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Effect of insecticides on the secondary spread of tomato severe rugose virus (ToSRV) and tomato chlorosis virus (ToCV) by *Bemisia tabaci* MEAM1, spread distance of ToSRV, and a nanotechnology approach to chemical management of the vector

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Thesis presented to obtain the degree of Doctor in Science. Area: Plant Pathology

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SUMMARY

RESUMO

Efeito de inseticidas na disseminação secundária do tomato severe rugose virus (ToSRV) e do tomato chlorosis virus (ToCV) por *Bemisia tabaci* MEAM1, distância de disseminação do ToSRV e uma abordagem nanotecnológica para o manejo químico do vetor

O tomate é considerado um dos principais produtos agrícolas do Brasil e do mundo. No entanto, vários fatores podem afetar os campos de tomateiros, como doenças causadas pelo tomato severe rugose virus (ToSRV) e tomato chlorosis virus (ToCV), ambos transmitidos por *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1). Estudos epidemiológicos demonstram que a chegada e o desenvolvimento das epidemias de ToSRV e ToCV nos campos de produção de tomate ocorrem principalmente através de infecções primárias, o que torna as fontes externas de inóculo extremamente importantes para as epidemias destas doenças. No entanto, a hipótese de que o inóculo que causa o desenvolvimento da epidemia pode estar próximo da plantação de tomateiros é pouco explorada até agora. Para tanto, o primeiro objetivo foi avaliar o voo de curta distância (10, 20, 30 e 50 metros) de *B. tabaci* MEAM1, através da marcação-liberação-recaptura dos insetos e a consequente transmissão do ToSRV para tomateiros colocados a distâncias crescentes do ponto de liberação do vetor. A eficácia de duas fontes de proteína (albumina de clara de ovo de galinha pasteurizada e extratos comerciais à base de soja como fontes de albumina e lecitina) e dois corantes (azul ou rosa) utilizados como marcadores de insetos também foi avaliada. Adultos virulíferos maracados de *B. tabaci* MEAM1 atingiram no máximo 30 m do ponto de liberação e nenhum tomateiro infectado com ToSRV foi detectado a 50 metros . As proteínas ou os corantes não afetaram a mortalidade de B. tabaci MEAM1, embora os testes de campo tenham mostrado que foram recapturados menos insetos marcados do que não marcados. Os resultados reforçam a suspeita de que a fonte de inóculo de ToSRV que suporta a epidemia deve estar próxima das áreas de cultivo dos tomateiros. No segundo objetivo estudou-se a hipótese de que como são improváveis fontes de inóculo ("reservoir") suficientemente fortes para sustentar elevadas incidências apenas através da disseminação primária, a disseminação secundária pode ocorrer, mesmo quando o vetor é eficientemente controlado com inseticidas. Avaliou-se as incidências ToSRV e ToCV em campos experimentais de tomateiros pulverizados alternadamente três vezes por semana com ciantraniliprole, acetamiprido e flupiradifurona. Foram utilizadas três áreas: plantas pulverizadas, não-pulverizadas e controle. As áreas com tomateiros pulverizados e não pulverizaosa consistiram em 90% de plantas sadias e 10% co-infectadas com ToSRV e ToCV, intercaladas aleatoriamente. Adultos avirulíferos de *B. tabaci* MEAM1 foram liberados semanalmente nas áreas com tomateiros pulverizaosa e não pulverizados. Na área controle não houve aplicação de inseticida nem liberação de insetos. O experimento foi repetido uma vez. Os sintomas foram avaliados semanalmente e testes moleculares foram realizados 70 dias após o transplante dos tomateiros para detecção dos vírus. As taxas médias de infecção por ToSRV e ToCV na área com tomateiros não pulverizados no primeiro e segundo experimentos foram de 95% e 81%, enquanto na área com tomateiros pulverizados, 6% e 7%, respectivamente. A área com tomateiros controle não apresentou plantas infectadas. Apesar da redução relevante, o controle químico não impediu completamente a disseminação secundária dos dois vírus. Embora a abordagem de manejo químico seja altamente eficiente na eliminação de adultos de *B. tabaci* MEAM1, os inseticidas apresentam algumas limitações de ação, permitindo taxas variáveis de transmissão desses vírus. A procura de medidas de controle seguras ao meio ambiente é crescente e novas abordagens para reduzir o volume e a frequência da aplicação de insecticidas nas culturas de tomateiros, mantendo a eficiência são fundamentais. O terceiro objetivo foi avaliar o desempenho do inseticida ciantraniliprole (CNAP) no controle de *B. tabaci* MEAM1 por meio do desenvolvimento de um nanoinseticida à base de zeína (ZeinCNAP). Após 48 horas de pulverização, a mortalidade do inseto pelo ZeinCNAP foi significativamente diferente à do CNAP comercial. Quando a dose do ZeinCNAP foi reduzida em 10 vezes, a mortalidade do inseto permaneceu igual ao CNAP comercial em dose completa. O peso da massa fresca, parâmetros fotossintéticos e estresse oxidativo, das plantas de tomate pulverizadas com ZeinCNAP não foram afetados, quando comparados com os das plantas não pulverizadas. O nanoinseticida desenvolvido apresenta efeitos promissores na mortalidade de *B. tabaci* MEAM1 e pode ser considerado seguro para tomateiros. Possivelmente, este estudo é o primeiro a avaliar os efeitos do ciantraniliprole nanoencapsulado em zeína na mortalidade de *B. tabaci* MEAM1.

Palavras-chave: Crinivirus, Begomovirus, Mosca-branca, Epidemiologia da doença, Nanotecnologia

ABSTRACT

Effect of insecticides on the secondary spread of tomato severe rugose virus (ToSRV) and tomato chlorosis virus (ToCV) by *Bemisia tabaci* MEAM1, spread distance of ToSRV, and a nanotechnology approach to chemical management of the vector

Tomato is considered one of the leading agricultural products in Brazil and the world. However, several factors can affect tomato crops, such as diseases caused by the tomato severe rugose virus (ToSRV) and tomato chlorosis virus (ToCV). Both viruses are transmitted by *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1). Epidemiological studies have shown that the arrival of the inoculum and the development of the ToSRV and ToCVepidemics in tomato fields occurs mainly through primary infections with the influx of viruliferous vector, which makes external sources of inoculum important in both virus epidemics. However, the hypothesis that the inoculum that causes epidemic development might be related near the tomato crop has yet to be explored. For that, the first objective was to evaluate the short-range flight (10, 20, 30, and 50 meters) of *B. tabaci* MEAM1, using mark-release-recapture insects, and consequently, the transmission of ToSRV to tomato plants placed at increasing distances from a release point. The efficacy of two protein sources (pasteurized chicken egg-white albumin and commercial soy-based extracts as sources of albumin and lecithin) and two dyes (blue or pink) used as insect markers was also evaluated. The whiteflies reached a maximum of 30 m from the release point, and no ToSRV-infected tomato plants were detected at 50 m. The proteins or the dyes did not affect *B. tabaci* MEAM1 mortality, although field tests showed that fewer marked than unmarked insects were recaptured. These findings reinforce the suspicion that the epidemic-supporting source of the ToSRV inoculum must be close to the tomato crop. The second objective studied the hypothesis that, as inoculum sources strong enough to sustain high incidences by primary dissemination alone are unlikely, secondary dissemination might occur, even with efficient vector control. The incidence of ToSRV and ToCV was evaluated in experimental tomato fields sprayed alternately thrice a week with cyantraniliprole, acetamiprid, and flupyradifurone. Three areas were used: sprayed, no-sprayed, and control. The sprayed and no-sprayed areas consisted of 90% healthy tomato plants and 10% co-infected with ToSRV and ToCV, randomly interspersed. Adults of aviruliferous *B. tabaci* MEAM1 were released weekly in the sprayed and no-sprayed tomato plants. In the control area, only healthy tomatoes were placed. The experiment was repeated once. Symptoms were evaluated weekly and molecular tests were performed 70 days after transplanting to detect virus infection. The average rates of ToSRV and ToCV infection in the no-sprayed tomato plants in the first and second assays were 95% and 81%, respectively, while in the sprayed tomato plants, they were 6% and 7%. The control area did not show infected plants. Despite the relevant reduction, chemical control could not wholly prevent the secondary spread of the two viruses. Although the chemical management approach is highly efficient in killing *B. tabaci* MEAM1, insecticides have some limitations in their action, allowing variable rates of virus transmission. The demand for environmentally friendly control measures is growing, and new approaches are essential to reduce the volume and frequency of insecticide application to tomato crops while maintaining efficiency. The third objective was to evaluate the performance of the insecticide cyantraniliprole (CNAP) by developing a zein-based nanoinsecticide (ZeinCNAP). After 48 h of spraying, ZeinCNAP insect mortality dis not differ significantly from commercial CNAP. When the dose of ZeinCNAP was reduced to 1/10, insect mortality remained almost the same as commercial CNAP in full dose. The fresh mass weight, photosynthetic parameters, and oxidative stress of the tomato plants treated with ZeinCNAP were not affected compared to untreated plants. The developed nanoinsecticide has promising effects on *B. tabaci* MEAM1 mortality and can be considered safe for tomato plants. To our knowledge, this study is the first to evaluate the effects of zein nanoencapsulated cyantraniliprole on whitefly mortality.

Keywords: Crinivirus, Begomovirus, Whitefly, Disease epidemiology, Nanotechnology

1. GENERAL INTRODUCTION

Tomato (*Solanum lycopersicum*, Solanaceae family) is considered one of the main agricultural products in Brazil and the world, mainly due to its social importance in economic and nutritional terms. China, India, Turkey, and the United States are the most significant producers (FAOSTAT, 2021). Brazil ranks ninth in the world, with an annual production of 3,809,986 tons in a planted area estimated at 54,502 hectares, produced mainly in the Southeast and Central West regions, in the states of Goiás, São Paulo, and Minas Gerais (IBGE, 2022).

Despite the high production volume, several negative factors can affect tomato crops. Water deficiency, nutritional imbalances, competition with weeds, soil compaction, pests, and diseases can affect the development and productivity of plants. Diseases are prominent among adverse factors, mainly due to their quantity and aggressiveness. The number of diseases that affect tomato crops is large. Those caused by viruses of the genera Crinivirus and Begomovirus deserve special attention. The Crinivirus genus includes 14 species containing the tomato chlorosis virus – ToCV, while the Begomovirus genus includes 628 species containing the tomato severe rugose virus – ToSRV (ICTV, 2023).

ToCV was first detected in greenhouse tomatoes in Florida, USA, causing what was described as “yellow leaf disorder” (Wisler et al., 1998b). Later, reports on the occurrence of ToCV emerged around the world, such as in Europe (Louro et al., 2000; Navas-Castillo et al., 2000), Asia (Tsai et al., 2004) and Africa (Mohamed et al., 2018). In Brazil, the first ToCV report occurred in 2008 in tomato plants from the region of Sumaré, in the state of São Paulo (Barbosa et al., 2008). The symptoms of ToCV in tomato plants and other hosts are characterized mainly by interveinal chlorosis in the lower leaves, upward leaf curling, and thickening of the leaf, which becomes brittle (Wintermantel and Wisler, 2006). The disease can also damage other important solanaceous crops, causing reductions of 30-52% in the development and yield of chilli pepper plants (Fortes et al., 2012; Mansilla-Córdova et al., 2018) and 48% in the dry mass of tomato plants (Bampi et al., 2019).

Symptoms of ToSRV infections in tomato plants are mosaic, leaf distortion, and reduced plant size (Inoue-Nagata et al., 2016). Tomato production can also be affected by ToSRV infection. Giordano et al. (2005) showed that early begomovirus infections in tomato seedlings cause a drop in productivity of up to 60%, while Inoue-Nagata et al. (2016) describe losses of up to 100%. ToSRV has so far, only been detected in Brazil.

Crinivirus and begomovirus are transmitted by cryptic species of *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1), Mediterranean (MED) and New World (NW), and

Trialeurodes vaporariorum, and *T. abutiloneus*, in a semi-persistent and persistent-circulative way (Wisler et al., 1998a; Navas-Castillo et al., 2000, Freitas et al., 2012), respectively.

Macedo et al. (2014) evaluated the incidence of ToCV in central Brazil and found a 20% rate of infection in 150 of the analyzed samples. From 2013 to 2017, Mituti et al. (2019) evaluated the incidence of viruses in several solanaceous crops in different Brazilian states and reported 20% crinivirus and 77% begomovirus in tomato samples. Assessments carried out in tomato fields in Sumaré region from 2018 to 2021 point to high incidence rates of ToSRV and ToCV, reaching values of up to 30% and 80%, respectively (unpublished data). It is essential to highlight that the epidemiological patterns data described in those works and the virus incidence values come from commercial fields treated by insecticides to control the vector.

Epidemiological studies have shown that the arrival of the virus and the epidemic development of diseases caused by ToCV and ToSRV in tomato crops occur mainly through primary infections by the continuous flow of viruliferous whiteflies (Barbosa et al., 2016; Bergamin Filho et al., 2016; Macedo et al., 2019). In this context, external sources of inoculum are considered extremely important for both virus epidemics. However, the influence of the distance between external sources of inoculum and tomato crops is unknown.

The vector flight behavior undoubtedly determines the ToCV and ToSRV spread process. Studies of the dispersal of *B. tabaci* are essential to provide the epidemiological knowledge necessary to develop good management practices. One of the ways to understand the dispersal dynamics of adults of *B. tabaci* MEAM1 and the transmission of viruses at short distances is through the monitoring of marked specimens (Narisu et al., 1999; Hagler and Jackson, 2001; Lavandero et al., 2004; Hagler and Machtley, 2016).

Understanding the location of the primary sources of inoculum and their influence distance is essential in disease management. By identifying them, eradication can reduce the primary transmission of both viruses, consequently reducing insecticide use. Currently, chemical insecticides to control *B. tabaci* are the most widely adopted method for managing both diseases. Insecticides act to reduce insect populations and consequently reduce the number of individuals capable of transmitting the virus in the field (Gilbertson et al., 2011). Although the insecticides cyantraniliprole, flupyradifurone, and pymetrozine reduce contact with the phloem and sap ingestion by the whitefly (Garzo et al., 2020), they still have limitations in preventing the transmission of both viruses, especially primary transmission (Maluta et al., 2021). The insecticides used on fields do not act immediately to stop the feeding behavior of the vector and, therefore do not prevent the virus from being inoculated into the phloem vessels (Wisler et al., 1998b; Garzo et al., 2020). Gouvêa et al. (2017) reported that the mortality of *B. tabaci* MEAM1 by cyantraniliprole (CNAP) was 54% 48 hours after applying the product to tomato plants. Maluta et al. (2021) found mortality rates for this insect around 40% three hours after the application of CNAP and flupyradifurone. Considering that ToSRV can be inoculated within only 5 minutes after feeding (Toloy et al., 2018) and ToCV within 5-20 minutes (Freitas, 2012; Wei et al., 2019), the delayed action of insecticides to kill the vector does not prevent virus transmission.

The following context is given: a) the use of chemical insecticides is the primary method to control the spread of both viruses through vector control; b) even with the high volumes and frequency of the insecticides applied in tomato commercial fields, variable incidence rates of the viruses are found; c) the influence of the primary source of inoculum is well described, but its presence near fields is still being determined. Despite all this information, the explanation of the variable incidences of viruses in commercial fields needs to be clarified. However, previous work (Macedo et al., 2019) showed that insecticides can not prevent primary ToCV infections but also secondary infections, which may explain the variation of incidence between fields. This role of secondary spread, even in the presence of insecticides, was never demonstrated experimentally in the field.

Due to the variable efficacy effects of insecticides, in conjunction with their undesirable side effects on agroecosystems, the necessity of new approaches to reduce virus transmission rates arises. Nanotechnology has emerged as a possible alternative to improve pesticide performance, especially in terms of efficiency of agrochemical delivery and utilization (Huang et al., 2019), as well as reducing the volume used in the field (Camara et al., 2019).

One of the strategies for using nanoparticles (NP) is encapsulating active ingredients (Gao et al., 2020) in various carriers to improve performance in controlling the target species. Polymeric nanoparticles can range from 1-1000 nm (Zielińska et al., 2020) and have been investigated for use in pesticides nanoformulations (Grillo et al., 2012; Pereira et al., 2014; Maruyama et al., 2016; Oliveira et al., 2018). One of the main advantages of using NP is reducing the applied volume while simultaneously guaranteeing or improving plant protection (Kah et al., 2018). This performance improvement is associated with changes in molecule characteristics such as reduced and uniform size, increased surface area to volume, reactivity, adjustability, and ease of coating (Elmer and White, 2018).

In association with pesticides, nanoparticles can be used more effectively against pests. Additionally, doses are significantly reduced when in nanoformulation form, decreasing the potential for adverse health effects of high doses, and are less expensive due to the reduction of the content of active ingredients content in the formulation (Kumar et al., 2014). Nanoformulations used to control insects with some efficacy have been described elsewhere, such as the use of garlic essential oil in rice grains against *Tribolium castaneum* beetle (Yang et al., 2009), imidacloprid in mulberry leaves for the control of the moth *Glyphodes pyloalis* (Memarizadeh et al., 2014), and thiamethoxam in tomato leaves for the control of the scale insect *Phenacoccus solenopsis* (Elabasy et al., 2020). For *B. tabaci*, only the use of combinations of nanoparticles and essential oils is described, which, although not improving the performance of the active ingredient in the insect's biological processes, allowed for a gradual release of the analyte, protecting the molecule against photodegradation and improving its solubility in water (Pereira et al., 2018; Peres et al., 2020).

This study aimed to clarify some aspects of the ToCV and ToSRV epidemiology and chemical control of the vector through insecticides. Were evaluated: i) flight behavior and ToSRV transmission by whiteflies in a near-source of inoculum arena; ii) importance of ToCV and ToSRV secondary spread by *B. tabaci* MEAM1 in a tomato field frequently insecticide-treated; iii) development of a new tool to control the vector, based on the use of nanotechnology.

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2. EVALUATION OF LOCAL MOVEMENT OF *Bemisia tabaci* MEAM1 AND TOMATO SEVERE RUGOSE VIRUS TRANSMISSION TO TOMATO PLANTS USING MARKED AND UNMARKED WHITEFLIES

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Abstract
Tomato golden mosaic disease, caused by the tomato severe rugose virus (ToSRV), is one of the most serious tomato diseases. ToSRV is transmitted by the whitefly *Bemisia tabaci* MEAM1, from a wide range of hosts. Introducing ToSRV in commercial fields probably can occur by transporting the virus from distant locations. However, our hypothesis is that the inoculum causing epidemic development might be related to nearby sources. This study evaluated the short-range flight distance of *B. tabaci* MEAM1 (marked with detectable proteins and powder dye), through recaptures in yellow sticky traps, and the transmission of ToSRV by viruliferous *B. tabaci* MEAM1 to tomato plants placed at increasing distances from a release point. Evaluated whiteflies reached a maximum of 30 m from the release point, and no ToSRV-infected tomato plants were detected at greater distances. These findings reinforce the suspicion that the epidemic-supporting source of ToSRV inoculum must be close to commercial cultivation areas. About the efficacy of the type of markers used, of the two protein sources tested (pasteurized chicken egg-white albumin and commercial soy-based extracts as sources of albumin and lecithin, respectively), albumin was considered easier to detect. Marking with these proteins or with two dyes (blue or pink) did not affect *B. tabaci* MEAM1 mortality, although field tests showed that fewer marked than unmarked insects were recaptured. Dye marking was considered more practical because of the ease of detection. The study validates a new tool to understand the movement of *B. tabaci* MEAM1, based on different marking methods.

Keywords: Begomovirus, whitefly marking, mark-release-recapture, disease spread

2.1 Introduction

Tomato golden mosaic disease, caused by the tomato severe rugose virus (ToSRV), is among the most important virus diseases affecting tomato (*Solanum lycopersicum*) crops in Brazil (Mituti et al., 2019; Souza et al., 2020). Symptoms of infected tomato plants include vein clearing, mosaic, leaf wrinkling, and stunting, with reduced yield (Barbosa et al., 2008; Inoue-Nagata et al., 2016).

ToSRV has a wide host range, naturally infecting 16 species of cultivated and non-cultivated plants (Nozaki et al., 2006; Souza-Dias et al., 2008; Barbosa et al., 2009; Barreto et al., 2013; Macedo et al., 2017a, 2017b; Moura et al., 2018; Duarte et al., 2021), and experimentally transmitted to plants of 16 other species (Barbosa et al., 2011; Michereff-Filho et al., 2012; Macedo et al., 2015), the majority in the family Solanaceae.

ToSRV is a member of the family *Geminiviridae*, genus *Begomovirus*, and has a bipartite genome composed of two single-stranded circular DNA molecules (Zerbini et al., 2017). It is transmitted in a persistent-circulative manner by the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) MEAM1 (Middle East-Asia Minor 1, formerly known as biotype B) (Inoue-Nagata et al., 2016).

B. tabaci MEAM1 is considered a supervector of plant viruses. Adults have piercing-sucking mouthparts and feed on cultivated and non-cultivated plants of 500 species in 74 families (Gilbertson et al., 2015). The same discuss that the number of successfully transmitted viruses by *B. tabaci* is due to certain characteristics of this vector, such as polyphagy, high reproductive rate (fecundity), establishment of large populations, and dispersal ability (i.e., short distances by flying and long distances via wind or in association with plants). These characteristics can directly influence the epidemiology of vector-borne diseases, mainly dispersal rates within and between tomato fields through changes in transmission dynamics.

External sources of inoculum are crucial in ToSRV epidemics. Epidemiological studies of tomato golden mosaic disease have shown that epidemics are predominantly governed by primary infections, through continuous influxes of viruliferous *B. tabaci* MEAM1 (Barbosa et al., 2016; Bergamin Filho et al., 2016; Macedo et al., 2019). However, the influence of the distance between external sources of ToSRV inoculum and tomato crops is not known. Vector flight behavior is certainly a factor determining this process. One of the ways to understand the dispersal dynamics of adults of *B. tabaci* MEAM1 at short distances is through the monitoring of specimens marked with powdered dyes (Narisu et al., 1999; Hagler and Jackson, 2001) or with proteins detectable in serological tests (Lavandero et al., 2004; Hagler and Machtley, 2016).

Studies of the dispersal of *B. tabaci* are essential to provide the epidemiological knowledge necessary to develop good management practices. This study aimed to determine the vector dispersal ability and its influence on the transmission distance of ToSRV by viruliferous adults of *B. tabaci* MEAM1 in the field, by releasing whiteflies in the center of an arena with tomato plants placed at increasing distances from a release point. The effectiveness of powdered dyes, chicken egg-white albumin, and lecithin to mark released adults of *B. tabaci* MEAM1 was also evaluated.

Conclusion

The movement of *B. tabaci* MEAM1 and transmission of ToSRV to tomato plants occurred up to 30 m from the release site. The locations with the highest concentration of recaptured insects overlap with the locations with the highest number of ToSRV-infected tomato plants. The results of the present study may partially explain observations of the incidence of ToSRV-infected tomato plants in different commercial fields in the Sumaré region, state of São Paulo, Brazil, during the last four years. In the same crop season, tomato fields located at distances from approximately 500 m from one another have shown different ToSRV incidences (1.6 to 28%) (unpublished data), suggesting that the infection appears to be governed by sources of inoculum and *B. tabaci* MEAM1 close to each field. These observations agree with epidemiological studies indicating that the spread of ToSRV in tomato crops in Brazil occurs predominantly through primary infections from a continuous influx of viruliferous *B. tabaci* MEAM1 from outside the field (Barbosa et al., 2016; Macedo et al., 2017c; Macedo et al., 2019). This indicates that close external sources of inoculum are essential for the occurrence of ToSRV epidemics in tomato commercial fields. Further studies using marked adults of *B. tabaci* MEAM1 on plants on the periphery of tomato fields may help to understand the association of primary infections with viruliferous insects coming from nearby sources of inoculum.

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3. SECONDARY DISSEMINATION OF TOMATO SEVERE RUGOSE VIRUS (TOSRV) AND TOMATO CHLOROSIS VIRUS (TOCV) IN TOMATO FIELDS UNDER THE EFFECT OF INSECTICIDES

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Abstract
Among several diseases, in Brazil, tomato (*Solanum lycopersicum*) crops can be affected by those caused by the begomovirus tomato severe rugose virus (ToSRV) and the crinivirus tomato chlorosis virus (ToCV), transmitted by *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1). The control of both diseases is mainly based on resistant hybrids (only for ToSRV) and frequent sprays of insecticides for vector control. Despite efficient vector control, infections with both viruses are still a reality. Primary transmission by migrant viruliferous whiteflies has been reported as the primary mechanism of introducing and spreading ToSRV and ToCV in tomato fields. However, as inoculum sources strong enough to sustain high incidences by primary dissemination alone are unlikely, secondary dissemination was hypothesized to occur, even with efficient vector control. This study aimed to evaluate the incidence of ToSRV and ToCV in experimental tomato fields sprayed alternately thrice a week with cyantraniliprole, acetamiprid, and flupyradifurone. Virus incidence in tomato plants was compared in three experimental areas: denominated sprayed, no-sprayed, and control. Tomatoes in the no-sprayed area were 2,600 m away from the tomatoes in the sprayed area and 3,350 m from the control area, while the plants in the no-sprayed area were 600 m away from the control area. The sprayed and no-sprayed areas consisted of 200 tomato plants, of which 180 were healthy, and 20 were co-infected with ToSRV and ToCV, randomly interspersed. Five hundred aviruliferous *B. tabaci* MEAM1 adults were released weekly in sprayed and no-sprayed tomato areas. In the control area, 200 healthy tomatoes served to monitor the natural incidence of the viruses (negative control). The experiments were carried out twice (2021 and 2022). Symptoms were evaluated weekly, and PCR and RT-PCR (for ToSRV and ToCV, respectively) tests were performed 70 days after transplanting to detect virus infection. Despite the relevant reduction, chemical control could not wholly prevent the secondary spread of the two viruses.

Keywords: Begomovirus, Crinivirus, Whitefly, Chemical control.

3.1 Introduction

Tomato (*Solanum lycopersicum*) is one of the plants most affected by diseases in different growing regions around the world. Among the various diseases, those caused by the begomovirus tomato severe rugose virus (ToSRV), which has been reported only in Brazil, and the crinivirus tomato chlorosis virus (ToCV) are predominant in the major tomato producing regions (Inoue-Nagata et al., 2016a).

ToSRV belongs to the Geminiviridae family, Begomovirus genus, and can be transmitted by cryptic species of *B. tabaci* in a persistent circulative manner. For *B. tabaci* MEAM1, the minimum virus acquisition and inoculation access periods are one and five minutes, respectively, with a latent period of approximately 12-15 h and a retention time of 25 days (Toloy et al., 2018). After tomato inoculation, the mean latent and incubation periods of ToSRV are approximately seven and 18 days, respectively (Favara et al., 2019). Infected tomato plants exhibit vein clearing, chlorosis, mosaic, and foliar wrinkle symptoms that affect plant development (Inoue-Nagata et al. 2016a, b). A reduction of 21.5% to 31.7% in the dry weight of the aerial part of tomato plants infected with ToSRV, compared to healthy plants has been reported (Bampi et al., 2019).

ToCV is a member of the Crinivirus genus, Closteroviridae family, and can be transmitted in a semipersistent manner by cryptic whitefly species of *B. tabaci* and by Trialeurodes abutiloneus and T. vaporariorum (Navas-Castillo et al., 2000; Wintermantel and Wisler, 2006). The minimum acquisition and inoculation access periods are five minutes for the relationships of *B. tabaci* MEAM1 with the Brazilian ToCV isolate (Freitas, 2012). The mean latent and incubation periods of ToCV in tomato plants are 13 and 30 days, respectively (Favara et al., 2019). Infected tomato plants exhibit mostly interveinal chlorosis in older leaves, which may also show bronzing or reddish spots and upward curved edges, eventually becoming thick and brittle. Symptoms begin at the base and progress toward apex of the plant, but rarely appear on younger leaves (Wintermantel and Wisler, 2006; Wintermantel et al., 2008).

It is relatively common to have mixed ToSRV and ToCV infections in tomato fields. In a survey conducted in tomato fields in Brasília and Goiás state, out of 150 plants analyzed, 48% were found to be infected with both viruses, 32% were infected only with ToCV, and 20% were infected only with ToSRV (Macedo et al., 2014). The mixed infections seem to have no interference in the development of the diseases caused by both viruses. Bampi et al. (2019) found no significant effects when tomato plants were co-infected with ToSRV and ToCV on virus titers and plant development compared to single infections.

Epidemiological studies have shown that the spatial and temporal dynamics of ToSRV and ToCV in tomato crops are similar. In all studies (Barbosa et al., 2016; Macedo et al., 2017; Macedo et al., 2019), the results show that epidemics are primarily governed by primary infections through continuous influxes of viruliferous whiteflies. However, the role of secondary field spread of both viruses needs to be better characterized. This work aimed to evaluate the secondary spread of ToCV and ToSRV in experimental tomato fields sprayed with insecticides thrice a week.

Conclusion

The present work showed that after two months of experimental evaluation, the mean incidence rates were 6% and 7.5% for ToSRV and ToCV, respectively. Even in low incidences, the infection rates of ToSRV and ToCV in experimental tomato fields sprayed with insecticides three times a week can represent a significant concern for producers due to yield loss and the environment. It is essential to note that those rates of virus spread reduction were at the cost of a high frequency and volume of insecticides. The wide use of chemicals leads to a scenario of loss of insecticide molecules' effectiveness and selection of resistant individuals (Hawkins et al., 2019). In a short- to medium-term period, the secondary infection may become the significant mechanism of spread even in the presence of larger amounts of insecticides, making the adverse effects of both diseases even worse.

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4. ZEIN-BASED NANOPARTICLE AS A NANOCARRIER OF THE INSECTICIDE CYANTRANILIPROLE TO CONTROL *Bemisia tabaci* MEAM1

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Abstract

Tomato is considered one of the most important crops in Brazil. Several diseases affect this vital crop. Among them, the diseases caused by viruses are concerning, especially those transmitted by *Bemisia tabaci* MEAM1. The primary method of managing these diseases is the chemical control of the vector through insecticides. Although this approach is highly efficient in killing insects, insecticides have some limitations in acting, allowing variable rates of virus transmission, that is, the insecticide action, in some cases, is not fast enough to prevent the introduction and spread of viruses into tomato crops. The demand for environmentally friendly control measures is growing. For that, new approaches to reduce the volume and frequency of insecticide application to tomato crops while maintaining efficiency are paramount. This study aims to evaluate the performance of cyantraniliprole insecticide (CNAP) by developing a zein-based nanoinsecticide (ZeinCNAP). The nanoformulation was synthesized using the nanoprecipitation method. Particle size, polydispersion, and zeta potential were obtained by dynamic light scattering over time. These measurements remained stable for 56 days. Transmission electron microscopy (TEM) was used to observe the morphology of the nanoparticles. The developed nanoinsecticide based on the zein platform and the active ingredient CNAP has promising effects on *B. tabaci* MEAM1mortality and can be considered safe for tomato plants, at least in a dose like that of the commercial formulation. To our knowledge, this study is the first to evaluate the effects of zein nanoencapsulated cyantraniliprole on the mortality of *B. tabaci* MEAM1.

Keywords: Chemical control, Nanoinsecticide, Whitefly, Protection, Tomato.

4.1 Introduction

The sucker polyphagous insect *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), also known as whitefly, remains one of the most economically important pests in crops around the world (Sammaritano et al., 2016; Perring et al., 2018). The insect can colonize a broad range of economically important plants, and when adult, the effects on plants include the reduction of the rate of photosynthesis due to sooty mold (Byrne and Bellows Jr., 1991) and the transmission of a large number of plant pathogenic viruses belonging to the genera begomovirus, carlavirus, crinivirus, ipomovirus, and torradovirus (Flock, 1981; Iwaki et al., 1982; Wisler et al., 1998; Maruthi et al., 2005; Amari et al., 2008).

Chemical insecticides are the most used method to control *B. tabaci* infestation in crops. Among the popular insecticides, active ingredient (a.i.) Cyazypyr, also called cyantraniliprole (CNAP), is a broad-spectrum anthranilic diamide insecticide and plays an important role in the control of this insect (Stansly et al., 2010; Barry et al., 2015). Insecticides are important to conventional agriculture; however, these chemicals can harm humans and other nontargeted organisms when misused (Köhler and Triebskorn, 2013). For example, the excessive use of these chemicals has shown side effects, such as the development of pest resistance (Sani et al., 2020).

Due to difficulties in creating new agricultural chemicals, research on the development of novel formulations is being conducted more intensively throughout the world (Saini et al., 2014). New methods to obtain better formulations are essential, focusing mainly on more safe and efficient pesticides, avoiding their adverse effects (Oliveira et al., 2014). In this context, nanoparticles can provide benefits for pesticide application. Nanotechnology offers great potential to revolutionize agriculture and can be used as an innovative tool for delivering agrochemicals efficiently (Ghormade et al., 2011, González et al., 2014). One of the strategies is based on entrapment of the a.i., which makes nanoformulations capable of reducing the concentration of applied pesticides due to a more precise targeted delivery (Ghormade et al., 2011).

Among the nanocarriers, zein is considered a promising platform. Zein is a protein derived from corn, considered a biodegradable compatible matrix, with great potential as a platform for the delivery of agrochemicals to plants (Salinas et al., 2021) and has been studied in combination with herbicides, fungicides, and insecticides. (Carvalho et al., 2023; Shi et al., 2023; Bonser et al., 2022). However, to our knowledge, no literature on insecticides combined with zein focuses on whitefly control. This work aimed to evaluate the effect of zein nanoparticles combined with the insecticide cyantraniliprole to control *Bemisia tabaci* MEAM1 and its possible health effects on tomato plants.

Conclusion

The newly developed nanoinsecticide based on the zein platform and the a.i. CNAP can be considered safe for tomato plants, at least in a dose like that of a commercial formulation.

The insecticide effect of ZeinCNAP on whitefly mortality was apparent and can be compared with commercial CNAP even at ten times less dose. CNAP movement to the systemic parts of the plant was not detected. The absorption of the CNAP was reduced when nanoformulated. However, the efficacy of CNAP increased. This study seems to be the first to evaluate the effects of zein nanoencapsulated CNAP on the mortality of *B. tabaci* MEAM1.

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