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INTERACTION AND MANAGEMENT OF BEGOMOVIRUSES AND WHITEFLIES IN WEEDS AND HORTICULTURE CROPS IN THE STATE OF SÃO PAULO AND SURVEY OF ORTHOTOSPOVIRUSES AND ASSOCIATED THRIPS IN THE ARICA Y PARINACOTA REGION - CHILE

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Advisors: Prof. Renate Krause Sakate (UNESP) and Prof. Inés Marlene Rosales Villavicencio (PUC-Chile).

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To my advisors, who believed and allowed me
to conduct my studies.
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ABSTRACT

The insects *Bemisia tabaci* (Gennadius) and *Frankliniella occidentalis* (Pergande) are pests that have managed to spread and establish in different regions of the world, disseminating several viruses, such as begomoviruses and orthotospoviruses. In order to contribute with key information for the development of management strategies for these pests in tomato (*Solanum lycopersicum* L.), cucumber (*Cucumis sativus* L.), and pepper (*Capsicum* spp.) crops, this thesis contemplates two studies involving the cryptic species of *B. tabaci* Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) in Brazil (chapters 1 and 2), and two studies of orthotospoviruses and thrips in Chile (Chapter 3 and 4). In chapter 1, the potential of invasive plants *Datura stramonium* and *Nicandra physaloides* L. (Gaertn.) as alternative hosts of MEAM1 and MED and for the tomato severe rugose virus (ToSRV) was studied, showing *N. physaloides* as a great alternative host of the virus when associated with MEAM1, whereas *D. stramonium* contributed more with the whitefly reproduction. In chapter 2, the causes related to recent MED infestations in cucumbers observed on São Paulo and Paraná states were investigated by means of comparison of biological performance and preference tests comparing MEAM1 vs. MED in commercial cucumber cultivars, showing that MED performed better in almost all commercial cultivars tested and that the performance is a key factor for the predominance of this whitefly in cucumber plants. In chapter 3, the putative new orthotospovirus Pepper necrotic spot virus was reported for the first time in Chile, infecting bell pepper fruits, suggesting its presence in northern production areas from Chile. Finally, in chapter 4, a survey of orthotospoviruses and associated thrips was carried out on tomato, bell pepper, and chili pepper plants in Arica y Parinacota (Northern Chile), where cases of the “spotted wilt” are increasing. The results showed the occurrence of an isolate of tomato spotted wilt virus, capable of infecting plants with the *Tsw* resistance gene, and the putative orthotospovirus pepper necrotic spot virus in Chile, both probably associated with *F. occidentalis,* occurring in all sampled locations.

Keywords: *Bemisia tabaci*; *Frankliniella occidentalis*; pepper necrotic spot virus; tomato spotted wilt virus; biological performance; preference; survey; PNSV; TSWV.

RESUMO EXPANDIDO

Os insetos *Bemisia tabaci* (Gennadius) e *Frankliniella occidentalis* (Pergande) são considerados super-vetores de virus de plantas, dentre os quais, destacam-se os *Begomovirus* e *Orthotospovirus*. Visando colaborar com informações para o desenvolvimento de estratégias de manejo para esses insetos e seus vírus associados nas culturas do tomate, pepino e pimentão, a presente tese traz dois estudos envolvendo as espécies crípticas Middle East-Asia Minor 1 (MEAM1) e Mediterranean (MED) no Brasil (capítulos 1 e 2), e dois capítulos envolvendo orthotospoviroses e tripes no chile (capítulos 3 e 4).

CAPÍTULO 1 - Avaliação de *Datura stramonium* e *Nicandra physaloides* como reservatórios do tomato severe rugose virus e de moscas brancas.

Tomato severe rugose virus (ToSRV) é um dos begomovirus mais prevalentes no Brasil, sendo transmitido pela mosca-branca *Bemisia tabaci* e ocorrendo principalmente em tomateiro. Práticas de controle cultural, como a rotação de culturas e o vazio sanitário, são corriqueiramente adotadas juntamente com outras práticas para controlar esse vírus, porém, sem muito sucesso. O potencial de plantas daninhas como hospedeiras alternativas e fonte de inóculo primário para a transmissão do ToSRV ainda é pouco estudado, e isso pode contribuir para falhas de controle cultural no campo.

Esse estudo teve como objetivo avaliar o papel das plantas daninhas *Datura stramonium* e *Nicandra physaloides* como hospedeiras alternativas do ToSRV e virificar a influência deste vírus na performance das espécies crípticas de *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1) e Mediterranean (MED).

Nicandra physaloides foi um melhor hospedeiro alternativo e, combinação com MEAM1, enquanto *D. stramonium* contribuiu mais como uma hospedeira alternativa das moscas brancas. A infecção viral melhorou a performance de MEAM1 e afetou negativamente desenvolvimento de MED em ambas as plantas hospedeiras. Esses dados sugerem que ambas as plantas daninhas possuem influência no patosistema do ToSRV e que o controle delas deve ser incluído em programas de manejo desse vírus.

CAPÍTULO 2 – Performance e preferência de *Bemisia tabaci* em pepineiro: Entendendo os recentes surtos da espécie críptica Mediterranean no Brasil.

Atualmente no Brasil, parte da produção de pepino está sendo feita sob cultivo protegido, em propriedades que também produzem pimentão e tomate, permitindo aos agricultores o aproveitamento da estrutura de tutora e do adubo remanescentes dos cultivos de pimentão e tomate, gerando economia. Por outro lado, nos últimos anos, essa prática tem contribuído para surtos populacionais de MED no estado de São Paulo, o que culminou no primeiro relato mundial do crinivirus *Tomato chlorosis virus* em pepino.

Nesse estudo foi comparada a preferência de *B. tabaci* MEAM1 e MED em uma situação contendo plantas de pepino (cv.Taiko), tomate (Sta. Clara) e pimentão (Magali) cultivados na mesma área, e a preferência e desempenho biológico de MEAM1 vs. MED em nove cultivares comerciais de pepineiro [cvs. Soldier, Taiko, Tsuyataro e Valent (tipo japonês), Durango, Aodai e Verde Comprido (tipo comum); e Pioneiro e Wisconsin SMR58 (tipo conserva)], avaliando também o potencial de cada cultivar para reduzir a população de moscas-brancas.

O pepineiro foi o hospedeiro mais preferido quando comparado ao tomate e pimentão no teste inicial de preferência para ambas as moscas-brancas, as quais não só frequentaram a planta, mas também foram capazes de se estabelecer e ovipositar.

A espécie críptica MED apresentou melhor desempenho biológico do que MEAM1 em quase todas as nove cultivares de pepino testadas, por apresentar melhor capacidade de sobrevivência, resultando em um número significativamente maior de adultos. Na avaliação de cultivares, apenas ‘Aodai’ se destacou, gerando uma alta mortalidade de ninfas de MEAM1, mostrando que pode ser uma boa opção a ser implantada em áreas com ausência de MED. Em contrapartida, nenhuma cultivar foi potencialmente satisfatória para a redução da população de MED, reforçando a necessidade de desenvolver estratégias de manejo específicas direcionadas a esta espécie de mosca branca.

CAPÍTULO 3 – Primeiro relato da espécie tentativa de orthotospovírus Pepper necrotic spot virus no Chile.

Em agosto de 2020 foram encontrados frutos de pimentão apresentando sintomas típicos da doença vira-cabeça (Manchas anelares e amadurecimento irregular) em feiras de rua e mercados da cidade de Santiago. Como nesse período a produção de pimentão se limita a região norte do país por questões climáticas, os frutos foram recolhidos para caracterização de possíveis viroses presentes.

O presente estudo teve como objetivo caracterizar espécies virais relacionadas com os sintomas de vira-cabeça nos pimentões comprados.

A caracterização das espécies virais foi feita por meio de RT-PCR usando primers genéricos e específicos para os orthotospovirus relacionados a doença conhecida por vira-cabeça. As sequencias obtidas foram submetidas a sequenciamento de Sanger. Os resultados revelaram a presença de uma espécie tentativa de orthotospovirus chamada Pepper necrotic spot virus, com 99.7% de identidade com o isolato T1, relatado no Peru.

Para confirmar a patogenicidade, fragmentos de um dos frutos foram usados para inoculação com extrato vegetal tamponado em plantas sadias das cultivares Coraza e Almuden de pimentão. Após 15 dias, sintomas de pontos necróticos e clorose foram observados, com o desenvolvimento de manchas anelares e mosaico ao longo dos dias. Todas as plantas sintomáticas foram positivas usando os mesmos primers genéricos e nenhum outro orthotospovirus foi detectado nas amostras positivas.

O PNSV foi primeiramente relatado no vale de La joya, aproximadamente 400 km da fronteira com o Chile. Até o momento apenas o trabalho de primeiro relato desse vírus está disponível e somente duas sequencias do RNA S estão presentes no GenBank. Esse é o primeiro relato do PNSV no Chile, e de acordo com a data em que os frutos de pimentão foram encontrados, suspeita-se que eles tenham sido cultivados no norte do Chile, e por isso ocorrência desse vírus deve ser melhor investigada nessa região.

CAPÍTULO 4 – Levantamento de orthotospoviroses e tripes associadas a pimenteiras em Arica y Parinacota – Chile.

Dentre os osthotospovirus causadores do “vira cabeça”, apenas as espécies *Tomato spotted wilt virus* e *Impatiens necrotic spot virus* foram detectadas no Chile, sempre em associação com *F. occidentalis.* Recentemente, surtos de “vira-cabeça” começaram a ser relatados por técnicos e agricultores da região de Arica y Parinacota, localizada no extremo norte do Chile, na divisa com o Peru.

Dada a importância dessa região como fornecedora de produtos hortícolas durante o inverno, esse estudo teve como objetivo mapear e caracterizar os agentes causais e tripes associadas aos surtos de “vira-cabeça” que estão ocorrendo em Arica y Parinacota.

Folhas jovens de plantas de pimentão e tomate, exibindo sintomas típicos de murcha e tripes associados, foram coletadas em áreas comerciais. Os vírus de plantas foram detectados em nível de espécie por RT-PCR, utilizando primers específicos ou genéricos direcionados a orthotospovírus e seus tripes associados foram identificados morfologicamente, com algumas amostras representativas submetidas a PCR, para confirmação da identificação.

A espécie *Tomato spotted wilt virus* e o Pepper necrotic spot virus (espécie tentativa) foram detectados em nove e 13 amostras, respectivamente. Todos os tripes coletados foram identificados como *F. occidentallis* e nenhuma outra espécie de tripes foi verificada nos exemplares analisados. A presença desses vírus é preocupante, mostrando a expansão do PNSV do Peru para novas áreas e sugerindo que o TSWV encontrado pode ser um isolado capaz de infectar pimentões resistentes, uma vez que a maioria das cultivares utilizadas nesta região possuem resistência ao TSWV. Além disso, a presença de apenas *F. occidentalis* sugere que esse inseto seja o provável vetor desses vírus. O sequenciamento de nova geração permitirá uma melhor caracterização de ambos os vírus.

Palavras-Chave: *Bemisia tabaci*; *Frankliniella occidentalis*; Pepper necrotic spot virus; *Tomato spotted wilt virus*; desempenho biológico; preferência; levantamento; PNSV; TSWV.

RESUMEN EXTENDIDO

Los insectos *Bemisia tabaci* (Gennadius) y *Frankliniella occidentalis* (Pergande) pueden ser considerados supervectores de virus de plantas, entre los cuales, destacan se los *Begomovirus* y *Orthotospovirus*. Con el fin de colaborar con información para el desarrollo de estrategias de manejo de estos insectos y sus virus asociados en los cultivos de tomate, pepino y pimiento, esta tesis trae dos estudios que involucran a las especies crípticas Middle east Asia Minor 1 (MEAM1) y Mediterranean (MED) de *B. tabaci* en Brasil (capítulos 1 y 2), y dos capítulos sobre Orthotospovirosis y trips en chile (capítulos 3 y 4).

CAPÍTULO 1 - Evaluación de las malezas *Datura stramonium* y *Nicandra physaloides* como reservoirs del tomato severe rugose virus y de mosquitas blancas.

El virus *Tomato severe rugose virus* (ToSRV) es uno de los begomovirus más prevalentes en Brasil, transmitido por la mosca blanca Bemisia tabaco, infectando principalmente el tomate. Las prácticas de control cultural, como la rotación de cultivos y el vacío sanitario, se adoptan comúnmente junto con otras prácticas para controlar este virus, sin embargo, sin mucho éxito. El potencial de las malezas como hospedadores alternativos y fuente de inóculo primario para la transmisión de ToSRV todavía está poco estudiado, y esto puede contribuir con las fallas en las prácticas de control cultural en el campo.

Este estudio tuvo como objetivo evaluar el papel de las malezas *D. stramonium* y *N. physaloides* como hospederos alternativos de ToSRV y verificar la influencia de este virus en el comportamiento de las especies crípticas de *Bemisia tabaci* MEAM1 y MED.

Nicandra physaloides fue un mejor hospedero alternativo cuando combinado con MEAM1, mientras que *D. stramonium* contribuyó más como hospedero alternativo para las mosquitas-blancas. La infección viral mejoró el rendimiento de MEAM1 y afectó negativamente el desarrollo de MED en ambas plantas hospederas. Estos datos sugieren que ambas malezas tienen influencia en el patosistema del ToSRV y que su control debe incluirse en los programas de manejo de este virus.

CAPÍTULO 2 - Comportamiento y preferencia de *Bemisia tabaci* en pepino: Comprensión de los recientes brotes de la especie críptica Mediterranean en Brasil.

Actualmente en Brasil, parte de la producción del pepino se realiza bajo cultivo protegido, en áreas que también producen pimiento y tomate, lo que permite a los agricultores aprovechar la estructura de conducción y el fertilizante restante de los cultivos de pimiento y tomate, generando ahorros. Por otro lado, en los últimos años, esta práctica ha contribuido con brotes poblacionales de MED en el estado de São Paulo, que culminaron con el primer reporte mundial del crinivirus *Tomato chlorosis virus* en pepino.

En este estudio, se comparó la preferencia de *B. tabaci* MEAM1 y MED en una situación con plantas de pepino (cv. Taiko), tomate (cv. Sta. Clara) y pimiento (cv.Magali) cultivadas en la misma zona, bien como la preferencia y performance de MEAM1 vs. MED en nueve cultivares comerciales de pepino [cvs. Soldier, Taiko, Tsuyataro y Valent (tipo japonés), Durango, Aodai y Verde Comprido (tipo común); y Pioneiro y Wisconsin SMR58 (tipo conservado)], evaluando también, el potencial de cada cultivar para reducir la población de mosca blanca.

El pepino fue el hospedero más preferido en comparación con los tomates y los pimientos en la prueba inicial de preferencia para ambas mosquitas blancas, que no solo frecuentaron la planta, sino que también podían asentarse y ovipositar. La especie críptica MED mostró un mejor rendimiento biológico que MEAM1 en casi todos los nueve cultivares de pepino evaluados, ya que tiene una mejor capacidad de sobrevivencia, lo que resultó en un número significativamente mayor de adultos. En la evaluación de cultivares, solo se destacó 'Aodai', generando una alta mortalidad de ninfas del MEAM1, siendo una buena opción para cultivos sin la presencia de MED. Por el contrario, ningún cultivar fue potencialmente satisfactorio para reducir la población de MED, lo que refuerza la necesidad de desarrollar estrategias de manejo específicas dirigidas a esta especie de mosca blanca.

CAPÍTULO 3 - Primer reporte del intento de Orthotospovirus Pepper necrotic spot virus en Chile.

En agosto de 2020, frutos de pimiento fueron encontrados en ferias y mercados de la ciudad de Santiago, presentando síntomas típicos del bronceado del pimento (manchas anulares y maduración irregular). Como en este período la producción de pimientos se limita por razones climáticas a la región norte del país, los frutos fueron recolectados para caracterizar la presencia de virus.

El presente estudio tuvo como objetivo caracterizar especies virales relacionadas con los síntomas en los pimientos comprados.

Las especies virales fueron caracterizadas mediante RT-PCR utilizando partidores genéricos y específicos para Orthotospovirus, relacionados con el bronceado del pimiento. Las bandas obtenidas fueron secuenciadas usando la técnica Sanger. Los resultados revelaron la presencia del orthotospovirus tentativa Pepper necrotic spot virus, con 99,7% de identidad con el aislado T1, reportado en Perú.

Para confirmar la patogenicidad, un extracto vegetal tamponado fue producido utilizando fragmentos de frutos infectados, e inoculado en plantas sanas de los cultivares Coraza y Almuden de pimiento. A los 15 días fueron observados síntomas de manchas necróticas y clorosis, con el desarrollo de manchas en anillo y mosaico a lo largo de los días. Todas las plantas sintomáticas fueron positivas utilizando los mismos partidores genéricos y no se detectó ningún otro orthotospovirus en las muestras positivas.

El PNSV fue encontrado por la primera vez en el valle de La Joya, aproximadamente a 400 km de la frontera con Chile. Hasta ahora, solamente el primer informe de este virus está publicado y solo dos secuencias del RNA S están presentes en el GenBank. Este es el primer reporte del PNSV en Chile, y de acuerdo a la fecha en que se encontraron los frutos del pimiento, se sospecha que fueron cultivados en el norte de Chile, por lo que se debería investigar mejor la ocurrencia de este virus en esa región.

CAPÍTULO 4 - Levantamiento de orthotospovirus y tripes asociados en plantas de pimiento en Arica y Parinacota - Chile.

Entre los orthotospovirus asociados al bronceado del tomate y pimiento, sólo se detectaron en Chile el *Tomato spotted wilt orthotospovirus* y *Impatiens necrotic spot orthotospovirus*, siempre en asociados con *F. occidentalis*. Recientemente, técnicos y agricultores de la región de Arica y Parinacota, ubicada en el extremo norte de Chile, comenzaron a reportar brotes de bronceado principalmente en pimientos.

Dada la importancia de esta región como proveedora de productos hortofrutícolas durante el invierno, este estudio tuvo como objetivo mapear y caracterizar los agentes causales y trips asociados a los brotes de “bronceado” que se están produciendo en Arica y Parinacota.

Hojas jóvenes con síntomas típicos de bronceado y tripes asociados fueron recolectados de plantas de pimiento y tomate. Los virus vegetales fueron detectados a nivel de especie mediante RT-PCR, utilizando partidores específicos o genéricos dirigidos a orthotospovirus y los trips asociados fueron identificados morfológicamente, con algunas muestras representativas sometidas a PCR, para confirmar la identificación.

Fueron detectados el *Tomato spotted wilt orthotospovirus* y el Pepper necrotic spot virus en nueve y 13 muestras, respectivamente. Todos los trips muestreados fueron identificados como *F. occidentallis* y no se identificaron otras especies de trips.

La presencia de estos virus es preocupante, mostrando la expansión del PNSV desde Perú a nuevas áreas y sugiriendo que el TSWV encontrado puede ser un aislado capaz de infectar pimientos resistentes, ya que la mayoría de los cultivares utilizados en esta región tienen resistencia al TSWV. Además, la presencia únicamente de *F. occidentalis* sugiere que este insecto es el probable vector de estos virus. La secuenciación de nueva generación permitirá una mejor caracterización de ambos virus.

Palabras clave: *Bemisia tabaco*; *Frankliniella occidentalis*; Pepper necrotic spot virus; *Tomato spotted wilt virus*; desempeño biológico; preferencia; levantamiento; PNSV; TSWV.

SUMMARY

GENERAL INTRODUCTION

The whitefly *Bemisia tabaci* and its associated viruses in Brazil.

Currently, the *B. tabaci* is considered a complex of at least 44 distinct cryptic species, which can differ in the transmission of some viruses, host preferences, temperature tolerance, resistance to insecticides, and endosymbiont composition (HOROWITZ et al., 2020; POLSTON; DE BARRO; BOYKIN, 2014). Among them, Middle East-Asia Minor 1 (MEAM1 or B Biotype) and Mediterranean (MED or Q biotype) are currently the most widespread and harmful to cultivated crops.

In America, *B. tabaci* was first reported in 1920 (BETHKE; PAINE; NUESSLY, 1991) mainly associated with weeds, and since 1970 started causing sporadic outbreaks in common beans (*Phaseolus vulgaris* L.) and soybeans [*Glycine Max* (L.) Merril], eventually transmitting viruses (FONTES; COLOMBO; LOURENÇÃO, 2010; MORALES, 2006).

The *B. tabaci* complex started to become more important in America in 1980 when MEAM1 was disseminated to several countries along with ornamental trading (INOUE-NAGATA; LIMA; GILBERTSON, 2016). The first introduction occurred in the United States at the end of the 1980s, dispersing rapidly to South American countries during the 1990s (KRAUSE-SAKATE et al., 2020). MEAM1 has adapted very well in solanaceous crops in Brazil, bringing begomoviruses from weeds to these crops. Since its introduction, more than 17 new begomoviruses were described, with an emphasis on the species *Tomato severe rugose virus* (ToSRV), that predominate in the south, southeast, and Midwest of Brazil, causing symptoms of dwarfing, mosaic, mottling, leaf rugose, epinasty, and chlorosis (Inoue-Nagata et al., 2016b; Macedo et al., 2018; Quadros et al.,2019).

The ToSRV is a bipartite begomovirus containing two 2600nts long single-stranded DNAs: The DNA-A, which is generally associated with transcription, replication, and the production of the coat protein; and the DNA-B, associated with virus movement (BROWN et al., 2012; ROJAS et al., 2005). It is believed that this predominance is related to a high symbiotic relationship between ToSRV and MEAM1 and its high range of hosts apart from tomato, comprising the potato (*Solanum tuberosum* L.), bell peppers (*Capsicum annuum* L.), Chilli peppers (*Capsicum baccatum* L.), Eggplants (*Solanum melongena* L.), soybeans, common beans; and the

weeds *Datura stramonium*, *Euphorbia heterophyla*, *Nicandra physaloides*, *Solanum americanum*, *Sida* spp. (BARBOSA et al., 2009; BARRETO et al., 2013; BEZERRA-AGASIE et al., 2006; MACEDO et al., 2017a, 2017b, 2015; MOURA et al., 2018).

According to Toloy et al. (2017), MEAM1 needs to feed at least 1 minute to acquire ToSRV particles, and after a latency period of 12 to 15h, just five minutes is sufficient to inoculate the virus in a new host. This time is much shorter than other Begomoviruses, such as Tomato rugose mosaic virus (ToRMV) (15 minutes of acquisition and 30 minutes for inoculation) or Tomato yellow vein streak virus (ToYVSV) (30 minutes for the acquisition and 10 minutes for virus inoculation) (FIRMINO et al., 2009; SANTOS; ÁVILA; RESENDE, 2003). Moreover, after 24 hours of acquisition, ToSRV stays retained and available to be inoculated throughout the entire life of the MEAM1 vector, while other species, such as TYLCV and *Chino del tomate virus*, are retained by 20 days and 4 to 7 days, respectively (FIRMINO et al., 2009).

The control measurements used for viral infections are based on the exclusion principle, employing healthy propagative material, eliminating vectors and implementing a crop-free period (HULL, 2014a). The crop-free period generated promising results in tomato crops in the Arana region (Israel) and in the United States, where significant reductions in the TYLCV viral inoculum were reported (GILBERTSON, 2011; UCKO; COHEN; BEN-JOSEPH, 1998). Currently, examples of this practice are applied in some areas from Brazil to control *B. tabaci* and ToSRV but do not show the same efficiency as other countries. This failure is related to the presence of alternative host crops to ToSRV, such as soybeans, potatoes, peppers, various weeds, and the climatic conditions favouring whitefly survival during the crop-free period (INOUE-NAGATA; LIMA; GILBERTSON, 2016).

Apart from MEAM1, other two cryptic species are present in Brazil: New World (NW or A biotype), which are native and colonize principally weeds and non-cultivated crops, and the Mediterranean (MED or Q Biotype), which was more recently reported, firstly in the Rio Grande do Sul State, in 2015, and then in São Paulo State, in 2017 (BARBOSA et al., 2015; MORAES et al., 2017). The introduction of MED can change the scenario of the commonly affected hosts, generating new outbreaks, and because of this, MED has been extensively studied.Firstly it was shown that MED is capable of transmitting the four most common whitefly-related viruses in Brazil, the carlavirus *Cowpea mild mottle virus* (CpMMV), the crinivirus *Tomato chlorosis virus* (ToCV), and

the begomoviruses *Bean golden mosaic virus* (BGMV), and ToSRV (BELLO et al., 2019). Moreover, it was shown that MED could perform better than MEAM1 in 'Magali-R' bell peppers and 'Pérola' common beans, presenting better survival rates and significantly more adults, displacing MEAM1 after 120 days (WATANABE et al., 2019).

Although MEAM1 is still predominating in Brazil (MORAES et al., 2018), MED was recently linked with outbreaks in São Paulo and Paraná states on cucumbers (*Cucumis sativus* L.) and bell peppers (*Capsicum annumm* L.), where the whitefly was considered a secondary pest (Bello et al., 2020b), which contributed to the first report of ToCV in cucumbers and the increase of this virus in bell peppers (Bello et al., 2020a).

In this regard, to contribute with key information about the behaviour and to develop management strategies against whiteflies, the present thesis brings two studies involving MEAM1 and MED in Brazil. In chapter 1 the potential of the weeds *D. stramonium* and *N. physaloides* of harboring MEAM1 and MED and tomato severe rugose virus (ToSRV) was studied; and in chapter 2, biological performance and preference tests comparing MEAM1 vs. MED were performed in cucumber commercial cultivars, to understand the causes related to the recent MED infestations in the states of São Paulo and Paraná.

Orthotospoviruses and thrips associated with the spotted wilt disease in solanaceous vegetables in Chile.

Orthotospoviruses stand out as one of the most devastating viruses for solanaceous vegetables worldwide due to the severe damage caused by them in plants and the difficulty in controlling their dissemination.

Virions from this genus vary from 80 to 120nm in size. They are composed of a phospholipidic membrane with embedded glycoproteins (Gn and Gc), containing inside three negative or ambisense ssRNA fragments: the S (Small, 2.9 kb), the M (Medium, 4.8 kb), and the L (Large, 8.9 kb), protected by capsid proteins. Attached in each fragment is an RNA-dependent RNA polymerase (RDRP), which will perform the negative strand first replication. Large RNA encodes RDRP (in the negative sense), while M RNA will encode the movement protein (NSm) (positive sense) and the glycoproteins Gn and Gc (negative sense). Finally, RNA S will encode the coat protein

(N) (Negative sense) and a gene silencing suppressor (NSs) in the positive sense of the RNA (Figure 1) (ICTV, 2020).

These viruses have a broad host range and are efficiently transmitted by several thrips species (GILBERTSON et al., 2015; SCHOLTHOF et al., 2011). *Orthotospovirus* genus is classified in the realm *Riboviria*, kingdom *Orthornavirae*, phylum *Negarnaviricota*, subphylum *Polyploviricotina*, Class *Ellioviricetes*, order *Bunyavirales*, and *Tospoviridae* family (ICTV, 2020). To date, 26 species are recognized by ICTV, divided into five phylogenetic clades with names represented by one key species: the Tomato spotted wilt orthotospovirus (TSWV), Soybean vein necrosis orthotospovirus (SVNV), Iris yellow spot orthotospovirus (IYSV), Watermelon silver mottle orthotospovirus (WSMoV) and Groundnut yellow spot orthotospovirus (GYSV) clades; according to the sequences of the N gene (capsid protein) (OLIVER; WHITFIELD, 2016; PAPPU; JONES; JAIN, 2009). The species from TSWV and SVNV clades are prevalent in America, which is why they are called "New World", while the IYSV, GYSV, WSMoV clades, being predominantly found in Asia, are known as "Old World" (Oliver and Whitfield 2016).

Among the diseases caused by orthotospoviruses, the spotted wilt disease stands out, being one of the main viral diseases in solanaceous vegetables, such as tomatoes and peppers (CHO et al., 1998). This disease can be caused by a complex of orthotospoviruses, comprising the species *Tomato spotted wilt virus* (TSWV), *Tomato chlorotic spot virus* (TCSV), *Groundnut ringspot virus* (GRSV), *Impatiens necrotic spot virus* (INSV), and *Chrysanthemum stem necrosis virus* (CSNV) are also frequently found in association with this disease, and their differentiation is possible only by molecular or serological techniques (LIMA; MICHEREFF FILHO, 2015).

The TSWV is the most widespread and was the first species described at the beginning of the 1930s. Its importance increased in the 1980s, following the world dispersion of the western flower thrips (*F. occidentalis*), generating, since 1994, annual management costs of more than 1 billion dollars (SCHOLTHOF et al., 2011). This species remained the only member of the orthotospovirus genus until the 1990s when, with the development and popularization of serological and molecular techniques, other species were reported in several places of the world (LAW; MOYER, 1990; OLIVER; WHITFIELD, 2016).

The symptoms in tomatoes are the bronzing of the upper sides of young leaves, which later develop into necrotic spots. The leaves become cupped downward, and

plants often show tip dieback. In bell peppers, the leaves often present distortions, mosaic, and chlorotic ring spots that can evolve to necrosis. In fruits, it is common to appear chlorotic or necrotic spots, generally with concentric rings, fruit deformation, and irregular ripening (LIMA; MICHEREFF FILHO, 2015).

Symptoms related to the spotted wilt may vary according to the time of infection (PAPPU; JONES; JAIN, 2009). For instance, in tomato and bell peppers, when the host is infected in the initial stages (until 25 days after transplant), the symptoms are more severe, usually resulting in generalized wilt, generally leading to plant death (PAPPU; JONES; JAIN, 2009). When the plant is infected in a later stage (from 30 to 60 days after transplant), the infection affects fruit production and quality without killing the host. When infection occurs during hosts' later stages (60 days after transplanting), the plant has a kind of age-related resistance, in which only mild symptoms are developed, and satisfactory amounts of fruit can be harvested (MORIONES et al., 1998). The aspects related to this phenomenon are not still wholly understood, but it may be related to a greater expression of defense genes in plant cells, such as PR proteins, as well as a decrease in the number of ribosomes, improving the defense response time to viral infections and delaying the translation of the viral genome (COLE et al., 2004; HULL, 2014b). Furthermore, the leaves of younger plants are thinner and have higher concentrations of nutrients such as nitrogen, affecting thrips feeding preference, and consequently, virus inoculation (SHRESTHA et al., 2015).

In nature, the spotted wilt disease is transmitted in a persistent-propagative manner by nine thrips (Thysanoptera: Thripidae) species from *Frankliniella* and *Thrips* genera (OLIVER; WHITFIELD, 2016).

The insect will be a vector only if it acquires the virus in the larval stages. First, the virus is acquired, reaches the insects' midgut's epithelial cells, and replicates. Then, viral particles spread through the insect by the hemolymph until finally reaching the salivary glands, where they will be available to be transmitted again (MONTERO-ASTÚA; ULLMAN; WHITFIELD, 2016). Moreover, it has been proven that Gn and Gc proteins play a fundamental role in the interaction between the virus and the insect, being vital for the transmission occurrence (NAGATA; DE AVILA, 2000).

All spotted wilt causal agents are present in South America (PAPPU; JONES; JAIN, 2009), and some species, such as GRSV and TCSV, originated in this continent. These species are currently in Paraguay, Brazil, and Argentina, predominating as spotted wilt causal agents in some regions of the last two countries (WILLIAMS et al.,

2001). Two other putative new orthotospoviruses, Pepper necrotic spot virus, and Alstroemeria necrotic streak virus, were first reported in Peru and Colombia, respectively, and are related with TSWV clade species (HASSANI-MEHRABAN et al., 2010; TORRES et al., 2012), but little information is available regarding them, and they were not formally associated with the spotted wilt disease.

To date, in Chile, only two species have been reported causing the spotted wilt disease: the TSWV in tomatoes, bell peppers, and the INSV in bell peppers (SEPÚLVEDA et al., 2005). A survey in 2005 showed that TSWV was one of the most common viruses, found in approximately 20% of the samples collected, in addition to reporting for the first time the occurrence of INSV in peppers, both of which were associated with the vector *F. occidentalis* (SEPÚLVEDA et al., 2005).

Apart from this study, no other surveys of orthotospoviruses and thrips vectors were published, and in several other important agricultural regions of Chile, the identity of the viruses associated with spotted wilt is unknown. Given the difficulties in controlling the spotted wilt by vector control and the presence of TSWV as a major spotted wilt causal agent, growers are using tomato and pepper cultivars with the *Sw-5* and *Tsw* resistance genes, respectively.

However, the gene *Tsw* is effective only against TSWV and is not yet known the effectiveness of the *Sw-5* gene against new orthotospoviruses (BOITEUX; DE ÁVILA, 1994). Currently, the spotted wilt is reaching epidemic levels in some regions of Arica y Parinacota, the northernmost region in Chile, and one of the leading suppliers of vegetables to Santiago Metropolitan region during the winter, suggesting the occurrence of a resistance-breaking isolate of TSWV or the INSV, or the introduction of new orthotospoviruses.

These facts suggest the importance of expanding the survey to other Chilean agricultural important regions, updating the spotted wilt species status, and better-characterizing orthotospoviruses present in this country.

In order to contribute with more information about the characterization and occurrence of orthotospoviruses in Chile, the chapter 3 of this thesis reported for the first time the occurrence of the putative new orthotospovirus Pepper necrotic spot virus in Chile, found in bell pepper fruits at a street market in Santiago Metropolitan Region; and the chapter four, surveyed orthotospoviruses and associated thrips in tomatoes, and peppers in Arica y Parinacota region, to better understand the causes related with the recently spotted wilt outbreaks.

CHAPTER 1 - Evaluation of *Datura stramonium* and *Nicandra physaloides* as reservoirs of tomato severe rugose virus and whiteflies

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ABSTRACT

Tomato severe rugose virus (ToSRV) is the most important begomovirus transmitted and spread by the whitefly *Bemisia tabaci* in tomato crops in Brazil. Cultural practices are being adopted, along with insecticides, for controlling this virus. However, little is known about the importance of weeds in the pathosystem, which can contribute to the failure of these practices. This work aimed to evaluate the role of *Datura stramonium* and *Nicandra physaloides* as alternative hosts of ToSRV and verify the viral influence on the biological performance of *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) cryptic species. *N. physaloides* was a better alternative host for ToSRV when combined with MEAM1 whiteflies, while *D. stramonium* was mostly a good host for whitefly reproduction. Viral infection improved MEAM1 performance on both host plants but affected MED performance negatively. These data suggest that both weeds can be of some importance for the pathosystem, and their control should be included in management programmes.

Keywords: *Begomovirus*, *Bemisia tabaci*, weeds.

1.1 Introduction

The *Bemisia tabaci* whitefly is one of the most important phytophagous pests worldwide, and it infests many plant species. It is currently considered a complex of cryptic species, in which Middle East-Asia Minor 1 (MEAM1 or B biotype) and Mediterranean (MED or Q biotype) are the most widespread, causing losses in many vegetables worldwide (De Barro *et al.*, 2011; Boykin and De Barro, 2014; Kanakala and Ghanim, 2019). In Brazil, four cryptic species are present: the indigenous New World 1 and New World 2 (NW1 and NW2 or A biotypes), which are mainly restricted to non-cultivated plants (Marubayashi *et al.*, 2013; Barbosa *et al.*, 2015); MEAM1, which was introduced in the early 1990s (Lourenção and Nagai, 1994) and is predominant in Brazil (Moraes *et al.*, 2018); and MED, first reported in Rio Grande do Sul State on sweet pepper crops (Barbosa *et al.*, 2015) and then in São Paulo State, having moved due to the international trade of ornamental plants (Moraes *et al.*, 2017). Currently, MED is spreading rapidly and can be found in five different Brazilian states (Moraes *et al.*, 2018).

The primary damage caused by whiteflies includes physiological disorders due to insect feeding, such as leaf necrotic lesions and irregular ripening in tomato, as well as leaf silvering in zucchini. In addition, whiteflies can secrete honeydew and further cause sooty mould growth (*Capnodium* sp.) (Lourenção and Nagai, 1994). These insects are also considered “supervectors” of several genera of plant viruses, among which *Begomovirus* is of great importance, representing 90% of the species transmitted by whiteflies (Gilbertson *et al.*, 2015).

Brazil is considered the largest centre of begomovirus diversity, and it has its own indigenous species. Since the introduction of MEAM1 in the mid-1990s, more than 17 new species have been reported infecting tomatoes and other solanaceous vegetables in Brazil (Inoue-Nagata *et al.*, 2016b; Macedo *et al.*, 2018; Quadros *et al.*, 2019). The strongest hypothesis is that some of these species infected weeds and wild hosts before being inoculated by MEAM1 (García-Arenal and Zerbini, 2019). Weed plants can thus easily serve as alternative hosts for several begomoviruses in the absence of tomato crops, and can also contribute to begomovirus variability, as the same plant can be infected by several virus species, contributing to the occurrence of recombination and rearrangements (García-Arenal and Zerbini, 2019). Moreover, the performance of whiteflies can be improved on virus-infected plants, with increased reproduction and survival rates, which can lead to faster and more efficient virus spread (Chen *et al.*, 2013; Shrestha *et al.*, 2017; Watanabe *et al.*, 2018).

Currently, the species *Tomato severe rugose virus* is considered the most widespread and important from the genus *Begomovirus* infecting tomato in the Brazilian southeast and midwest regions, in which 75% of all tomato production in 2017 was concentrated (IBGE, 2019). Tomato severe rugose virus is a bipartite begomovirus that causes symptoms of rugosity, interveinal chlorosis, stunting, and mosaic in tomato crops. Additionally, this virus has also been found naturally infecting potatoes (Solanum tuberosum), which develop yellow mosaic symptoms; peppers (Capsicum spp.), which develop symptoms of chlorotic spots, rugosity, and mild leaf distortion; and soybeans and common beans, in which the virus is symptomless (Inoue-Nagata *et al.*, 2016b; Macedo *et al.*, 2017a, 2017b). Moreover, several weeds are known hosts of ToSRV, such as *Crotalaria* sp., *Datura stramonium*, *Euphorbia heterophylla*, *Sida* spp., *Nicandra physaloides*, and *Malva* spp., developing symptoms of yellow mosaic, leaf distortion, and stunting (Barbosa *et al.*, 2009; Barreto *et al.*, 2013; Macedo *et al.*, 2015).

This virus is transmitted in a nonpropagative, persistent circulative manner by the whitefly *B. tabaci*, with a latent period of 12–15 hr to reach the salivary glands. A recent study showed that MEAM1 needs a few minutes to acquire and transmit ToSRV, but 24 hr acquisition and inoculation access periods are needed for full efficiency of transmission (Toloy *et al.*, 2018). The management of ToSRV is based on several cultural practices, such as crop rotation, crop-free periods, and weed management. However, the main strategy used today is based on vector control with insecticides (Gilbertson *et al.*, 2011; Lapidot *et al.*, 2014). The Brazilian state of Goiás has implemented a tomato-free period for managing *B. tabaci* and ToSRV spread, but this policy has not been efficient due to the capacity of ToSRV to infect other vegetable and weed species (Inoue-Nagata *et al.*, 2016b; Macedo *et al.*, 2017a, 2017b).

Although it is known that several weeds can serve as hosts for ToSRV and whiteflies, there are few studies to evaluate how good they are as inoculum sources of ToSRV. In addition, there is no information about MEAM1 and MED performance on ToSRV-infected weeds and their efficiency of transmission from these plants. Moreover, little is known about the impact that the recent introduction of MED will bring to Brazilian crops, although recent findings have shown their ability to transmit ToSRV to tomatoes from other cultivated hosts (Bello *et al.*, 2019).

Therefore, this work aimed to evaluate the role of two solanaceous weeds: Datura stramonium (jimsonweed) and Nicandra physaloides (apple of Peru), which are quite common on farms in southeastern Brazil and are proven to host ToSRV. The study involved investigating their influence on the ToSRV pathosystem, verifying the capacity of each weed to retain the virus, and to be a source of inoculum for viral reinfection in tomatoes in a crop-free period situation. Moreover, this study also evaluated the biological performance of MEAM1 and MED in healthy and ToSRV-infected plants of each host.

1.2 Material and methods

1.2.1 Establishment and identification of whitefly colonies

The population of MEAM1 was collected from cabbage (*Brassica oleracea*) (GenBank accession number MH186145) in Campinas, SP, Brazil (22°52′14″S, 47°04′38″W) and maintained in cabbage. The MED population was collected from

begonias (*Begonia* spp.) (GenBank accession number MH047295) in Santa Isabel, SP, Brazil (23°22′20″S, 46°10′35″W) and maintained in bell pepper (*Capsicum annuum*). Both populations were then transferred to cotton plants to develop new offspring and maintained inside insect-proof rearing cages in a greenhouse, at 25 ± 2°C, with natural light incidence.

For species identification, DNA from whiteflies of each colony was extracted using the Chelex protocol (Walsh *et al.*, 1991), and used as template for PCR with the primer pair C1-J-2195 and TL2-N-3014 (Simon *et al.*, 1994), for *mtCOI* gene amplification. This was followed by restriction fragment length polymorphism (RFLP) analysis of the amplicons using *Taq*I (Bosco *et al.*, 2006). The purity of the colonies was monitored monthly

1.2.2 Source and detection of ToSRV isolate

Tomato severe rugose virus isolate was collected from infected tomato plants in Campinas, SP, Brazil, and maintained in tomato plants inside insect-proof cages. For ToSRV detection, DNA was extracted from approximately 50 mg of fresh leaf tissue from infected plants using the CTAB protocol described by Doyle and Doyle (1987). PCR was performed using a GoTaq Green Master Mix kit (Promega), following the manufacturer's instructions. The degenerate primers PrV324 and PrC889, which amplify a 579 bp fragment of the coat protein of begomoviruses, were used for this reaction, with annealing at 60 °C for 20 s (Wyatt and Brown, 1996). All PCR products were subjected to agarose gel electrophoresis (1%), stained with ethidium bromide (Sigma-Aldrich) and visualized under UV light.

Random positive ToSRV samples were selected, purified (QIAquick Gel Extraction Kit, Qiagen) and sequenced in both directions using the primers described above, for comparison with sequences available in GenBank using the BLASTn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch).

1.2.3 Whitefly performance in healthy and ToSRV infected *D. stramonium* and *N. physaloides*

Performance assays of each whitefly species were evaluated by the number of eggs laid, number of eggs hatched, number of adults emerged, survival rate, and

development time on each host, using healthy and ToSRV-infected plants for comparison.

All assays were conducted in insect-proof cages (45 × 45 × 55 cm) and maintained in growth rooms at 26 ± 2 °C and 14 hr artificial light. Twenty adults (10 male and 10 female) of each whitefly species were transferred to clip cages (2 × 2 × 3 cm) and attached to the abaxial side of the leaf, on 15 leaves (15 replications). After 48 hr, the clip cages and all adult insects were removed, leaving only the eggs, which were counted using a magnifying glass (Zeiss) with a 20× magnification. The hatched eggs were counted after 7 days, using the same magnifying glass as before, and the adult emergence was counted daily and collected with a manual insect aspirator every morning (08:00), from the 14th day after egg counting until no more adults were observed.

Development time was measured in days, encompassing immature stages and the adult emergence period, which were used together to calculate the total development time for each treatment. The survival rate was obtained by calculating the percentage of adults generated from all eggs laid ([no. of adults/no. of eggs] ×100). Four plant hosts with 7–9 true leaves were used in each treatment, totalling a maximum of four leaves (replicates) in each plant tested.

1.2.4 Transmission assays

For inoculation, adult whiteflies were transferred to insect-proof cages containing ToSRV-infected tomato plants for an acquisition access period (AAP) of 24 hr. After that, whiteflies (10 adults per plant) were transferred to healthy seedlings (at the 2-true-leaves stage) confined in insect-proof cages for a 24hr inoculation access period (IAP) under the same controlled conditions mentioned before. After the IAP, seedling plants were treated with a mixture of Oberon (0.03%; Bayer, Brazil; a.i., Spiromesifen) and Cartap BR 500 (0.25%; Ihara, Brazil; a.i., cartap hydrochloride) applied with a manual sprayer, in order to eliminate all the remaining eggs, nymphs, and adults. DNA was extracted from every plant of each treatment for ToSRV detection by PCR, as described previously. For the negative control, virus-free adult whiteflies were collected from each population (*B. tabaci* MEAM1 and MED) and transferred to 10 healthy seedlings of each host for mock-inoculation. All the experiments were carried out in greenhouses at 28 ± 2 °C and repeated twice with 15 replicates of each host, totalling 30 plants.

Transmission assays were conducted separately with each *B. tabaci* species by using ToSRV-infected cv. Santa Clara tomatoes that showed symptoms of severe rugosity as initial sources of inoculum. Inoculations were performed in three steps: (a) each weed host (*D. stramonium* and *N. physaloides*) was inoculated with ToSRV from infected tomato plants; (b) from each group of infected weed hosts, one plant was used as a source of inoculum to perform transmission to new seedlings of the same species, thus simulating the conditions of a tomato-free period in the field; and (c) healthy Santa Clara tomato plants were then back-inoculated to simulate the capacity of ToSRV reinfection from the alternative host. Additionally, ToSRV inoculations using only tomato plants were carried out as controls of the inoculation processes.

1.2.5 Data analysis

All data had the homogeneity of variances and normality assessed with the F-Snedecor and Shapiro–Wilk tests, respectively. The number of eggs laid, eggs hatched and adults emerged were transformed to log(x) before analyses in order to reduce the heteroscedasticity, and then those values were submitted to analysis of variance (ANOVA) by using Minitab 16 software (Minitab Inc.) to verify whether there were significant differences ($p < .05$) among the treatments. All experiments were conducted with a completely randomized design. Double factorial analysis was performed. After the ANOVA, means were submitted to Tukey's test ($\alpha = .05$).

The data of ToSRV transmission by MEAM1 and MED were analysed with a $\chi 2$ test ($p < .05$) using a general log-linear model (GLM). The analysis was performed using the software package R v. 3.1.0 (R Development Core Team, 2013).

1.3 Results

1.3.1 Numbers of whitefly eggs, nymphs and adults on healthy and ToSRV-infected *D. stramonium*

Comparing MED and MEAM1 on healthy *D. stramonium* plants, no significant differences were observed in the number of eggs and nymphs ($F = 2.29$, $df = 1, 56$, $p = .13$ and $F = 1.61$, $df = 1, 56$, $p = .21$, respectively), whereas with the viral infection, a greater number of eggs and nymphs were observed for MEAM1 ($F = 5.45$, $df = 1, 56$,

p = .02 and *F* = 4.46, *df* = 1, 56, *p* = .03, respectively). The number of eggs and nymphs of MEAM1 did not differ between healthy and ToSRV-infected plants (*F* = 0.06, *df* = 1, 56, *p* = .81 and *F* = 0.16, *df* = 1, 56, *p* = .69, respectively), whereas for MED, significant reductions of 51.88% and 53.61%, respectively, were observed with ToSRV infection for eggs and nymphs (*F* = 16.68, *df* = 1, 56, *p* = .0001 and *F* = 14.29, *df* = 1, 56, *p* = .0004, respectively) (Table 1).

In healthy plants, adult emergence was significantly lower for MEAM1 than for MED (*F* = 135.57, *df* = 1, 56, *p* = .0001), whereas in infected plants, the opposite occurred, with significantly more MEAM1 adults having emerged in comparison with MED (*F* = 17.01, *df* = 1, 56, *p* = .0001). These changes are related to host viral infection, which dramatically increased the emergence of MEAM1 adults (*F* = 95.41, *df* = 1, 56, *p* = .0001), but caused a significant decrease in MED adult emergence (*F* = 6.00, *df* = 1, 56, *p* = .0001) (Table 1).

Table 1. Mean number of eggs, nymphs and adults of two whitefly cryptic species on healthy and ToSRV-infected *Datura Stramonium*.

Stage	**Species**	**Healthy**	**ToSRV-infected**
Eggs	MEAM1	151.33 aA	146.20 aA
	MED	184.13 aA	95.53 bB
Nymphs	MEAM1	140.06 aA	131.93 aA
	MED	165.86 aA	88.93 bB
Adults	MEAM1	3.40 bB	127.33 aA
	MED	151.13 aA	75.00 bB

Note: Means followed by the same lowercase letter within columns and uppercase letter within rows indicate no significant ($p < 0.05$) difference between treatments according to an ANOVA followed by Tukey's test. Data were transformed by log(x) before analysis.

1.3.2 Numbers of whitefly eggs, nymphs and adults on healthy and ToSRV-infected *N. physaloides*

Comparing MED and MEAM1 on healthy *N. physaloides*, no significant differences were observed in the numbers of eggs or nymphs (*F* = 0.07, *df* = 1, 56, *p* = .79 and *F* = 0.01, *df* = 1, 56, *p* = .91, respectively). In contrast, on infected plants, greater numbers of eggs and nymphs were seen for MEAM1 compared to MED (*F* = 23.03, *df* = 1,56, *p* < .0001 and *F* = 30.06, *df* = 1, 56, *p* < .0001, respectively). Moreover,

the numbers of eggs and nymphs of MEAM1 increased significantly on ToSRV-infected plants when compared with the healthy ones (F = 9.22, df = 1, 56, p = .0036 and F = 8.43, df = 1, 56, p = .0053, respectively), whereas for MED, a significant reduction could be observed with ToSRV infection (F = 4.07, df = 1, 56, p = .048 and F = 6.34, df = 1, 56, p = .015, respectively) (Table 2). Adult emergence was significantly lower for MED in comparison with MEAM1 on both healthy and ToSRV-infected plants (F = 16.73, df = 1, 56, p = .0001 and F = 85.16, df = 1, 56, p < .0001, respectively). Significant differences were also observed in the numbers of MEAM1 adults on healthy and ToSRV-infected plants, with more whiteflies emerging from ToSRV-infected plants (F = 6.00, df = 1, 56, p = .017), whereas for MED, a decrease in adults was observed on infected plants (F = 7.23, df = 1, 56, p = .0094) (Table 2).

Table 2. Mean number of eggs, nymphs and adults of two whitefly cryptic species on healthy and ToSRV-infected *Nicandra Physaloides*

Stage	**Species**	**Healthy**	**ToSRV-infected**
Eggs	MEAM1	119.33 aB	173.80 aA
	MED	123.93 aA	87.73 bB
Nymphs	MEAM1	107.60 aB	148.33 aA
	MED	106.06 aA	70.73 bB
Adults	MEAM1	102.73 aB	138.13 aA
	MED	43.60 bA	4.73 bB

Note: Means followed by the same lowercase letter within columns and uppercase letter within rows indicate no significant ($p < 0.05$) difference between treatments according to an ANOVA followed by Tukey's test. Data were transformed by log(x) before analysis.

1.3.3 Whitefly development times and survival rates on healthy and ToSRV-infected *D. stramonium* and *N. physaloides*

On *D. stramonium*, ToSRV infection significantly reduced the development time of immature stages of MEAM1 by 7 days (from 20 to 13 days) (F = 77.72, df = 1, 56, p < .0001) and caused a 6-day increase in the adult emergence period (from 4 to 10 days) (F = 38.47, df = 1, 56, p < .0001), which also resulted in an increase in the number of adults. By contrast, viral infection did not cause differences in MED developmental stages. In addition, no significant differences between MED and MEAM1 were observed in the total duration of development. In contrast, for *N.*

physaloides, the total development time was shorter for MED in comparison with MEAM1 in both healthy (19 vs. 25 days, $F = 23.42$, $df = 1, 56$, $p < .0001$) and ToSRV-infected plants (16 vs. 26 days, $F = 64.49$, $df = 1, 56$, $p < .0001$). This was primarily observed during the adult emergence period, which lasted 9 days less in comparison with MEAM1 (3 vs.12 days, $F = 93.46$, $df = 1, 56$, $p < .0001$). Despite having a shorter cycle in *N. physaloides*, fewer MED adults were generated (Table 2). Regarding survival rate in healthy *D. stramonium*, more MED survived than MEAM1 (81.6% vs. 2.8%, $F = 197.41$, $df = 1, 56$, $p < .001$). There was no difference in survival of MED between healthy and virus-infected plants, whereas MEAM1 survival was significantly favoured in virus-infected plants (increase of 86.3%, $F = 237.02$, $df = 1, 56$, $p < .0001$), surpassing the survival rate seen for MED (89.1% vs. 84.4%, $F = 0.71$, $df = 1, 56$, $p = .4023$) (Table 3a).

In *N. physaloides*, the survival rate of MEAM1 was superior in both healthy and ToSRV-infected plants (average of 84.61% vs. 20.30%, $F = 117.65$, $df = 1, 56$, $p < .0001$) and, with the viral infection, a general decrease in survival rate occurred (average of 59.4%–45.51%), principally affecting MED ($F = 5.49$, $df = 1, 56$, $p = .0227$) (Table 3b).

Table 3. Survival rate (%) of two whitefly cryptic species on healthy and ToSRV-infected plants of *Datura Stramonium* (a) and *Nicandra Physaloides* (b).

(a)	***Species***	**Healthy**	**ToSRV-infected**
D. stramonium	MEAM1	2.8 bB	89.1 aA
	MED	81.6 aA	84.4 aA

(b)	***Species***	**Healthy**	**ToSRV-infected**	**Average**
N. physaloides	MEAM1	86.7	82.5	84.61 a
	MED	32.0	8.6	20.30 b
	Average	59.4a	45.51b	

Note: (a) Means followed by the same lowercase letter within columns and uppercase letter within rows indicate no significant ($p < 0.05$) difference between treatments according to an ANOVA followed by Tukey's test. Data were transformed by log(x) before analysis. (b) Means followed by different letter indicate significant difference ($p < 0.05$) between treatments according to ANOVA followed by Tukey's test.

1.3.4 Transmission assays

MEAM1 had a better transmission rate than MED (36.1% vs. 3.3%, $\chi2 = 2.6$, $p < .001$) when ToSRV was transmitted from tomato to *D. stramonium*, whereas in transmissions from *D. stramonium* to *D. stramonium* (0% vs. 0%, $\chi2 = 2.2$, $p \approx 1.0$) and from *D. stramonium* to tomato (3% vs. 0%, $\chi2 = 1.9$, $p = .066$), no significant differences were observed (Table 4). MEAM1 showed a better transmission rate than MED in transmissions from tomato to *N. physaloides* (25.6% vs. 3.3%, $\chi2 = 0.8$, $p < .001$) and from *N. physaloides* to *N. physaloides* (43.3% vs. 6.7%, $\chi2 = 3.4$, $p < .001$), while from *N. physaloides* to tomato, no significant differences were observed (23.3% vs. 13.3%, $\chi2 = 1.7$, $p = .066$) (Table 4). *N. physaloides* developed severe leaf rugosity, chlorotic lesions, and stunting, while *D. stramonium* developed leaf mild mottle.

Table 4. Transmission of ToSRV in *D. stramonium* and *N. physaloides* with MEAM1 and MED whiteflies.

Transmission step	MEAM1		MED		Significance
	Infected/Inoculated	% of infection	Infected/Inoculated	% of infection	
Tomato-Datura	13/36	36.1	1/30	3.3	*
Datura-Datura	0/30	0	0/32	0	ns
Datura-Tomato	1/30	3.3	0/30	0	ns
Tomato-Nicandra	11/43	25.6	1/30	3.3	*
Nicandra-Nicandra	13/30	43.3	2/30	6.7	*
Nicandra-Tomato	7/30	23.3	4/30	13.3	ns
Tomato-Tomato	26/30	86.66	4/30	13.3	*

Note: Asterisk indicates significant difference for transmission between MEAM1 and MED in each weed host (P< 0,05); ns indicates no significant difference.

1.4 Discussion

Data obtained in this study shows that both *D. stramonium* and *N. physaloides* may have an important role in the ToSRV/whitefly pathosystem, with *N. physaloides* showing a better capacity for hosting ToSRV, and *D. stramonium* contributing more towards whitefly reproduction. The relationship between ToSRV and MEAM1 seems to be more efficient than ToSRV and MED, because MEAM1 performance was improved on both ToSRV-infected weeds, while MED performance decreased in comparison with healthy plants. Moreover, MEAM1 showed higher efficiency in

transmitting ToSRV in most host plant combinations tested, including from tomato to tomato, which may indicate MEAM1 as a more important vector of ToSRV than MED.

It has already been reported that virus-infected plants can influence vector performance and preference. A study from China involving MED whiteflies, tomato yellow leaf curl virus (TYLCV) and *D. stramonium*, showed that MED preferred settling on TYLCV-infected rather than healthy *D. stramonium*. In addition, they had a better performance on infected plants, increasing their oviposition, body length, longevity, fecundity, and survival rate (Chen *et al.*, 2013). Another study from China, involving a whitefly species, tomato chlorosis virus (ToCV), and tomatoes, suggested that MED is a better vector than MEAM1, because MED had more virus accumulation, retention, and better performance on ToCV-infected plants. Additionally, nonviruliferous MED preferred to settle on ToCV-infected plants and viruliferous MED preferred to settle on healthy ones, contributing to the virus spreading (Shi *et al.*, 2018). A study from Brazil showed that ToCV is harmful for MEAM1, and dramatically decreases its adult emergence and survival rates (Watanabe *et al.*, 2018). These results help to explain the interaction between whiteflies and plants infected with viruses, in which each whitefly species has different behaviour according to the virus and host plant.

Regarding ToSRV transmission, it was observed that MEAM1 transmitted it very well, while MED transmitted the virus to only a few plants. Therefore, MEAM1 performance increased from healthy to ToSRV-infected plants on both weeds, whereas MED performance decreased. MEAM1 can be considered a better vector of ToSRV, suggesting that this whitefly species is better adapted to ToSRV than MED. This would be logical, as MEAM1 has been exposed to the virus for over 20 years, while MED has recently been introduced in Brazil and has only recently been exposed to ToSRV, which is a native Brazilian virus.

It is also important to highlight that MED has already been reported to be a good vector of many Brazilian viruses, including ToSRV (Bello *et al.*, 2019). However, as we have obtained different results, more studies are necessary to understand the relationship between these whiteflies and ToSRV. For example, some studies suggest that the good vectors are the ones that acquire higher concentrations and retain the virus for a long period (Fiallo-Olivé *et al.*, 2020). For some begomoviruses, such as papaya leaf curl China virus (PaLCuCNV) (Guo *et al.*, 2015, 2018) and tomato yellow leaf curl China virus (TYLCCNV) (Jiu *et al.*, 2006), this pattern involving high virus retention and high transmission rate occurs. Nevertheless, to date, there is no

information about the real interaction of ToSRV with MEAM1 and MED regarding this pattern.

In Brazil, ToSRV can be found in the southeast and Midwest regions, and is considered, currently, the most important virus infecting tomato. When tomato is prematurely infected, symptoms and losses caused by the disease are higher (Giordano *et al.*, 2005), and can reach 100% (Bergamin-Filho *et al.*, 2016). It is also known that MEAM1 has been the main vector of this virus since its introduction (Inoue-Nagata *et al.*, 2016a). As our results show here, MEAM1 has better performance on ToSRV-infected plants, indicating that both *D. stramonium* and *N. physaloides* can contribute to an increase in MEAM1 population and, consequently, virus spreading. Moreover, the performance and transmission data presented in this study suggest that *N. physaloides* has more potential to harbour and be an important source of ToSRV inoculum in the field than *D. stramonium*.

Currently, the main management strategy used in Brazil for ToSRV is the chemical control of its whitefly vector. However, it has been reported that this method is not efficient when the whitefly population is high (Inoue-Nagata *et al.*, 2016a). Another option in Brazil is the use of resistant cultivars. There are several genes reported to confer resistance to tomato plants for begomoviruses, such as *Ty-1*, *Ty-2*, *Ty-3*, *Ty-4*, *Ty-5*, *ty-5*, *tcm-1*, and *tgr-1*. Initially, these genes were developed to reduce the impact of TYLCV, a monopartite begomovirus (Inoue-Nagata *et al.*, 2016a); however, as ToSRV is a bipartite species, these genes do not confer a high resistance to the Brazilian virus, which can also lead to great losses in high inoculum pressure situations (Boiteux *et al.*, 2007; Aguilera *et al.*, 2011). Additionally, another technique adopted in order to reduce the inoculum source of ToSRV is to establish a crop-free period (Salati *et al.*, 2002). Ideally, when reducing the tomato cultivation, the virus presence in the field will also be reduced, as tomato is considered to be the main inoculum source, although weed plants have also been reported to be infected with ToSRV (Silva *et al.*, 2010; Barreto *et al.*, 2013; Inoue-Nagata *et al.*, 2016a). Our data support this statement, suggesting that weeds are very important in ToSRV epidemiology, not only functioning as virus reservoirs, but also increasing the MEAM1 population. Thus, several management strategies must be combined in order to reduce the whitefly population as well as the virus inoculum source.

In summary, our study showed that *D. stramonium* and *N. physaloides* are important in virus dissemination and whitefly reproduction. Therefore, an integrated

pest management strategy should be used, such as the establishment of crop-free periods, weed management, and vector chemical control, in order to ensure healthy and safe farming. Furthermore, whitefly monitoring is very important in order to know which cryptic species are present in the area, which would be helpful for finding the best management strategy with chemical products.

More studies involving whitefly species and ToSRV in other weeds or alternative hosts are also necessary in order to better understand their contributions to ToSRV.

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References

Aguilera, J.G., Hurtado, F.D., Xavier, C.A.D., Laurindo, B.S., Nick, C., Gil, M.A. *et al*. (2011) Identificação dos genes *Ty-2* e *Ty-3* de resistência a begomovírusem genótipos de tomateiro. *Pesquisa Agropecuária Brasileira*, 46, 772–775.

Barbosa, J.C., Barreto, S.S., Inoue-Nagata, A.K., Resi, M.S., Firmino, A.C., Bergamin Filho, A. *et al*. (2009) Natural infection of *Nicandra physaloides* by tomato severe rugose virus in Brazil. *Journal of General Plant Pathology*, 75, 440–443.

Barbosa, L.F., Yuki, V.A., Marubayashi, J.M., De Marchi, B.R., Perini, F.L., Pavan, M.A. *et al*. (2015) First report of *Bemisia tabaci* Mediterranean (Q biotype) species in Brazil. *Pest Management Science*, 71, 501–504.

Barreto, S.S., Hallwass, M., Aquino, O.M. and Inoue-Nagata, A.K. (2013) A study of weeds as potential inoculum sources for a tomato-infecting begomovirus in central Brazil. *Phytopathology*, 103, 436–444.

Bello, V.H., Watanabe, L.F.M., Santos, B.R., Marabayashi, J.M., Yuki, V.A., De Marchi, R.B. *et al*. (2019) Evidence for increased efficiency of virus transmission by populations of Mediterranean species of *Bemisia tabaci* with high *Hamiltonella* prevalence. *Phytoparasitica*, 47, 293–300.

Bergamin-Filho, A., Inoue-Nagata, A.K., Bassanezi, R.B., Belasque, J., Amorim, L., Macedo, M.A. *et al*. (2016) The importance of primary inoculum and area-wide disease management to crop health and food security. *Food Security*, 8, 221–238.

Boiteux, L.S., Oliveira, V.R., Silva, C.H., Makashima, N., Inoue-Nagata, A.K., Fonseca, M.E.N. *et al*. (2007) Reaction of tomato hybrids carrying the *Ty-1* locus to Brazilian bipartite begomovirus species. *Horticultura Brasileira*, 25, 20–23.

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

Boykin, L.M. and 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. *et al*. (2013) Virus infection of a weed increases vector attraction to and vector fitness on the weed. *Scientific Reports*, 3, 2253.

De Barro, P.J., Liu, S.S., Boykin, L.M. and Dinsdale, A.B. (2011) *Bemisia tabaci*: A statement of species status. *Annual Review of Entomology*, 56, 1–19.

Doyle, J.J. and Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19, 11–15.

Fiallo-Olivé, E., Pan, L.L., Liu, S.S. and Navas-Castillo, J. (2020) Transmission of begomoviruses and other whitefly-borne viruses: dependence on the vector species. *Phytopathology*, 110, 10–17.

García-Arenal, F. and Zerbini, F.M. (2019) Life on the edge: Geminiviruses at the interface between crops and wild plant hosts. *Annual Review of Virology*, 6, 411–433.

Gilbertson, R.L., Rojas, M. and Natwick, E. (2011) Development of integrated pest management (IPM) strategies for whitefly (*Bemisia tabaci*)-transmissible geminiviruses. In: Thompson, W.M.O. (Ed.) *The Whitefly, Bemisia tabaci (Homoptera: Aleyrodidae) Interaction with Geminivirus-Infected Host Plants: Bemisia tabaci, Host Plants and Geminiviruses*. Dordrecht, Netherlands: Springer, Netherlands, pp. 323–356.

Gilbertson, R.L., Batuman, O., Webster, C.G. and Adkins, S. (2015) Role of the insect dupervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. *Annual Review of Virology*, 2, 67–93.

Giordano, L.B., Fonseca, M.E.N., Silva, J.B.C., Inoue-Nagata, A.K. and Boiteux, L.S. (2005) Efeito da infecção precoce por begomovirus com genoma bipartido em características de frutos de tomate industrial. *Horticultura Brasileira*, 23, 815–818.

Guo, T., Guo, Q., Cui, X.Y., Liu, Y.Q., Hu, J. and Liu, S.S. (2015) Comparison of transmission of Papaya leaf curl China virus among four cryptic species of the whitefly *Bemisia tabaci* complex. *Scientific Reports*, 5, 15432.

Guo, T., Zhao, J., Pan, L.L., Geng, L., Lei, T., Wang, X.W. *et al*. (2018) The level of midgut penetration of two begomoviruses affects their acquisition and transmission by two species of *Bemisia tabaci*. *Virology*, 515, 66–73.

IBGE (2019) *Produção Agrícola Municipal*. Rio de Janeiro, Brazil: IBGE.

Inoue-Nagata, A.K., Carvalho, C.M., Zerbini, F.M., Rezende, J.A.M., Krause-Sakate, R. and Nagata, T. (2016a) Vírus transmitidos por moscas-brancas no Brasil: Vetores, principais doenças e manejo. *Revisão Anual de Patologia de Plantas*, 24, 8–9.

Inoue-Nagata, A.K., Lima, M.F. and Gilbertson, R.L. (2016b) A review of geminivirus diseases in vegetables and other crops in Brazil: current status and approaches for management. *Horticultura Brasileira*, 34, 8–18.

Jiu, M., Zhou, X.P. and Liu, S.S. (2006) Acquisition and transmission of two begomoviruses by the B and a non-B Biotype of *Bemisia tabaci* from Zhejiang, China. *Journal of Phytopathology*, 154, 587–591.

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

Lapidot, M., Legg, J.P., Wintermantel, W.M. and Polston, J.E. (2014) Management of whitefly-transmitted viruses in open-field production systems. *Advances in Virus Research*, 90, 147–206.

Lourenção, A.L. and Nagai, H. (1994) Surtos populacionais de *Bemisia tabaci* no estado de São Paulo. *Bragantia*, 53, 53–59.

Macedo, M.A., Michereff Filho, M., Navas-Castillo, J. and Inoue-Nagata, A.K. (2015) Host range and whitefly transmission efficiency of Tomato severe rugose virus and Tomato golden vein virus in tomato plants. *Tropical Plant Pathology*, 40, 405–409.

Macedo, M.A., Barreto, S.S., Costa, T.M., Maliano, M.R., Rojas, M.R., Gilbertson, R.L. *et al*. (2017a) First report of common beans as a non-symptomatic host of tomato severe rugose virus in Brazil. *Plant Disease*, 101, 261.

Macedo, M.A., Barreto, S.S., Costa, T.M., Rocha, G.A., Dianese, E.C., Gilbertson, R.L. *et al*. (2017b) First report of tomato severe rugose virus, a tomato-infecting begomovirus, in soybean plants in Brazil. *Plant Disease*, 101, 1959.

Macedo, M.A., Albuquerque, L.C., Maliano, M.R., Souza, J.O., Rojas, M.R., Inoue-Nagata, A.K. *et al*. (2018) Characterization of tomato leaf curl purple vein virus, a new monopartite New World begomovirus infecting tomato in northeast Brazil. *Archives of Virology*, 163, 737–743.

Marubayashi, J.M., Yuki, V.A., Rocha, K.C.G., Mituti, T., Pelegrinotti, F.M., Ferreira, F.Z. *et al*. (2013) At least two indigenous species of the *Bemisia tabaci* complex are present in Brazil. *Journal of Applied Entomology*, 137, 113–121.

Moraes, L.A., Marubayashi, J.M., Yuki, V.A., Ghanim, M., Bello, V.H., De Marchi, B.R. *et al*. (2017) New invasion of *Bemisia tabaci* Mediterranean species in Brazil associated to ornamental plants. *Phytoparasitica*, 45, 517–525.

Moraes, L.A., Muller, C., Bueno, R.C.O.F., Santos, A., Bello, V.H., De Marchi, B.R. *et al*. (2018) Distribution and phylogenetics of whiteflies and their endosymbiont relationships after the Mediterranean species invasion in Brazil. *Scientific Reports*, 8, 14589.

Quadros, A.F.F., Silva, J.P., Xavier, C.A.D., Zerbini, F.M. and Boari, A.J. (2019) Two new begomoviruses infecting tomato and *Hibiscus* sp. In the Amazon region of Brazil. *Archives of Virology*, 164, 1897–1901.

R Development Core Team (2013) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/. [Accessed 7 January 2019].

Salati, R., Nahkla, M.K., Rojas, M.R., Guzamn, P., Jaquez, J., Maxwell, D.P. *et al*. (2002) Tomato yellow leaf curl virus in the Dominican Republic: Characterization of an infectious clone, virus monitoring in whiteflies, and identification of reservoir hosts. *Phytopathology*, 92, 487–496.

Shi, X., Tang, X., Zhang, X., Zhang, D., Li, F., Yan, F. *et al*. (2018) Transmission efficiency, preference and behavior of *Bemisia tabaci* MEAM1 and MED under the influence of tomato chlorosis virus. *Frontiers in Plant Science*, 8, 2271.

Shrestha, D., McAuslane, H.J., Adkins, S.T., Smith, H.A., Dufault, N., Colee, J. *et al*. (2017) Host-mediated effects of semipersistently transmitted Squash vein yellowing virus on sweetpotato whitefly (Hemiptera: Aleyrodidae) behavior and fitness. *Journal of Economic Entomology*, 110, 1433–1441.

Silva, A.K.F., Santos, C.D.G. and Nascimento, A.K.Q. (2010) Transmissão de begomovírus de plantas daninhas para tomateiros pela mosca-branca. *Planta Daninha*, 28, 507–514.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. and 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.

Toloy, R.S., Mituti, T., Freitas, D.M.S., Maluta, N.K.P., Silva, T.N.Z., Lopes, J.R.S. *et al*. (2018) Features of the relationship between Tomato severe rugose begomovirus and *Bemisa tabaci* MEAM1 reveal that the virus is acquired during a probe lasting only one minute. *European Journal of Plant Pathology*, 151, 541–547.

Walsh, P.S., Metzger, D.A. and 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. and Krause-Sakate, R. (2018) Performance of *Bemisia tabaci* MEAM 1 and *Trialeurodes vaporariorum* on Tomato chlorosis virus (ToCV) infected plants. *Journal of Applied Entomology*, 142,1008–1015.

Wyatt, S.D. and Brown, J.K. (1996) Detection of subgroup III geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. *Phytopathology*, 86, 1288–1293.

CHAPTER 2 - Performance and preference of *Bemisia tabaci* on cucumbers: Understanding the recent outbreaks of Mediterranean cryptic species in Brazil

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ABSTRACT

Currently, in Brazil, part of the cucumber production is cultivated in greenhouses, in rotation with bell pepper and tomato. However, this kind of cultivation seems to be contributing to heavy infestations of *Bemisia tabaci* Mediterranean cryptic species (MED) in cucumbers, which is indeed related to the recent detection of tomato chlorosis virus (ToCV) in this crop. Thus, the present study investigated the settling preference of *B. tabaci* Middle east Asia-Minor 1 (MEAM1) and MED cryptic species for cucumber, bell pepper, and tomato plants cultivated in the same area. In addition, the settling preference and biological performance of MEAM1 and MED on representative cucumber commercial cultivars were also compared. Cucumber was the most preferred host when compared with tomato and bell pepper for both whiteflies, which were also capable of establishing and ovipositioning in all cucumber cultivars tested. The Mediterranean species showed better adaptability than MEAM1 in almost all cucumber cultivars tested, by showing a better capacity of survival and resulting in a more significant number of adults. When evaluating cultivars, 'Aodai' stood out for presenting high MEAM1 nymph mortality, showing that it may be a good option to be implemented in areas with the absence of MED. In contrast, no cultivars were potentially satisfactory for use in the management of MED, reinforcing the need to develop specific management strategies directed for this cryptic whitefly species.

Keywords: Whitefly behaviour; *Cucumis sativus*; Fitness; Settling-preference; Biological performance.

2.1 Introduction

Cucumber (*Cucumis sativus* L.) is important horticulturally, being used not only for food but also in the cosmetic and pharmaceutical industries (Carvalho et al., 2013; Santi et al., 2014). More than 50% of Brazilian cucumber production is located in the southeast region, mainly in the state of São Paulo, with a cultivated area of 1644 ha in 2019 (CEPEA, 2018; IEA, 2020). To meet temperature requirements (above 20 ◦C) and maintain production throughout the year, part of the cucumber crop is cultivated in greenhouses (Oliveira et al., 1995; Reis et al, 1991, 1992), in rotation with other vegetables, such as tomatoes and bell peppers. With this practice, cucumbers are

planted right after the harvest and complete removal of tomatoes or bell peppers, which enables the utilisation of the training system and the remaining fertiliser in the soil that was already implemented in previous crops, generating better profits for producers. However, although this cultivation system has advantages for growing, it also can promote ideal conditions for pest development, including the whitefly *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae), which can feed on all these crops.

Bemisia tabaci is considered one of the most important agricultural pests globally (Navas-Castillo et al., 2011). Besides being highly polyphagous, this insect can cause physiological disorders and loss of vigour while facilitating the growth of sooty mould (*Capnodium* spp.) associated with the excretion of honeydew. However, *B. tabaci* stands out by its ability to transmit hundreds of plant viruses, from the genera *Begomovirus, Carlavirus, Crinivirus, Ipomovirus, Torradovirus and Polerovirus* (De Barro et al., 2011; Ghosh et al., 2019; Navas-Castillo et al., 2011).

Bemisia tabaci is now considered a complex of distinct cryptic species, in which the Middle East-Asia Minor 1 (MEAM1, former B biotype) and Mediterranean (MED, former Q Biotype) are the most invasive and destructive species worldwide (Boykin and De Barro, 2014; Kanakala and Ghanim, 2019; Tay et al., 2017).

Middle east Asia-Minor 1 was reported for the first time in Brazil in the early 1990s, and over the past 28 years, this species became the most prevalent whitefly species in this country (Lourenção and Nagai, 1994; Moraes et al., 2018). By contrast, MED was recently introduced. It was first reported in the Rio Grande do Sul State in 2014 (southeastern region) (Barbosa et al., 2015) and more recently in the Paraná and São Paulo States (South and Southeast Regions) (Moraes et al., 2018).

Since its first introduction, MED has been monitored through several surveys and analyses of samples from different locations of Brazil. Until 2018, this cryptic species was limited only to ornamental crops, with some sporadic occurrence in vegetables and weeds in the surrounding areas (Moraes et al, 2017, 2018). However, outbreaks caused by MED in bell pepper (*Capsicum annuum* L.) and cucumber cultivated under greenhouse conditions in the Paraná and São Paulo States have been reported since 2018 (Bello et al., 2020b). Furthermore, MED, like MEAM1, is capable of transmitting the most critical whitefly-associated viruses in Brazil (Bello et al., 2019), and a study has reported the natural infection of ToCV in greenhouse-cultivated cucumber plants in association with MED species occurrence for the first time (Bello et al., 2020a). This virus is predominantly viewed as a pathogen of solanaceous crops,

having been previously reported in cucurbits only once, infecting a single squash plant (*Cucurbita moschata* D.), in Costa Rica (Solorzano-Morales ´ et al., 2011). Since its first report in Brazil, no other ToCV surveys were carried out in cucumbers, and the prevalence of this virus and its association with *B. tabaci* is still unknown. However, the situation of the high MED population plus the recent ToCV report in cucumber suggests that a study to better understanding this relationship is necessary for our conditions.

Therefore, the present study aimed to explain the recent MED outbreaks in greenhouse cultivated cucumbers that have been occurring in Paraná and the São Paulo States. The preference dynamics of MEAM1 and MED in cucumber, bell pepper, and tomato crops in the same area were assessed. Then, a second assay was performed comparing the settling preference and performance of MEAM1 and MED on nine representative cucumber commercial cultivars while also looking for materials already available in the market that are effective in reducing whitefly populations to integrate them into management programs.

2.2 Material and methods

2.2.1 Identification and establishment of whitefly colonies

For this study, previously identified whitefly populations (Gorayeb et al., 2020) were used. The population of MEAM1 was collected from cabbage (*Brassica oleracea*) (GenBank accession number *MH186145*) in Campinas, São Paulo, Brazil (22◦ 52′ 14″ S, 47◦ 04′ 38″ W), and the MED population was collected from begonias (*Begonia* spp.) (GenBank accession number *MH047295*) in Santa Isabel, SP, Brazil (23◦ 22′ 20″ S, 46◦ 10′ 35″ W).

Both were transferred to insect-proof cages containing cotton plants (*Gossypium hirsutum*) and were maintained in greenhouse conditions, with 25 ± 2 ◦C and natural light. To ensure the purity of each colony, the population identity was checked monthly by molecular analysis of a portion of the mtCOI gene using the primer pair C1-J-2195-FW (5′–TTGATTTTTTGGTCATCCAGAAGT–3′) and C1-J-2195-RV (5′–TCCAATGCACTAATCTGCCATATTA–3′) (Frohlich et al., 1999; Simon et al., 1994) followed by restriction fragment length polymorphism (RFLP) analysis of the amplicons using *TaqI* (Bosco et al.,2006).

2.2.2 Settling preference tests

Two separate settling preference tests were performed in this study: one evaluating whitefly preference among cucumber (cv. Taiko), bell pepper (cv. Magali R) and tomato (cv. Santa Clara) plants, and another using representative standard commercial cucumber cultivars: ‘Aodai’, ‘Durango’ and ‘Verde Comprido’; Japanese type cultivars: ‘Soldier’, ‘Taiko’, ‘Tsuyataro’ and ‘Valent’; and the canning type cultivars, ‘Pioneiro’ and ‘Wisconsin SMR-58’.

Individual plants of each cultivar (3–4 true leaves stage) were placed randomly and equidistant inside insect-proof cages (100 × 60 × 50 cm), preventing contact among them. All cages were then submitted to an artificial infestation with approximately 100 non-sexed whitefly adults (no more than 7 days old) per plant. Each cage represented a replicate, with all assays repeated twice in time with four replicates each, totalling eight replicates. Adult whitefly counting per cultivar was determined at 1, 3, 6, 12, 24 and 48 h after infestation using a dental mirror with a handle under dim light to prevent whitefly movement during each evaluation. Following each counting, the plants were fully randomised again, to avoid small variations related to their position inside the cage.

After the last evaluation (48 h), all adults were removed, and the oviposition per plant was assessed, using a 1cm^2 template, and counting the number of eggs inside it at ten random locations on the abaxial side of the leaves. From those counts, an average number of eggs per cm^2 was generated for each plant and used for statistical analysis. All settling preference assays were conducted in greenhouses with 28 ± 2 °C and natural light.

2.2.3 Whitefly performance on commercial cucumber cultivars

For this test, new plants of the same cucumber cultivars previously used in settling preference tests were used. The performance was assessed through whitefly oviposition, hatchability [(number of nymphs/number of eggs) × 100], number of adults, survival rate [(number of adults/number of eggs) × 100], and development time. The tests were conducted in insect-proof rearing cages (45 × 45 × 55 cm) inside controlled environment rooms with a light period of 14 h per day and a temperature of 28 ± 2 °C.

Initially, ten adult couples of each cryptic species (no more than 7 days old) were introduced in clip cages (2 × 2 × 3 cm) and attached to the abaxial leaf side (one clip cage per leaf) of each cultivar for 48 h for oviposition. Subsequently, insects were removed together with the clip-cages, and the eggs were counted using a magnifying glass (Zeiss®), with a magnification of 20X. The egg hatching was evaluated after seven days, and adults were counted and collected daily (at 8:00 a.m. for standardisation) with a manual insect aspirator, starting on the day when the first adult emerged, until all whiteflies had emerged as adults. Total developmental time was measured in days, corresponding to each phase of the whitefly cycle, from egg-laying until the emergence of the first adult (immature stages) and during the adult emergence period.

All performance tests were conducted twice (two repetitions) with ten replications (a leaf with one clip cage attached) each, totalling 20 replications per cultivar, with no more than four clip cages per plant.

2.2.4 Data analyses

Statistical analyses were performed using the software Infostat (Di Rienzo et al., 2008). All data had the homogeneity of variances and normality assessed with the Levene's and Shapiro-Wilk tests, respectively. Repeated-measures ANOVAs were used to test significance in all settling preference tests, whereas one-way ANOVAs were used to compare whitefly oviposition after all settling preference tests. Means were ranked using Scott & Knott's test. Due to the absence of normality conditions in data from performance assays (number of eggs, hatchability, number of adults, and survival rate), cultivars were compared using the ranked Kruskal-Wallis test, and the comparison of MEAM1 and MED performance was assessed employing the Wilcoxon-Mann Whitney test.

2.3 Results

2.3.1 Whitefly settling preference among cucumber, bell pepper, and tomato

When evaluating the settling preference of MEAM1, the number of adults was significantly different among the hosts ($F = 23.58$, $df = 2, 141$, $p < 0.01$), with the

cucumber being the most attractive host, followed by the tomato and bell pepper (Table 1). Similarly, for MED, cucumber presented a higher quantity of adults, differing significantly from tomato and bell pepper ($F = 11.46$, $df = 2, 141$, $p < 0.01$), that presented similar infestations.

Regarding the oviposition, a fewer number of MEAM1 eggs were observed in bell pepper plants when compared to that on cucumber and tomato plants ($F = 15.90$, $df = 2, 21$, $p < 0.01$) (Table 1). In contrast, for MED, there were no significant differences in eggs laid between the three hosts ($F = 1.59$, $df = 2, 21$, $p = 0.23$). However, evaluating in a global way it is likely that there is a greater number of eggs in the cucumber, since the leaf surface of this plant is greater than that of peppers and tomatoes.

2.3.2 Whitefly settling preference in cucumber cultivars

When testing whitefly settling preference in cucumber cultivars, significant differences were observed for MEAM1 ($F = 22.05$, $df = 8, 423$, $p < 0.01$) in which, ‘Durango’ and ‘Valent’ were the most infested cultivars whereas ‘Soldier’ and ‘Tsuyataro’ were the least preferred (Table 2). For MED, significant differences were also observed ($F = 6.96$, $df = 8, 423$, $p < 0.01$), in which ‘Aodai’ and ‘Valent’ were less preferred in comparison with the remaining cultivars (Table 2).

Similarly, differences were also observed for MEAM1 oviposition ($F = 3.64$, $df = 8, 63$, $p < 0.01$), in which ‘Soldier’ was the one with fewest eggs laid whereas the remaining cultivars did not present significant differences (Table 2). By contrast, for MED, no significant differences were observed for oviposition among the cucumber cultivars tested ($F = 0.97$, $df = 8, 63$, $p = 0.46$) (Table 2).

Table 1. Settling preference and oviposition[1] of MEAM1 and MED on tomato, bell pepper and cucumber in free choice tests.

	Host	nº of Adults							nº of eggs/cm² (Mean ± S.E.)
		1h	3h	6h	12h	24h	48h	Significance	
MEAM1	Tomato 'Sta Clara'	56.9	67.2	88.2	88.5	86.7	79.5	b	4.6 ± 1.0 a
	Bell Pepper 'Magali'	53.0	51.9	45.6	49.6	38.0	24.6	c	1.4 ± 0.3 b
	Cucumber 'Taiko'	73.9	85.4	113.0	118.0	124.9	134.2	a	8.9 ± 1.8 a
	Host	nº of Adults							nº of eggs/cm² (Mean ± S.E.)
		1h	3h	6h	12h	24h	48h	Significance	
MED	Tomato 'Sta Clara'	50.2	57.7	64.7	70.8	55.0	64.4	b	4.2 ± 1.1 a
	Bell Pepper 'Magali'	40.4	51.0	52.7	59.1	64.5	60.7	b	2.5 ± 0.4 a
	Cucumber 'Taiko'	85.9	105.8	107.0	104.5	112.1	108.0	a	6.3 ± 1.4 a

[1]Each value represents the average of 10 random counts per plant. Means followed by the same letter indicate no significant ($p < .05$) difference between treatments according to an analysis of variance followed by Scott & Knott's test. Data were transformed by $(x)^{1/3}$ before analysis.

Table 2. Settling preference and oviposition[1] of MEAM1 and MED on cucumber cultivars after free choice tests.

Whitefly	Cultivar	nº of Adults							nº of eggs/cm²
		1h	3h	6h	12h	24h	48h	Significance	(Mean ± S.E.)
MEAM1	Soldier	18.8	26.5	37.0	36.9	31.4	42.8	d	1.1 ± 0.2 b
	Valent	97.5	123.1	147.3	129.4	126.0	135.9	a	3.8 ± 1.2 a
	Tsuyataro	18.1	24.0	42.1	29.5	31.0	30.6	d	3.1 ± 0.9 a
	Taiko	54.6	82.0	75.3	75.8	80.9	79.4	b	2.8 ± 0.4 a
	Durango	108.1	135.6	120.9	116.9	126.4	141.8	a	3.0 ± 0.4 a
	Aodai	46.6	63.5	60.9	56.6	64.9	67.6	c	3.5 ± 0.6 a
	Verde Comprido	29.4	52.1	58.0	58.9	59.8	70.5	c	2.3 ± 0.3 a
	Wisconsin SMR 58	54.4	70.1	69.1	65.8	81.5	73.0	b	5.1 ± 0.5 a
	Pioneiro	67.4	106.6	98.6	107.3	99.1	105.9	b	4.4 ± 0.8 a
MED	Soldier	47.0	54.8	63.5	49.0	62.0	72.9	a	1.5 ± 0.4 a
	Valent	60.4	61.5	63.9	52.4	55.8	48.0	b	0.3 ± 1.4 a
	Tsuyataro	77.9	95.9	98.8	76.3	98.9	102.6	a	1.3 ± 0.7 a
	Taiko	87.3	86.5	82.1	59.4	76.0	68.6	a	0.6 ± 0.5 a
	Durango	66.5	66.6	72.6	52.3	63.4	54.9	a	0.8 ± 0.7 a
	Aodai	27.5	38.3	37.5	38.3	47.1	50.3	b	1.4 ± 0.7 a
	Verde Comprido	104.6	110.6	104.5	96.8	103.4	127.1	a	1.7 ± 1.5 a
	Wisconsin SMR 58	66.3	67.9	67.4	63.4	68.8	63.0	a	1.3 ± 1.4 a
	Pioneiro	96.3	90.9	91.5	76.4	82.9	86.6	a	1.9 ± 1.1 a

[1]Each value represents the average of 10 random counts per plant. Means followed by the same letter indicate no significant ($p < .05$) difference between treatments according to an analysis of variance followed by Scott & Knott's test. Data were transformed by $(x)^{1/3}$ before analysis.

2.3.3 Whitefly oviposition, hatchability, number of adults and survival rates on commercial cucumber cultivars

When comparing the performance of MEAM1 and MED in each cucumber cultivar, significant differences were observed, especially in the number of adults and survival rates.

Mediterranean whiteflies exhibited a higher survival rate when compared to that of MEAM1 in ‘Verde comprido’ ($H = 23.18$, $P < 0.01$), ‘Aodai’ ($H = 29.27$, $P < 0.01$), ‘Durango’ ($H = 14.75$, $P < 0.01$), ‘Pioneiro’ ($H = 18.38$, $P < 0.01$), ‘Taiko’ ($H = 13.43$, $P = 0.0002$), ‘Wisconsin SMR58’ ($H = 15.70$, $P < 0.01$) and ‘Soldier’ ($H = 3.79$; $P = 0.04$), while a higher MEAM1 survival rate was observed only for ‘Tsuyataro’ ($H = 10.19$; $P < 0.01$) (Table 3)

Regarding the number of adults, greater values were observed for MED in ‘Verde comprido’ ($H = 17.69$, $P < 0.01$), ‘Aodai’ ($H = 29.27$, $P < 0.01$), ‘Durango’ ($H = 7.32$, $P < 0.01$), ‘Pioneiro’ ($H = 16.46$, $P < 0.01$) and ‘Taiko’ ($H = 20.28$, $P < 0.01$) whereas no significant differences were observed on ‘Wisconsin SMR58’ ($H = 1.32$, $P = 0.25$), ‘Soldier’ ($H = 1.72$; $P = 0.19$), ‘Valent’ ($H = 3.33$, $P = 0.07$) and ‘Tsuyataro’ ($H = 0.35$; $P = 0.55$) (Table 3).

Considering only MEAM1 performance among all cultivars, significant differences could be observed in oviposition ($H = 53.17$, $P < 0.01$), hatchability ($H = 30.51$, $P < 0.01$), number of adults ($H = 52.18$, $P < 0.01$) and survival rate ($H = 82.66$, $P < 0.01$), in which ‘Aodai’ (common type) stood out for its nymph mortality, resulting in almost no adults per leaf (Table 4).

When testing the performance of only MED on cucumber cultivars, significant differences were also observed in all parameters evaluated: oviposition ($H = 26.21$, $P < 0.01$), hatchability ($H = 43.32$, $P < 0.01$), emergence of adults ($H = 41.29$, $P < 0.01$) and survival rate ($H = 61.5$, $P < 0.01$), with emphasis on ‘Soldier’ and ‘Tsuyataro’, which presented the lowest numbers of MED adults (Table 5).

Table 3. Comparison of MED and MEAM1 performance on cucumber commercial cultivars

Parameters	Whitefly species	Cultivars								
		Verde comprido	Aodai	Durango	Pioneiro	Wisconsin SMR 58	Taiko	Soldier	Valent	Tsuyataro
Nº of eggs	MEAM1	109.5 a	189.5 a	87.0 a	112.5 a	104.0 a	75.0 b	80.0 a	53.0 b	50.5 b
	MED	86.5 a	116.5 b	83.5 a	119.0 a	72.5 b	108.5 a	72.5 a	96.5 a	83.5 a
Hatchability (%)	MEAM1	83.6 b	92.3 a	81.1 a	94.0 a	97.9 a	76.8 b	96.5 a	82.1 a	97.6 a
	MED	100.0 a	91.8 a	95.1 a	96.8 a	100.0 a	90.5 a	95.2 a	84.4 a	77.6 b
Nº of adults	MEAM1	34.0 b	1.5 b	35.0 b	35.0 b	47.5 a	27.0 b	33.5 a	36.5 a	40.5 a
	MED	74.0 a	64.0 a	60.5 a	100.0 a	61.0 a	101.5 a	44.5 a	56.0 a	45.0 a
Survival rate (%)	MEAM1	35.0 b	0.8 b	42.8 b	28.4 b	64.9 b	53.6 b	53.3 b	67.4 a	82.9 a
	MED	100.0 a	77.9 a	78.6 a	79.0 a	100.0 a	89.1 a	72.2 a	61.7 a	62.0 b

Medians followed by the same letter in columns indicate no significant statistical difference between MEAM1 and MED after Wilcoxon-Mann-Whitney test (5% of significance).

Table 4. Performance of MEAM1 on cucumber commercial cultivars

Cultivars	Nº of eggs	Hatchability (%)	Nº of adults	Survival rate (%)
Verde Comprido	109.5 bc	83.6 cd	34.0 b	35.0 d
Aodai	189.5 a	92.3 bcd	1.5 c	0.8 e
Durango	87.0 bc	81.1 bcd	35.0 ab	42.8 d
Pioneiro	112.5 ab	94.0 ab	35.0 ab	28.4 d
Wisconsin SMR 58	104.0 b	97.9 a	47.5 a	64.9 abc
Taiko	75.0 cd	76.8 a	27.0 b	53.6 cd
Soldier	80.0 cd	96.5 abc	33.5 ab	53.3 bcd
Valent	53.0 d	82.1 cd	36.5 ab	67.4 ab
Tsuyataro	50.5 d	97.6 d	40.5 ab	82.8 a

Medians followed by the same letter in columns indicate no significant statistical difference after Kruskal-Wallis ranked test (5% of significance).

Table 5. Performance of MED on cucumber commercial cultivars

Cultivars	Nº of eggs	Hatchability (%)	Nº of adults	Survival rate (%)
Verde Comprido	86.5 bcde	100.0 ab	74.0 abc	100.0 a
Aodai	116.5 abc	91.8 cde	64.0 bcd	77.9 def
Durango	83.5 bcde	95.1 bc	60.5 cde	78.6 cde
Pioneiro	119.0 a	96.8 abc	100.0 ab	79.0 bcd
Wisconsin SMR 58	72.5 e	100.0 a	61.0 cd	100.0 a
Taiko	108.5 ab	90.5 cd	101.5 a	89.1 abc
Soldier	72.5 e	95.2 abc	44.5 e	72.2 ef
Valent	96.5 abcd	84.4 de	56.0 de	61.7 f
Tsuyataro	83.5 cde	77.6 e	45.0 de	62.0 f

Medians followed by the same letter in columns indicate no significant statistical difference after Kruskal-Wallis ranked test (5% of significance).

2.3.4 Whitefly developmental time on commercial cucumber cultivars

When comparing the total developmental time of each of the cryptic species in cucumber cultivars, significant differences were observed for ‘Durango’ ($H = 6.81$, $P = 0.01$) and ‘Soldier’ ($H = 8.93$, $P < 0.01$), in which the MED cycles were 2.5 days (25 × 27.5 days) and 3.5 days shorter (22 × 25.5 days), respectively. In both cases, the differences observed were related to the adult emergence period, which was 13 days (MEAM1) versus 10 days (MED) ($H = 12.65$, $P < 0.01$), for ‘Durango’; and 10 days (MEAM1) versus 8 days (MED) (H

= 5.16, P = 0.02) for ‘Soldier’. For all other cultivars, no significant differences were observed, and the total developmental time ranged from 25 to 27 days.

2.4 Discussion

A notable shift has been observed in the whitefly dynamics after MED detection in the Paraná and São Paulo States, in which MED outbreaks have become common in greenhouse-grown cucumber, increasing production losses, and management costs (Bello et al., 2020b). The data obtained in this study showed that both MED and MEAM1 preferred cucumber plants to colonise and oviposit instead of tomato and bell pepper plants, which may explain their establishment on cucumber (Table 1).

It is already known that whiteflies have good reproduction and development on tomato and bell pepper, which are more preferred by MEAM1 and MED, respectively (Sun et al., 2013; Watanabe et al., 2019). Moreover, previous works have reported cucurbits, like Squash (*Cucurbita pepo* L.) and cucumbers as more preferred hosts for whiteflies than solanaceous plants (Bird and Krüger, 2006; Schuster, 2003; Sharma and Budha, 2015). However, those studies were performed either with MEAM1 or without detecting whitefly cryptic species.

Our data indicated that the cucumber crop is even more preferred by both MEAM1 and MED under Brazilian conditions and may play an important role in the field. As the cultivation of cucumber, tomato, and bell pepper in the same or nearby areas is frequent in Brazil, the whitefly population remains elevated during the year, a fact that was recently confirmed by Bello et al. (2020b) in the São Paulo and Paraná States.

This data can also be related to the recent ToCV transmission to cucumber since tomato has been usually found infected by ToCV in Brazil (Coêlho et al., 2019; Macedo et al., 2019; Mituti et al., 2019). The ToCV found in cucumber was associated with heavy MED infestation (Bello et al., 2020a) in areas also containing high ToCV incidence in greenhouse-grown tomato near cucumber greenhouses, indicating the probable migration and ToCV transmission between these crops.

Moreover, this study showed that both whiteflies were capable of establishing and ovipositing in all cucumber cultivars tested, without distinction among their different types (canning, Japanese and common), presenting variations among cultivars, but having a considerable number of adults in all of them (Table 2), demonstrating that all cultivars tested are suitable to be infested by both whiteflies and inoculated with ToCV. Different cucumber cultivar types such as cvs. ‘Alaska’ (Japanese type, fresh consumption) (Bird and Krüger, 2006) and ‘Bhaktapur Local’ (used for canning and fresh consumption) (Sharma and Budha, 2015) have been reported as suitable and preferred hosts of whiteflies over other crops. In Brazil, despite the whitefly being considered a pest of cucumber since the introduction of MEAM1, the control employed is mostly chemical (Carvalho et al., 2013), with no research focused on the development of resistant cultivars (Novaes et al., 2020).

However, the recent increase in the importance of this pest in cucumbers is changing this scenario, and recent studies have been carried out searching for genotypes with degrees of resistance (Novaes et al., 2020). Despite this, since the presence of ToCV in cucumbers has been associated with a large population of whiteflies, the use of the least preferred cultivars (Tsuyataro and Soldier, for MEAM1; Valent and Aodai for MED, see Table 2) may help to reduce ToCV transmission. Orfanidou et al. (2016) showed that in tomato plants, an infestation of only five adults of MED was sufficient to transmit ToCV at a rate of 20%, which increased according to greater use of insects, reaching 100% transmission with an infestation of at least 40 adults. In this sense, a better understanding of the number of whitefly adults required for ToCV transmission between cucumbers and field tests using the cultivars mentioned above will generate key information to support their use in host-mediated management.

Performance tests allowed the evaluation of whitefly viability and reproduction in each cultivar, showing that MED had better adaptability in almost all of the tested cucumber cultivars when compared with MEAM1. This fact was mainly related to the survival rate, which was superior for MED in eight of the nine cultivars, resulting in a higher number of adults at the very end of sampling (Table 3). These results can be related to MED prevalence in the recent infestations observed in greenhouse conditions (Bello et al., 2020b); that is, by having a better capacity to survive in cucumber, over time, MED ends up better adapting itself to

the crop and may displace MEAM1. Similar results were also observed in Brazil for ‘Magali-R’ bell pepper and ‘Pérola’ common beans, in which MED presented a better survival rate and a higher number of adults than MEAM1, displacing it on these crops after 120 days (Watanabe et al., 2019).

Nevertheless, other factors, such as the insecticide active compounds and doses, as well as viral infections, must be considered in further studies of MED prevalence in cucumbers to complement our results, since they can help this cryptic species to prevail (Pan et al., 2015).

It is well known that MED is less susceptible to some insecticides than MEAM1 (Horowitz et al., 2020; Pan et al., 2015; Sun et al., 2013), and in Brazil, since this method is the primary means used to control of whiteflies, it can act by selecting MED and accelerating the displacement of MEAM1. For example, in Israel, a study comparing the population dynamics of MEAM1 and MED on cotton showed that MEAM1 was prevalent in the absence of insecticide spraying. However, when insecticides were used, MED predominated over MEAM1 (Horowitz and Ishaaya, 2014). Another study from China revealed that, on tomato and eggplant fields, in the absence of insecticide spraying, MEAM1 was the prevalent species. However, after some months of chemical control, MED displaced MEAM1 and became the predominant whitefly cryptic species (Sun et al., 2013).

Virus infection can also change whitefly behaviour, influencing its performance and preference. In China, for example, MED is believed to have been a major player in the dissemination of tomato yellow leaf curl virus (TYLCV, Begomovirus), given the indirect mutualistic relationship between them (Pan et al., 2013). A study showed that MED preferred to settle-in and oviposited more in TYLCV-infected *Datura stramonium* (L.) than on the healthy plants, also presenting a better performance, with larger body lengths, fecundity, longevity, and survival rates when compared with MED whiteflies reared on healthy plants (Chen et al., 2013). Another study from Spain showed that after acquiring TYLCV, MED had a significant reduction in its nymphal stage durations, and greater longevity of male adults, with an improvement in the oviposition on infected tomato plants (Maluta et al., 2014).

Similarly, the already mentioned ToCV also has a synergic relationship with MED. A study in China showed that ToCV infection in tomato plants

increased fecundity and decreased the developmental time of MED, as well as reduced MEAM1 survival significantly (Shi et al., 2018). The same reduction was observed later in Brazil, in which it was reported a 92.4% drop in MEAM1 adult emergence on ToCV-infected ‘Santa Clara’ tomato plants (Watanabe et al., 2018). Curiously, ToCV was first reported in cucumber associated with MED species under greenhouses conditions (Bello et al., 2020b).

The data from this study has serious implications for the management of whitefly, with cucumber acting as an important host for whitefly development. Recently, Gorayeb et al. (2020), demonstrated the importance of the solanaceous *Datura stramonium* and *Nicandra physaloides* (L.) as alternative hosts for both MED and MEAM1, connecting the presence of these species with failures in the practice of a tomato-free period for whitefly management in some regions of Brazil. In the case of cucumbers, a higher number of plants would be employed, which will assist with an even more significant impact, the maintenance of whiteflies in the area, showing that the practice of successive cultivations of cucumber, pepper, and tomato, or even their cultivation in the same area, is not indicated for regions with high whitefly populations. Moreover, the different behaviours observed for MEAM1 and MED in this study strengthen the need to routinely monitor whitefly populations by molecular identification, followed by studies to identify management measures targeted for each cryptic species (Pan et al., 2015; Sun et al., 2013).

The cultivars here considered “effective” were those which contributed to the reduction of the whitefly population, showing a median of adults per leaf smaller than the number of whiteflies initially employed in each clip cage (ten whiteflies). Thus, ‘Aodai’ stood out for its high MEAM1 nymph mortality, resulting in an insignificant number of adults per leaf (Table 4), indicating that this cultivar may be used in areas infested with only MEAM1. More recently, a study performed in Brazil, with cucumber genotypes, showed an expression of antibiosis by the genotype ‘Wellington’, which prolonged the developmental time and reduced significantly the emergence of MEAM1 adults (Novaes et al., 2020). Similarly, high nymph mortality for MEAM1 was observed in *C. annuum* ‘Amaxito’, ‘Tabaquero’ and ‘Simojovel’, which was related to defence responses of the plants related to host enzymatic activity (Latournerie-Moreno et al., 2015).

In contrast, for MED, although differences were observed among the tested cultivars, substantial levels of adult emergence were still observed (Table 5); that is, none of them can be considered to reduce the whitefly population, strengthening the necessity of searching for other resistant materials focused on MED management.

In summary, this study showed that cucumbers are excellent whitefly alternative hosts, contributing to the accumulation of large populations of both MEAM1 and MED in areas where tomato and bell peppers are also cultivated. Once established, the whitefly performance is an essential factor that contributes to MED predominance in greenhouse cultivated cucumber infestations, probably displacing MEAM1 in the field, which ends up colonising other hosts less preferred by MED.

Additionally, more studies involving cultivars resistant for MED should be performed to find out a good option to decrease MED populations, as well as studies including ToCV, cucumber, and whiteflies, to understand their relationship better.

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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., Krause-Sakate, R., 2015. First report of Bemisia tabaci Mediterranean (Q biotype) species in Brazil. Pest Manag. Sci. 71, 501–504. https://doi.org/10.1002/ps.3909.

Bello, V.H., Gorayeb, E.S., Watanabe, L.F.M., De Marchi, B.R., Ribeiro-Junior, M.R., Vicentin, E., da Silva, F.B., Krause-Sakate, R., 2020a. First report of tomato chlorosis virus infecting cucumber in Brazil. Plant Dis. 104, 603. https://doi.org/10.1094/ PDIS-07-19-1490-PDN.

Bello, V.H., Watanabe, L.F.M., Fusco, L.M., De Marchi, B.R., Da Silva, F.B., Gorayeb, E.S., Moura, M.F., de Souza, I.M., Muller, C., Salas, F.J.S., Yuki, V.A., Bueno, R.C.O.F., Pavan, M.A., Krause-Sakate, R., 2020b. Outbreaks of bemisia tabaci Mediterranean species in vegetable crops in São Paulo and Paran´ a states, Brazil. Bull. Entomol. Res. 53, 1–10. https://doi.org/10.1017/S0007485319000841.

Bello, V.H., Watanabe, L.F.M., Santos, B.R., Marubayashi, J.M., Yuki, V.A., De Marchi, B. R., 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. Phytoparasitica 47, 293–300. https://doi.org/10.1007/s12600-019-00729-y.

Bird, T.L., Krüger, K., 2006. Response of the polyphagous whitefly Bemisia tabaci Bbiotype (Hemiptera: Aleyrodidae) to crop diversification – influence of multiple sensory stimuli on activity and fecundity. Bull. Entomol. Res. 96, 15–23. https://doi.org/10.1079/ber2005398.

Bosco, D., Loria, A., Sartor, C., Cenis, J.L., 2006. PCR-RFLP identification of Bemisia tabaci biotypes in the Mediterranean Basin. Phytoparasitica 34, 243–251. https://doi.org/10.1007/BF02980951.

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. Front. Ecol. Evol. 2, 1–5. https://doi.org/10.3389/fevo.2014.00045.

Carvalho, A.D.F., Amaro, G.B., Lopes, J.F., Vilela, N.J., Filho, M.M., Andrade, R., 2013. A cultura do pepino. Circ. T´ ec. 113, 18. Cepea, 2018. Pequenos Mercados, Grandes Oportunidades. Hortifruti Bras, p. 34.

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, 2253. https://doi.org/10.1038/srep02253.

Coêlho, L.M., Lima, A.T.M., Inoue-Nagata, A.K., Rizo, A.A.I., de Carvalho Pontes, N., Golynski, A., de Albuquerque, L.C., 2019. Genetic structure analyses of the tomatoinfecting crinivirus Tomato chlorosis virus indicates low genetic variability in the p22 gene and evidence of segregation based on sampling location in Brazil. Trop. Plant Pathol. 44, 292–296. https://doi.org/10.1007/s40858-019-00280-5.

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/annurevento-112408-085504.

Di Rienzo, J., Casanoves, F., Balzarini, M., Gonzalez, L., Tablada, M., Robledo, C., 2008. InfoStat Version ´ 2011. Grupo InfoStat - Universidad Nacional de Córdoba.

Frohlich, D.R., Torres-Jerez, I., Bedford, I.D., Markham, P.G., Brown, J.K., 1999. A phylogeographical analysis of the Bemisia tabaci species complex based on mitochondrial DNA markers. Mol. Ecol. 8, 1683–1691. https://doi.org/10.1046/j.1365-294x.1999.00754.x.

Ghosh, S., Kanakala, S., Lebedev, G., Kontsedalov, S., Silverman, D., Alon, T., Mor, N., Sela, N., Luria, N., Dombrovsky, A., Mawassi, M., Haviv, S., Czosnek,

H., Ghanim, M., 2019. Transmission of a new Polerovirus infecting pepper by the whitefly bemisia tabaci. J. Virol. 93, 1–14. https://doi.org/10.1128/JVI.00488-19.

Gorayeb, E.S., Bello, V.H., Cruciol, G.C.D., Watanabe, L.F.M., Dovigo, L.H., Sartori, M.M. P., Pavan, M.A., Krause-Sakate, R., 2020. Evaluation of Datura stramonium and Nicandra physaloides as reservoirs of tomato severe rugose virus and whiteflies. Plant Pathol. 69, 569–575. https://doi.org/10.1111/ppa.13139.

Horowitz, A.R., Ghanim, M., Roditakis, E., Nauen, R., Ishaaya, I., 2020. Insecticide resistance and its management in Bemisia tabaci species. J. Pest. Sci. 93, 893–910. https://doi.org/10.1007/s10340-020-01210-0 (2004).

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.

IEA, 2020. Estatística da Produção Paulista. Estatística da Produção Paulista. URL. http://ciagri.iea.sp.gov.br/nia1/subjetiva.aspx?cod_sis=1&idioma=1.

Kanakala, S., Ghanim, M., 2019. Global genetic diversity and geographical distribution of Bemisia tabaci and its bacterial endosymbionts. PloS One 14, e0213946. https://doi. org/10.1371/journal.pone.0213946.

Latournerie-Moreno, L., Ic-Caamal, A., Ruiz-Sánchez, E., Ballina-Goméz, H., IslasFlores, I., Chan-Cupul, W., González-Mendoza, D., 2015. Survival of *Bemisia tabaci* and activity of plant defense-related enzymes in genotypes of *Capsicum annuum* L. Chil. J. Agric. Res. 75, 71–77. https://doi.org/10.4067/S0718-58392015000100010.

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/S000687051994000100006.

Macedo, M.A., Inoue-Nagata, A.K., Silva, T.N.Z., Freitas, D.M.S., Rezende, J.A.M., Barbosa, J.C., Michereff-Filho, M., Nascimento, A.R., Lourenção, A.L., Bergamin Filho, A., 2019. Temporal and spatial progress of the diseases caused by the crinivirus tomato chlorosis virus and the begomovirus tomato severe rugose virus in tomatoes in Brazil. Plant Pathol. 68, 72–84. https://doi.org/10.1111/ppa.12920.

Maluta, N.K.P., Garzo, E., Moreno, A., Lopes, J.R.S., Fereres, A., 2014. Tomato yellow leaf curl virus benefits population growth of the Q biotype of bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae). Neotrop. Entomol. 43, 385–392. https://doi.org/10.1007/s13744-014-0223-z.

Mituti, T., Moura, M.F., Macedo, M.A., Silva, T.N.Z., Pinto, L.R., Costa, H., KrauseSakate, R., Inoue-Nagata, A.K., Nunes, G.G., Lima, M.F., Rezende, J.A.M., 2019. Survey of begomoviruses and the crinivirus, tomato chlorosis virus, in solanaceous in Southeast/Midwest of Brazil. Trop. Plant Pathol. 44, 468–472. https://doi.org/10.1007/s40858-019-00294-z.

Moraes, L.A., Marubayashi, J.M., Yuki, V.A., Ghanim, M., Bello, V.H., De Marchi, B.R., da Fonseca Barbosa, L., Boykin, L.M., Krause-Sakate, R., Pavan, M.A., 2017. New invasion of Bemisia tabaci Mediterranean species in Brazil associated to ornamental plants. Phytoparasitica 45, 517–525. https://doi.org/10.1007/s12600-017-0607-9.

Moraes, L.A., Muller, C., Bueno, R.C.O. de 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, 14589. https://doi.org/10.1038/s41598-018-32913-1.

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

Novaes, N.S., Lourenção, A.L., Bentivenha, J.P.F., Baldin, E.L.L., Melo, A.M.T., 2020. Characterization and potential mechanisms of resistance of cucumber genotypes to Bemisia tabaci (Hemiptera: Aleyrodidae). Phytoparasitica 48, 643–657. https://doi.org/10.1007/s12600-020-00826-3.

Oliveira, A.C.B., Araujo, M.L., Leal, N.R., 1995. Avaliação do comportamento de três cultivares de pepino submetidas ao cultivo sob estrutura de proteção e a céu aberto na região ao Norte Fluminense-RJ. Hortic. Bras. 13, 99.

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 Dis. 100, 2043–2049. https://doi.org/10.1094/PDIS-01-16-0054-RE.

Pan, H., Chu, D., Liu, B., Shi, X., Guo, L., Xie, W., Carrière, Y., Li, X., Zhang, Y., 2013. Differential effects of an exotic plant virus on its two closely related vectors. Sci. Rep. 3, 2230. https://doi.org/10.1038/srep02230.

Pan, H., Preisser, E.L., Chu, D., Wang, S., Wu, Q., Carrière, Y., Zhou, X., Zhang, Y., 2015. Insecticides promote viral outbreaks by altering herbivore competition. Ecol. Appl. 25, 1585–1595. https://doi.org/10.1890/14-0752.1.

Reis, N.V.B., Horino, Y., Oliveira, C.A.S., Boiteux, L.S., 1991. Influência da radiação ao fotossinteticamente ativa (RFA) sobre os componentes de produção de nove genótipos de pepino plantado a céu aberto e sob estufas plásticas. Hortic. Bras. 9, 55.

Reis, N.V.B., Horino, Y., Oliveira, C.A.S., Boiteux, L.S., Lopes, J.F., 1992. Influência de temperatura - graus-dia - sobre a produção de pepino sob cultivo protegido e a céu aberto. Hortic. Bras. 10, 65.

Santi, A., Scaramuzza, W.L., Soares, D.M., Tieppo, R.C., Krause, W., Scaramuzza, J.F., Dallacort, R., Krause, W., Tieppo, R.C., 2014. Desempenho e orientação do crescimento do pepino japonês em ambiente protegido. Hortic. Bras. 31, 649–653. https://doi.org/10.1590/s0102-05362013000400023.

Schuster, D.J., 2003. Preference of *Bemisia argentifolii* (Homoptera: Aleyrodidae) for selected vegetable hosts relative to tomato. J. Agric. Urban Entomol. 20, 59–67.

Sharma, M., Budha, P.B., 2015. Host preference vegetables of tobacco whitefly bemisia tabaci (Gennadius, 1889) in Nepal. J. Instr. Sci. Technol. 20, 133–137. https://doi.org/10.3126/jist.v20i1.13936.

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. https://doi.org/10.1093/aesa/87.6.651.

Solórzano-Morales, A., Barboza, N., Hernández, E., Mora-Umana, F., Ramírez, P., Hammond, R.W., 2011. Newly discovered natural hosts of tomato chlorosis virus in Costa Rica. Plant Dis. 95, 497. https://doi.org/10.1094/PDIS-11-10-0836.

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.

Tay, W.T., Elfekih, S., Polaszek, A., Court, L.N., Evans, G.A., Gordon, K.H.J., De Barro, P. J., 2017. Novel molecular approach to define pest species status and tritrophic interactions from historical Bemisia specimens. Sci. Rep. 7, 429. https://doi.org/10.1038/s41598-017-00528-7.

Watanabe, L.F.M., Bello, V.H., De Marchi, B.R., Sartori, M.M.P., Pavan, M.A., KrauseSakate, R., 2018. Performance of Bemisia tabaci MEAM 1 and Trialeurodes vaporariorum on Tomato chlorosis virus (To CV) infected plants. J. Appl. Entomol. 142, 1008–1015. https://doi.org/10.1111/jen.12559.

Watanabe, L.F.M., Bello, V.H., De Marchi, B.R., Silva, F.B. da, Fusco, L.M., Sartori, M.M., Pavan, M.A., Krause-Sakate, R., 2019. Performance and competitive displacement of Bemisia tabaci MEAM1 and MED cryptic species on different host plants. Crop Protect. 124, 104860.
https://doi.org/10.1016/j.cropro.2019.104860.

CHAPTER 3 – First report of the putative orthotospovirus Pepper necrotic spot virus in Chile.

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3.1 Disease note

In August of 2020, several bell peppers presenting irregular ripening and ring spots, typically found on orthotospovirus-infected pepper plants, were found in Markets from Santiago, Chile. In this period of the year, most of the bell peppers are provided by the northern region of Arica y Parinacota, due to the winter conditions. Therefore, those symptomatic fruits can be related to an increase of orthotospoviruses in this region.

To better investigate the causal agents of this disease, two bell pepper fruits exhibiting ring spots and irregular maturation (figure 1A) were bought in a street market from Santiago metropolitan region and analyzed molecularly.

Approximately 100mg of fruit tissue of each sample was used for total RNA extraction, using the Tri reagent® (Sigma-Aldrich), following the manufacturer's instructions. The extracted RNA was used as a template for cDNA synthesis using the SuperScript® First-Strand Synthesis System (Invitrogen™) following the manufacturer's instructions. The PCRs were performed using the Platinum™ Taq DNA Polymerase (Invitrogen™) with the addition of 1Mm of orthotospovirus generic primers BR60 (5' CCCGGATCCTGCAGAGCAATTGTGTCA 3') and BR65 (5' ATCAAGCCTTCTGAAAGTCAT 3') (EIRAS et al., 2001).

The PCR products were analyzed on 1% agarose gel and stained with Gelred (Sigma-Aldrich). Bands of the expected size (455pb) were purified using QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions and sent for sequencing at the Laboratório de Secuenciación y Tecnologías Omicas of the Pontifica Universidad Católica de Chile. The sequences obtained were compared with those existing in GenBank, using the BlastN tool (https: // blast. ncbi.nlm.nih.gov).

The sequences found (Appendix A) revealed the presence of the putative orthotospovirus Pepper necrotic spot virus (PNSV) in both samples, which were named isolates S1 and S3, presenting 98.8 and 97.7% of identity, respectively, with the isolate T1 (HE584763), reported infecting bell peppers in Peru.

To prove pathogenicity, one PNSV-infected sample was used to inoculate mechanically six healthy bell pepper plants (Three of cv. Almuden and three of cv. Coraza), using phosphate buffer (0.05 M sodium phosphate and 0.02 M sodium sulfite, pH 7.0). Four other plants (two of each cultivar) were mock-inoculated. After 15 days, the development of symptoms was observed weekly until 90 days after inoculation. After 50 days, new extractions and RT-PCRs were performed in inoculated plants to confirm virus infection.

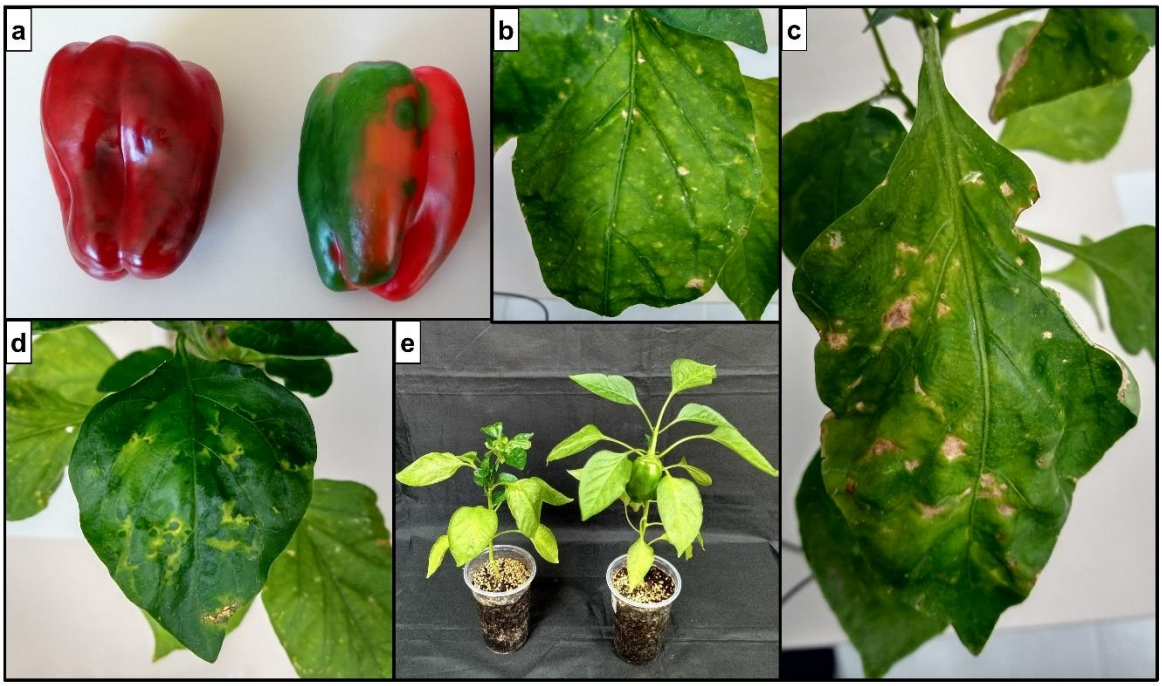
Two plants of each PNSV-inoculated cultivars (66,6% of infection) developed symptoms. Mild mottling and necrosis were observed after 15 days, with the development of ring spots after 25 days and symptoms of leaf distortion and mosaic after 60 days (Figure 1). All infected plants resulted in positive reactions using universal orthotospovirus primers, confirming the inoculation. No other orthotospoviruses were found in the inoculated plants, and no symptoms or viruses were observed in the mock-inoculated plants.

This virus was firstly reported in La Joya valley, Peru, approximately 400km from the Chilean border, in 2012 (Torres et al., 2012). So far, only two sequences from the Small RNA segment are present in GenBank, and this virus is not yet accepted as an Orthtotospovirus by ICTV. The occurrence of PNSV in Chile shows that this virus is spreading to new areas and that greater attention should be paid to it. This virus starts causing mottling and necrotic spots (Figure 1b and c), but as the infected plant develops, the symptoms become the same as those caused by other spotted wilt-related viruses, such as ring spots, irregular

ripening (PAPPU; JONES; JAIN, 2009) (Figure 1a); therefore, this virus should be formally included in the complex of species causing this disease.

Given the importance as a vegetable supplier to the metropolitan region during the winter and the localization along the Peruvian border, it is probable that these fruits came from Arica y Parinacota. A survey in this region is necessary to confirm the occurrence of PNSV in the field and prevent it from spreading to other bell pepper-producing regions.

Figure 1. PNSV-infected bell pepper fruits from Santiago and symptoms developed after Koch's postulate. A, bell pepper fruits exhibiting symptoms of ring spots and irregular maturation. B, symptoms of mild mottling and necrotic spots at 15 days after inoculation. C, Symptoms of ring spots and necrotic spots 25 days after inoculation. D, Symptoms of mosaic and leaf crinkle 60 days after inoculation. E, Comparison of healthy (left) and PNSV-infected bell pepper 'Coraza' plants, 50 days after inoculation.



References

Eiras, M., R.O. Resende, A. Missiaggia, and A.C. de Ávila. 2001. RT-PCR and Dot Blot hybridization methods for a universal detection of tospoviruses. Fitopatologia Brasileira 26: 170–175. https://doi.org/10.1590/S0100-41582001000200009

Pappu, H.R., R.A.C Jones, and R.K. Jain. 2009. Global status of tospovirus epidemics in diverse cropping systems: Successes achieved and challenges ahead. Virus Research 141: 219–236. https://doi.org/10.1016/j.virusres.2009.01.009

Torres, R., J. Larenas, C. Fribourg, and J. Romero. 2012. Pepper necrotic spot virus, a new tospovirus infecting solanaceous crops in Peru. Archives of Virology 157: 609–615. https://doi.org/10.1007/s00705-011-1217-3.

CHAPTER 4 – Survey of orthotospoviruses and associated thrips in pepper crops of Arica y Parinacota – Chile.

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ABSTRACT

Currently, only *Tomato spotted wilt orthotospovirus,* and *Impatiens necrotic spot orthotospovirus* were detected in bell peppers in Chile, always associated with *Frankliniella occidentalis*. Recently, outbreaks of spotted wilt started to be reported by growers and technicians from Arica y Parinacota, north of Chile. Given the importance of this region as a vegetable supplier to Southern regions during the winter, this study aimed to map and characterize orthotospoviruses and their associated thrips in bell pepper plants from Arica y Parinacota. Young leaves of bell pepper and tomato plants, exhibiting typical symptoms of spotted wilt and associated thrips vectors, were collected from commercial areas. Plant viruses were detected at the species level by RT-PCR, using specific or generic primers targeting orthotospoviruses. Their associated thrips were identified morphologically, with some representative samples submitted to PCR, using primers for ITS or MtCOI genes, confirming the species identity. Tomato spotted wilt virus, and the putative new orthotospovirus Pepper necrotic spot virus were detected in nine and 13 samples, respectively. All collected thrips were identified as *F. occidentallis,* and no other potential vectors were found in the locales sampled. The presence of these viruses is worrying, showing the expansion of PNSV from Peru to new areas and suggesting that the TSWV found may be a resistance-breaking isolate since most of the cultivars used in this region are TSWV-resistant. Moreover, the presence of only *F. occidentalis* suggests that this insect is the probable vector of these viruses. High throughput sequencing should be performed with both viruses to characterize both viruses better.

4.1 Introduction

In 2019, a total area of 77,243 ha was destined for horticultural crops in Chile. From that, 5,328 ha and 993 ha were destined for tomato and bell pepper cultivation, respectively (ODEPA, 2020). Despite having satisfactory yields for supplying the domestic market in the summer, the price of those vegetables can increase by 300% during the winter, when colder regions are unable to produce those crops (IPINZA, 2014). During this period, the Arica y Parinacota region

stands out for having good soil and climate conditions that allow the cultivation of these vegetables throughout the year, making it one of the main suppliers of tomatoes and peppers to the Metropolitan Region (ODEPA, 2020).

However, such good climatic conditions, combined with the rapid growth of agriculture, generated a suitable environment for the introduction and establishment of plant viruses and their insect vectors, which, from 2007, started to generate significant losses in the production of solanaceous crops (SEPÚLVEDA et al., 2011). Several studies were carried out in the subsequent years, mainly with potyviruses and begomoviruses and their vectors, which significantly improved Arica y Parinacota vegetable production (ROJAS-BERTINI, 2019; SEPÚLVEDA et al., 2011).

Nevertheless, in the last years, producers and technicians started to report the occurrence of typical symptoms of spotted wilt, mainly in bell pepper and chili pepper plants, indicating an increase in cases of this disease in the region (Rosales, M. Personal communication, 2020). This could also be observed in the market, where fruits with ringspot symptoms were frequently observed. During collections carried out in 2020, incidences of 30-100% were observed, showing that this disease was becoming epidemic in some areas.

The spotted wilt disease is caused by an *Orthotospovirus* viral complex of species (OLIVER; WHITFIELD, 2016) that formally comprises the *Tomato spotted wilt orthotospovirus* (TSWV), *Tomato chlorotic spot orthotospovirus* (TCSV), *Groundnut ringspot orthotospovirus* (GRSV), *Chrysanthemum stem necrosis orthotospovirus* (CSNV) and *Impatiens necrotic spot orthotospovirus* (INSV) (KUO et al., 2014; LIMA; MICHEREFF FILHO, 2015), but other orthotospoviruses, such as the putative species pepper necrotic spot virus, can also cause the same symptoms (See chapter 3). The differentiation of those viruses is possible only with molecular characterization.

To date, nine thrips species (Thysanoptera: Thripidae) from *Frankliniella* and *Thrips* genera are related with the transmission of the spotted wilt virus complex, with an emphasis to *F. occidentalis* and *F. schultzei*, that are the most widespread and less species-specific in viral transmission (OLIVER; WHITFIELD, 2016).

Despite TCSV and GRSV predominate as spotted wilt causal agents in some South American countries, such as Brazil and Argentina (WILLIAMS et al.,

2001), the last survey of viruses done in bell peppers in the Coquimbo region showed the presence of only TSWV and INSV in association with this disease (Sepúlveda et al., 2005). Tomato spotted wilt virus was one of the most common viruses found in approximately 20% of the collected samples associated only with *F. occidentalis* (SEPÚLVEDA et al., 2005). Moreover, the same study found for the first time INSV in bell pepper, but only in 3% of the samples collected, and no other significant reports of this virus were published(SEPÚLVEDA et al., 2005).

Inside this scenario, and given the difficulties in controlling the spread of orthotospoviruses through the control of the thrips vector, the leading management mechanism adopted in Chile has been the use of tomato and bell pepper resistant varieties, possessing the *Sw-5* and *Tsw* genes, respectively (DE OLIVEIRA et al., 2018; ROJAS-BERTINI, 2019).

A recent report showed the occurrence of a possible resistance-breaking TSWV isolate in bell peppers from Arica y Parinacota, which can also be related to this new epidemic of spotted wilt disease (ROJAS-BERTINI, 2019). However, this phenomenon can also be related to the introduction of other species of the spotted wilt complex, such as TCSV and GRSV, that are not recognized by the *Tsw* resistance gene, or even with the INSV that is already present in Chile (BOITEUX; DE ÁVILA, 1994; SEPÚLVEDA et al., 2005).

Additionally, the putative orthotospovirus Pepper necrotic spot virus was recently reported in bell peppers found in markets from Santiago metropolitan region (see chapter 3), which increased the suspicious of the presence of this virus in Arica y Parinacota.

These facts strengthen the importance of expanding the surveys to the Arica y Parinacota region, seeking to obtain a more updated and in-depth characterization of orthotospoviruses and their vectors, and prevent the spread of new orthotospovirus species to other Chilean regions.

In this regard, the present study reports the prevalence and geographical distribution of TSWV and PNSV in bell peppers and Chili peppers at three locations at the Arica y Parinacota region and identifies associated thrips that may be related to the transmission of these viruses.

4.2 Material and methods

4.2.1 Sampling of infected plants and thrips

The collections were carried out in November 2020 at eight commercial areas of peppers and/or tomatoes. A total of 74 samples (62 samples of *Capsicum annuum*, nine of *Capsicum baccatum*, and three of *Solanum lycopersicum*) containing leaves or fruits with typical symptoms of spotted wilt (ring spots, fruit, and leaf deformation, Figure 1) were collected from the valleys of Azapa (50 samples of *C. annuum*, five samples of *C. baccatum* and three samples of *S. lycopersicum*), Lluta (four samples of *C. baccatum*), and in the Pampa Concordia region (12 samples of *C. annuum*) (figure 2). All samples were packed in paper bags and kept in an ultra-freezer (-80 °C) until the moment of processing.

Adult thrips samples were collected with manual aspirators directly from symptomatic plants. Then, specimens were stored in 1.5 ml tubes containing 1 ml of 95% ethanol, and maintained in a freezer at -20 ° C until processing. A total of 220 adult thrips were collected from valleys of Azapa (140 adults from *C. annuum* and 20 from *C. baccatum*), Lluta (20 adults from *C. annuum* and ten from *C. baccatum*), and Pampa Concórdia (30 adults from *C. annuum).* No adult thrips were found in *S. lycopersicum* plants during scoutings.

Figure 1. Symptoms of ring spots and fruit deformation (a), leaf crinkle, necrosis (b and c). and ring spots in leaves (d) observed in the bell peppers sampled in Arica y Parinacota.

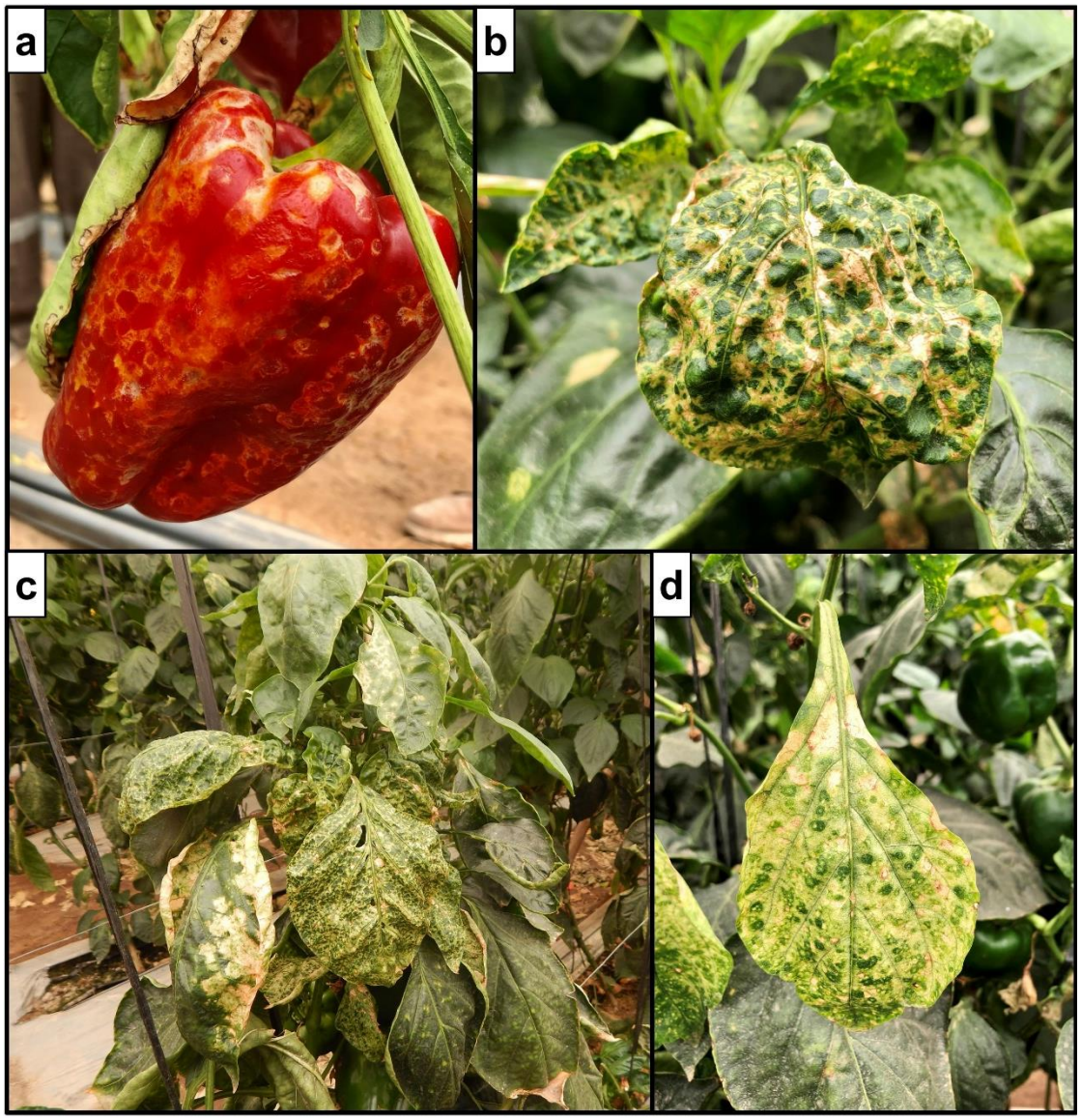
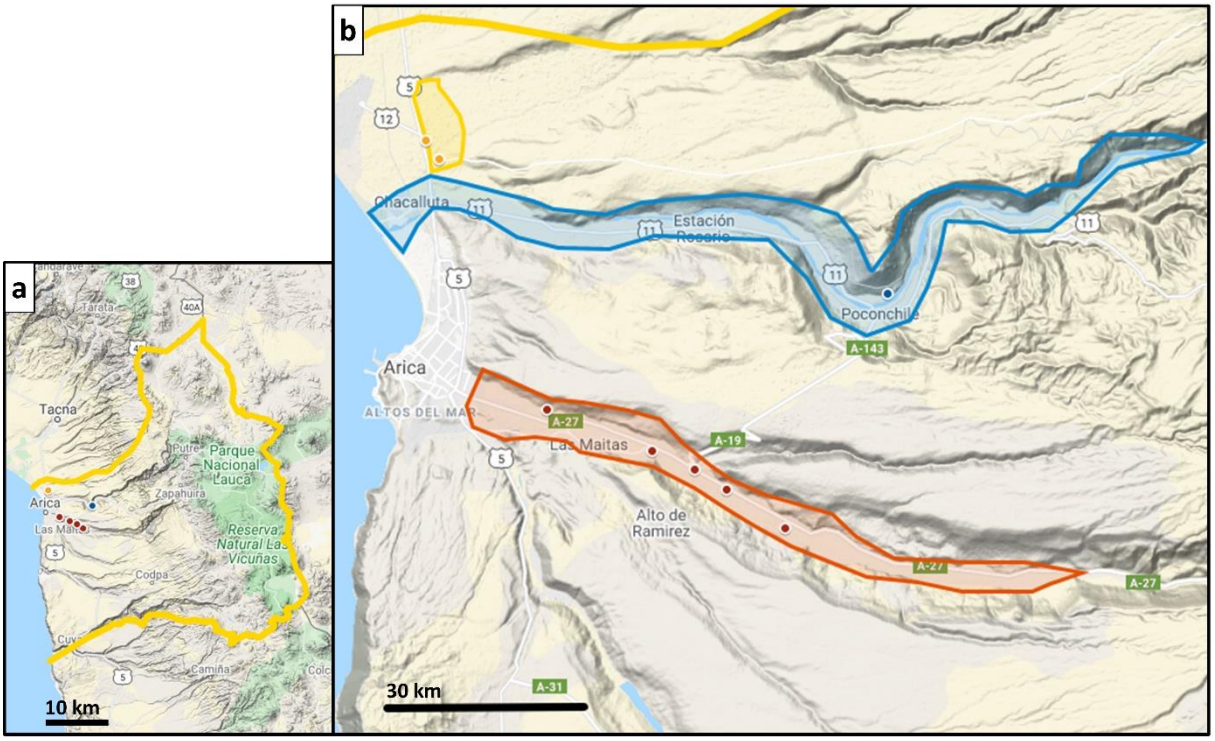


Figure 2. Map of Arica y Parinacota (a), and the locales sampled (b). In yellow, Pampa Concórdia; in blue, Lluta Valley; and in red, Azapa Valley. The dots represent the locattions sampled.



Elaborated by the author for this study, using google Earth Software (https://www.google.com/earth/).

4.2.2 Orthotospovirus detection

For virus detection, approximately 100mg of plant tissue of each sample was used for total RNA extraction, using the Tri reagent® (Sigma-Aldrich), following the manufacturer's instructions. The extracted RNA was used as a template for cDNA synthesis using the SuperScript® First-Strand Synthesis System (Invitrogen) following the manufacturer's instructions.

The PCRs were performed using the Platinum™ Taq DNA Polymerase (Invitrogen) with the addition of 1Mm of each primer used in the reaction. For each sample, five PCRs were done using universal orthotospovirus primers or specific primers targeting each virus species (Table 1).

All primers (both developed in this study and those from other studies) were submitted to blast search (https://blast.ncbi.nlm.nih.gov) to evaluate species specificity and were analysed with IDT oligo analyser software

(https://www.idtdna.com/pages/tools/oligoanalyzer), seeking to examine the occurrence of secondary structures, self-and heterodimer formation.

The PCR product was analyzed on 1% agarose gel and stained with Gelred (Sigma-Aldrich). Bands of the expected size from representative samples were purified QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions and were sent for sequencing at the Laboratorio de Secuenciación y Tecnologías Omicas of the Pontificia Universidad Católica de Chile. The sequences obtained were compared with those existing in GenBank, using the BlastN tool (https://blast. ncbi.nlm.nih.gov).

4.2.3 Identification of associated thrips

All collected thrips were analysed morphologically, followed by molecular analysis for species confirmation. The parameters considered for the morphological identification followed the recommendations from identification keys proposed by De Borbón and Zamar (2018) and Moritz et al. (2009).

For molecular identification, the total DNA of each thrips was extracted using the modified chelex protocol, in which each adult was homogenized in a 1.5 ml tube, containing 50µL of a solution of chelex 100 (Sigma-Aldrich) at 5%, followed by the incubation at 94 °C for 5 minutes and finally centrifugation at 14000 rpm for 5 minutes (BOONHAM et al., 2002).

Two microliters of extracted DNA were used as a template for a PCR, using the Platinum™ Taq DNA Polymerase (Invitrogen), with specific primer pairs which amplify regions of ribosomal DNA of the species *Frankliniella occidentalis*, *F. schultzei*, and *T. tabaci* and a thrips universal primer pair (Table 1).

Table 1. List of primers used in this study.

Target Species	Target gene/region	Primer name		Sequence	Expected amplicon size (bp)	Annealing temperature (°C)
Impatiens necrotic spot orthotospovirus[1]	N gene	F	N 2021F	5' ACACAACACAAAGCAAACCAAGCTCAAATC 3'	960	54
		R	N2980c	5′-TCACGAATATAACATCATATAATCCAAAGC-3′		
Groundnut ringspot orthotospovirus [2]	N gene	F	GRSVJ1	5' GTAGTGGTCCATAGCAATGC 3'	800	54
		R	BR60	5' CCCGGATCCTGCAGAGCAATTGTGTCA 3'		
Tomato chlorotic spot orthotospovirus [3]	N gene	F	TCSVJ1F	5' GGCTTCTTTCCTTACTCCGAAC 3'	872	60
		R	TCSVJ1R	5' CTCAGCAACACAAATCATCACA 3'		
Tomato spotted wilt orthotospovirus [4]	RDRP gene	F	L1	5' TTAAGCAAGTTCTGTGAG TT 3'	276	50
		R	L2	5' ATGTCTAAGGTTAAGCTCACTA 3'		
Orthotospovirus universal primers [5]	N gene	F	BR60	5' CCCGGATCCTGCAGAGCAATTGTGTCA 3'	453	55
		R	BR65	5' ATCAAGCCTTCTGAAAGTCAT 3'		
F. occidentalis [6]	ITS2	F	Focc1U5	5’ GGTCGCTTCACCGCTTCCRCCCGTAAA 3’	236	55
		R	Focc2D5	5’ CAAAGTGCGAGAAAATAAAATGCAAACT 3’		
F. schultzei [7]	28S	F	AG75	5’ GGTGTCGTCAGCTCGTGC 3’	779	55
		R	AG76	5’ GAAGCAAGCCGGAGTTCTC 3’		
T. tabaci [6]	ITS2	F	Ttab971U	5’ AGAAACGATTACCAGACTGCCCAAG 3’	420	55
		R	Ttab1D	5’ AGTGATGCAGCACAACACATTCCAC 3’		
Thrips universal primers [8]	MtCOI	F	mtD-7.2F	5’ ATTAGGAGCHCCHGAYATAGCATT 3’	433	56
		R	mtD-9.2R	5’ CAGGCAAGATTAAAATATAAACTTCTG 3’		

[1]Kuo et al., 2014; [2] Cruciol et al., 2019; [3]Designed by the author for this study; [4]Thomas *et al.*, 2004; [5]Eiras et al., 2001; [6] Yeh et al., 2014; [7]Jangra et al., 2020; [8]Brunner *et al.*, 2002**.**

All primers were submitted to blast search (https://blast.ncbi.nlm.nih.gov) to evaluate species specificity and were analysed with IDT oligo analyser software (https://www.idtdna.com/pages/tools/oligoanalyzer), seeking to examine the occurrence of secondary structures, self-and heterodimer formation.

All PCR products were subjected to electrophoresis in an agarose gel (1.0%), stained with Gelred (Sigma-Aldrich), and visualized under UV light. The correct size bands of representative samples were purified (QIAquick Gel Extraction Kit Qiagen) and sent for sequencing. at the Laboratorio de Secuenciación y Tecnologías Omicas of the Pontificia Universidad Católica de Chile. The corresponding sequences were then compared with those on GenBank, using the BlastN tool (https://blast.ncbi.nlm.nih.gov) for species confirmation.

4.3 Results

4.3.1 Orthotospovirus detection in Arica y Parinacota

Virus-infected plants were found in all sampled localities, only in Capsicum plants. Tomato spotted wilt virus was observed in nine samples, with sequences ranging from 96.9 to 97.4% of identity with an isolate from South Korea (MF159046) (Appendix B). Surprisingly, the putative new orthotospovirus PNSV was detected in 12 samples (Appendix C), using the degenerated primer pair, ranging from 93.5 to 100% of identity with the Strain T1 of this virus (HE584763), first reported in Peru (Torres et al. 2012) (Table 2). Three samples from the same location in the Azapa valley presented mixed infection of TSWV and PNSV (Table 2). No positive results were observed for GRSV, INSV, and TCSV.

Table 2. Orthotospoviruses detected in Arica y Parinacota collected on *Capsicum* plants.

Location (Coordinates)	Sample	Host (cultivar)	Pathogen Detected	
			TSWV	PNSV
Azapa Valley (18° 31' 42" S, 70° 9' 45.5" W)	AP5	*C. annuum* (Coraza)		X
	AP6	***C. annuum* (Coraza)**	**X**	**X**
	AP7	*C. annuum* (Coraza)		X
	AP10	*C. annuum* (Coraza)	X	
	AP12	*C. annuum* (Coraza)		X
	AP16	***C. annuum* (Goliath)**	**X**	**X**
	AP17	***C. annuum* (Goliath)**	**X**	**X**
	AP19	*C. annuum* (Goliath)		X
	AP20	*C. annuum* (Goliath)	X	
	AP23	*C. annuum* (Goliath)		X
Azapa Valley (18° 31' 10.6" S, 70° 11' 5.2" W)	AP55	*C. annuum* (Aquiles)		X
	AP59	*C. annuum* (Aquiles)		X
	AP66	*C. annuum* (Aquiles)	X	
Pampa concordia (18° 22' 36.8" S, 70° 17' 40.9" W)	AP24	*C. annuum* (Coraza)		X
	AP27	*C. annuum* (Coraza)		X
Lluta valley (18° 26' 32.1" S, 70° 3' 47.9" W)	AP41	*C. baccatum* (Escabeche)	X	
	AP42	*C. baccatum* (Escabeche)	X	
	AP43	*C. baccatum* (Escabeche)	X	

Samples in bold are mixed infected with PNSV and TSWV.

4.3.2 Thrips identification in Arica y Parinacota

Morphological analysis revealed only *Frankliniella occidentalis* (Pergande) in all areas sampled, which was later confirmed by molecular analysis. Two representative sequences (Appendix D) of the ITS region were 100% identical with *F. occidentalis* sequences from USA, China, Mexico, Taiwan, and Poland. Three other representative sequences from the MtCOI gene (Appendix D) showed 98.4% of identity with *F. occidentalis* samples from China and India. No adults of *Thrips tabaci* and *F. schultzei* were found in any samples collected (Table 3).

Table 3. Thrips identified in Arica y Parinacota collected from *Capsicum* plants.

Locale	Coordinates	Cultivar (host plant)	Environment	Nº of thrips analyzed	% of species (number)		
					F. occidentalis	*F. schultzei*	*T. tabaci*
Azapa valley	18° 31' 10.6" S, 70° 11' 5.2" W	Aquiles *(C. annuum)*	GH	20	100	0	0
		Coraza (*C. annuum*)	GH	10	100	0	0
		Painita (*C. annuum*)	GH	10	100	0	0
	18° 31' 42" S, 70° 9' 45.5" W	Coraza (*C. annuum*)	GH	30	100	0	0
		Goliath (*C. annuum*)	GH	30	100	0	0
	18° 32' 17.5" S, 70° 8' 45.6" W	Confidaro (*C. annuum*)	GH	20	100	0	0
	18° 33' 25.8" S, 70° 6' 58.9" W	Coraza (*C. annuum*)	GH	20	100	0	0
		Infierno (*C. baccatum*)	GH	20	100	0	0
Lluta Valley	18° 26' 32.1" S, 70° 3' 47.9" W	Escabeche (*C. baccatum*)	OF	10	100	0	0
		Airone (*C. annuum*)	GH	20	100	0	0
Pampa Concórdia	18° 22' 36.8" S, 70° 17' 40.9" W	Coraza (*C. annuum*)	GH	20	100	0	0
	18° 22' 2.3" S, 70° 18' 5.2" W	Coraza (*C. annuum*)	GH	10	100	0	0

GH, Greenhouse. OF, Open field.

4.4 Discussion

The discovery of PNSV-infected bell pepper fruits for sale in Santiago's street markets (see chapter 3) gave us attention to the possibility of PNSV-infected plants in Chilean agricultural areas. The fact that the plants were found on the market during late winter/early autumn indicated that these fruits were probably originated from Arica y Parinacota, since this region is the leading vegetable supplier during this period.

Arica y Parinacota is an important agricultural region for Chile for its climate conditions, permitting several cultivations of horticulture crops throughout the year and providing vegetables to colder regions during the winter. The bell pepper stands out and is commonly produced in the regions of Azapa valley and Pampa Concórdia, with some production also occurring in Lluta valley. In the last years, the spotted wilt became the most critical disease in bell peppers from this region, and a survey was necessary to understand better the species of viruses and thrips associated with the disease among the cultivated areas.

Our study revealed two orthotospoviruses: *Tomato spotted wilt orthotospovirus* and Pepper necrotic spot virus, probably in association with the western flower thrips (*F. occidentalis*), the only thrips species found in the studied areas.

Tomato spotted wilt orthotospovirus is considered the predominant species associated with the spotted wilt in Chile. A survey conducted in the Coquimbo region showed this virus's predominance in association with *F. occidentalis* in bell peppers. Besides that, the same study revealed the presence of *Impatiens necrotic spot orthotospovirus* but in lower incidences (SEPÚLVEDA et al., 2005). Based on this and in other non-published surveys, the control of spotted wilt has been based on insecticide applications and the use of bell pepper or tomato resistant cultivars, possessing the *Tsw* and *Sw-5* genes, respectively.

The presence of TSWV in plants with resistance genes is worrisome, strengthening the suspicion that resistance-breaking variants of TSWV (RB-TSWV) are already present in Arica y Parinacota. Rojas-Bertini (2019) has already observed this same fact, finding TSWV infection in pepper plants with the *Tsw* gene.

Resistance-breaking variants of TSWV are already present in several countries, such as United States, Italy, Spain, Argentina, and Turkey (CRESCENZI; VIGGIANO; FANIGLIULO, 2013; DELIGOZ; ARLI SOKMEN; SARI, 2014; FERRAND et al., 2015; MACEDO; ROJAS; GILBERTSON, 2018; MARGARIA; CIUFFO; TURINA, 2004;

ROGGERO; MASENGA; TAVELLA, 2002). The resistance-breaking is related to genetic changes in the NSs gene affecting the plant's capacity to recognize the virus (DE OLIVEIRA et al., 2018). The sequencing of the complete TSWV genome will be carried out to characterize the occurrence of resistance.

Pepper necrotic spot virus was found in 66% of viruliferous plants and is already widespread in the regions of Pampa Concórdia and Azapa valley, which are the main producers of pepper in Arica y Parinacota. As we suspected, the recent increase in cases of spotted wilt in Arica y Parinacota is related to the introduction of a new virus species, and this virus will probably be prevalent in the region due to the high pressure of cultivars with the TSW gene, which confers resistance only to TSWV (DE OLIVEIRA et al., 2018).

There is only one study related to PNSV, restricted to the region where it was first reported (TORRES et al., 2012). Only two RNA S sequences are present on GenBank, and this virus is not yet accepted in the *Orthotospovirus* genus by ICTV (ICTV, 2020). Despite being generic for orthotospovirus, the primer pair used to detect PNSV in our study (EIRAS et al., 2001) was not designed for this species, and therefore, some negative samples may also be infected with this virus.

The complete sequencing of PNSV RNAs will generate key information to confirm its inclusion in the *Orthotospovirus* genus and better understand the mechanisms involved in PNSV emergence, as well as allow the design of specific primers that can be used for more sensitive detection of this virus in other samples collected in Arica y Parinacota.

When evaluating thrips species, only the western flower thrips (*F. occidentalis*) was found in all samples, suggesting that this insect can be vectoring the virus. This finding also strengthens this insect's association with bell peppers in northern Chile, corroborating with a survey in the Coquimbo region, where only the western flower thrips were found in that crop (SEPÚLVEDA et al., 2005). The western flower thrips is considered a super-vector of viruses transmitting more than 30 virus species, including the five orthotospoviruses commonly associated with the spotted wilt disease (GILBERTSON et al., 2015; OLIVER; WHITFIELD, 2016). The characteristics of having a small size, cryptic habits, high reproductive potential, and high dispersal make it challenging to control this pest and contain virus spread (HE et al., 2020).

The occurrence of only one thrips species may be related to environmental factors, such as orthotospovirus infection and the application of insecticides.

Orthotospovirus-infection affects thrips performance, behaviour and dynamics indirectly, changing plant volatiles and insect defense responses (OLIVER; WHITFIELD, 2016). Studies from the USA and the Netherlands showed that TSWV-infection improved survival and developmental time of *F. occidentalis* (BAUTISTA et al., 1995; MARIS et al., 2004). Another study from China showed that *F. occidentalis* developmental time, mating behavior, fecundity, and offspring sex allocation was changed by TSWV-infection facilitating virus transmission (WAN et al., 2020). By contrast, TSWV was shown to be harmful to *Frankliniella fusca*, increasing its developmental time and reducing survival and adult size (STUMPF; KENNEDY, 2005).

In Arica y Parinacota the cultivation is extremally intensive. Some producers perform three bell pepper cultivations in the same year, leading to a continuous use of insecticides, which can select some resistant insects and help them survive by killing natural enemies. To date, the Insecticide Resistance Action Committee (IRAC) reported *F. occidentalis* resistance against 11 major active ingredients, with few compounds still effective against this pest. This is probably happening in Arica y Parinacota, where no other pests or natural enemies were observed in sampled plants.

So far, this study was effective in amplifying the knowledge about the orthotospovirus present in Arica y Parinacota, giving key information about species and possible vectors present in the most critical bell pepper and tomato production areas. This study is also generating new questions and directions for future research, which will help to chart a path for spotted wilt management.

References

Bautista, R.C., R.F. Mau, J.J. Cho, and D.M. Custer. 1995. Potential of tomato spotted wilt tospovirus plant hosts in Hawaii as virus reservoirs for transmission by *Frankliniella occidentalis* (Thysanoptera: Thripidae). Phytopathology 85: 953–958. https://doi.org/10.1094/Phyto-85-953.

Boiteux, L.S., and A.C. de Ávila. 1994. Inheritance of a resistance specific to tomato spotted wilt tospovirus in Capsicum chinense 'PI 159236.' Euphytica 75: 139–142. https://doi.org/10.1007/BF00024541.

Boonham, N., P. Smith, K. Walsh, J. Tame, J. Morris, N. Spence, J. Bennison, and I. Barker. 2002. The detection of *Tomato spotted wilt virus* (TSWV) in individual thrips using real time fluorescent RT-PCR (TaqMan). Journal of Virological Methods 101: 37–48. https://doi.org/10.1016/S0166-0934(01)00418-9.

Brunner, P.C., C. Fleming, and J.E. Frey. 2002. A molecular identification key for economically important thrips species (Thysanoptera: Thripidae) using direct sequencing and a PCR-RFLP-based approach. Agricultural and Forest Entomology 4: 127–136. https://doi.org/10.1046/j.1461-9563.2002.00132.x

Crescenzi, A., A. Viggiano, and A. Fanigliulo. 2013. Resistance breaking tomato spotted wilt virus isolates on resistant pepper varieties in Italy. Communications in. Agricultural and Applied. Biological. Sciences 78: 609–612.

Cruciol, G.C.D., M.F. Moura, M.R. Ribeiro-Junior, V.Y. Egawa, E.P. de Souza, M.A Pavan, and R. Krause-Sakate. 2019. First report of groundnut ringspot virus in scarlet eggplant in Brazil. Journal of Plant Pathology 101: 399. https://doi.org/10.1007/s42161-018-0171-2

De Borbón, C.M., and M.I. Zamar. 2018. Two new species of *Frankliniella* (Thysanoptera: Thripidae) from Argentina with a key to species from Argentina and Chile. Zootaxa 4369: 419–431. https://doi.org/10.11646/zootaxa.4369.3.7

de Oliveira, A.S., L.S. Boiteux, R. Kormelink, and R.O. Resende. 2018. The *Sw-5* Gene Cluster: Tomato Breeding and Research Toward Orthotospovirus Disease Control. Frontiers in Plant Science 9. https://doi.org/10.3389/fpls.2018.01055

Deligoz, I., M., Arli Sokmen, and S. Sari. 2014. First report of resistance breaking strain of *Tomato spotted wilt virus* (Tospovirus; Bunyaviridae) on resistant sweet pepper cultivars in Turkey. New Disease Reports 30: 26. https://doi.org/10.5197/j.2044-0588.2014.030.026.

Eiras, M., R.O. Resende, A. Missiaggia, and A.C. de Ávila. 2001. RT-PCR and Dot Blot hybridization methods for a universal detection of tospoviruses. Fitopatologia Brasileira 26: 170–175. https://doi.org/10.1590/S0100-41582001000200009.

Ferrand, L., M.L. García, R.O. Resende, P.A. Balatti, and E. Dal Bó. 2015. First Report of a Resistance-breaking Isolate of *Tomato spotted wilt virus* Infecting Sweet Pepper Harboring the Tsw Gene in Argentina. Plant Disease 99: 1869. https://doi.org/10.1094/PDIS-02-15-0207-PDN.

Gilbertson, R.L., O, Batuman, C.G. Webster, and S. Adkins. 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.

He, Z., J.F. Gu, S.R. Reitz, Z.R. Lei, and S.Y. Wu. 2020. A global invasion by the thrip, Frankliniella occidentalis: Current virus vector status and its management. Insect Science 27, 626–645. https://doi.org/10.1111/1744-7917.12721.

ICTV, I. comitee on T. of viruses, 2020. Virus Taxonomy: 2019 Release [WWW Document]. URL: https://talk.ictvonline.org/taxonomy/.

Ipinza, A.F., 2014. El mercado del tomate para consumo fresco. ODEPA, Santiago, Chile.

Jangra, S., A. Mittal, H. Dhall, R.K. Jain, and A. Ghosh. 2020. A multiplex PCR assay for rapid identification of major tospovirus vectors reported in India. BMC Genomics: 21, 170. https://doi.org/10.1186/s12864-020-6560-x.

Kuo, Y.W., R.L. Gilbertson, T. Turini, E.B. Brennan, R.F Smith, and S.T. Koike. 2014. Characterization and epidemiology of outbreaks of impatiens necrotic spot virus on lettuce in Coastal California. Plant Disease 98: 1050–1059. https://doi.org/10.1094/PDIS-07-13-0681-RE.

Lima, M.F. and M. Michereff-Filho. 2015. Vira-cabeça do tomateiro: sintomas, epidemiologia, transmissão e medidas de manejo. Technical report 110. Brazilian Agricultural Research Corporation (Embrapa hortaliças), Brasilia.

Macedo, M.A., M.R. Rojas, R.L. Gilbertson. 2018. First Report of a Resistance-Breaking Strain of Tomato Spotted Wilt Orthotospovirus Infecting Sweet Pepper with the Tsw Resistance Gene in California, U.S.A. Plant Disease 103: 1048. https://doi.org/10.1094/PDIS-07-18-1239-PDN.

Margaria, P., M. Ciuffo, and M. Turina. 2004. Resistance breaking strain of Tomato spotted wilt virus (Tospovirus; Bunyaviridae) on resistant pepper cultivars in Almería, Spain. Plant Pathology 53: 795. https://doi.org/https://doi.org/10.1111/j.1365-3059.2004.01082.x.

Maris, P.C., N.N. Joosten, R.W. Goldbach, D. Peters. 2004. *Tomato spotted wilt virus* infection improves host suitability for its vector *Frankliniella occidentalis*. Phytopathology 94: 706–711. https://doi.org/10.1094/PHYTO.2004.94.7.706.

Moritz, G.B., C. O'Donnell, and M.P. Parrella. 2009. Pest thrips of North America - associated with domestic and imported crops. [WWW Document]. CBIT Publ. URL https://thripsnet.zoologie.uni-halle.de/key-server-neu/data/0a08090e-0e03-4a0e-8502-070105080e05/media/Html/index.html

ODEPA, 2020. Estadísticas productivas. URL: https://www.odepa.gob.cl/estadisticas-del-sector/estadisticas-productivas.

Oliver, J.E. and A.E. Whitfield. 2016. The Genus *Tospovirus*: Emerging Bunyaviruses that Threaten Food Security. Annual Review of Virology 3: 101–124. https://doi.org/10.1146/annurev-virology-100114-055036.

Roggero, P., V. Masenga, and L. Tavella. 2002. Field Isolates of *Tomato spotted wilt virus* Overcoming Resistance in Pepper and Their Spread to Other Hosts in Italy. Plant Disease 86: 950–954. https://doi.org/10.1094/PDIS.2002.86.9.950.

Rojas-Bertini, C.A. 2019. Characterization of viral agents present in tomato crops in Arica and Parinacota Region of Northern Chile. Ponthificial Catholic University of Chile.

Sepúlveda, P.R., S.P. Larraín, E.C. Quiroz, A.P. Rebufel, and S.F. Graña 2005. Identificación e Incidencia de Virus en Pimiento en la Zona Centro Norte de Chile y su Asociación con Vectores. Agricultura Técnica 65: 235-245. https://doi.org/10.4067/S0365-28072005000300001.

Sepúlveda, R.P., Rosales, I.M., Rojas, C., Medina, C. V., Sepúlveda, G., Brown, J.K., Mora, R., 2011. Vírus transmitidos por insectos vetores en tomate en la región de Arica y Parinacota: Situación actual y manejo, 1st ed. Intituto nacional de investigaciones agropecuárias - INIA, Ururi.

Stumpf, C.F. and G.G. Kennedy. 2005. Effects of tomato spotted wilt virus (TSWV) isolates, host plants, and temperature on survival, size, and development time of Frankliniella fusca. Entomologia Experimentalis Applicata 114: 215–225. https://doi.org/10.1111/j.1570-7458.2005.00251.x.

Thomas, J.E., M.W. Schwinghamer, J.N. Parry, M. Sharman, M.A. Schilg, and E.K. Dann. 2004. First report of *Tomato spotted wilt virus* in chickpea (*Cicer arietinum*) in Australia. Australasian Plant Pathology 33: 597–599. https://doi.org/10.1071/AP04065.

Torres, R., J. Larenas, C. Fribourg, and J. Romero. 2012. Pepper necrotic spot virus, a new tospovirus infecting solanaceous crops in Peru. Archives of Virology 157: 609–615. https://doi.org/10.1007/s00705-011-1217-3.

Wan, Y., S. Hussain, A. Merchant, B. Xu, W. Xie, S. Wang, Y. Zhang, X. Zhou, and Q. Wu. 2020. *Tomato spotted wilt orthotospovirus* influences the reproduction of its insect vector, western flower thrips, *Frankliniella occidentalis*, to facilitate transmission. Pest Management Science 76: 2406–2414. https://doi.org/10.1002/ps.5779.

Williams, L.V., P.M.L Lambertini, K. Shohara, and E.B Biderbost. 2001. Occurrence and Geographical Distribution of Tospovirus Species Infecting Tomato Crops in Argentina. Plant Disease 85: 1227–1229. https://doi.org/10.1094/PDIS.2001.85.12.1227.

Yeh, W.B., M.J. Tseng, N.T. Chang, S.Y. Wu, and Y.S. Tsai. 2014. Development of Species-Specific Primers for Agronomical Thrips and Multiplex Assay for Quarantine Identification of Western Flower Thrips. Journal of Economic Entomology 107: 1728–1735. https://doi.org/10.1603/EC14027.

FINAL CONSIDERATIONS

- Both *N. physaloides* and *D. stramonium* can contribute with ToSRV patosystem. *N. physaloides* was a better alternative host for ToSRV when combined with MEAM1 whiteflies, while *D. stramonium* was mostly a good host for whitefly reproduction. Tomato severe rugose virus-infection improved MEAM1 performance on both host plants but affected MED performance negatively, strengthening the symbiotic relationship between this virus and MEAM1 that was developed over the years.

- Cucumber was more preferred by both MEAM1 and MED in comparison with tomato and bell pepper and both Whiteflies were capable to settle in and oviposit in all cucumber cultivars. Mediterranean whiteflies performed better in almost all cucumber commercial cultivars tested, which can be related with MED predominance in this crop in the field. Among all cucumber cultivars tested, only ‘Aodai’ showed the potential to decrease MEAM1 population, whereas none of the tested cultivars were satisfactory for decreasing MED population.

- The putative orthotospovirus Pepper necrotic spot virus is now present in Chile, and its symptoms are indistinguishable from those caused by other viruses from the spotted wilt complex.

- *Tomato spotted wilt virus* and PNSV are causing the spotted wilt epidemic in Arica y Parinacota. These viruses need to be better characterized to guide the development of management strategies.Only *F. occidentalis* was observed in all locales sampled and is probably acting as a vector of the orthotospoviruses found in Arica y Parinacota.

REFERENCES

BARBOSA, J. C. *et al.* Natural infection of Nicandra physaloides by Tomato severe rugose virus in Brazil. **Journal of General Plant Pathology**, Tokyo, v. 75, n. 6, p. 440-443, Oct. 2009.

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

BARRETO, S. S. *et al.* Study of Weeds as Potential Inoculum Sources for a Tomato-Infecting Begomovirus in Central Brazil. **Phytopathology**, St. Paul, v. 103, n. 5, p. 436-444, Jan. 2013.

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

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**, Cambridge, v. 53, n. 1, p. 1-10, Aug. 2020b.

BELLO, V. H. *et al.* Evidence for increased efficiency of virus transmission by populations of Mediterranean species of Bemisia tabaci with high Hamiltonella prevalence. **Phytoparasitica**, Rishon LeZion, v. 47, p. 293-300, Apr. 2019.

BETHKE, J. A.; PAINE, T. D.; NUESSLY, G. S. Comparative Biology, Morphometrics, and Development of Two Populations of Bemisia tabaci (Homoptera: Aleyrodidae) on Cotton and Poinsettia. **Annals of the Entomological Society of America**, Oxford, v. 84, n. 4, p. 407-411, Jul. 1991.

BEZERRA-AGASIE, I. C. *et. al.* First Report of Tomato severe rugose virus in Chili Pepper in Brazil. **Plant Disease**, St. Paul, v. 90, n. 1, p. 114, Jan. 2006.

BOITEUX, L. S.; DE ÁVILA, A. C. Inheritance of a resistance specific to tomato

spotted wilt tospovirus in Capsicum chinense ‘PI 159236’. **Euphytica**, Amsterdam, v. 75, n. 1–2, p. 139–142, Jan. 1994.

BROWN, J. K. *et al.* Family Geminiviridae. *In*: KING, A. M. Q. *et al.* (org.). **Virus Taxonomy: Ninth report of the International Committee on Taxonomy of Viruses.** 9. ed. London: Elsevier, 2012. p. 351–373.

CHO, J. *et al.* Approaches for controlling tomato spotted wilt virus. *In*: HADIDI, A.; KHETARPAL, R. K.; KOGANEZAWA, H. (org.). **Plant Virus Disease Control**. St. Paul: APS Press, 1998. p. 547–564.

COLE, Anthony B. *et al.* Temporal Expression of PR-1 and Enhanced Mature Plant Resistance to Virus Infection Is Controlled by a Single Dominant Gene in a New Nicotiana Hybrid. **Molecular Plant-Microbe Interactions**, St. Paul, v. 17, n. 9, p. 976–985, Feb. 2004.

FIRMINO, A. C. *et al.* Tomato yellow vein streak virus: relationship with Bemisia tabaci biotype B and host range. **Scientia Agricola**, Piracicaba, v. 66, n. 6, Dec. 2009.

FONTES, F. V. H. M.; COLOMBO, C. A.; LOURENÇÃO, A. L. Caracterização molecular e divergência genética de Bemisia tabaci (Genn.) (Hemiptera: Aleyrodidae) em diferentes culturas e locais de cultivo. **Neotropical Entomology**, Santo Antônio de Goiás, v. 39, n. 2, p. 221–226, Abr. 2010.

GILBERTSON, R. L. Implementation and success of host-free periods for managing tomato-infecting begomoviruses in developing countries. *In*: APS IPPC JOINT MEETING, 2011, Honolulu. **Anais** [...]. Honolulu: Am Phytopath Society, 2011. p. S210. Disponível em: https://www.apsnet.org/meetings/Documents/2011_Meeting_Abstracts/s11ma17.htm. Acess on: 10 Mar. 2021.

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**, Palo Alto, v. 2, n. 1, p. 67–93, Nov. 2015.

HASSANI-MEHRABAN, A. *et al.* A distinct tospovirus causing necrotic streak on Alstroemeria sp. in Colombia. **Archives of virology**, Berlin, v. 155, n. 3, p. 423–428, Feb. 2010.

HOROWITZ, A. R. *et al.* Insecticide resistance and its management in Bemisia tabaci species. **Journal of Pest Science**, Berlin, v. 93, p. 893–910, Feb. 2020.

HULL, R. Ecology, Epidemiology, and Control of Plant Viruses. *In*: HULL, Roger (org.). **Plant Virology**. 5th. ed. London: Elsevier, 2014a. Chapter 14, p. 809–876.

HULL, R. Symptoms and Host Range. *In*: HULL, R. **Plant Virology** (org.). 5th ed. Boston: Academic Press, 2014b. Chapter 4, p. 145–198.

INOUE-NAGATA, A. K.; LIMA, M. F.; GILBERTSON, R. L. A review of geminivirus diseases in vegetables and other crops in Brazil: current status and approaches for management. **Horticultura Brasileira**, Recife, v. 34, n. 1, p. 8–18, Mar. 2016.

KRAUSE-SAKATE, R. *et al.* Population Dynamics of Whiteflies and Associated Viruses in South America: Research Progress and Perspectives. **Insects**, Basel, v.11, n.12, 847, Nov. 2020.

LAW, M. D.; MOYER, J. W. A tomato spotted wilt-like virus with a serologically distinct N protein. **Journal of General Virology**, London, v. 71, n. 4, p. 933–938, Apr. 1990.

LIMA, M. F.; MICHEREFF-FILHO, M. **Vira-cabeça do tomateiro: sintomas, epidemiologia, transmissão e medidas de manejo.** Brasilia. Available at: https://www.embrapa.br/hortalicas/busca-de-publicacoes/-/publicacao/1026816/vira-cabeca-do-tomateiro-sintomas-epidemiologia-transmissao-e-medidas-de-manejo. Acess on: 15 Apr. 2021.

MACEDO, M. A. *et al.* First Report of Common Beans as a Non-Symptomatic Host of Tomato severe rugose virus in Brazil. **Plant Disease**, St. Paul, v. 101, n. 1, p. 261,

Jan. 2017a.

MACEDO, M. A. *et al.* First Report of Tomato severe rugose virus , a Tomato-Infecting Begomovirus, in Soybean Plants in Brazil. **Plant Disease**, St. Paul, v. 101, n. 11, p. 1959–1959, Nov. 2017b.

MACEDO, M. A.; ROJAS, M. R.; GILBERTSON, R. L. First Report of a Resistance-Breaking Strain of Tomato Spotted Wilt Orthotospovirus Infecting Sweet Pepper with the Tsw Resistance Gene in California, U.S.A. **Plant Disease**, St. Paul, v. 103, n. 5, p. 1048, Mar. 2019.

MACEDO, M. A. *et al.* Host range and whitefly transmission efficiency of Tomato severe rugose virus and Tomato golden vein virus in tomato plants. **Tropical Plant Pathology**, Brasilia, v. 40, n. 6, p. 405–409, Oct. 2015.

MONTERO-ASTÚA, M.; ULLMAN, D. E.; WHITFIELD, A. E. Salivary gland morphology, tissue tropism and the progression of tospovirus infection in Frankliniella occidentalis. **Virology**, Basel, v. 493, p. 39–51, Jun. 2016.

MORAES, L. A. *et al.* New invasion of Bemisia tabaci Mediterranean species in Brazil associated to ornamental plants. **Phytoparasitica**, Rishon LeZion, v. 45, n. 4, p. 517–525, Aug.2017.

MORAES, L. A. *et al.* Distribution and phylogenetics of whiteflies and their endosymbiont relationships after the Mediterranean species invasion in Brazil. **Scientific Reports**, London, v. 8, n. 1, 14589, Oct. 2018.

MORALES, F. J. History and Current Distribution of Begomoviruses in Latin America. **Advances in Virus Research**, London, v. 67, p. 127–162, Oct. 2006.

MORIONES, E. *et al.* Effect of plant age at time of infection by tomato spotted wilt tospovirus on the yield of field-grown tomato. **European Journal of Plant Pathology**, Amsterdam, v. 104, p. 295-300, Apr.1998.

MOURA, M. F. *et al.* First report of tomato severe rugose virus in eggplant. **Journal of Plant Pathology**, Turin, v. 100, n. 3, p. 599, Jul. 2018.

NAGATA, T.; DE AVILA, A. C. Transmission of Chrysanthemum stem necrosis virus, a recently discovered tospovirus, by two thrips species. **Journal of Phytopathology**, Berlin, v. 148, p. 123-125, Jan. 2000.

OLIVER, J. E.; WHITFIELD, A. E. The Genus Tospovirus : Emerging Bunyaviruses that Threaten Food Security. **Annual Review of Virology**, Palo Alto, v. 3, n. 1, p. 101–124, Sep. 2016.

PAPPU, H. R.; JONES, R. A. C.; JAIN, R. K. Global status of tospovirus epidemics in diverse cropping systems: Successes achieved and challenges ahead. **Virus Research**, Amsterdam, v. 141, n. 2, p. 219–236, May. 2009.

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

ROJAS, M. R. *et al.* Exploiting Chinks in the Plant's Armor: Evolution and Emergence of Geminiviruses. **Annual Review of Phytopathology**, Palo Alto, v. 43, n. 1, p. 361–394, May. 2005.

SANTOS, C. D. G.; ÁVILA, A. C.; RESENDE, R. O. Estudo da interação de um begomovírus isolado de tomateiro com a mosca branca. **Fitopatologia Brasileira**, Brasilia, v. 28, p. 664–673, Dez. 2003.

SCHOLTHOF, K. B. G. *et al.* Top 10 plant viruses in molecular plant pathology. **Molecular Plant Pathology**, London, v. 12, n. 9, p. 938–954, Oct 2011.

SEPÚLVEDA, P. R. *et al.* Identificación e Incidencia de Virus en Pimiento en la Zona Centro Norte de Chile y su Asociación con Vectores. **Agricultura Técnica**, Chillán, v. 65, n. 3, Sep. 2005.

SHRESTHA, A. *et al.* Effects of Thrips Density, Mode of Inoculation, and Plant Age on Tomato Spotted Wilt Virus Transmission in Peanut Plants. **Environmental Entomology**, Oxford, v. 44, n. 1, p. 136–143, Feb. 2015.

TORRES, R. *et al.* Pepper necrotic spot virus, a new tospovirus infecting solanaceous crops in Peru. **Archives of Virology**, Berlin, v. 157, n. 4, p. 609–615, Jan. 2012.

UCKO, O.; COHEN, S.; BEN-JOSEPH, R. Prevention of virus epidemics by a crop-free period in the arava region of Israel. **Phytoparasitica**, Rishon LeZion, v. 26, n. 4, p. 313–321, Dec. 1998.

WATANABE, L. F. M. *et al.* Performance and competitive displacement of Bemisia tabaci MEAM1 and MED cryptic species on different host plants. **Crop Protection**, Amsterdam, v. 124, 104860, Oct. 2019.

WILLIAMS, L. V. *et al.* Occurrence and Geographical Distribution of Tospovirus Species Infecting Tomato Crops in Argentina. **Plant Disease**, St. Paul, v. 85, n. 12, p. 1227–1229, Dec. 2001.

APPENDIX A - PNSV Sequences from chapter three.

Isolate PNSV S1: 5’ AATATTCAACGTCAGCACATCAGTCTTACAAATCATCACAT
TAGTAGAATAAGAAGTGATCACGGGATACAGAGTTGCACTTTCTTACCCTAAATT
GCATATATTGAAAGCACACCAAATCTTCTTCAGCCATCATGTCTAAGGTTAAATT
GACAAAGGAAAATATCATTGCCTTGTTAACCCAATCGGCAGAAGTTGAATTTGAA
GAAGATCAGAATCAAACTGCATTCAACTTCAAAACTTTCTGCCATGATAATCTTG
ACCTGCTCAAGAAGATGAACATAACTTCATGCTTGACATTCCTGAAAAACCGTCA
GAGTATCATGAAGGTGGTCAAGCAAAGTGATTTTACCTTTGGTAAAATCACTATC
AAAAAGACTTCAGACAGGATAGGAGCGACTGATATGACTTTCAGAAGGCTTGAT
AAATCTTGTTGATGGCAGGTCTGATTATAATTAGGGAAGTTTTGAAGGAGAAAGC
AGGAGGAGAAAAATCCTCTC 3’

Isolate PNSV S3: 5’ ATTCTTTCAAACGTCTGACACTCAGTCTTACAATCATCAC
ATTAGTAGAATAAGAAGTGATCACGGGATACAGAGTTGCACTTTCTTACCCTAAA
TTGCATATATTGAAAGCACACCAAATCTTCTTCAGCCATCATGTCTAAGGTTAAAT
TGACAAAGGAAAATATCATTGCCTTGTTAACCCAATCGGCAGAAGTTGAATTTGA
AGAAGATCAGAATCAAACTGCATTCAACTTCAAAACTTTCTGCCATGATAATCTT
GACCTGCTCAAGAAGATGAACATAACTTCATGCTTGACATTCCTGAAAAACCGTC
AGAGTATCATGAAGGTGGTCAAGCAAAGTGATTTTACCTTTGGTAAAATCACTAT
CAAAAAGACTTCAGACAGGATAGGAGCGACTGATATGACTTTCAGGGGCTTGAT
AGTCTCGCATGGCGGTCAAGATTATTTTGGTTGTTTGTTCCGAAGTCCAATGCA
GTTTGATTCTGAATCTTTTGGAAGTTTAACTTCTGCCGTTTTGGGGTTAACCGGG
CACTGGTTATTTTCCCTTT 3’

APPENDIX B - *Tomato spotted wilt orthotospovirus* sequences obtained on chapter 4.

TSWV L segment Isolate AP6: 5’ GCGGAGGGGGGGCTGCTCTTTGCTATCATA
TTAATCCAATTTTAACTACTGGTGAAGCTCAGTTTAATGATAGAATGCATTTTAAT
GTATGTTAAATTGAAGAAGGTTTGTATACCAACAGACATTTTTTTGAATCTAAAAA
AAGCTCAAGGAACTTTCGGGCAAAATGAAACTGCCATAGGACTTTTGACTAAGG
GTTTGACGACAAACACATACCCTGTTAGCATGAATTGGTTGCAAGGCAATTAA 3’.

TSWV L Segment Isolate AP10: 5’ GCGCAGGAGCGGCGGCTATTAGCTATCAT
ATTAATCCAATTTTAACTACTGGTGAAGCTCAGTTTAATGATAGAATGCATTTTAA
TGTATGTTAAATTGAAGAAGGTTTGTATACCAACAGACATTTTTTTGAATCTAAAA
AAAGCTCAAGGAACTTTCGGGCAAAATGAAACTGCCATAGGACTTTTGACTAAG
GGTTTGACGACAAACACATACCCTGTTAGCATGAATTGGTTGCAAGGCAATTAA
3’.

APPENDIX C - Pepper necrotic spot virus sequences obtained on chapter 4.

PNSV_S_Segment_Isolate_AP5: 5’ ATCACCGATAAACAAGTGAATACAAAAAAA
TGGATGACTTTCAGAAGGCTTGATAATGCATATATTGAATGCACAAAAAATCTTC
TTCAGCCATCATGTCTAAGGTTAAATTGACAAAGGAAAATATCATTGCCTTGTTA
ACCCAATCGGCAGAAGTTGAATTTGAAGAAGATCAGAATCAAACTGCATTCAACT
TCAAAACTTTCTGCCATGATAATCTTGACCTGCTCAAGAAGATGAACATAACTTC
ATGCTTGACATTCCTGAAAAACCGTCAGAGTATCATGAAGGTGGTCAAGCAAAG
TGATTTTACCTTTGGTAAAATCACTATCAAAAAGACTTCAGACAGGATAGGAGCG
ACTGATATGACTTTCAGAAGGCTTGATAACA 3’.

PNSV_S_Segment_Isolate_AP6: 5’ ATCATCACATTAGTAGAATAAGAAGTGATCA
CGGGATACAGAGTTGCACTTTCTTACCCTAAATTGCATATATTGAAAGCACACCA
AATCTTCTTCAGCCATCATGTCTAAGGTTAAATTGACAAAGGAAAATATCATTGC
CTTGTTGACCCAATCGGCAGAAGTTGAATTTGAAGAAGATCAGAATCAAACTGC
ATTCAACTTCAAAACTTTCTGCCATGATAATCTTGACCTGCTCAAGAAGATGAAC
ATAACTTCATGCTTGACATTCCTGAAAAACCGTCAGAGTATCATGAAGGTGGTCA
AGCAAAGTGATTTTACCTTTGGTAAAATCACTATCAAAAAGACTTCAGACAGGAT
AGGAGCTACTGATATGACTTTCAGAAGGCTTGATAA 3’.

PNSV_S_Segment_Isolate_AP7: 5’ TTCAAATTCAACTTCTGCCGATTGGGTCAA
CAAGGCAATGATATTTTCCTTTGTCAATTAAACCTTAGACATGATGGCTAAACAA
GATTTGGTGTCGAGTCAATATATTCAATTTAGGGTAAGAAAGTGCAACTCAGGAT
CCCGCGATCCCTTCTTATTCTACTAATGTGATGATCTGTAAGCCTGAGTGCTGAG
GTTTTGAATAAAATTGACACGTATGCATTCT 3’.

PNSV_S_Segment_Isolate_AP12: 5’ ACTCAGCACTCAGTCTTACAAATCATCACA
TTAGTAGAATAAGAAGTGATCACGGGATACAGAGTTGCACTTTCTTACCCTAAAT
TGCATATATTGAAAGCACACCAAATCTTCTTCAGCCATCATGTCTAAGGTTAAATT
GACAAAGGAAAATATCATTGCCTTGTTGACCCAATCGGCAGAAGTTGAATTTGAA
GAAGATCAGAATCAAACTGCATTCAACTTCAAAACTTTCTGCCATGATAATCTTG
ACCTGCTCAAGAAGATGAACATAACTTCATGCTTGACATTCCTGAAAAACCGTCA
GAGTATCATGAAGGTGGTCAAGCAAAGTGATTTTACCTTTGGTAAAATCACTATC
AAAAAGACTTCAGACAGGATAGGAGCTACTGATATGACTTT 3’.

PNSV_S_Segment_Isolate_AP16: 5’ TATCATCACATTAGTAGAATAAGAAGTG
ATCACGGGATACAGAGTTGCACTTTCTTACCCTAAATTGCATATATTGAAAGCAC
ACCAAATCTTCTTCAGCCATCATGTCTAAGGTTAAATTGACAAAGGAAAATATCA
TTGCCTTGTTGACCCAATCGGCAGAAGTTGAATTTGAAGAAGATCAGAATCAAAC
TGCATTCAACTTCAAAACTTTCTGCCATGATAATCTTGACCTGCTCAAGAAGATG
AACATAACTTCATGCTTGACATTCCTGAAAAACCGTCAGAGTATCATGAAGGTGG
TCAAGCAAAGTGATTTTACCTTTGGTAAAATCACTATCAAAAAGACTTCAGACAG
GATAGGAGCTACTGATATGACTTTCAGAAGGCTTGATA 3’.

PNSV_S_Segment_Isolate_AP17: 5’ ACGGGATACGGATTAGTACTTTCTTGCCCG
AAATTGCATATATTGAAAGCACACCAAATCTTCTTCAGCCATCATGTCTAAGGTT
AAATTGACAAAGGAAAATATCATTGCCTTGTTAACCCAATCGGCAGAAGTTGAAT
TTGAAGAAGATCAGAATCAAACTGCATTCAACTTCAAAACTTTCTGCCATGATAA
TCTTGACCTGCTCAAGAAGATGAACATAACTTCATGCTTGACATTCCTGAAAAAC
CGTCAGAGTATCATGAAGGTGGTCAAGCAAAGTGATTTTACCTTTGGTAAAATCA
CTATCAAAAAGACTTCAGACAGGATAGGAGCGACTGATATGACTTTCAGAAGGC
TTGATAAA 3’.

PNSV_S_Segment_Isolate_AP19: 5’ ATAATCACATTAGTAGAATAAGAAGTGATC
ACGGGATACAGAGTTGCACTTTCTTACCCTAAATTGCATATATTGAAAGCACACC
AAATCTTCTTCAGCCATCATGTCTAAGGTTAAATTGACAAAGGAAAATATCATTG
CCTTGTTAACCCAATCGGCAGAAGTTGAATTTGAAGAAGATCAGAATCAAACTGC
ATTCAACTTCAAAACTTTCTGCCATGATAATCTTGACCTGCTCAAGAAGATGAAC
ATAACTTCATGCTTGACATTCCTGAAAAACCGTCAGAGTATCATGAAGGTGGTCA
AGCAAAGTGATTTTACCTTTGGTAAAATCACTATCAAAAAGACTTCAGACAGGAT
AGGAGCGACTGATATGACTTTCAGAAGGGCTTGATA 3’.

PNSV_S_Segment_Isolate_AP23: 5’ ATCAAGCCTTCTGAAAGTCATATCAGTCG
CTCCTATCCTGTCTGAAGTCTTTTTGATAGTGATTTTACCAAAGGTAAAATCACTT
TGCTTGACCACCTTCATGATACTCTGACGGTTTTTCAGGAATGTCAAGCATGAAG
TTATGTTCATCTTCTTGAGCAGGTCAAGATTATCATGGCAGAAAGTTTTGAAGTT
GAATGCAGTTTGATTCTGATCTTCTTCAAATTCAACTTCTGCCGATTGGGTTAAC

AAGGCAATGATATTTTCCTTTGTCAATTTAACCTTAGACATGATGGCTGAAGAAG
ATTT 3’.

PNSV_S_Segment_Isolate_AP24: 5’ AGTAGAATAAGAAGTGATCACGGGATACA
GAGTTGCACTTTCTTACCCTAAATTGCATATATTGAAAGCACACCAAATCTTCTTC
AGCCATCATGTCTAAGGTTAAATTGACAAAGGAAAATATCATTGCCTTGTTGACC
CAATCGGCAGAAGTTGAATTTGAAGAAGATCAGAATCAAACTGCATTCAACTTCA
AAACTTTCTGCCAAGATAATCTTGACCTGCTCAAGAAGATGAACATAACTTCATG
CTTGACATTCCTAAAAAACCGTCAGAGTATCATGAAGGTGGTCAAGCAAAGTGA
TTTTACCTTTGGTAAAATCACTATCAAAAAGACTTCAGACAGGATAGGAGCTACT
GATATGACTTTCAGAAGGCTTGATAAA 3’.

PNSV_S_Segment_Isolate_AP27: 5’ CACATTAGTAGAATAAGAAGTGATCACGG
GATACAGAGTTGCACTTTCTTACCCTAAATTGCATATATTGAAAGCACACCAAAT
CTTCTTCAGCCATCATGTCTAAGGTTAAATTGACAAAGGAAAATATCATTGCCTT
GTTGACCCAATCGGCAGAAGTTGAATTTGAAGAAGATCAGAATCAAACTGCATT
CAACTTCAAAACTTTCTGCCAAGATAATCTTGACCTGCTCAAGAAGATGAACATA
ACTTCATGCTTGACATTCCTGAAAAACCGTCAGAGTATCATGAAGGTGGTCAAG
CAAAGTGATTTTACCTTTGGTAAAATCACTATCAAAAAGACTTCAGACAGGATAG
GAGCTACTGATATGACTTTCAGAAGGCTTGATA 3’.

PNSV_S_Segment_Isolate_AP55: 5’ GCATATATTGAAAGCACACCAAATCTTCTTC
AGCCATCATGTCTAAGGTTAAATTGACAAAGGAAAATATCATTGCCTTGTTAACC
CAATCGGCAGAAGTTGAATTTGAAGAAGATCAGAATCAAACTGCATTCAACTTCA
AAACTTTCTGCCATGATAATCTTGACCTGCTCAAGAAAATGAACATAACTTCATG
CTTGACATTCCTGAAAAACCGTCAGAGTATCATGAAGGTGGTCAAGCAAAGTGA
TTTTACTTTTGGTAAAATCACTATCAAAAAGACTTCAGACAGGATAGGAGCGACT
GATATGACTTTCAGAAGGCTTGATAAA 3’.

PNSV_S_Segment_Isolate_AP59: 5’ CAAGCCTTCTGAAAGTCATATCAGTCGCTC
CTATCCTGTCTGAAGTCATTTTGATAGTGATTTTACCAAAAGTAAAATCACTTTAC
TTGACCACCTTCATGATACTTCGAGGGTTTTTCAGGAATGTCAAGCAAGAAGTTA
TGTTCATTTTCTTGAGCAGGTCAAGATTATCAGGGCAGAAAGTTGTGAAATTGAA

TGCCGTTTGATTTTGATCGTCTTCAAATTCAACTTTTGCGGGTAGGGGTAACAAG
GCAATGATATTTTCCTTTGTCAATTTAACCTTAGACAAGA 3’.

APPENDIX D - Thrips sequences obtained on chapter 4.

F. occidentalis #1 Arica y Parinacota ITS: 5’ TCGAAACGCAAAGTGCGAGAAA
ATAAAATGCAAACTGCGCGGTTGCTCTTTGTAAGGCATACCTCCAAGTCTTCAAG
ACGGGCGGCGCGACGTCGAGTCCGAGAACAAGAAACGTCACACACCCGCCGC
TTTTGGCAGAACGTCTGGAGGCT 3’.

F. occidentalis #3 Arica y Parinacota ITS: 5’ TCGAAACGCAAAGTGCGAGAAAAT
AAAATGCAAACTGCGCGGTTGCTCTTTGTAAGGCATACCTCCAAGTCTTCAAGA
CGGGCGGCGCGACGTCGAGTCCGAGAACAAGAAACGTCACACACCCGCCGCT
TTTGGCAGAACGTCTGGAGGCT 3’.

F. occidentalis #70 Arica y Parinacota MtCOI: 5’ GCAAATCAAACAGTGCTGGTATA
AAACTGGGTCTCCACCTCCTCTCGGATCAAAGAAGGATGTATTTAAGTTTCGGT
CTGTTAATAATATTGTAATAGCTCCTGCTAAAACTGGTAACGACAATAATAATAAA
ATAGCTGTTAAAATAACTGATCAAACAAATAAAGTTATCTTTTCCGTTGTTAATTTT
TTGATCTTTAAATTTAAAATCGTTGTAATAAAATTTAAAGCTCCTAGAATTGAAGA
AATACCTGCTAAATGAAGGGAAAAAATAGTTAAATCTACTGATGGTCCAGAGTGA
TAAAAAGTTGACAAAGGTGGGTAAACTGTTCATCCTGTTCCTGCACCATCTTTTG
ATAAACCTATAATTAACAATGTTAAAGAGGGTGGAAGAAGTCAAAATCTTATGTT
ATTAAGTCGAGGAAATGCTATATCTGGGCCTCCCTAAATAAAAAAAGGGGGGGG
GGGACATAATAACCCCCGAGGTTGACTTCTTCCCGCGTCTGGGAACATTGTTTA
ATTATAGGGTTTATCA 3’.

F. occidentalis #137 Arica y Parinacota MtCOI: 5’ GCAGAATCAAACAGTGCTG
GTATAAAACTGGGTCCCCACCTCCTCTCGGATCAAAGAAGGATGTATTTAAGTTT
CGGTCTGTTAATAATATTGTAATAGCTCCTGCTAAAACTGGTAACGACAATAATA
ATAAAATAGCTGTTAAAATAACTGATCAAACAAATAAAGTTATCTTTTCCGTTGTT
AATTTTTTGATCTTTAAATTTAAAATCGTTGTAATAAAATTTAAAGCTCCTAGAATT
GAAGAAATACCTGCTAAATGAAGGGAAAAAATAGTTAAATCTACTGATGGTCCAG
AGTGATAAAAAGTTGACAAAGGTGGGTAAACTGTTCATCCTGTTCCTGCACCATC
TTTTGATAAACCTATAATTAACAATGTTAAAGAGGGTGGAAGAAGTCAAAATCTTA
TGTTATTAAGTCGAGGAAATGCTATATCAGGGCCTCCCCTAATAGA 3’

F. occidentalis #211 Arica y Parinacota MtCOI: 5’ TGCTGGTATAAAACTGGGTC
CCCACCTCCTCTCGGATCAAAGAAGGATGTATTTAAGTTTCGGTCTGTTAATAAT
ATTGTAATAGCTCCTGCTAAAACTGGTAACGACAATAATAATAAAATAGCTGTTAA
AATAACTGATCAAACAAATAAAGTTATCTTTTCCGTTGTTAATTTTTTGATCTTTAA
ATTTAAAATCGTTGTAATAAAATTTAAAGCTCCTAGAATTGAAGAAATACCTGCTA
AATGAAGGGAAAAAATAGTTAAATCTACTGATGGTCCAGAGTGATAAAAAGTTGA
CAAAGGTGGGTAAACTGTTCATCCTGTTCCTGCACCATCTTTTGATAAACCTATA
ATTAACAATGTTAAAGAGGGTGGAAGAAGTCAAAATCTTATGTTATTAAGTCGAG
GAAATGCTAT 3’.