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EDUARDO SILVA GORAYEB

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.

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.

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