerged. More adults emerged in the first days for MEAM1 on tomato and for MED on common bean. Adult emergence on sweet pepper was constantly higher for MED compared to MEAM1. In soybean, a unique different pattern of adult emergence was observed, suggesting that this plant host favours MEAM1 emergence only in the first week after the first emergence. In cotton, MED and MEAM1 species had a similar pattern with a decreasing emergence over time.

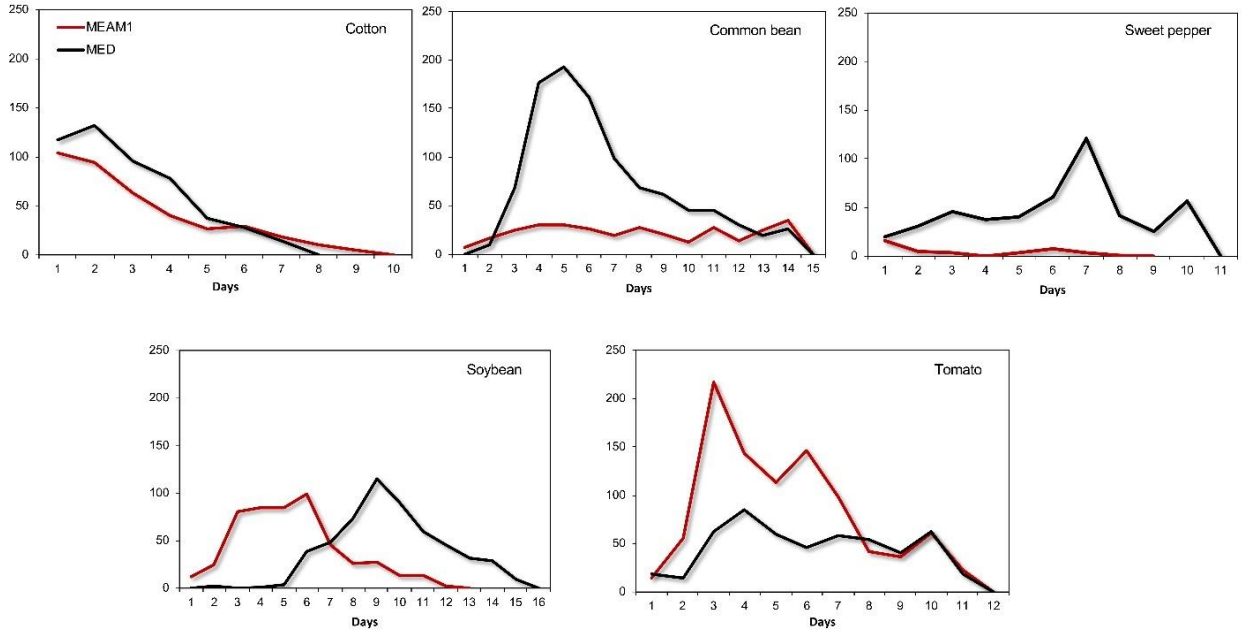


Fig. 1. Number of adults emerged for MEAM1 and MED per day on each host plant.

1.3.2 Competitive displacement between MEAM1 and MED

A complete displacement of one species by another was observed only for sweet pepper, common bean and tomato (Fig. 2). The MED species displaced MEAM1 in sweet pepper and in common bean, while MEAM1 displaced MED in tomato plants. Our results suggest that soybean and cotton did not favour any of the species, as no displacement was observed after 150 days of evaluation.

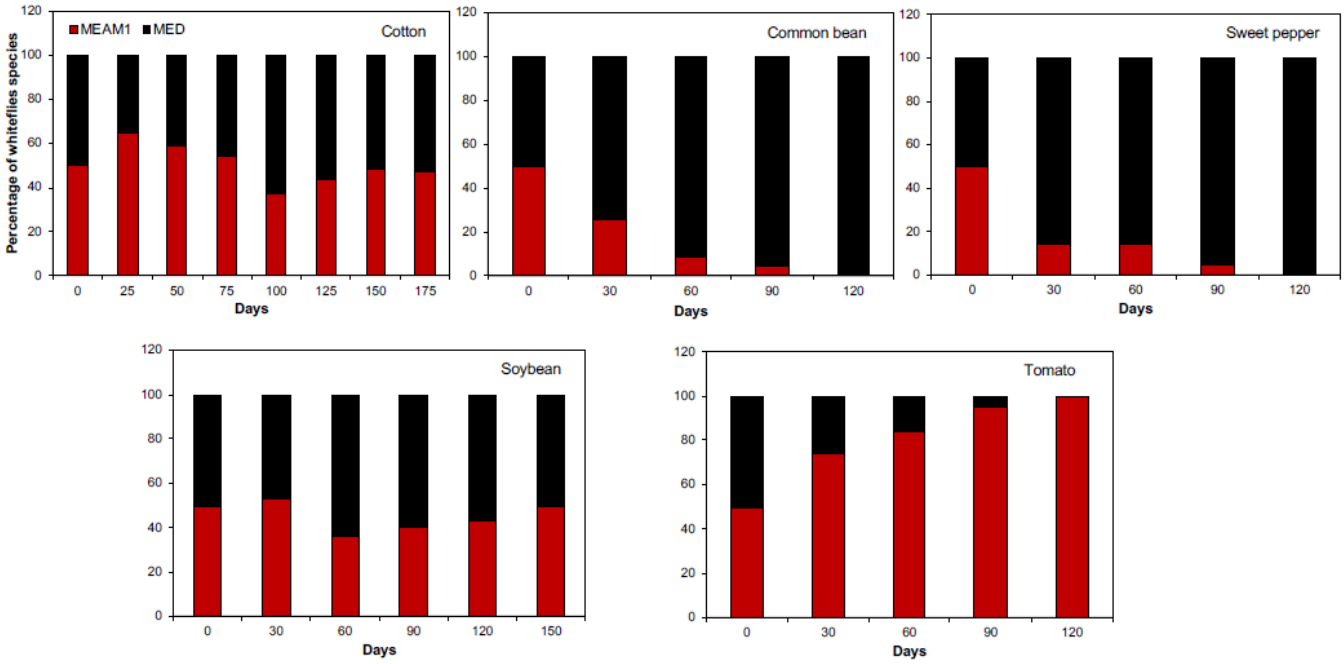


Fig. 2. Competitive displacement between MEAM1 and MED on each host plant.

1.4 DISCUSSION

The data obtained in both performance and competitive displacement assays revealed that the MED species is highly adapted to common bean and sweet pepper, while MEAM1 is more adapted to tomato plants, suggesting that growers need to be alert to the occurrence of MED in these crops. Brazil is among the largest growers and consumers of common bean in the world, with a production of 3.3 million tons under open field conditions in the season 2017/18 (Coêlho, 2017). Bean production in the country relies on three main growing seasons: the “rainy season,” when beans are sown from August through November (49.4% of the total production), the “dry season”, which usually lasts from mid-February to mid-March (45.3% of the total production), and the “irrigated crop” or “winter crop,” where the beans are sown between April and July (5.3% of the total production). The latter is mainly cultivated in the central-western and south-eastern regions of the country (de FARIA et al., 2016). The large areas cultivated with common bean throughout the year could contribute to the adaptation of MED in open fields in Brazil, since our study demonstrates that common bean is the most suitable host for MED development among the tested host plants. This can have direct impacts on the ecology and dissemination of MED to other crops, since common beans, soybeans and cotton are grown in nearby areas in the central-western region

of Brazil. The migration of MED populations from common bean to soybean and cotton might be an issue of concern to growers.

Common bean is also heavily affected by whitefly-transmitted viruses (Inoue-Nagata et al., 2016a, 2016b), namely the *Bean golden mosaic virus* (BGMV, *Begomovirus*) and the *Cowpea mild mottle virus* (CPMMV, *Carlavirus*), which are widely distributed in Brazil (Inoue-Nagata et al., 2016a, 2016b). The *Bemisia tabaci* MED cryptic species is an efficient vector of BGMV and CPMMV (Bello et al., 2019) and can contribute to an increased occurrence of these viruses in the field. Soybean can also be infected with BGMV and CPMMV (Inoue-Nagata et al., 2016a). To date, there are no data about the damage caused by BGMV in soybean, but studies with CPMMV are in progress and indicate that the virus causes considerable losses, depending on soybean genotypes. Additionally, common bean could serve as a source of inoculum of BGMV and CPMMV to soybean, compromising its yield. Soybean is the most important crop in Brazil, moving approximately US$ 36.96 billion (R$ 142.64 billion) in 2017/18; any factor that affects its productivity has therefore a high impact on the Brazilian economy. Our results demonstrate that MEAM1 and MED do not outcompete each other on cotton and soybean. In addition, common bean might be a key crop to the build-up of MED populations and, consequently, its migration from common bean to other crops, therefore contributing to MEAM1 displacement in the field. Also, virus transmission might be an important parameter to investigate in future studies.

In Brazil, sweet pepper is grown throughout the year in the open field and under greenhouse conditions, with a production average of 350,000 tons on 13,000 ha (Eduardo et al., 2012). In our experiments, sweet pepper plants were clearly a better host for MED than for MEAM1, as previously reported for China (Sun et al., 2013). Furthermore, in Brazil, there are different species of begomoviruses that infect sweet pepper, such as *Tomato severe rugose virus* (ToSRV), *Tomato golden vein virus* (TGVV) and *Tomato yellow vein streak virus* (ToYVSV), and the crinivirus *Tomato chlorosis virus* (ToCV) reported in this culture. However, since no significant yield losses have yet been associated with viruses in this crop, so far, *B. tabaci* MEAM1 did not occur frequently in sweet pepper (Inoue-Nagata et al., 2016a), probably contributing to the low incidence of these viruses. The recent introduction of MED raises concerns about a possible outbreak of begomoviruses and the crinivirus in this crop.

Regarding the tomato crop, our results showed that MEAM1 displaced MED, as observed in China (Sun et al., 2013). The MEAM1 cryptic species has been responsible for the emergence of numerous different begomoviruses since its introduction into Brazil due to its polyphagous characteristic of colonising both tomato and weeds and, consequently, transmitting viruses from native weeds to the tomato crop (Inoue-Nagata et al., 2016b, 2016a). Currently, MEAM1 is the main vector of ToCV and ToSRV viruses in Brazil. Some studies have indicated that the virus ToSRV and the vector MEAM1 work together to spread the virus. The virus adapts to the vector, and the ToSRV-viruliferous MEAM1 prefers the volatiles of a healthy tomato plant (Fereres et al., 2016; Maluta et al., 2017). However, another study has shown that ToCV- and ToSRV-infected plants can have a deleterious effect on nymph viability, but no effect in the reproductive parameters in emerged adults for MEAM1, with no contribution to virus spread (Maluta et al., 2018).

A study from China has revealed that non-viruliferous MED prefer ToCV-infected plants, while viruliferous MED prefer healthy plants, offering insights into the ToCV epidemiology (Shi et al., 2018). When reared on ToCV-infected tomato plants, MED has a higher survival rate and a shorter development time than MEAM1, indicating that the spread of ToCV in China could be related to MED species (Shi et al., 2018). However, another study from China has shown that ToCV-infected plants could be harmful to MED, decreasing the fecundity and longevity of the adults and increasing the development time when compared to healthy plants (Li et al., 2018). These contradictory results indicate that the effect of ToCV-infected plants on MED may be affected by MED populations, the virus isolate and the tomato cultivars used. Thus, it would be also important to perform studies in Brazil to elucidate the behaviour of MED on ToCV-infected tomato plants. For the MEAM1 cryptic species, it has already been observed that ToCV-infected plants greatly influence its performance, dramatically decreasing the number of adults generated and their survival rate in Brazil (Watanabe et al., 2018). This previous study was important to the understanding of the relationship between virus and the MEAM1 vector in Brazil and the next goal may be the investigation of the new invasive MED species, since it is an efficient vector of our main viruses (Bello et al., 2019).

In addition, the MED cryptic species is an excellent vector of the most important begomovirus in the world, the *Tomato yellow leaf curl virus* (TYLCV) (Lefeuvre et al., 2010). This virus has not been detected in Brazil yet, but it has already been reported

in Venezuela (Zambrano et al., 2007). Studies have shown that MED has a better performance than MEAM1, feeds more on TYLCV-infected plants and was responsible for the rapid spread of this virus in China (Chen et al., 2013; Liu et al., 2013; Pan et al., 2012). If TYLCV was introduced in Brazil, MED would potentially be a key vector for its dissemination in tomato and sweet
pepper plants.

For cotton plants, we conclude that both MED and MEAM1 cryptic species are well adapted to this crop. However, it is important to highlight that in Brazil, cotton is grown close to common bean areas. Our results show that MED is highly adapted to common bean. As a consequence, the MED population could be increased on common bean crops and can migrate to nearby crops, such as cotton. This is a valid concern and requires adequate management, since MED is less susceptible to insecticides than MEAM1. A study on cotton in Israel has demonstrated that in areas where MEAM1 was predominant, MED displaced MEAM1 after insecticide application. When the application of insecticides ceased, MEAM1 was capable of displacing MED and to predominate again (Horowitz and Ishaaya, 2014), indicating that insecticide application in the field might select MED populations over MEAM1.

This has also been observed in a field study in China, which investigated the competition between MEAM1 and MED cryptic species on tomato and eggplants. Initially, with no insecticide spraying, MEAM1 predominated over MED, as expected. However, a few months after the spraying, MED displaced MEAM1 and predominated in the area (Sun et al., 2013), indicating that insecticide application can directly influence the predominant species in the field.

In summary, common bean was the most suitable host plant for MED, indicating that it may be a key crop in the spread of MED under field conditions in Brazil. Consequently, this crop could be responsible for the establishment of MED in other important crops such as soybean and cotton. To avoid the spread of MED populations throughout the country, constant monitoring and identification of whitefly populations in the fields and greenhouses are necessary, combined with adequate management strategies tailored to each *B. tabaci* cryptic species present in a given area.

Acknowledgments

This project was financially supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - process number 2017/21588-7) and Conselho

Nacional de Desenvolvimento Científico e Tecnológico (CNPq - process number 405684/2018-5). This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. L.M.F. is a recipiente of CNPq scholarship. M.A.P. and R.K.S. are CNPq research fellows.

1.5 REFERENCES

Barbosa, L. da F., Yuki, V.A., Marubayashi, J.M., De Marchi, B.R., Perini, F.L., Pavan, M. A., de Barros, D.R., Ghanim, M., Moriones, E., Navas-Castillo, J., 2015. First report of Bemisia tabaci Mediterranean (Q biotype) species in Brazil. Pest Manag. Sci. 71, 501–504.

Bedford, I.D., Briddon, R.W., Brown, J.K., Rosell, R.C., Markham, P.G., 1994. Geminivirus transmission and biological characterisation of Bemisia tabaci (Gennadius) biotypes from different geographic regions. Ann. Appl. Biol. 125, 311–325.

Bello, V.H., Watanabe, L.F.M., Santos, B.R., Marubayashi, J.M., Yuki, V.A., Marchi, B.R. De, Pavan, M.A., Krause-sakate, R., 2019. Evidence for Increased Efficiency of Virus Transmission by Populations of Mediterranean Species of Bemisia Tabaci with High Hamiltonella Prevalence.

Bertin, S., Luigi, M., Parrella, G., Giorgini, M., Davino, S., Tomassoli, L., 2018. Survey of the distribution of Bemisia tabaci (Hemiptera: Aleyrodidae) in Lazio region (Central Italy): a threat for the northward expansion of Tomato leaf curl New Delhi vírus (Begomovirus: geminiviridae) infection. Phytoparasitica 46, 171–182.

Chen, G., Pan, H., Xie, W., Wang, S., Wu, Q., Fang, Y., Shi, X., Zhang, Y., 2013. Virus infection of a weed increases vector attraction to and vector fitness on the weed. Sci. Rep. 3, 3–8. https://doi.org/10.1038/srep02253.

Coêlho, JaD., 2017. Produção de grãos: feijão, milho e soja. Caderno Setorial ETENE 1–13.

De Barro, P.J., Liu, S.-S., Boykin, L.M., Dinsdale, A.B., 2011. Bemisia tabaci: a statement of species status. Annu. Rev. Entomol. 56, 1–19. https://doi.org/10.1146/annurev-ento-112408-085504.

de FARIA, J.C., Aragao, F.J.L., Souza, T., Quintela, E.D., Kitajima, E.W., RIBEIRO, S. da G., 2016. Golden mosaic of common beans in Brazil: management with a transgenic approach. Embrapa Arroz e Feijão-Artigo em periódico indexado.

Eduardo, L., Barretto, P., Alberto, C., Santos, D.O.S., Souza, E.Q.D.E., 2012. Pimentão. Série Agric. Fam. Coleção Passo a Passo – Pimentão.

Fereres, A., Peñaflor, M.F.G.V., Favaro, C.F., Azevedo, K.E.X., Landi, C.H., Maluta, N.K. P., Bento, J.M.S., Lopes, J.R.S., 2016. Tomato Infection by Whitefly-Transmitted Circulative and Non-circulative Viruses Induce Contrasting Changes in Plant Volatiles and Vector Behaviour. Viruses 8. https://doi.org/10.3390/v8080225.

Horowitz, A.R., Ishaaya, I., 2014. Dynamics of biotypes B and Q of the whitefly Bemisia tabaci and its impact on insecticide resistance. Pest Manag. Sci. 70, 1568–1572. https://doi.org/10.1002/ps.3752.

Horowitz, A.R., Kontsedalov, S., Khasdan, V., Ishaaya, I., 2005. Biotypes B and Q of Bemisia tabaci and their relevance to neonicotinoid and pyriproxyfen resistance. Arch. Insect Biochem. Physiol. 58, 216–225.

Inoue-Nagata, Carvalho, C.M., Zerbini, F.M., Rezende, J.A.M., Krause-Sakate, R., Nagata, T., 2016. Vírus transmitidos por moscas-brancas no brasil: vetores, principais doenças e manejo. Revisão Anu. Patol. plantas 24, 7–29.

Inoue-Nagata, Lima, M.F., Gilbertson, R.L., 2016. A review of geminivirus diseases in vegetables and other crops in Brazil: current status and approaches for management. Hortic. Bras. 34, 8–18. https://doi.org/10.1590/S0102-053620160000100002.

Jiao, X., Xie, W., Wang, S., 2012. Host Preference and Nymph Performance of B and Q Putative Species of Bemisia Tabaci on Three Host Plants, pp. 423–430. https://doi.org/10.1007/s10340-012-0441-2.

Kanakala, S., Ghanim, M., 2019. Global Genetic Diversity and Geographical Distributionof Bemisia Tabaci and its Bacterial Endosymbionts.

Konjevic, A., 2018. First Interception of Bemisia Tabaci Mediterranean (Q Biotype) in Serbia 1, pp. 1–5. https://doi.org/10.1111/jen.12508.

Lapidot, M., Legg, J.P., Wintermantel, W.M., Polston, J.E., 2014. Management of whitefly-transmitted viruses in open-field production systems. In: Advances in Virus Research. Elsevier, pp. 147–206.

Lefeuvre, P., Martin, D.P., Harkins, G., Lemey, P., Gray, A.J.A., Meredith, S., Lakay, F., Monjane, A., Lett, J.M., Varsani, A., Heydarnejad, J., 2010. The spread of tomato yellow leaf curl virus from the middle east to the world. PLoS Pathog. 6. https://doi.org/10.1371/journal.ppat.1001164.

Li, J., Ding, T.B., Chi, H., Chu, D., 2018. Effects of Tomato chlorosis virus on the performance of its key vector, Bemisia tabaci, in China. J. Appl. Entomol. 142, 296–304.

Liu, B., Preisser, E.L., Chu, D., Pan, H., Xie, W., Wang, S., Wu, Q., Zhou, X., Zhang, Y., 2013. Multiple forms of vector manipulation by a plant-infecting virus: Bemisia tabaci and tomato yellow leaf curl virus. J. Virol. 87, 4929–4937.

Liu, X., Zhang, Y., Xie, W., Wu, Q., Wang, S., 2016. The Suitability of Biotypes Q and B of Bemisia Tabaci (Gennadius) (Hemiptera: Aleyrodidae) at Different Nymphal Instars as Hosts for Encarsia Formosa Gahan (Hymenoptera: Aphelinidae). https://doi.org/10.7717/peerj.1863.

Lourenção, A.L., Nagai, H., 1994. Surtos populacionais de Bemisia tabaci no Estado de São Paulo. Bragantia 53, 53–59.

Maluta, N., Fereres, A., Roberto, J., Lopes, S., 2018. Plant - mediated indirect effects of two viruses with different transmission modes on Bemisia tabaci feeding behavior and fitness. J. Pest. Sci. 2004 https://doi.org/10.1007/s10340-018-1039-0.

Maluta, N.K.P., Fereres, A., Lopes, S., 2017. Settling Preferences of the White Fl Y Vector Bemisia Tabaci on Infected Plants Varies with Virus Family and Transmission Mode 1–10. https://doi.org/10.1111/eea.12631.

Marubayashi, J.M., Yuki, V.A., Rocha, K.C.G., Mituti, T., Pelegrinotti, F.M., Ferreira, F.Z., Moura, M.F., Navas-Castillo, J., Moriones, E., Pavan, M.A., 2013. At least two indigenous species of the Bemisia tabaci complex are present in Brazil. J. Appl. Entomol. 137, 113–121.

Minitab, I., 2010. Minitab 16 Statistical Software. PA Minitab, Inc, State Coll. URL [Computer software]. www.minitab.com.

Moraes, L.A., Marubayashi, J.M., Yuki, V.A., Ghanim, M., Bello, V.H., De Marchi, B.R., Barbosa, L.F., Boykin, L.M., Krause-Sakate, R., Pavan, M.A., 2017. New invasion of Bemisia tabaci Mediterranean species in Brazil associated to ornamental plants. https://doi.org/10.1007/s12600-017-0607-9.

Moraes, L.A., Muller, C., de Bueno, R.C.O.F., Santos, A., Bello, V.H., De Marchi, B.R., Watanabe, L.F.M., Marubayashi, J.M., Santos, B.R., Yuki, V.A., Takada, H.M., de Barros, D.R., Neves, C.G., da Silva, F.N., Gonçalves, M.J., Ghanim, M., Boykin, L., Pavan, M.A., Krause-Sakate, R., 2018. Distribution and phylogenetics of whiteflies and their endosymbiont relationships after the Mediterranean species invasion in Brazil. Sci. Rep. 8, 1–13. https://doi.org/10.1038/s41598-018-32913-1.

Navas-Castillo, J., Fiallo-Olivé, E., Sánchez-Campos, S., 2011. Emerging virus diseases transmitted by whiteflies. Annu. Rev. Phytopathol. 49, 219–248. https://doi.org/10.1146/annurev-phyto-072910-095235.

Omondi, A.B., Kyerematen, R.A., Danquah, E.Y., 2005. Host Preference and Suitability of Some Selected Crops for Two Biotypes of Bemisia Tabaci in Ghana 393–400.

Pan, H., Chu, D., Yan, W., Su, Q., Liu, B., Wang, S., Wu, Q., 2012. Rapid spread of tomato yellow leaf curl virus in China is aided differentially by two invasive. Whiteflies 7, 1–9. https://doi.org/10.1371/journal.pone.0034817.

Pignati, W.A., Lima, F.A.N., de S. e Lara, S.S., de Correa, M.L.M., Barbosa, J.R., Leão, L.H. da C., Pignatti, M.G., 2017. Distribuição espacial do uso de agrotóxicos no Brasil: uma ferramenta para a Vigilância em Saúde. Ciência Saúde Coletiva 22, 3281–3293. https://doi.org/10.1590/1413-812320172210.17742017.

Shi, X., Tang, X., Zhang, X., Zhang, D., Li, F., Yan, F., Zhang, Y., Zhou, X., Liu, Y., 2018. Transmission efficiency, preference and behavior of Bemisia tabaci MEAM1 and MED under the influence of tomato chlorosis virus. Front. Plant Sci. 8, 1–9. https://doi.org/10.3389/fpls.2017.02271.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87, 651–701.

Skaljac, M., Zanic, K., Ban, S.G., Kontsedalov, S., Ghanim, M., 2010. Co-infection and localization of secondary symbionts in two whitefly species. BMC Microbiol. 10, 1.

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. Bull. Entomol. Res. 103, 344–353. https://doi.org/10.1017/S0007485312000788.

Sun, D.B., Xu, J., Luan, J.B., Liu, S.S., 2011. Reproductive incompatibility between the B and Q biotypes of the whitefly Bemisia tabaci in China: genetic and behavioural

evidence. Bull. Entomol. Res. 101, 211–220. https://doi.org/10.1017/S0007485310000416.

Walsh, P.S., Metzger, D.A., Higuchi, R., 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10, 506–513.

Watanabe, L.F.M., Bello, V.H., De Marchi, B.R., Sartori, M.M.P., Pavan, M.A., Krause-Sakate, R., 2018. Performance of Bemisia tabaci MEAM1 and Trialeurodes vaporariorum on Tomato chlorosis virus (ToCV) infected plants. J. Appl. Entomol. 1, 1–8. https://doi.org/10.1111/jen.12559.

Xu, H.Y., Yang, N.W., Chi, H., Ren, G.D., Wan, F.H., 2018. Comparison of demographic fitness and biocontrol effectiveness of two parasitoids, Encarsia sophia and Eretmocerus hayati (Hymenoptera: aphelinidae), against Bemisia tabaci (Hemiptera: Aleyrodidae). Pest Manag. Sci. 74, 2116–2124. https://doi.org/10.1002/ps.4908.

Xu, J., Lin, K.K., Liu, S.S., 2010. Performance on Different Host Plants of an Alien and na Indigenous Bemisia Tabaci from China 2003, pp. 771–779. https://doi.org/10.1111/j.1439-0418.2010.01581.x.

Zambrano, K., Carballo, O., Geraud, F., Chirinos, D., Fernández, C., Marys, E., 2007. First report of Tomato yellow leaf curl virus in Venezuela. Plant Dis. 91, 768.

Zang, L., Chen, W., Liu, S., 2006. Comparison of Performance on Different Host Plants between the B Biotype and a Non-B Biotype of Bemisia Tabaci from Zhejiang, pp. 221–227. China. https://doi.org/10.1111/j.1570-7458.2006.00482.x.

CAPÍTULO 2 – Performance of *Bemisia tabaci* MEAM1 and *Trialeurodes vaporariorum* on *Tomato chlorosis virus* (ToCV) infected plants

Luís Fernando Maranho Watanabe[1] | Vinicius Henrique Bello[1] | Bruno Rossitto De Marchi[1] | Maria Márcia Pereira Sartori[2] | Marcelo Agenor Pavan[1] | Renate Krause-Sakate[1]

[1]Department of Plant Protection, Faculdade de Ciências Agronômicas, School of Agriculture, São Paulo State University (UNESP), Botucatu, SP, Brazil
[2]Department of Production and Plant Breeding, Faculdade de Ciências Agronômicas, School of Agriculture, São Paulo State University (UNESP), Botucatu, SP, Brazil

Correspondence
Luís Fernando Maranho Watanabe
Department of Plant Protection, Faculdade de Ciências Agronômicas, São Paulo State University (UNESP), São Paulo, Brazil.
Email: luiswatanabe92@hotmail.com

Received: 13 April 2018 | Accepted: 23 July 2018
DOI: 10.1111/jen.12559

Funding information
Fundação de Amparo à Pesquisa do Estado de São Paulo, Grant/Award Number: 2017/21588-7

ABSTRACT

Whiteflies are important agricultural pests of several crops and cause great economic losses, mainly by the transmission of plant viruses. Among the different species of whiteflies, *Trialeurodes vaporariorum* and *Bemisia tabaci* MEAM1 (B biotype) transmit the crinivirus *Tomato chlorosis virus* (ToCV). Previous studies report that virus-infected plants can influence the attractiveness and the behaviour of different species of whiteflies. In this study, we evaluated the number of eggs, egg hatching rate and emergence of adults of both *T. vaporariorum* and *B. tabaci* MEAM1 on ToCV-infected and healthy tomatoes. In addition, ToCV transmission assays were conducted with both whitefly species. ToCV infection did not influence the number of eggs or egg hatching rate of *T. vaporariorum*; whereas the emergence of adults was reduced by 37.3% on ToCV-infected tomatoes. By contrast, ToCV-infected tomatoes strongly affected the *B. tabaci* MEAM1 egg hatching rate and emergence of adults with reductions of 41.8% and 92.4%, respectively. Regarding virus transmission, *T. vaporariorum* transmitted ToCV with lower efficiency (35.7%) than *B. tabaci* MEAM1 (78.6%). Our data suggest that the performance of *T. vaporariorum* is less affected than that of *B. tabaci* MEAM1 when feeding on ToCV-infected tomato plants.

KEYWORDS: adults, crinivirus, development, eggs, transmission, whitefly

2.1 INTRODUCTION

Whiteflies are important, widely distributed pests of several agricultural crops (Anderson et al., 2004; Lapidot, Legg, Wintermantel, & Polston, 2014) that cause direct and indirect damage by feeding on the plant phloem and producing “honeydew” (Colvin et al., 2006; Prijovic, Marcic, Drobnjakovic, Medjo, & Peric, 2013) as well as transmitting plant viruses (Navas-Castillo, Fiallo-Olivé, & Sánchez-Campos, 2011), respectively.

In the last two decades, the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) has emerged as one of the most prominent pests worldwide, colonizing over 600 host plants (Polston, De Barro, & Boykin, 2014) and transmitting more than 300 species of virus (Gilbertson, Batuman, Webster, & Adkins, 2015). Molecular phylogenetic analyses based on the mitochondrial cytochrome oxidase I (mtCOI) gene and crossing experiments between biotypes of *B. tabaci* indicate that they are a cryptic species complex composed of at least 43 morphologically indistinguishable species (Boykin & De Barro, 2014; De Barro, Liu, Boykin, & Dinsdale, 2011; Firdaus et al., 2013; Kanakala & Ghanim, 2015; Marubayashi et al., 2013; Sun, Xu, Luan, & Liu, 2011).

Bemisia tabaci MEAM1 (known as B biotype) was first reported in the state of São Paulo, Brazil, at the beginning of 1990s (Lourenção & Nagai, 1994) and is the prevalent *B. tabaci* species in Brazil. *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) is an important pest of vegetable and ornamental crops (López et al., 2012) and prefers greenhouse conditions in which the life cycle is faster than that in the open field (Simmonds, Manlove, Blaney, & Khambay, 2002). Despite the preference for greenhouses, outbreaks of *T. vaporariorum* have been reported under field conditions in Brazil (Lourenção, Alves, Fugi, & Matos, 2008). *Bemisia tabaci* MEAM1 and *T. vaporariorum* species transmit different genera of viruses. While *B. tabaci* transmits *Begomovirus*, *Carlavirus*, *Crinivirus*, *Ipomovirus* and *Torradovirus* (Gilbertson et al., 2015; Navas-Castillo et al., 2011), *T. vaporariorum* has been shown to transmit only the genera *Crinivirus* and *Torradovirus* (Navas-Castillo, Lõpez-Moya, & Aranda, 2014; Navas-Castillo et al., 2011; van der Vlugt et al., 2015).

Tomato chlorosis virus (ToCV) (genus *Crinivirus*, family Closteroviridae) is an economically important virus worldwide (Orfanidou, Dimitriou, Papayiannis, Maliogka, & Katis, 2014) transmitted semi-persistently and was identified for the first time in tomato in 1996 (Wisler, Li, Liu, Lowry, & Duffus, 1998). Over the past two decades,

Tomato chlorosis virus (ToCV) has spread to North America, South America, Europe, Africa, the Middle East and Asia (Accotto et al., 2001; Arruabarrena et al., 2014; Fiallo-Olivé, Hamed, Moriones, & Navas-Castillo, 2011; Hirota et al., 2010; Segev, Wintermantel, Polston, & Lapidot, 2004; Wisler et al., 1998). This virus can cause great damage, reducing tomato yields by 100% (Lozano, Moriones, & Navas-Castillo, 2006; Velasco, Simón, Janssen, & Cenis, 2008). In Brazil, ToCV was first reported in 2006 in São Paulo State infecting tomato (Barbosa et al., 2008). Years later, ToCV was also reported infecting tomato in five additional states of Brazil: Minas Gerais (MG), Rio de Janeiro (RJ), Espírito Santo (ES), Goiás (GO) and Bahia (BA) (Barbosa, Costa, Gioria, & Rezende, 2011). The damage caused by ToCV in tomatoes in Brazil can reach 52% depending on the genotypes (Córdova, 2015; Inoue-Nagata et al., 2016). In addition to tomato, ToCV also infects sweet pepper (Navas-Castillo et al., 2011), potato (Freitas, Nardin, Shimoyama, Souza-Dias, & Rezende, 2012), eggplant and scarlet eggplant (Fonseca et al., 2016).

Previous studies report that *B. tabaci* is a better vector of ToCV than *T. vaporariorum*, with higher transmission efficiency and longer persistence in the whitefly (Wintermantel & Wisler, 2006). Other studies have shown that ToCV has a role in whitefly behaviour. Healthy plants are more attractive to *B. tabaci* MEAM1 than ToCV-infected plants because of their volatiles (Fereres et al., 2016). In addition, *B. tabaci* MEAM1 and Mediterranean (MED) fecundity, development time and survival were affected when feeding on ToCV-infected plants (Shi et al., 2018).

Thus, the goal of this study was to understand the influence of ToCV-infected tomato on the number of eggs, egg hatching rate and emergence of adults of *T. vaporariorum* and *B. tabaci* MEAM1. In addition, transmission assays were performed to evaluate and compare the transmission efficiencies of ToCV by both whiteflies species on tomato plants.

2.2 MATERIALS AND METHODS

2.2.1 Whitefly colonies and identification

The population of *T. vaporariorum* was collected from arrud plants (*Ruta graveolens*) in Atibaia, São Paulo, and taken to the Virology Laboratory of Faculdade de Ciências Agronômicas, UNESP, Botucatu, to start a colony on the same host plant, *R. graveolens*. The population of *B. tabaci* MEAM1 was collected on cabbage (*Brassica oleracea* L.) in Campinas, São Paulo, and maintained on cabbage. For

identification of whiteflies, total DNA extraction by the Chelex protocol (Walsh, Metzger, & Higuchi, 1991) was performed, and the
DNA was used as template for PCR analyses using primers C1-J-2195 and TL2-N-3014 of the mtCOI gene (Simon et al., 1994) for *B. tabaci* and primers TvapF and WFrev (Scott, Workman, Drayton, & Burnip, 2007) for *T. vaporariorum*. The populations were maintained in insect-proof rearing cages in the greenhouse, and the purity was monitored every 6 months by molecular analyses. Whiteflies from each species used in these experiments were newly emerged (48 hr postemergence).

2.2.2 Obtaining the ToCV isolate

The ToCV isolate was collected on tomato plants from Lins, São Paulo, for further molecular analyses. Total RNA was extracted using Trizol® (Invitrogen, Brazil) from all tomato plants, individually. Afterwards, extracted RNA was submitted to a RT-PCR using primers HS-11/HS-12 (Dovas, Katis, & Avgelis, 2002), which amplified a conserved region of a heat shock protein (HSP-70). The RT-PCR was used to make a Nested-PCR using ToC-5/ToC-6 specific primers of ToCV (Dovas et al., 2002), which amplified a fragment with approximately 463 bp. The virus isolate was maintained in insect-proof cages. To maintain the ToCV isolate, transmissions from ToCV-infected plants to healthy plants were performed every 2 months with whiteflies as described in section 2.4.

2.2.3 Whitefly performance on ToCV-infected and healthy tomatoes

All assays were conducted using two plants in insect-proof rearing cages (45 × 45 × 55 cm). At first, ten whitefly couples were transferred to a clip cage (5 × 5 × 5 cm) and attached on a leaf, totalling ten leaves (ten replications). After 48 hr, the clip cages and whitefly adults were removed, and only the laid eggs remained. The eggs were counted using a magnifying glass (Zeiss®) and maintained for further evaluation of egg hatch rate, emergence of adults, development time and survival rate. Daily evaluations were performed during the entire assay to determine the exact time between each phase of the whitefly life cycle. Newly emerged adults were collected with a manual insect aspirator and immediately counted. All tomatoes used in this assay were cv. Santa Clara. Two tomato plants with 5–7 true leaves stage were used for each treatment. The temperature was maintained at 28 ± 2°C.

2.2.4 Tomato chlorosis virus transmission assays

Tomato chlorosis virus transmission assays were performed on tomato with *T. vaporariorum* and *B. tabaci* MEAM1. Whiteflies were transferred to a cage with ToCV-infected plants for the acquisition access period (AAP) of 24 hr. Then, whiteflies were transferred to healthy plants (10 adults/plant) confined in a leaf-clip cage for the inoculation access period (IAP) of 24 hr. Following the inoculation, whiteflies were aspirated using a manual insect aspirator, and the plants were sprayed with insecticides (pyriproxyfen and acetamiprid) to kill all eggs, nymphs and adults of remaining whiteflies. After 30 days, the plants were monitored to determine the presence of virus and the purity based on the molecular analyses described above. All experiments were performed using tomato cv. Santa Clara with 14 plants (14 replications) for each species. The temperature was controlled at 28 ± 2°C.

For the negative control, virus-free adult whiteflies were collected from each population (*T. vaporariorum* and *B. tabaci* MEAM1) and transferred to a healthy tomato plant for mock-inoculation.

2.2.5 Data analysis

The numbers of eggs, eggs hatched and adults emerged were transformed by log(x) before analysis to reduce heteroscedasticity and then were submitted to analysis of variance (ANOVA) using Statview software (Concepts and StatView 1987) to determine whether significant differences ($p < 0.05$) occurred between treatments. Then, means were tested using Tukey's test ($\alpha = 5\%$).

Data of ToCV transmission by *T. vaporariorum* and *B. tabaci* MEAM1 were analyzed by chi-square test ($p < 0.01$) using a general log-linear model (GLM). The analysis was performed using the software package R 3.1.0 (R Development Core Team, 2008).

2.3 RESULTS

2.3.1 Whitefly performance on ToCV-infected and healthy tomatoes

The mean number of eggs laid on healthy and ToCV-infected tomato plants was significantly higher for *B. tabaci* MEAM1 than that for *T. vaporariorum* (Table 1).

Table 1 – Mean of number of eggs of whiteflies species on healthy and ToCV-infected tomatoes

Whiteflies species	Tomato plants		Average
	Healthy	ToCV-infected	
T. vaporariorum	36.2	37.6	36.9 b
B. tabaci MEAM1	106.8	69.9	88.35 a

Means followed by the same letter indicate no significant ($p < 0.05$) differences between treatments according to an ANOVA followed by a Tukey test. Data was transformed by log(x) before analysis.

Tomato chlorosis virus did not influence the egg hatch rate for *T. vaporariorum*. By contrast, ToCV-infected tomatoes strongly affected the egg hatch rate of *B. tabaci* MEAM1. In addition, the *B. tabaci* MEAM1 egg hatch rate was significantly higher than that of *T. vaporariorum* on healthy tomatoes, but the rate did not differ on ToCV-infected tomatoes (Table 2).

Table 2 – Mean of egg-hatching rate of whiteflies species on healthy and ToCV-infected tomatoes

Whiteflies species	Tomato plants	
	Healthy	ToCV-infected
T. vaporariorum	31.6 aB	31.8 aA
B. tabaci MEAM1	103.9 aA	60.4 bA

Means followed by the same uppercase letter within columns and lowercase letter within lines indicate no significant ($p < 0.05$) differences between treatments according to an ANOVA followed by a Tukey test. Data was transformed by log(x) before analysis.

There was no significant difference between the number of *T. vaporariorum* adults that emerged on ToCV-infected tomatoes and healthy tomatoes, although the mean number on healthy tomatoes was higher than that on ToCV-infected tomatoes (Table 3). By contrast, the emergence of *B. tabaci* MEAM1 adults was significantly higher on healthy tomato plants than that on ToCV-infected tomato plants. However, on ToCV-infected tomato plants, the number of *T. vaporariorum* was significantly higher than of *B. tabaci* MEAM1 (Table 3).

Table 3 – Mean of number of adults emerged of whiteflies species on healthy and ToCV-infected tomatoes

Whiteflies species	Tomato plants	
	Healthy	ToCV-infected
T. vaporariorum	29.5 aB	18.5 aA
B. tabaci MEAM1	94.8 aA	7.2 bB

Means followed by the same uppercase within columns and lowercase within lines indicate no significant ($p < 0.05$) differences between treatments according to an ANOVA followed by a Tukey test. Data was transformed by log(x) before analysis.

In the daily evaluations of the whitefly life cycle (Figure 1), *T. vaporariorum* adults started to emerge earlier than those of *B. tabaci* MEAM1. The peak of emergence for *T. vaporariorum* was on the third day on both healthy and ToCV-infected tomato plants. *Bemisia tabaci* MEAM1 peak emergence on healthy plants was on the third day after the first adult emergence, and the adults continued emerging for 12 days. The emergence of MEAM1 adults on ToCV-infected tomato plants was constantly low compared to *B. tabaci* MEAM1 on healthy plants.

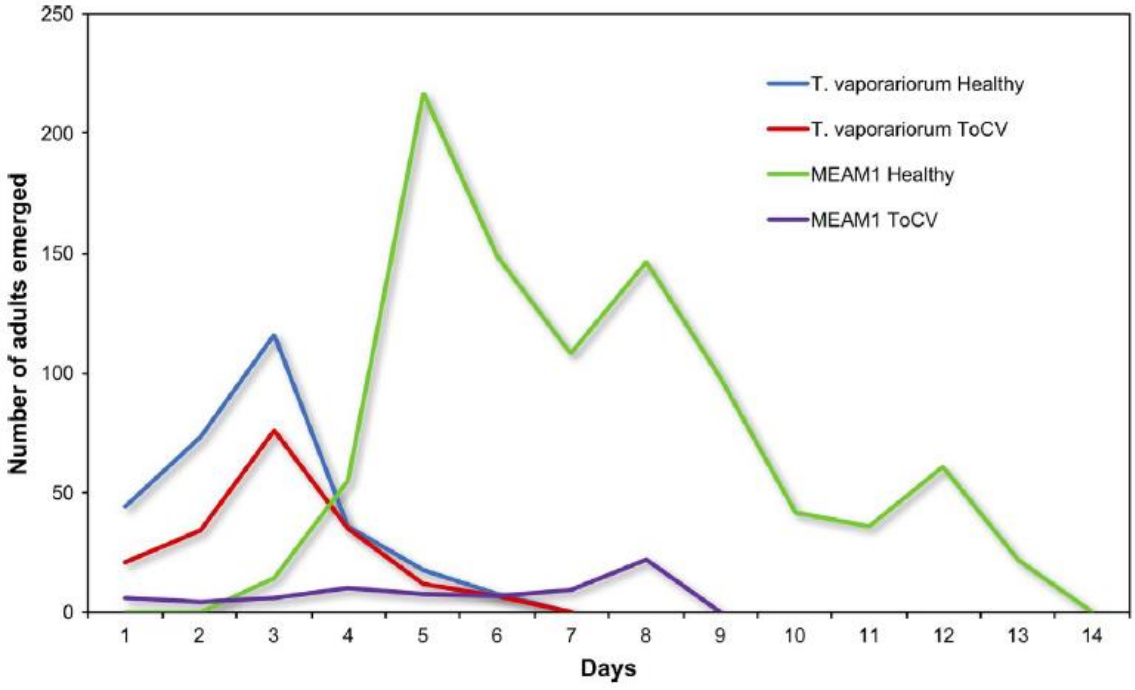


Figure 1. Number of adults emerged of *Trialeurodes vaporariorum* and *Bemisia tabaci* MEAM1 on ToCV-infected and healthy tomato over fourteen days.

Regarding development time, the life cycle of *T. vaporariorum* on healthy and ToCV-infected tomatoes was not different. By contrast, the *B. tabaci* MEAM1 life cycle was significantly longer on healthy tomato plants than that on ToCV-infected plants (Figure 2a). In addition, development time of *T. vaporariorum* was significantly faster than *B. tabaci* MEAM1 on both ToCV-infected and healthy tomatoes (Figure 2a). The difference in the survival rate [(number of adults emerged/number of eggs laid)*100] was significant in each species when comparing healthy with ToCV-infected tomato plants. In addition, the difference was significant between species on both healthy and ToCV-infected plants (Figure 2b).

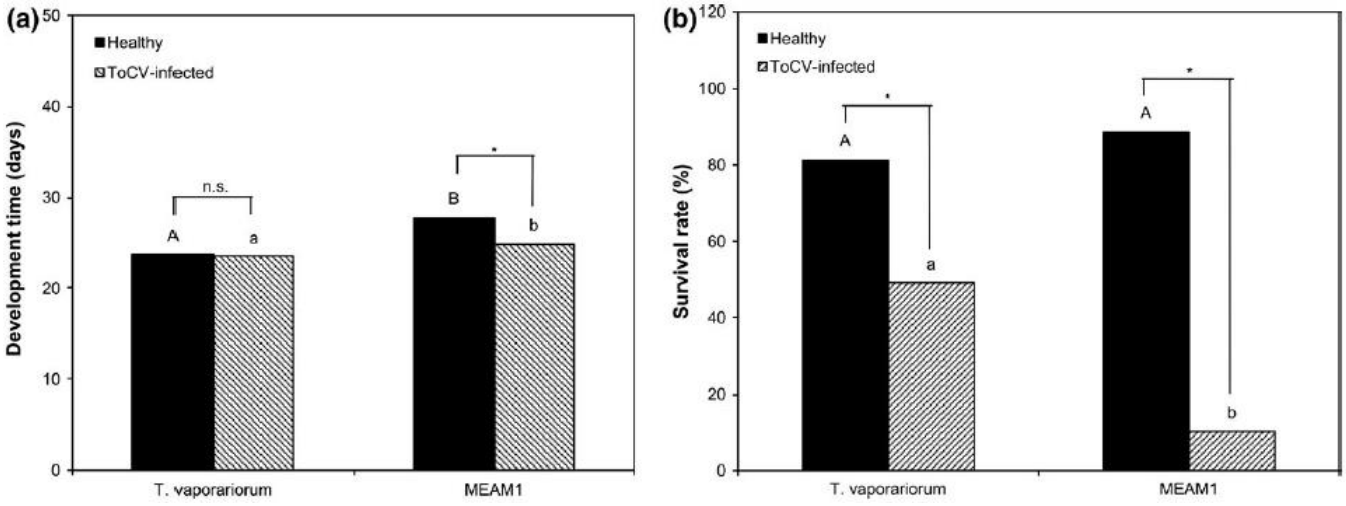


Figure 2. (a) Development time. (b) Survival rate of *Trialeurodes vaporariorum* and *Bemisia tabaci* MEAM1 on healthy and ToCV-infected tomato. In each panel, means followed by different uppercase letters are significantly different for *T. vaporariorum* vs. MEAM1 on healthy tomato plants ($p < 0.05$); means followed by different lowercase letters are significantly different for *T. vaporariorum* vs. MEAM1 on ToCV-infected tomato plants ($p < 0.05$); asterisk indicates a significant difference for *T. vaporariorum* or MEAM1 on healthy vs. ToCV-infected tomatoes plants; and n.s. indicates no significance for *T. vaporariorum* or MEAM1 on healthy vs. ToCV-infected tomatoes plants ($p < 0.05$).

2.3.2 ToCV transmission assays

The efficiency of ToCV transmission by *B. tabaci* MEAM1 was 78.6% (11 infected plants of 14), whereas the efficiency of *T. vaporariorum* was 35.7% (5 infected plants of 14) (Figure 3).

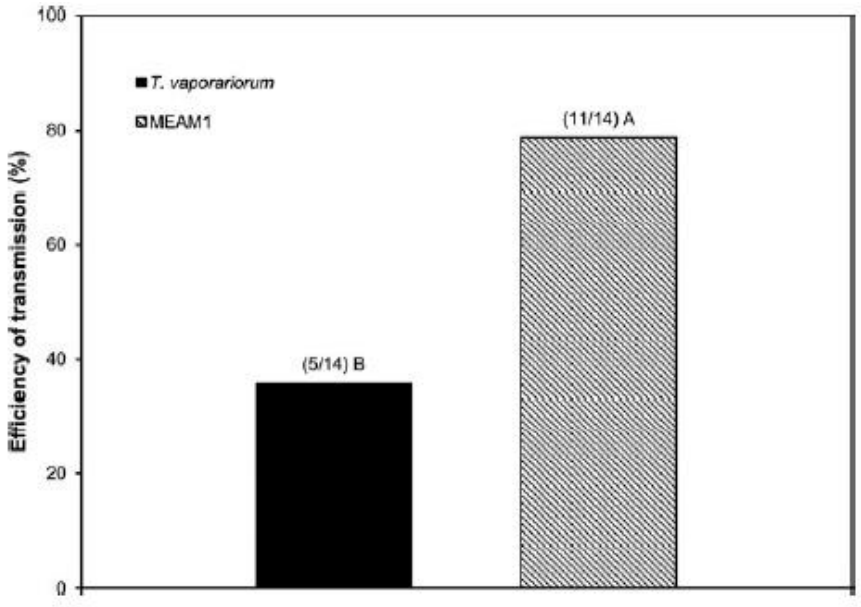


Figure 3. Efficiency of transmission of ToCV by *Trialeurodes vaporariorum* and *Bemisia tabaci* MEAM1. The results show the percentage of infected plants (infected plants/total number of plants tested) 30 days after the inoculation. Means followed by different letters indicate significant difference between treatments ($p < 0.01$).

2.4 DISCUSSION

In this study, we found that ToCV-infected plants affected the performance of *B. tabaci* MEAM1 much more than that of *T. vaporariorum*. The number of *B. tabaci* MEAM1 adults emerged and the survival rate was heavily affected, decreasing 92.4% and 78.8%, respectively. In contrast, only *T. vaporariorum* survival rate was affected on ToCV-infected plants, decreasing 32.5%. In addition, *T. vaporariorum* development time was faster than *B. tabaci* MEAM1 in both healthy and infected plants, although ToCV positively influenced *B. tabaci* MEAM1 development time.

This dramatic decrease in the performance of *B. tabaci* MEAM1 compared with that of *T. vaporariorum* may have serious implications in the epidemiology of ToCV. In the field, the number of newly emerged *B. tabaci* MEAM1 adults that could feed on ToCV-infected tomato plants would be reduced and, as a consequence, fewer adults would transmit ToCV to healthy plants. Therefore, *T. vaporariorum* would be expected to generate more adults than *B. tabaci* MEAM1, which could acquire virus from ToCV-infected tomato plants and transmit it. In addition, the faster development time of *T. vaporariorum* compared to *B. tabaci* MEAM1 on both ToCV-infected and healthy plants may also contribute to virus transmission. This favours faster dissemination of *T.*

vaporariorum in fields and might help this species to establish before *B. tabaci* MEAM1. The data obtained in this study suggest that tomato crops where *T. vaporariorum* populations are present, could favour epidemics of ToCV due to the better performance of *T. vaporariorum* on infected plants compared to *B. tabaci* MEAM1.

However, there are other factors that could influence a ToCV epidemics. A previous study has found that *B. tabaci* MEAM1 transmits ToCV more efficiently than *T. vaporariorum* (Wintermantel & Wisler, 2006). Herein, we also found *B. tabaci* MEAM1 as a better vector of ToCV compared to *T. vaporariorum* but the efficiency of transmission from our data for *T. vaporariorum* was 35.7% while Wintermantel & Wisler, 2006 reported only 4% of transmission efficiency, using 10 insects in their assays. Another important parameter for epidemiology that must be considered is the virus retention time in the vector. *Tomato chlorosis virus* persists in *B. tabaci* MEAM1 for 2 to 3 days (Shi et al., 2018; Wintermantel & Wisler, 2006) while only 1 day in *T. vaporariorum* (Wintermantel & Wisler, 2006). Thus, considering transmission efficiency and virus retention time, *B. tabaci* MEAM1 is more likely to lead a ToCV epidemics. *Tomato chlorosis virus* is an important *Crinivirus* infecting tomato in greenhouses and in the field. Previous studies report that ToCV can cause losses as high as 52% on tomato in Brazil (Córdova, 2015; Inoue-Nagata et al., 2016). A recente survey has found that T. vaporariorum is primarily distributed in the south and southeast region, whereas *B. tabaci* MEAM1 is widely distributed across the country (L. A. De Moraes, C. Muller, R. C. F. O. Bueno, A. C. Santos, V. H. Bello, B. R. De Marchi, L. F. M. Watanabe, J. M. Marubayashi, B. R. Santos, V. A. Yuki, H. M. Takada, D. R. De Barros, C. G. Neves, F. N. Da Silva, M. J. Gonçalves, M. Ghanim, L. M. Boykin, M. A. Pavan., & R. Krause-Sakate, unpublished). Because *T. vaporariorum* has improved performance under mild temperature (19–22°C) (Manzano & van Lenteren, 2009), southern regions of Brazil should be more likely to experience ToCV by this vector. In contrast, the *B. tabaci* MEAM1 has an improved performance under high temperatures (25–30°C) (Nava-Camberos, Riley, & Harris, 2001). It indicates that under specific conditions of greenhouses, mild temperatures with high ToCV incidence, the virus could help *T. vaporariorum* to has an advantage over the *B. tabaci* MEAM1.

Both whiteflies are present in Brazil for at least 25 years (Lourenção & Nagai, 1994; Russell, 1963), more than a decade before the first report of the crinivirus ToCV in 2006 (Barbosa et al., 2008). More recently, in 2014, *B. tabaci* MED species was also identified in Brazil and brought new concerns about plant virus epidemics. Studies

evaluating the performance of vectors feeding on virus-infected plants are valuable to predict and to understand epidemics of plant viruses as well as pest outbreaks. A recent study in China revealed that feeding on ToCV-infected plants influences the performance of both invasive species *B. tabaci* MED and *B. tabaci* MEAM1. *Bemisia tabaci* MED showed better performance on ToCV-infected plants than on healthy plants, with increases in fecundity and survival rate and a decrease in development time. In contrast, ToCV-infected tomato plants exhibited negative effects on the *B. tabaci* MEAM1 species compared to healthy plants, including a decreasing survival rate. In addition, *B. tabaci* MED species was reported to be able to retain ToCV for at least 4–6 days (Orfanidou, Pappi, Efthimiou, Katis, & Maliogka, 2016; Shi et al., 2018), longer than that reported for MEAM1. These results indicate that ToCV may help *B. tabaci* MED species to increase in abundance and to also become widespread in China (Shi et al., 2018). However, another study from China showed that ToCV-infected plants decreased the fecundity and female adult longevity of *B. tabaci* MED and increased the development time, indicating that ToCV causes negative effects in the vector performance (Li, Ding, Chi, & Chu, 2018). Our results revealed a greater decrease in the performance of *B. tabaci* MEAM1 on ToCV-infected tomato plants compared to the results from China with the same whitefly species. The study from China showed a survival rate around 30% for *B. tabaci* MEAM1 while our data indicates only 10%. The differences in performance from *B. tabaci* MEAM1 populations found in Brazil and China might also occur for *B. tabaci* MED species. Therefore, to evaluate the effects of ToCV in the performance of *B. tabaci* MED species from Brazil, further studies would be necessary using local populations.

Furthermore, the whitefly preference on ToCV-infected or healthy plants is an interesting parameter to investigate. Shi et al., 2018 showed that nonviruliferous *B. tabaci* MEAM1 were more attracted by ToCV-infected plants than by healthy plants while viruliferous *B. tabaci* MEAM1 were attracted by both plants equally. Curiously, Fereres et al., 2016 showed that nonviruliferous *B. tabaci* MEAM1 were more attracted by healthy plants and viruliferous showed no preference. To date, there are no studies about the attractiveness of *T. vaporariorum* on ToCV-infected and healthy tomato plants.

The transmission assays conducted in this study had lower numbers of replicates compared to other studies (Shi et al., 2018; Wintermantel & Wisler, 2006), as our goal was only to prove the ability of the evaluated populations to transmit ToCV.

By contrast, the performance assays had a similar number of replicates (10 replicates) compared to previous studies of whitefly performance (12 replicates) (Chen et al., 2013), as this was the main goal of the present study.

In summary, the data obtained in this study suggest that *T. vaporariorum* can have an advantage over *B. tabaci* MEAM1 on ToCV-infected tomato plants, establishing earlier and disseminating faster in the presence of ToCV-infected tomato plants. Moreover, the data obtained herein will contribute to better understanding the effects of plant viruses in the whitefly vector population. More studies on the roles of ToCV in *T. vaporariorum* and *B. tabaci* MEAM1 are required to determine whether *T. vaporariorum*, in fact, has an advantage over *B. tabaci* MEAM1 under high ToCV infection conditions.

ACKNOWLEDGEMENTS

This project was financially supported by FAPESP (process number 2017/21588-7). L.F.M.W. and V.H.B. are recipients of CAPES scholarships. B.R.D.M. is a recipient of CNPq scholarship. M.M.P.S., M.A.P. and R.K.S. are CNPq research fellows.

AUTHOR CONTRIBUTION

L. F. M. Watanabe and R. Krause-Sakate conceived research. L. F. M. Watanabe and V. H. Bello conducted experiments. L. F. M. Watanabe, V. H. Bello and B. R. De Marchi contributed material. L. F. M. Watanabe and M. M. P. Sartori conducted statistical analyses. L. F. M. Watanabe and B. R. De Marchi wrote the manuscript. R. Krause-Sakate and M. A. Pavan secured funding. All authors read
and approved the manuscript.

ORCID

Luís Fernando Maranho Watanabe: http://orcid.org/0000-0002-3113-3223

Bruno Rossitto De Marchi: http://orcid.org/0000-0002-4401-2594

2.5 REFERENCES

Accotto, G. P., Vaira, A. M., Vecchiati, M., Finetti Sialer, M. M., Gallitelli, D., & Davino, M. (2001). First report of tomato chlorosis virus in Italy. Plant Disease, 85, 1208. https://doi.org/10.1094/PDIS.2001.85.11.1208C.

Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R., & Daszak, P. (2004). Emerging infectious diseases of plants: Pathogen pollution, climate change and agrotechnology drivers. Trends in Ecology & Evolution, 19, 535–544. https://doi.org/10.1016/j.tree.2004.07.021.

Arruabarrena, A., Rubio, L., González-Arcos, M., Maeso, D., de Fonseca, M. E. N., & Boiteux, L. S. (2014). First report of tomato chlorosis vírus infecting tomato crops in Uruguay. Plant Disease. 98, 1445. https://doi.org/10.1094/PDIS-11-13-1153-PDN.

Barbosa, J. C., Costa, H., Gioria, R., & Rezende, J. A. M. (2011). Occurrence of tomato chlorosis virus in tomato crops in five Brazilian states. Tropical Plant Pathology, 36, 256–258.

Barbosa, J. C., Teixeira, A. P. M., Moreira, A. G., Camargo, L. E. A., Filho, A. B., Kitajima, E. W., & Rezende, J. A. M. (2008). First report of tomato chlorosis virus infecting tomato crops in Brazil. Plant Disease, 92, 1709. https://doi.org/10.1094/PDIS-92-12-1709C.

Boykin, L. M., & De Barro, P. J. (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., … Zhang, Y. (2013). Virus infection of a weed increases vector attraction to and vector fitness on the weed. Scientific Reports, 3, 3–8.

Colvin, J., Omongo, C. A., Govindappa, M. R., Stevenson, P. C., Maruthi, M. N., Gibson, G., … Muniyappa, V. (2006). Host-plant viral infection effects on arthropod-vector population growth, development and behaviour: management and epidemiological implications. Advances in Virus Research, 67, 419–452. https://doi.org/10.1016/S0065-3527(06)67011-5.

Concepts and StatView. (1987). StatView II. Berkeley, CA: Abacus Concepts Inc.

Córdova, P. J. M. (2015). Resistência de genótipos de tomateiro à infecção com o Tomato chlorosis virus e tolerância à doença.

De Barro, P. J., Liu, S.-S., Boykin, L. M., & Dinsdale, A. B. (2011). Bemisia tabaci: A statement of species status. Annual Review of Entomology, 56, 1–19. https://doi.org/10.1146/annurev-ento-112408-085504.

Dovas, C. I., Katis, N. I., & Avgelis, A. D. (2002). Multiplex detection of criniviruses associated with epidemics of a yellowing disease of tomato in Greece. Plant Disease, 86, 1345–1349. https://doi.org/10.1094/PDIS.2002.86.12.1345.

Fereres, A., Peñaflor, M. F. G. V., Favaro, C. F., Azevedo, K. E. X., Landi, C. H., Maluta, N. K. P., … Lopes, J. R. S. (2016). Tomato infection by whitefly-transmitted circulative and non-circulative viruses induce contrasting changes in plant volatiles and vector behaviour. Viruses, 8(8), 225. https://doi.org/10.3390/v8080225.

Fiallo-Olivé, E., Hamed, A. A., Moriones, E., & Navas-Castillo, J. (2011). First report of tomato chlorosis virus infecting tomato in Sudan. Plant Disease, 95, 1592. https://doi.org/10.1094/PDIS-08-11-0631.

Firdaus, S., Vosman, B., Hidayati, N., Jaya Supena, D. E., Visser, R., & van Heusden, A. W. (2013). The Bemisia tabaci species complex: Additions from different parts of the world. Insect Science, 20, 723–733. https://doi.org/10.1111/1744-7917.12001.

Fonseca, M. E. N., Boiteux, L. S., Lima, M. F., Mendonça, J. L., Costa, A. F., Fontes, M. G., … González-Arcos, M. (2016). First report of tomato chlorosis virus infecting eggplant and scarlet eggplant in Brazil. Plant Disease, 100, 867. https://doi.org/10.1094/PDIS-09-15-1087-PDN.

Freitas, D. M. S., Nardin, I., Shimoyama, N., Souza-Dias, J. A. C., & Rezende, J. A. M. (2012). First report of tomato chlorosis virus in potato in Brazil. Plant Disease, 96, 593. https://doi.org/10.1094/PDIS-12-11-1068-PDN.

Gilbertson, R. L., Batuman, O., Webster, C. G., & 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. https://doi.org/10.1146/annurev-virology-031413-085410.

Hirota, T., Natsuaki, T., Murai, T., Nishigawa, H., Niibori, K., Goto, K., … Okuda, S. (2010). Yellowing disease of tomato caused by tomato chlorosis virus newly recognized in Japan. Journal of General Plant Pathology, 76, 168–171. https://doi.org/10.1007/s10327-010-0219-4.

Inoue-Nagata, A. K., Carvalho, C. M., Zerbini, F. M., Rezende, J. A. M., Krause-Sakate, R., & Nagata, T. (2016). Vírus transmitidos por moscas-brancas no brasil: Vetores, principais doenças e manejo. Revisão Anual de Patologia de Plantas, 24, 7–29.

Kanakala, S., & Ghanim, M. (2015). Advances in the genomics of the whitefly Bemisia tabaci: An insect pest and a virus vector, In C. Raman, M. R. Goldsmith & T. A. Agunbiade (Eds.), Short Views on Insect Genomics and Proteomics (pp. 19–40). Basel, Switzerland: Springer. https://doi.org/10.1007/978-3-319-24235-4.

Lapidot, M., Legg, J. P., Wintermantel, W. M., & Polston, J. E. (2014). Management of whitefly-transmitted viruses in open-field production systems, In G. Lobenstein & N. Katis (Eds.), Advances in Virus Research (pp. 147–206). Oxford, UK: Elsevier.

Li, J., Ding, T. B., Chi, H., & Chu, D. (2018). Effects of tomato chlorosis virus on the performance of its key vector, Bemisia tabaci, in China. Journal of Applied Entomology, 142, 296–304. https://doi.org/10.1111/jen.12477.

López, Y. I. Á., Martínez-Gallardo, N. A., Ramírez-Romero, R., López, M. G., Sánchez-Hernández, C., & Délano-Frier, J. P. (2012). Cross-kingdom effects of plant-plant signaling via volatile organic compounds emitted by tomato (Solanum lycopersicum) plants infested by the greenhouse whitefly (Trialeurodes vaporariorum). Journal of Chemical Ecology, 38, 1376–1386. https://doi.org/10.1007/s10886-012-0201-z.

Lourenção, A. L., Alves, A. C., Fugi, C. G. Q., & Matos, E. S. (2008). Outbreaks of Trialeurodes vaporariorum (West.) (Hemiptera: Aleyrodidae) under field conditions in the State of São Paulo, Brazil. Neotropical Entomology, 37, 89–91. https://doi.org/10.1590/S1519-566X2008000100014.

Lourenção, A. L., & Nagai, H. (1994). Surtos populacionais de Bemisia tabaci no Estado de São Paulo. Bragantia, 53, 53–59. https://doi.org/10.1590/S0006-87051994000100006.

Lozano, G., Moriones, E., & Navas-Castillo, J. (2006). Complete nucleotide sequence of the RNA2 of the crinivirus tomato chlorosis virus. Archives of Virology, 151, 581–587. https://doi.org/10.1007/s00705-005-0690-y.

Manzano, M. R., & van Lenteren, J. C. (2009). Life history parameters of Trialeurodes vaporariorum (Westwood) (Hemiptera: Aleyrodidae) at different environmental conditions on two bean cultivars. Neotropical Entomology, 38, 452–458. https://doi.org/10.1590/S1519-566X2009000400002.

Marubayashi, J. M., Yuki, V. A., Rocha, K. C. G., Mituti, T., Pelegrinotti, F. M., Ferreira, F. Z., … Pavan, M. A. (2013). At least two indigenous species of the Bemisia tabaci complex are present in Brazil. Journal of Applied Entomology, 137, 113–121. https://doi.org/10.1111/j.1439-0418.2012.01714.x.

Nava-Camberos, U., Riley, D. G., & Harris, M. K. (2001). Temperature and host plant effects on development, survival, and fecundity of Bemisia argentifolii (Homoptera: Aleyrodidae). Environmental Entomology, 30, 55–63. https://doi.org/10.1603/0046-225X-30.1.55.

Navas-Castillo, J., Fiallo-Olivé, E., & Sánchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. Annual Review of Phytopathology, 49, 219–248. https://doi.org/10.1146/annurev-phyto-072910-095235.

Navas-Castillo, J., Lõpez-Moya, J. J., & Aranda, M. A. (2014). Whitefly-transmitted RNA viruses that affect intensive vegetable production. Annals of Applied Biology, 165, 155–171. https://doi.org/10.1111/aab.12147.

Orfanidou, C. G., Dimitriou, C., Papayiannis, L. C., Maliogka, V. I., & Katis, N. I. (2014). Epidemiology and genetic diversity of criniviruses associated with tomato yellows disease in Greece. Virus Research, 186, 120–129. https://doi.org/10.1016/j.virusres.2013.12.013.

Orfanidou, C. G., Pappi, P. G., Efthimiou, K. E., Katis, N. I., & Maliogka, V. I. (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. https://doi.org/10.1094/PDIS-01-16-0054-RE.

Polston, J. E., De Barro, P., & Boykin, L. M. (2014). Transmission specificities of plant viruses with the newly identified species of the Bemisia tabaci species complex. Pest Management Science, 70, 1547–1552. https://doi.org/10.1002/ps.3738.

Prijovic, M., Marčić, D., Drobnjaković, T., Među, I., & Perić, P. (2013). Life history traits and population growth of greenhouse whitefly on diferente tomato genotypes. Pesticid Phytomed, 28, 239–245. https://doi.org/10.2298/PIF1304239P.

R Development Core Team. (2008). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Russell, L. M. (1963). Hosls and distribution of five species of Trialeurodes (Homoptera: Aleyrodidae). Annals of the Entomological Society of America, 56, 149–153. https://doi.org/10.1093/aesa/56.2.149.

Scott, I. A. W., Workman, P. J., Drayton, G. M., & Burnip, G. M. (2007). First record of Bemisia tabaci biotype Q in New Zealand. New Zealand Plant Protection, 60, 264–270.

Segev, L., Wintermantel, W. M., Polston, J. E., & Lapidot, M. (2004). First report of tomato chlorosis virus in Israel. Plant Disease, 88, 1160. https://doi.org/10.1094/PDIS.2004.88.10.1160A.

Shi, X., Tang, X., Zhang, X., Zhang, D., Li, F., Yan, F., … 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.

Simmonds, M. S. J., Manlove, J. D., Blaney, W. M., & Khambay, B. P. S. (2002). Effects of selected botanical insecticides on the behaviour and mortality of the glasshouse whitefly Trialeurodes vaporariorum and the parasitoid Encarsia formosa. Entomologia Experimentalis et Applicata, 102, 39–47. https://doi.org/10.1046/j.1570-7458.2002.00923.x.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., & Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America, 87, 651–701. https://doi.org/10.1093/aesa/87.6.651.

Sun, D. B., Xu, J., Luan, J. B., & Liu, S. S. (2011). Reproductive incompatibility between the B and Q biotypes of the whitefly Bemisia tabaci in China: Genetic and behavioural evidence. Bulletin of Entomological Research, 101, 211–220. https://doi.org/10.1017/S0007485310000416.

Velasco, L., Simón, B., Janssen, D., & Cenis, J. L. (2008). Incidences and progression of tomato chlorosis virus disease and tomato yellow leaf curl virus disease in tomato under different greenhouse covers in southeast Spain. Annals of Applied Biology, 153, 335–344. https://doi.org/10.1111/j.1744-7348.2008.00262.x.

van der Vlugt, R. A. A., Verbeek, M., Dullemans, A. M., Wintermantel, W. M., Cuellar, W. J., Fox, A., & Thompson, J. R. (2015). Torradoviruses. Annual Review of Phytopathology, 53, 485–512. https://doi.org/10.1146/annurev-phyto-080614-120021.

Walsh, P. S., Metzger, D. A., & Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques, 10, 506–513.

Wintermantel, W. M., & Wisler, G. C. (2006). Vector specificity, host range, and genetic diversity of tomato chlorosis virus. Plant Disease, 90, 814–819. https://doi.org/10.1094/PD-90-0814.

Wisler, G. C., Li, R. H., Liu, H., Lowry, D. S., & Duffus, J. E. (1998). Tomato chlorosis virus: A new whitefly-transmitted, phloem-limited, bipartite closterovirus of tomato. Phytopathology, 88, 402–409.

CAPÍTULO 3 – Cassava common mosaic virus in cassava plants: assessment and distribution, and obtainment of a new Brazilian isolate in São Paulo State

Luís Fernando Maranho Watanabe[1], Marcos Roberto Ribeiro Junior[1], Angélica Maria Nogueira[1], Julio Massaharu Marubayashi[1], Felipe Barreto da Silva[1], Cristiane Müller[2], Renate Krause Sakate[1]

[1]Department of Plant Protection, Universidade Estadual Paulista “Julio de Mesquita Filho” (UNESP), Botucatu, São Paulo, Brazil.
[2]Corteva™ Agrisciences, Mogi Mirim, São Paulo, Brazil.

Correspondence
Luís Fernando Maranho Watanabe
Department of Plant Protection, Faculdade de Ciências Agronômicas, São Paulo State University (UNESP), São Paulo, Brazil.
Email: luiswatanabe92@hotmail.com

ABSTRACT

Cassava (*Manihot esculenta* Crantz) is one of the most important crops in source of calories, providing food for 800 million people around the world. In South America, Brazil is the main producer. In Brazil, several viruses have been reported, being cassava common mosaic virus (CsCMV) the most important. Surveys performed in Paraná state, Brazil, and in Argentina showed an incidence of CsCMV of 96% and 85.2%, respectively, for cassava plants collected. The damage caused by this virus can reach losses of 30%. To better understand the actual situation of this virus in São Paulo State, a survey was perfomed collecting cassava leaves from 11 different areas in São Paulo State. In one are no CsCMV was detected but on the other 10, five had 100% of infection, followed by 89, 82, 75, 69 and 67% of infection in the other five areas. Additionally, we obtained a complete genome from Brazil (BR2019), that was distantly related to the previous Brazilian isolate reported (colocar numero de acesso), being closer to CsCMV reported in China (MT038420). Our data indicate that CsCMV is still an important virus infecting cassava in Brazil and efforts need to be done to reduce the damage caused by the virus.

3.1 INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most important crops in source of calories, providing food for 800 million people around the world (LEGG et al., 2015). In 2018, the area harvested of cassava in the world was 24.6 million hectares, with a production of 277.8 million of tons and yield of 11,297.3 kg/ha (FAOSTAT, 2018). Africa is the continent with the highest production, reaching 177.9 million tons, being Nigeria the most important country, with 59.4 million tons (FAOSTAT, 2018).

In South America, Brazil is the main cassava grower, producing around 20 million tons in 2018 (CONAB, 2018). Although its growing is mostly concentrated on small farmers with low-income, the efficiency of production is extremely high, being possible to keep using this system. The three main Brazilian growers regions include the North, South and Northeast, which reached 6.68 million, 4.79 million and 4.56 million tons, respectively in 2019. The Southeast produces 2 million tons, but the yield is one of the highest in Brazil, with 18,018 kg/ha.

Regarding phytosanitary issues, cassava plants are heavily affected by viruses, in which, currently, at least 15 have been reported around the world (LEGG et al., 2015). The cassava mosaic disease (CMD), caused by the *Cassava mosaic virus*

species, genus *Begomovirus* and the cassava brown streak disease (CBSD), caused by *Cassava brown streak virus* and *Ugandan cassava brown streak virus* species, genus *Ipomovirus*, all transmitted by the whitefly *Bemisia tabaci*, are the most important virus in Africa, causing huge losses and making the cassava inconsumable (LEGG et al., 2014, 2015). In Brazil, the viruses already reported are the cassava common mosaic virus (CsCMV) (COSTA, 1940; KITAJIMA et al., 1965; SILVA; KITAJIMA; OLIVEIRA, 1963), cassava vein mosaic virus (CsVMV) (COSTA, 1940; DE KOCHKO et al., 1998), cassava frogskin-associated virus (CsFSaV) (CALVERT et al., 2008), cassava symptomless virus (CsSLV) (KITAJIMA; COSTA, 1979) and cassava American latent virus (CsALV) (WALTER et al., 1989).

Cassava common mosaic virus is classified into the *Potexvirus* genus based on the morphology, genome, and serology. Symptoms of mosaic and chlorosis in cassava leaves have been reported by the infection of this virus (CALVERT; CUERVO IBÁÑEZ; LOZANO, 2012). CsCMV has 6376 nucleotides and is composed by a ssRNA potentially encoded RNA dependent RNA polymerase (RdRp), triple gene block 1-3 (TGB1-3) and coat protein (CP) (CALVERT et al., 1996). Surveys previously performed in Paraná state, Brazil, and in Argentina showed an incidence of CsCMV of 96% and 85.2%, respectively, for cassava plants collected (SILVA et al., 2011; ZANINI et al., 2018). In addition, the damage in the aerial part yield, root yield and starch yield have been reported in causing losses of 30% in two varieties. Nevertheless, tolerant varieties have also been reported (VENTURINI et al., 2016).

The transmission of CsCMV has never been associated with vectors. Thus, to date, as other potexvirus, CsCMV is correlated with mechanical transmission (LEGG et al., 2015). Another important parameter that must be considered is the vegetative propagation used for cassava. This type of propagation increases the accumulation of the virus, affecting the roots yield and quality (CARVAJAL-YEPES et al., 2014). Moreover, the infected cassava seed is an inoculum source for disseminating the virus in other fields.

Currently, the detection of the CsCMV is usually performed by using ELISA test or RT-PCR with generic primers Potex 5 and Potex 1RC, described by Van der Vlugt & Berendsen, 2002. By using this pair of primers is possible to investigate the variability among different isolates. For example, in Argentina, CsCMV variability has been investigated by Zanini *et al.*, 2018, in which the isolates shared 77.1% to 80.3%

nucleotide identity, indicating that different strains are present infecting cassava there. In Brazil, there is no information about the variability among isolates.

Another relevant information is about the variability among isolates from different countries. Yu *et al.*, 2020 have compared the CsCMV genome from China with a CsCMV from Brazil and other potexvirus, such as hydrangea ringspot virus, nandina mosaic virus, tulip virus X and plantago asiatica mosaic virus. The Chinese CsCMV shared 88% of nucleotide similarity with the Brazilian CsCMV. This Brazilian sequence was obtained by Calvert *et al.*, 1996. After more than 20 years, a new isolate from Brazil has not been reported yet and there is no information about the stability or variability of the virus in Brazil as well as among all other isolates in the GenBank.

Despite CsCMV is important in Brazil, there is lack of knowledge about its incidence in São Paulo state fields, about the variability from different isolates in Brazil and about the variability among all isolates from GenBank. Thus, the goals of this study were to verify the presence of the virus in São Paulo cassava field and to analyse the variability among the isolates in a global context.

3.2 MATERIAL AND METHODS

3.2.1 Field collection and virus detection

Samples of leaves from cassava plants were collected between December 2019 and February 2020 in different regions from São Paulo state. For each area, at least 22 plants were randomly collected, kept in plastic bags, transported to the Department of Plant Protection from UNESP Botucatu, and stored at -80°C for further analysis. Then, RNA extraction was performed by using the adapted Bertheau protocol (BERTHEAU et al., 2020). Pieces of cassava leaves were individually macerated within mortar and pistil in PBS-tween buffer and transferred to a 1.5 mL microtube. The samples were centrifuged at 13,000 rpm for 10 min at 4°C. A total of 200 µL of the supernatant were transferred to a new 1.5 mL microtube, mixed with 20 µL of SDS and submitted to a water bath at 55°C for 15 min. Then, 100 µL of potassium acetate were added, and the tubes were vortexed and left in ice for 5 min, followed by a centrifugation at 13,000 rpm for 5 min at 4°C. The supernatant was transferred to a new 1.5 mL microtube, and 700 µL of sodium iodide and 5 µL of silicon were added. The mix was vortexed and kept at room temperature for 10 min and followed by a centrifugation at 5,000 rpm for 1 min. The supernatant was discarded and washed twice by adding 500 µL of wash solution and centrifuging at 5,000 rpm for 1 min. Then,

the pellet was dried in vacuum, resuspended with 400 µL of DEPC water and left in water bath at 55°C for 5 min. Then, the samples were centrifuged at 13,000 rpm for 5 min and the supernatant was transferred to a new microtube and stored at -80°C for further single step RT-PCR analysis. The RT-PCR was performed using the primers CsCMV 3509F (CAA TCA CCG GAC TTT GGG GA) and CsCMV 3804R (AGG CTG TGA AGT CAT TGG CA), designed in this study. Randomly, one positive sample was Sanger sequenced for virus confirmation.

3.2.2 Bayesian phylogenetic analysis of the complete genome, RdRp and cp genes of CsCMV

A positive sample was selected randomly to be sent to high-throughput sequencing (HTS) and sequence analysis for obtaining the complete genome of the CsCMV. The total RNA was extracted following PureLink® viral RNA/DNA mini kit (Invitrogen, by Thermo Fisher Scientific and used for construction of a cDNA library utilizing the Complete ScriptSeq Kit (Epicenter, Illumina) and transcriptome sequencing with Illumina HiSeq2500 platform (Roossinck et al. 2015) at the Center of Functional Genomics (ESALQ/USP, Piracicaba, Brazil). Adapter sequence removal and quality trimming were performed with CLC Genomics Workbench software version 9.0.3. The raw data was trimmed with quality scores limit set to 0.01, ambiguous limit set to 2. The assembly parameters consisted of mismatch cost (2), insertion cost (3), deletion cost (3), length fraction (0.5), similarity fraction (0.9) and minimum contig length of either 500 bp. Then, using the Geneious software version 11.1.5 (KEARSE et al., 2012), the CsCMV complete genome was obtained by mapping the contigs sequences to the reference genome sequence (MT038420) retrieved from Genbank.

Three different Bayesian phylogenetic trees were built using the complete sequences, the RdRp and the CP genes. In order to build the dataset, all complete genome sequences of CsCMV from GenBank were used: China (MT038420, MN243731 and MN428639), Argentina (KT002439), Colombia (KT002435), Brazil (NC_001658) and the isolate from this study (MT279196). It also was added the potato virus x (NC_011620) as an outgroup. The sequences were aligned using Muscle v.3.8.425 within the Geneious v.11.1.5 software, and phylogenetic analysis was performed using Mr. Bayes 3.2.2 (RONQUIST; HUELSENBECK, 2003). The program MrModeltest v.2.2 (NYLANDER, 2004) was used to select the nucleotide substitution model using the Akaike information criterion (AIC). A total of 10 million generations,

excluding 25% from the resulting trees as burnin, with two independent runs were performed simultaneously. Phylogenetic trees were visualized by FigTree v1.4.4 software.

3.3. RESULTS

3.3.1 Assessments of the incidence of CsCMV in cassava plants in São Paulo state

Eleven collection sites were evaluated for the presence of CsCMV, including six from Mogi Mirim (samples ID 1, 2, 3, 5, 6 and 7), two from Taquarituba (samples ID 10 and 11), one from Presidente Prudente (Sample ID 4), one from Santa Maria da Serra and one from Pirassununga counties (Figure 1). The CsCMV was detected in all locations, except in one area from Taquarituba (Table 1). The percentage of infection ranged from 67% (24/36) in area 10 to 100% in area 1 (31/31), area 2 (55/55), area 3 (58/58), area 7 (69/69), and area 8 (86/86). (Table 1). The positive samples were usually found with symptoms of mosaic as illustrated in Figure 2.

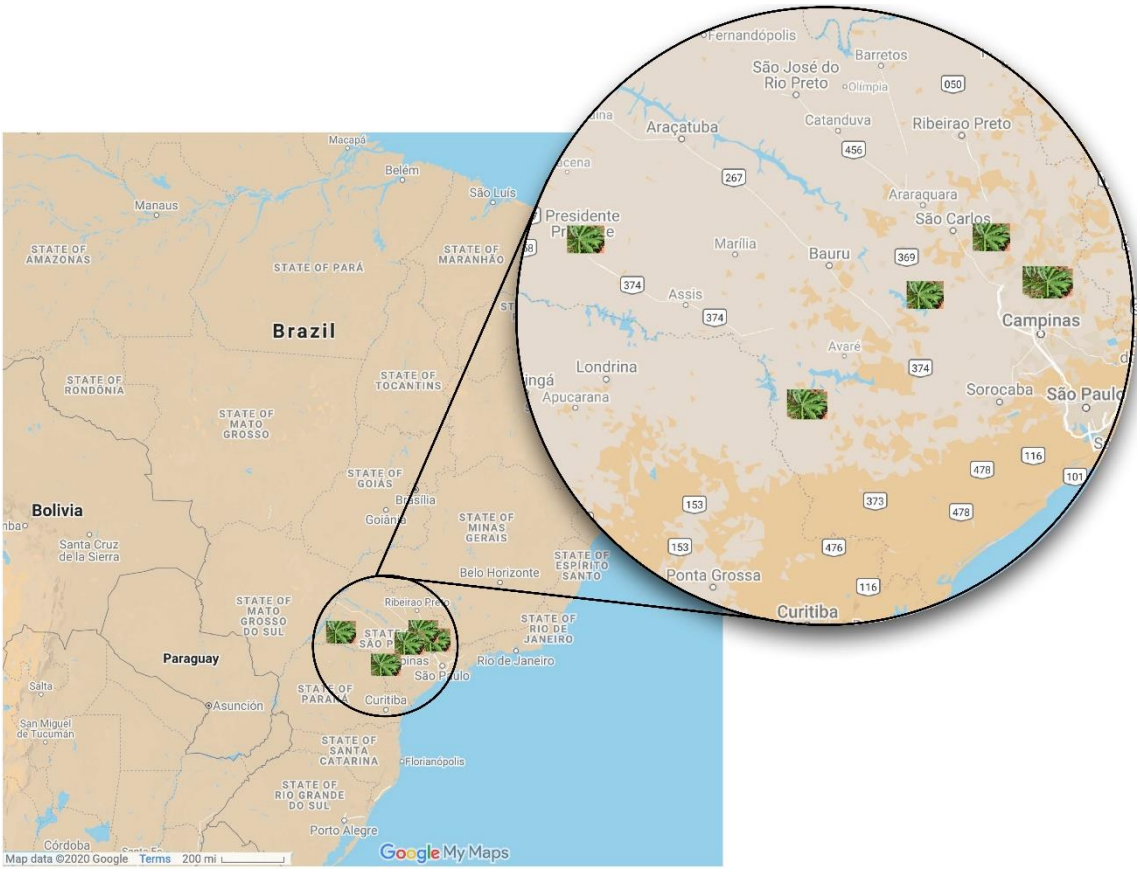


Figure 1. Map of the regions where cassava plants were collected for investigating the incidence of the CsCMV.

Table 1: Survey of cassava plants in São Paulo State for CsCMV detection.

Cassava plants collected in São Paulo State					
#ID	**Date**	**County**	**Coordinates**	**PCR positive / Samples analysed**	**Percentage of infection (%)**
1	05/12/2019	Mogi Mirim	22°26'54"S 47°02'25"W	31/31	100%
2	05/12/2019	Mogi Mirim	22°27'34"S 47°00'29"W	55/55	100%
3	05/12/2019	Mogi Mirim	22°28'51"S 46°59'07"W	58/58	100%
4	09/01/2020	Presidente Prudente	22°05'24"S 51°21'57"W	18/22	82%
5	30/01/2020	Mogi Mirim	22°26'31"S 47°03'31"W	47/63	75%
6	30/01/2020	Mogi Mirim	22°26'15"S 46°58'53"W	64/72	89%
7	30/01/2020	Mogi Mirim	22°27'27"S 46°56'02"W	69/69	100%
8	30/01/2020	Pirassununga	22°04'12"S 47°31'23"W	86/86	100%
9	31/01/2020	Santa Maria da Serra	22°34'40"S 48°08'53"W	42/61	69%
10	07/02/2020	Taquarituba	23°30'37"S 49°16'48"W	24/36	67%
11	07/02/2020	Taquarituba	23°32'01"S 49°15'17"W	0/24	0%
Total	-	-	-	494/577	85.6%

Figure 2. Symptoms of mosaic from cassava plants naturally infected by the CsCMV in the field in São Paulo State.

3.3.2 Obtaining the complete genome

A total of 40,261,228 reads (average length, 98.2 bp) were generated after trimming, of which 18,222,279 were mapped to the cassava common mosaic virus (NC001658) genome, covering the entire sequence with a mean coverage depth of 267456.0× and 98,5% nucleotide identity. No other virus sequences were identified in HTS sequence data. The final sequence obtained (MT279196) was 6,373 nt long, with a 5’ UTR of 77 nt and 3’ UTR of 114 nt [excluding the poly(A) tail], and ORFs (Open Reading Frame) RdRp, TGB1, TGB2, TGB3 and CP similar to the observed for CsCMV complete genomes previously deposited in GenBank.

3.3.3 Bayesian phylogenetic analysis of the complete genome, RdRp and CP genes of CsCMV

The three phylogenetic trees grouped the isolates in three major clades (Figures 3A, 3B and 3C). The first group was composed by isolates from Argentina (KT002439), China (MT038420) and from this study denominated as BR2019 (MT279196), the second one was composed by isolates from China (MN243731 and MN428639), and the third composed by isolates from Brazil (NC_001658) and Colombia (KT002435) (Figures 3A, 3B and 3C). The previous sequenced Brazilian isolate (NC_001658) did not grouped with the BR2019 isolate. Additionally, although the three groups formed, they have not had a phylogenetic relationship perfectly congruent, in which it changed according to the genomic region analysed (Figures 3A, 3B and 3C).

We also performed the comparison pair to pair using SDT analysis of the complete genome, and sequences showed identities from 88.7% to 93.4% among them. A maximum identity of BR2019 (MT279196) isolate was obtained with an isolate from China (MT038420), while the minimum identity of BR2019 (MT279196) isolate was with the previous sequenced Brazilian isolate (NC_001658) (Figure 4).

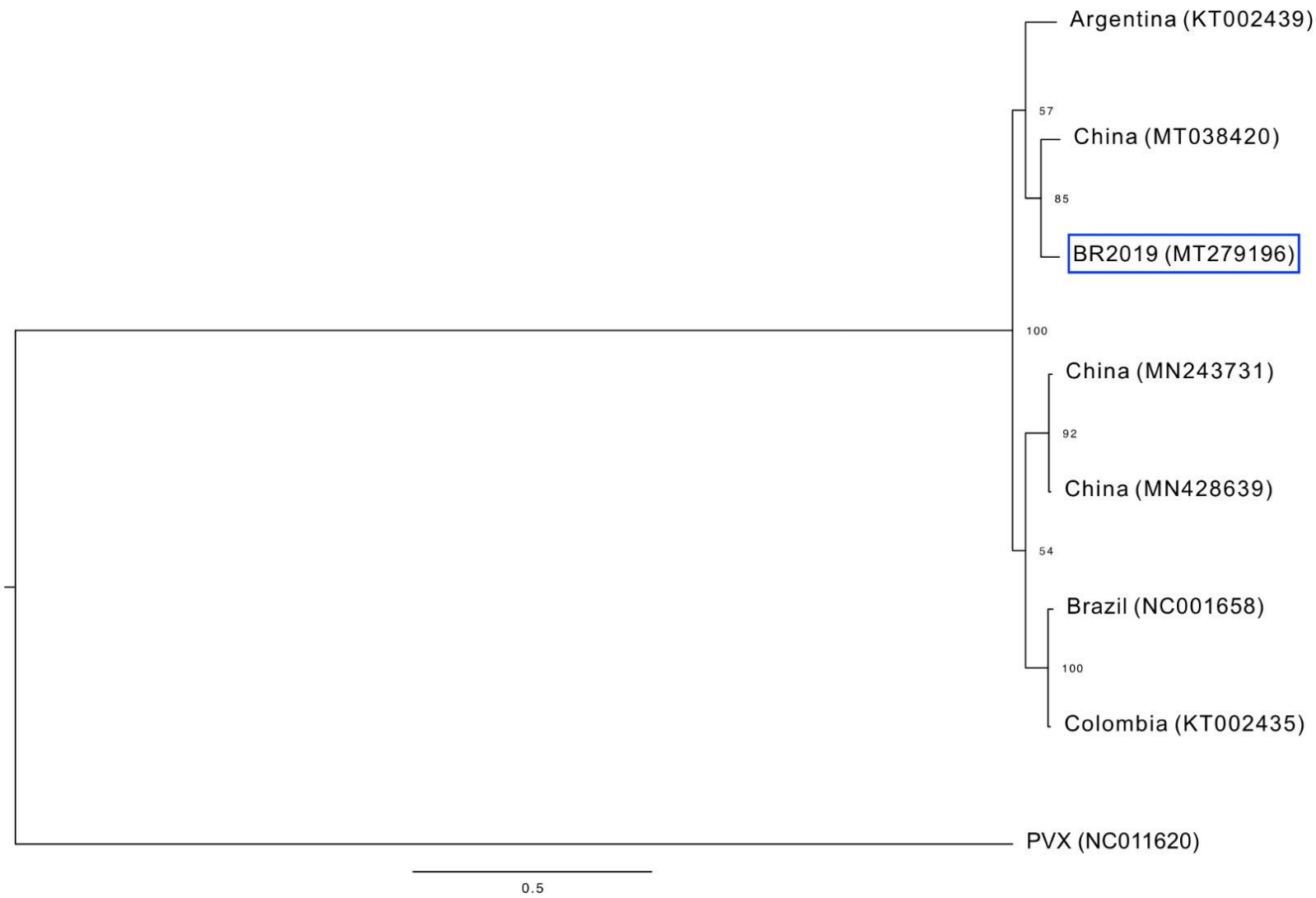


Figure 3A. Phylogenetic tree based on the complete genome of different CsCMV isolates available in GenBank using Bayesian analysis (Mr. Bayes V.3.2.2, 10 million generations). Potato virus X (PVX) was added as outgroup.

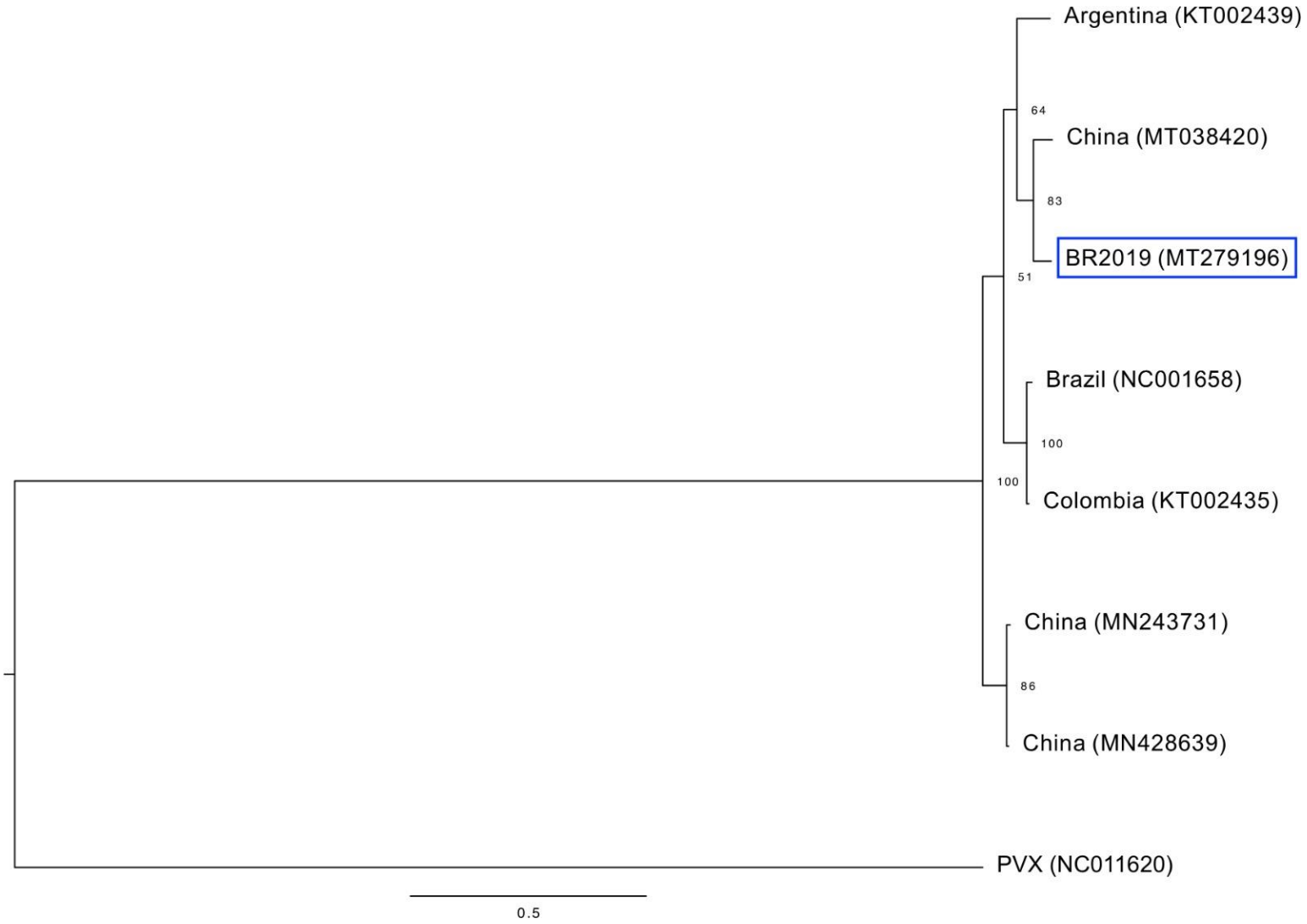


Figure 3B. Phylogenetic tree based on RdRp gene of different CsCMV isolates available in GenBank using Bayesian analysis (Mr. Bayes V.3.2.2, 10 million generations). Potato virus X (PVX) was added as outgroup.

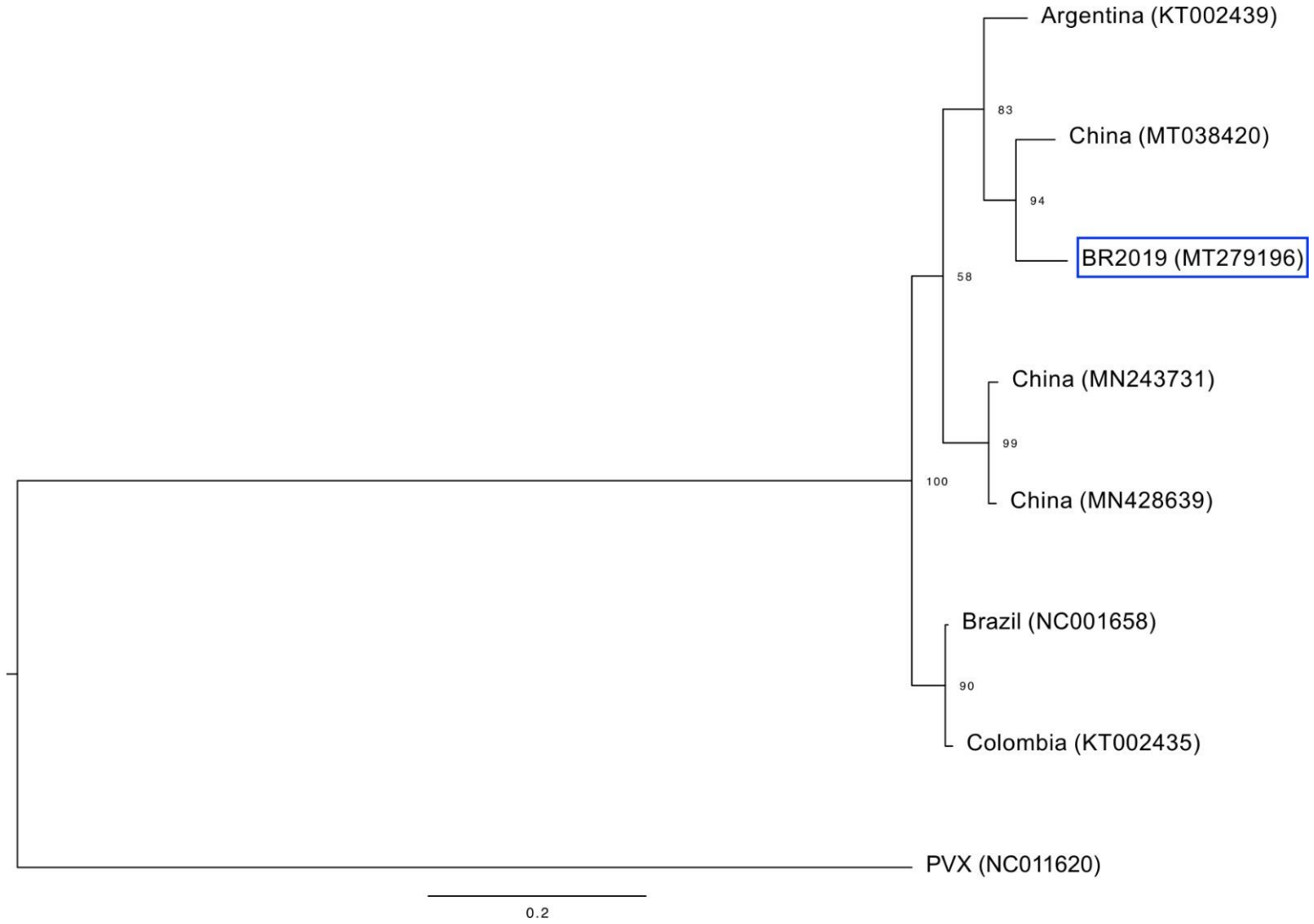


Figure 3C. Phylogenetic tree based on CP gene of different CsCMV isolates available in GenBank using Bayesian analysis (Mr. Bayes V.3.2.2, 10 million generations). Potato virus X (PVX) was added as outgroup.

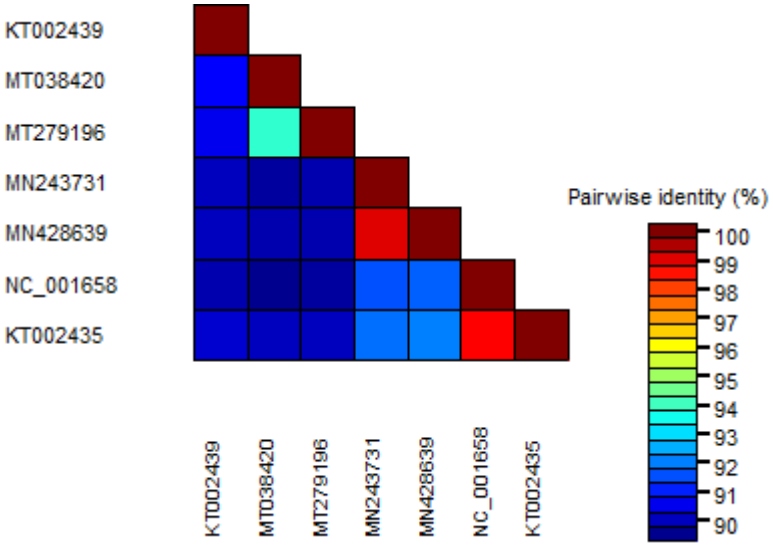


Figure 4. Genome pairwise identity based on complete sequence of different CsCMV isolates available in GenBank using SDT v1.2.

3.4 DISCUSSION

Herein, we found CsCMV infecting cassava in Brazil in 10 different areas from 11 areas sampled in São Paulo State, with the infection rate varying from 67% to 100%, showing that the virus is widely widespread in the São Paulo fields. In one area the virus was not found, which caught our attention, because it may have a significant importance that can explain the absence of the virus. In all areas where positive samples were found, cassava plants presented severe symptoms of mosaic. In addition, a new Brazilian isolate obtained in this study was closely related to isolates from China and from Argentina and, curiously, was not related to the previous sequenced Brazilian isolate.

The cassava common mosaic disease (CCMD), caused by the CsCMV, was considered a secondary disease, with minor importance, but which has recently been classified as a re-emergent virus in South America (Yu *et al.*, 2020). According to surveys performed years ago, in northwest region from Paraná State (Brazil), the incidence of the virus has reached 96%, being 101 positive samples from a total of 106 (Silva *et al.*, 2011). In Argentina, Zanini *et al.*, 2018 found CsCMV as predominant in the northeast region, where the incidence was 85.2%, from a total of 726 samples collected. In our study, we found a virus infecting rate varying from 67% to 100% depending on the area.

Cassava plants are propagated vegetatively, through stakes. This method of propagation helps pathogens to be spread, because once the plant source is infected, the stakes will keep being infected. This may explain why the incidence of the virus is so high in the field. However, it is possible to obtain virus-free stakes, preventing to spread viruses in the field. A study from Uganda revealed that techniques including hot water therapy and meristem tip culture techniques can be used for removing viruses of infected stakes, making them virus-free stakes (Nakabonge *et al*., 2020). Moreover, some companies in Brazil have also been working with virus-free stakes of cassava, which will certainly help the producers to improve their yield, once CsCMV can cause losses about 30%, affecting mainly the roots, aerial part, and starch yields (Venturini *et al.*, 2016). Another hypothesis is that a resistant variety might have been used at area 11, once CsCMV was not found. However, as we did not get the information about which variety was been used, further investigations are necessary in that area for obtaining more information about the use of virus-free stakes or a possible resistance for CsCMV.

In this study, we also obtained the complete genome of an isolate of CsCMV by NGS, denominated BR2019 (MT279196). Three different phylogenetic trees were built, including the complete genome, RdRp, and CP genes (Figures 3A, 3B and 3C). Curiously, in all trees, the isolate from this study BR2019 (MT279196) was closely related with an isolate from China (MT038420) and did not show relation with the Brazilian isolate (NC_001658) sequenced by Calvert *et al*., 1996, that was closely related to the isolate from Colombia (KT002435) (Figures 3A, 3B and 3C).

Cassava plants are originally from Brazil being cultivated for many years. During the 1990s, the first Brazilian complete genome was obtained (Calvert *et al.*, 1996), but since then, no further sequencing has been performed for CsCMV in the country. Due to the vegetative propagation method, the previous Brazilian isolate theoretically would keep being disseminated in Brazil, and it seems logical that the BR2019 isolate would be closer to the previous one. Nevertheless, the BR2019 isolate was grouped with an isolate from China, hypothesizing that this new isolate was introduced into Brazil. In addition, the results suggest that possibly the isolate BR2019 belongs to a phylogenetically distinct lineage in relation to the Brazilian isolate initially reported. However, although this close relationship with the Chinese isolate, the lack of database regarding the complete genome of CsCMV may induce this result. It may be possible that if there were more sequences available in GenBank, other groups would be built,

which could change the clades in the phylogenetic trees. Moreover, although we did not find an isolate similar to the old Brazilian isolate, it is likely that it has been infecting cassava in Brazil yet. Then, more surveys and new sequences would help in understanding the CsCMV situation and how many isolates are present in the country. Different isolates may also have influence in the losses caused by the virus to cassava plants, which makes it essential to investigate the isolates and their damage to the crop.

3.5 REFERENCE

Bertheau Y, Fréchon D, Toth IK et al., 2020. DNA amplification by polymerase chain reaction (PCR) To cite this version: HAL Id: hal-02698181., 39–59.

Calvert LA, Cuervo Ibáñez M, Lozano I, 2012. Cassava viral diseases of South America. In: International Center for Tropical Agriculture.

Calvert LA, Cuervo M, Lozano I, Villareal N, Arroyave J, 2008. Identification of three strains of a virus associated with cassava plants affected by frogskin disease. Journal of phytopathology 156, 647–653.

Calvert LA, Cuervo MI, Ospina MD, Fauquet CM, Ramirez B-C, 1996. Characterization of cassava common mosaic virus and a defective RNA species. Journal of General Virology 77, 525–530.

Carvajal-Yepes M, Olaya C, Lozano I, Cuervo M, Castaño M, Cuellar WJ, 2014. Unraveling complex viral infections in cassava (Manihot esculenta Crantz) from colombia. Virus Research 186, 76–86.

CONAB, 2018. Parâmetros de análise de mercado da raiz de mandioca e derivados - médias mensais. Análise Mensal, 6.

Costa AS, 1940. Observações sobre o mosaico comum eo mosaico das nervaduras da mandioca (Manihot utilissima Pohl.). Journal de Agronomia (Piracicaba) 3, 239–251.

FAOSTAT FAO, 2018. Crop statistics.

Kearse M, Moir R, Wilson A et al., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.

Kitajima EW, Costa AS, 1979. Rhabdovirus-like particles in tissues of five different plant species. Fitopatologia Brasileira 4, 1979.

Kitajima EW, Wetter C, Oliveira AR, Silva DM, Costa AS, 1965. Morfologia do vírus do mosaico comum da mandioca. Bragantia 24, 247–260.

De Kochko A, Verdaguer B, Taylor N, Carcamo R, Beachy RN, Fauquet C, 1998. Cassava vein mosaic virus (CsVMV), type species for a new genus of plant double stranded DNA viruses? Archives of virology 143, 945–962.

Legg JP, Kumar PL, Makeshkumar T et al., 2015. Cassava virus diseases: biology, epidemiology, and management. In: Advances in virus research. Elsevier, 85–142.

Legg J, Somado EA, Barker I et al., 2014. A global alliance declaring war on cassava viruses in Africa. Food Security 6, 231–248.

Nakabonge G, Nangonzi R, Tumwebaze BS, Kazibwe A, Samukoya C, Baguma Y, 2020. Production of virus-free cassava through hot water therapy and two rounds of meristem tip culture. Cogent Food & Agriculture 6, 1800923.

Nylander J, 2004. MrModeltest v. 2.2 b. Department of Systematic Zoology, Uppsala University, Sweden.

Ronquist F, Huelsenbeck JP, 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Silva JM, Carnelossi PR, Bijora T et al., 2011. Immunocapture-RT-PCR detection of Cassava common mosaic virus in cassava obtained from meristem-tip culture in Paraná state. Tropical Plant Pathology 36, 271–275.

Silva DM, Kitajima EW, Oliveira AR, 1963. Obtencao do virus do mosaico comun da mandioca purificado. Ciencia e cultura 15, 304.

Venturini MT, Araújo T da S, Abreu EFM et al., 2016. Crop losses in Brazilian cassava varieties induced by the Cassava common mosaic virus. Scientia Agricola 73, 520–524.

Van der Vlugt RAA, Berendsen M, 2002. Development of a general potexvirus detection method. European Journal of Plant Pathology 108, 367–371.

Walter B, LADEVEZE I, Etienne L, Fuchs M, 1989. Some properties of a previously undescribed virus from cassava: Cassava American latent virus. Annals of applied biology 115, 279–289.

Yu N tong, Yang Y, Li J hua, Li W li, Liu Z xin, 2020. Complete genome sequence of a distinct isolate of cassava common mosaic virus (CsCMV) infecting cassava in Hainan, China. European Journal of Plant Pathology 158, 583–587.

Zanini AA, Cuellar WJ, Celli MG et al., 2018. Distinct strains of the re-emergent Cassava common mosaic virus (genus: Potexvirus) infecting cassava in Argentina. Plant Pathology 67, 1814–1820.

CAPÍTULO 4 – Atividades Realizadas Durante o Intercâmbio na Oklahoma State University (OSU), Estados Unidos

Durante meu curso de doutorado realizado na UNESP, campus de Botucatu, entre 2017 e 2020, fui contemplado com uma Bolsa de Doutorado-Sanduíche no Exterior (PDSE)" da CAPES, e realizei treinamento durante 6 meses no laboratório denominado de "National Institute for Microbial Forensics & Food and Agricultural Biosecurity (NIMFFAB)", incluído no "Departament of Entomology and Plant Pathology" da Oklahoma State University (OSU), Stillwater, Oklahoma, EUA., OSU, sob orientação do Prof. Dr. Francisco M. Ochoa Corona, docente nesta instituição.

Durante esse período, trabalhei com desenhos de primers e com diagnose de vírus em videira, utilizando softwares gratuitos e acessíveis a todos, como o Primer3, Primer Blast, mfold, entre outros. Também obtive conhecimento da técnica de qPCR e HRM, extrações de DNA/RNA, PCR, RT-PCR, sequenciamento Sanger e "Next Generation Sequencing (NGS)" de diferentes amostras de videira infectadas por vírus, obtendo uma grande quantidade de dados de bioinformática, que seria utilizado posteriormente, em um software, denominado de "E-probe Diagnostic Nucleic-acid Analysis (EDNA)" desenvolvido pelos próprios pesquisadores da OSU, incluindo o Dr. Francisco e o Dr. Andres Espindola. O EDNA tem como objetivo a diagnose rápida, em uma única análise e de maneira rápida e precisa, de diversos patógenos, incluindo vírus, bactérias, fungos, entre outros, que infectam determinada cultura. O intuito é utilizá-la como ferramenta na diagnose de pragas quarentenárias, onde pode-se diagnosticar doenças que estão infectando determinada amostra e que não estão presentes no país ou estado que se está analisando. Dessa forma, a introdução de uma nova doença seria evitada, auxiliando a produção agrícola.

Foi obtido um banco de dados de RNA/DNA para os vírus grapevine leafroll-associated virus 3 (GLRaV-3) e grapevine leafroll-associated virus 4 (GLRaV-4), vírus de RNA, e o grapevine red blotch virus (GRBV), vírus de DNA, que vem sendo utilizado pelos pesquisadores da OSU para desenvolvimento de protocolos de diagnose. Considero que este período de intercâmbio proporcionou um grande aprendizado e amadurecimento profissional.

CONSIDERAÇÕES FINAIS

- As espécies invasivas MEAM1 e MED do complexo *B. tabaci* são adaptadas de acordo com os hospedeiros, onde MEAM1 é mais adaptada ao tomateiro e MED é mais adaptada ao pimentão e feijoeiro;
- Para as culturas da soja e do algodoeiro, MEAM1 e MED são adaptadas de forma semelhante, uma vez que não houve predominância de nenhuma das espécies;
- O ToCV influencia na performance das espécies *B. tabaci* MEAM1 e *T. vaporariorum*, porém mais drasticamente a performance de MEAM1;
- MEAM1 é um vetor mais eficiente de ToCV do que *T. vaporariorum*;
- *Trialeurodes vaporariorum* pode ser um vetor importante de ToCV em ambientes com alta incidência desse vírus, especialmente em climas mais amenos;
- O CsCMV está bem distribuído na cultura da mandioca no Estado de São Paulo, visto que a presença do vírus foi encontrada em 10 das 11 áreas estudadas.
- O isolado BR2019 de CsCMV obtido neste estudo apresentou maior relacionamento filogenético com um proveniente da China.

REFERÊNCIAS

ACCOTTO, G. P. et al. First report of Tomato chlorosis virus in Italy. **Plant Disease**, v. 85, n. 11, p. 1208, 2001.

ALBUQUERQUE, L. C. et al. Further characterization of tomato-infecting begomoviruses in Brazil. **Archives of Virology**, v. 157, n. 4, p. 747–752, 2012.

ALMEIDA, A. M. R. et al. Necrose da haste da soja. **Serie Documentos**, v. 221, p. 1–48, 2003.

ARRUABARRENA, A. et al. First report of Tomato chlorosis virus infecting tomato crops in Uruguay. **Plant disease**, v. 98, n. 10, p. 1445, 2014.

BARBOSA, J. C. et al. First report of Tomato chlorosis virus infecting tomato crops in Brazil. **Plant Disease**, v. 92, n. 12, p. 1709, 2008.

BARBOSA, J. C. et al. Characterization and experimental host range of a Brazilian tomato isolate of Tomato severe rugose virus. **Journal of Phytopathology**, v. 159, n. 9, p. 644–646, 2011.

BARBOSA, J. C.; TEIXEIRA, L. D. D.; REZENDE, J. A. M. First Report on the Susceptibility of Sweet Pepper Crops to Tomato chlorosis virus in Brazil. **Plant Disease**, v. 94, n. 3, p. 374, 3 fev. 2010.

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

BELLO, V. H. et al. First Report of Tomato Chlorosis Virus Infecting Cucumber in Brazil. **Plant Disease**, v. 104, n. 2, p. 603, 23 set. 2019.

BELLO, V. H. et al. Outbreaks of Bemisia tabaci Mediterranean species in vegetable crops in São Paulo and Paraná States, Brazil. **Bulletin of Entomological Research**, 2020a.

BELLO, V. H. et al. First Report of Tomato Chlorosis Virus Infecting Cucumber in Brazil. **Plant Disease**, v. 104, n. 2, p. 603, fev. 2020b.

BERTHEAU, Y. et al. DNA amplification by polymerase chain reaction (PCR) To cite this version : HAL Id : hal-02698181. p. 39–59, 2020.

BONDAR, G. **Aleyrodideos do Brasil**. Bahia: Boletim do Laboratório de Patologia Vegetal do Estado da Bahia, 1928.

BROWN, J. K.; FROHLICH, D. R.; ROSELL, R. C. The sweetpotato or silverleaf whiteflies: biotypes of Bemisia tabaci or a species complex? **Annual review of entomology**, v. 40, n. 1, p. 511–534, 1995.

CALVERT, L. A. et al. Characterization of cassava common mosaic virus and a defective RNA species. **Journal of General Virology**, v. 77, n. 3, p. 525–530, 1996.

CALVERT, L. A. et al. Identification of three strains of a virus associated with cassava plants affected by frogskin disease. **Journal of phytopathology**, v. 156, n. 11-12, p. 647–653, 2008.

CALVERT, L. A.; CUERVO IBÁÑEZ, M.; LOZANO, I. Cassava viral diseases of

South America. In: [s.l.] International Center for Tropical Agriculture, 2012.

CARVAJAL-YEPES, M. et al. Unraveling complex viral infections in cassava (Manihot esculenta Crantz) from colombia. **Virus Research**, v. 186, p. 76–86, 2014.

CASTILLO-URQUIZA, G. P. et al. Six novel begomoviruses infecting tomato and associated weeds in Southeastern Brazil. **Archives of Virology**, v. 153, n. 10, p. 1985–1989, 2008.

CONAB. Parâmetros de análise de mercado da raiz de mandioca e derivados - médias mensais. **Análise Mensal**, n. 61, p. 6, 2018.

COSTA, A. S. Observações sobre o mosaico comum eo mosaico das nervaduras da mandioca (Manihot utilissima Pohl.). **Journal de Agronomia (Piracicaba)**, v. 3, n. 3, p. 239–251, 1940.

COSTA, A. S. Studies on Abutilon mosaic in Brazil. **Phytopathologische Zeitschrift**, v. 24, p. 97–112, 1955.

COSTA, A. S. Increase in the populational density of Bemisia tabaci, a threat of widespread virus infection of legume crops in Brazil. In: BIRD, J.; MARAMOROSCH, K. (Eds.). . **Tropical diseases of legumes**. New York: Academic, 1975.

COSTA, A. S.; BENNETT, C. W. White-fly-transmitted mosaic of Euphorbia prunifolia. **Phytopathology**, v. 40, n. 3, p. 266–283, 1950.

COSTA, A. S.; GASPAR, J. O.; VEGA, J. Mosaico angular do feijoeiro Jalo causado por um carlavirus transmitido pela mosca branca Bemisia tabaci. **Fitopatologia brasileira**, v. 8, n. 2, p. 325–337, 1983.

COSTA, A. S.; OLIVEIRA, A. R.; SILVA, D. M. Transmissao mecanica do mosaico dourado do tomateiro. **Fitopatologia**, 1975.

COSTA, T. M. et al. The recombinant isolate of cucurbit aphid-borne yellows virus from Brazil is a polerovirus transmitted by whiteflies. **Plant Pathology**, 2020.

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

DE KOCHKO, A. et al. Cassava vein mosaic virus (CsVMV), type species for a new genus of plant double stranded DNA viruses? **Archives of virology**, v. 143, n. 5, p. 945–962, 1998.

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.

FAOSTAT, F. A. O. **Crop statistics**, 2018.

FERNANDES, J. J. et al. Biological and molecular properties of Tomato rugose mosaic virus (ToRMV), a new tomato-infecting begomovirus from Brazil. **Plant Pathology**, v. 55, n. 4, p. 513–522, 2006.

FIALLO-OLIVÉ, E. et al. First report of Tomato chlorosis virus infecting tomato in Sudan. **Plant Disease**, v. 95, n. 12, p. 1592, 2011.

FLORES, E.; SILBERSCHMIDT, K.; KRAMER, M. Observações de “clorose

infecciosa” das malváceas em tomateiros do campo. **O Biológico**, v. 26, p. 65–69, 1960.

FONSECA, M. E. N. et al. First Report of Tomato chlorosis virus Infecting Eggplant and Scarlet Eggplant in Brazil. **Plant Disease**, v. 100, n. 4, p. 867, 2016.

FORTES, I. M.; MORIONES, E.; NAVAS-CASTILLO, J. Tomato chlorosis virus in pepper: Prevalence in commercial crops in southeastern Spain and symptomatology under experimental conditions. **Plant Pathology**, v. 61, n. 5, p. 994–1001, 2012.

FORTES, I. M.; NAVAS-CASTILLO, J. Potato, an experimental and natural host of the crinivirus Tomato chlorosis virus. **European Journal of Plant Pathology**, v. 134, n. 1, p. 81–86, 2012.

FREITAS, D. M. S. et al. First report of Tomato chlorosis virus in potato in Brazil. **Plant disease**, v. 96, n. 4, p. 593, 2012.

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 2019.

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.

HIROTA, T. et al. Yellowing disease of tomato caused by Tomato chlorosis virus newly recognized in Japan. **Journal of general plant pathology**, v. 76, n. 2, p. 168–171, 2010.

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; 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.

KANAKALA, S.; GHANIM, M.; KANAKALA, S. AND GHANIM, M. Global genetic diversity and geographical distribution of Bemisia tabaci and its bacterial endosymbionts. **PLoS ONE**, v. 14, n. 3, 2019.

KEARSE, M. et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. **Bioinformatics**, v. 28, n. 12, p. 1647–1649, 2012.

KITAJIMA, E. W. et al. Morfologia do vírus do mosaico comum da mandioca. **Bragantia**, v. 24, n. UNICO, p. 247–260, 1965.

KITAJIMA, E. W.; COSTA, A. S. Rhabdovirus-like particles in tissues of five different plant species. **Fitopatologia Brasileira**, v. 4, n. 55, p. 1979, 1979.

LEE, W. et al. Taxonomic Status of the Bemisia tabaci Complex (Hemiptera: Aleyrodidae) and Reassessment of the Number of Its Constituent Species. **PLoS ONE**, v. 8, n. 5, 2013.

LEGG, J. et al. A global alliance declaring war on cassava viruses in Africa. **Food Security**, v. 6, n. 2, p. 231–248, 2014.

LEGG, J. P. et al. Cassava virus diseases: biology, epidemiology, and management. In: **Advances in virus research**. [s.l.] Elsevier, 2015. v. 91p. 85–142.

LIU, H. Y.; WISLER, G. C.; DUFFUS, J. E. Particle lengths of whitefly-transmitted criniviruses. **Plant Disease**, v. 84, n. 7, p. 803–805, 2000.

LOURENÇÃO, A. L. et al. Outbreaks of Trialeurodes vaporariorum (West.) (Hemiptera: Aleyrodidae) under field conditions in the State of São Paulo, Brazil. **Neotropical entomology**, v. 37, n. 1, p. 89–91, 2008.

LOURENÇÃO, A. L.; NAGAI, H. Surtos populacionais de Bemisia tabaci no Estado de São Paulo. **Bragantia**, v. 53, n. 1, p. 53–59, 1994.

LOZANO, G.; MORIONES, E.; NAVAS-CASTILLO, J. Complete nucleotide sequence of the RNA2 of the crinivirus tomato chlorosis virus. **Archives of virology**, v. 151, n. 3, p. 581–587, 2006.

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.

MANSILLA-CÓRDOVA, P. J. et al. Screening tomato genotypes for resistance and tolerance to Tomato chlorosis virus. **Plant Pathology**, v. 67, n. 5, p. 1231–1237, 2018.

MANZANO, M. R.; VAN LENTEREN, J. C. Life history parameters of Trialeurodes vaporariorum (Westwood) (Hemiptera: Aleyrodidae) at different environmental conditions on two bean cultivars. **Neotropical entomology**, v. 38, n. 4, p. 452–458, 2009.

MARUBAYASHI, J. M. et al. At least two indigenous species of the Bemisia tabaci complex are present in Brazil. **Journal of Applied Entomology**, v. 137, n. 1–2, p. 113–121, 2013.

MATYIS, J. C. et al. Purificação e morfologia do vírus do mosaico dourado do tomateiro. **Summa Phytopathologica**, v. 1, p. 267–275, 1975.

MORAES, L. A. DE et al. New invasion of Bemisia tabaci Mediterranean species in Brazil associated to ornamental plants. v. 1, 2017.

NAVAS-CASTILLO, J.; FIALLO-OLIVÉ, E.; SÁNCHEZ-CAMPOS, S. Emerging virus diseases transmitted by whiteflies. **Annual review of phytopathology**, v. 49, p. 219–48, 2011.

NYLANDER, J. MrModeltest v. 2.2 b. **Department of Systematic Zoology, Uppsala University, Sweden**, 2004.

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.

RIBEIRO, S. G. et al. Distribution and genetic diversity of tomato-infecting begomoviruses in Brazil. **Archives of Virology**, v. 148, n. 2, p. 281–295, 2003.

ROCHA, C. S. et al. Brazilian Begomovirus Populations Are Highly Recombinant, Rapidly Evolving, and Segregated Based on Geographical Location. **Journal of Virology**, v. 87, n. 10, p. 5784–5799, 2013.

RONQUIST, F.; HUELSENBECK, J. P. MrBayes 3: Bayesian phylogenetic inference under mixed models. **Bioinformatics**, v. 19, n. 12, p. 1572–1574, 2003.

SEGEV, L. et al. First report of Tomato chlorosis virus in Israel. **Plant Disease**, v. 88, n. 10, p. 1160, 2004.

SILVA, D. M.; KITAJIMA, E. W.; OLIVEIRA, A. R. Obtencao do virus do mosaico comun da mandioca purificado. **Ciencia e cultura**, v. 15, p. 304, 1963.

SILVA, J. M. et al. Immunocapture-RT-PCR detection of Cassava common mosaic virus in cassava obtained from meristem-tip culture in Paraná state. **Tropical Plant Pathology**, v. 36, n. 5, p. 271–275, 2011.

STOBBE, A. H. et al. E-probe Diagnostic Nucleic acid Analysis (EDNA): A theoretical approach for handling of next generation sequencing data for diagnostics. **Journal of Microbiological Methods**, v. 94, n. 3, p. 356–366, 2013.

SUEKANE, R. et al. Danos da mosca-branca Bemisia tabaci (Genn.) e distribuição vertical das ninfas em cultivares de soja em casa de vegetação. **Arquivos do Instituto Biológico**, v. 80, n. 2, p. 151–158, 2013.

VAN DER VLUGT, R. A. A.; BERENDSEN, M. Development of a general potexvirus detection method. **European Journal of Plant Pathology**, v. 108, n. 4, p. 367–371, 2002.

VELASCO, L. et al. Incidences and progression of tomato chlorosis virus disease and tomato yellow leaf curl virus disease in tomato under different greenhouse covers in southeast Spain. **Annals of applied biology**, v. 153, n. 3, p. 335–344, 2008.

VENTURINI, M. T. et al. Crop losses in Brazilian cassava varieties induced by the Cassava common mosaic virus. **Scientia Agricola**, v. 73, n. 6, p. 520–524, 2016.

VYSKOČILOVÁ, S. et al. An integrative approach to discovering cryptic species within the Bemisia tabaci whitefly species complex. **Scientific Reports**, v. 8, n. 1, p. 1–13, 2018.

WALTER, B. et al. Some properties of a previously undescribed virus from cassava: Cassava American latent virus. **Annals of applied biology**, v. 115, n. 2, p. 279–289, 1989.

WINTERMANTEL, W. M.; WISLER, G. C. Vector Specificity, Host Range, and Genetic Diversity of Tomato chlorosis virus. **Plant Disease**, v. 90, n. 6, p. 814–819, 2006.

WISLER, G. C. et al. Tomato Chlorosis Virus : A New Whitefly-Transmitted , Phloem-Limited , Bipartite Closterovirus of Tomato. 1998.

YU, N. TONG et al. Complete genome sequence of a distinct isolate of cassava common mosaic virus (CsCMV) infecting cassava in Hainan, China. **European Journal of Plant Pathology**, v. 158, n. 2, p. 583–587, 2020.

ZANINI, A. A. et al. Distinct strains of the re-emergent Cassava common mosaic virus (genus: Potexvirus) infecting cassava in Argentina. **Plant Pathology**, v. 67, n. 8, p. 1814–1820, 2018.