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Interactions between insect vectors – host plants – phytopathogenic microorganisms – natural enemies in Hemiptera Aleyrodidae, vectors of phytopathogenic viruses, to strengthen eco-compatible control of important diseases in horticultural crops

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Index

1 Introduction 1

1.1 Organisms – environment interactions: an ecological framework 1

1.1.1 Dynamics of aquatic ecosystems 4

1.1.2 The terrestrial ecosystems and their mechanisms 6

1.2 Multitrophic interactions in agroecosystems 8

1.2.1 Aboveground interactions...................................9

1.2.2 Relations between soil organisms and microorganisms 11

1.2.3 Aboveground – Belowground links..................13

1.3 Whiteflies: problematic pests and vectors of viruses in agriculture 15

1.3.1 Whiteflies as protagonists in multitrophic interactions with plants, pathogens and natural enemies..........17

1.4 State of knowledge and need for this research 26

1.4.1 Knowledge gap..26

1.4.2 Research questions 27

2 Geographic distribution of *Bemisia tabaci* species in Sicily and patterns in facultative endosymbiont community composition 29

2.1 Abstract...30

2.2 Keywords...31

2.3 Introduction 31

2.4 Materials and methods...34

2.4.1 Sample collection 34

2.4.2 Molecular identification of *B. tabaci* species and its endosymbionts 35

2.5 Results 37

2.6 Discussion..41

2.7 Acknowledgements 45

3 *Bemisia tabaci* (Hemiptera: Aleyrodidae): what relationships with and morpho-physiological effects on the plants it develops on?..46

3.1 Simple summary 47

3.2 Abstract...47

3.3 Keywords...48

3.4 Introduction 48

3.5 Materials and methods..49
  3.5.1 Data analysis...52

3.6 Results 52

3.7 Discussion..57

3.8 Conclusions 59

3.9 Declarations...60
  3.9.1 Author contributions...60
  3.9.2 Funding...60
  3.9.3 Institutional review board statement...........................60
  3.9.4 Informed consent statement..60
  3.9.5 Data availability statement 60
  3.9.6 Conflicts of interest 60

4 Can *Macrolophus pygmaeus* (Hemiptera: Miridae) mitigate the damage caused to plants by *Bemisia tabaci* (Hemiptera: Aleyrodidae)?..61

4.1 Simple summary 62

4.2 Abstract...62

4.3 Keywords..63

4.4 Introduction 63

4.5 Materials and methods..67
  4.5.1 Insects and plants...67
  4.5.2 Experimental design and sampling...................67
  4.5.3 Data analysis...70

4.6 Results 70

4.7 Discussion...76

4.8 Declarations..79
  4.8.1 Author contributions...79
  4.8.2 Funding...79
  4.8.3 Institutional review board statement.................79
  4.8.4 Informed consent statement..............................79
  4.8.5 Data availability statement 80
  4.8.6 Conflicts of interest 80

5 Tomato leaf curl New Delhi virus Spain strain is not transmitted by *Trialeurodes vaporariorum* and is inefficiently transmitted by *Bemisia tabaci* Mediterranean between zucchini and the wild cucurbit *Ecballium elaterium*..................................81

5.1 Abstract...82

5.2 Keywords..82

5.3 Introduction 82

5.4 Materials and methods..85
  5.4.1 Virus isolate and plant agroinoculation 85
  5.4.2 Virus detection..85
  5.4.3 Whitefly transmission.......................................86

5.5 Results and discussion..87
  5.5.1 ToLCNDV-ES is not transmitted by *T. vaporariorum* between zucchini plants 87

5.5.2 ToLCNDV-ES is not efficiently transmitted by *B. tabaci* MED between zucchini and *E. elaterium*..................88

5.6 Acknowledgements 90

6 Conclusion...92

Research highlights

Research questions answered by the study

- *Bemisia tabaci* MED presence is increasing in both greenhouse and field crops as well as on wild weed species in all areas of Sicily where intensive horticultural production is performed.
- *B. tabaci* MED populations are prevalent in the southeastern part of Sicily, while *B. tabaci* MEAM1 populations are mainly concentrated in the western areas of the island.
- The present study represents the first report of whitefly endosymbionts sequences from Sicily and Italy.
- *B. tabaci* MED harms the morphology and physiology of both eggplant and tomato plants, significantly impacting height, indirect chlorophyll content, leaf area and shoot dry weight of plants.
- *Macrolophus pygmaeus* presence overall reduces the negative effects of *B. tabaci* MED on the morphology and physiology of the host plants.
- *Trialeurodes vaporariorum* cannot be considered a threat as a virus vector on cucurbit crops since the whitefly cannot transmit Tomato Leaf Curl New Delhi Virus (ToLCNDV) between zucchini plants.
- *B. tabaci* MED is not an efficient vector of ToLCNDV between zucchini plants and wild *Ecballium elaterium* plants. Therefore, *E. elaterium* does not represent a risk to neighbouring cultivated zucchini plants as it does not act as a reservoir for the begomovirus.

New research questions stimulated by the study

- The findings of this research highlight the need to deeply understand the nature of the symbiosis between whiteflies and their facultative endosymbiont community.
- Based on the results obtained in this study, further investigations are needed to study deeply how phytophagy by *M. pygmaeus* can, directly and indirectly, affect host plants and whether *B. tabaci* can suppress or resist plant defences stimulated by the zoophytophagous predator.

- Results achieved in the experiments here reported open new needs to fully understand the transmission mechanisms performed by *B. tabaci* MED, to know why the whitefly fails to efficiently transmit ToLCNDV from wild *E. elaterium* to cultivated zucchini plants and vice versa.

Abstract

In the agricultural context, where complex relations occur between organisms belonging to various trophic levels and the environment, all components involved cannot be considered individually as they considerably depend on each other. Plants are the central element of many food webs, that develop both above and below the ground, and of several multitrophic interactions involving symbionts, pollinators, pathogens, pests and their natural enemies. Among pests, which represent one of the most critical trophic levels in agroecosystems, whiteflies (Hemiptera: Aleyrodidae) are considered one of the main obstacles to agricultural production on a global scale. They attack many crops in almost every cultivated region of the world, and very often are responsible for the transmission of devastating virus diseases. Because of the various damages these pests cause to plants, many studies have focused on reducing losses deriving from whiteflies' infestations to agricultural yield over the years. Nevertheless, in the insect vector – host plant – phytopathogenic microorganism – natural enemy systems, it is little known how the organisms involved affect each other, and thus how whiteflies interact with different trophic levels of their food webs. This being considered, the present work has been carried out to understand the nature of the abovementioned multitrophic interactions and, consequently, to provide helpful information for the sustainable management of these pests and their related viruses in agriculture. In particular, and as a starting base of the whole study, the research focused on the species composition and endosymbiont community of the *Bemisia tabaci* (Gennadius) complex in Sicily. In addition, the relations that link some solanaceous and cucurbits, *B. tabaci* MED and *Trialeurodes vaporariorum* (Westwood), *Macrolophus pygmaeus* (Rambur), and Tomato Leaf Curl New Delhi Virus (ToLCNDV) have been investigated.

Keywords: multitrophic interactions, whiteflies, taxonomy, endosymbionts, vegetable host plants, plant morphology and physiology, mirid predator, zoophytophagy, biological control, transmission, begomovirus, wild plants.

Riassunto

Nel contesto agricolo, dove si verificano complesse relazioni tra gli organismi e il loro ambiente circostante, tutti i componenti coinvolti non possono essere considerati singolarmente poiché notevolmente dipendenti gli uni dagli altri. Le piante costituiscono l'elemento centrale di molte reti alimentari, che si sviluppano sia sopra che sotto il suolo, e di numerose interazioni multitrofiche che coinvolgono simbionti, impollinatori, microrganismi patogeni, parassiti e i loro nemici naturali. Tra i fitofagi, che rappresentano uno dei livelli trofici più importanti negli agroecosistemi, gli Aleirodidi o “mosche bianche” (Hemiptera: Aleyrodidae) sono considerati uno dei principali ostacoli alla produzione agricola su scala globale. Essi attaccano molte colture di quasi tutte le regioni coltivate del mondo e molto spesso sono responsabili della trasmissione di devastanti malattie fitosanitarie. A causa dei vari danni che tali fitofagi causano alle piante, nel corso degli anni molti studi si sono concentrati sulla possibilità di riduzione delle perdite da loro determinate alle produzioni agrarie. Ciononostante, è ancora poco noto come gli organismi coinvolti nei sistemi insetto vettore – pianta ospite – microrganismo fitopatogeno – nemico naturale si influenzano reciprocamente, e quindi come gli Aleirodidi interagiscono con i diversi livelli trofici delle loro reti alimentari. A tal proposito, il presente lavoro è stato realizzato per comprendere la natura delle suddette interazioni multitrofiche e, di conseguenza, per fornire informazioni utili per la gestione sostenibile di questi parassiti e dei virus ad essi correlati in agricoltura. In primo luogo, e come base conoscitiva di premessa al restante lavoro di tesi, le ricerche si sono focalizzate sulla composizione specifica e sulla comunità di endosimbionti del complesso *Bemisia tabaci* (Gennadius) presente in Sicilia. Inoltre, sono state analizzate le relazioni che legano alcune solanacee e cucurbitacee, *B. tabaci* MED e *Trialeurodes vaporariorum* (Westwood), *Macrolophus pygmaeus* (Rambur), e Tomato Leaf Curl New Delhi Virus (ToLCNDV).

Parole chiave: interazioni multitrofiche, mosche bianche, tassonomia, endosimbionti, piante orticole, morfologia e fisiologia delle piante, predatore miride, zoofitofagia, controllo biologico, trasmissione, begomovirus, piante spontanee.

1 Introduction

1.1 *Organisms – environment interactions: an ecological framework*

In recent years, the interest in the environment and the mechanisms of production of resources useful for humanity is growing due to the increasing human population, the consumption of renewable and non-renewable sources, and the significant, rapid changes occurring in the world (Díaz et al., 2006). Studies on the environment are crucial to managing our planet’s resources through investigating interactions connecting biotic systems, of which humans are an integral part, with the physical systems on which they depend (Chapin et al., 2011). The structures that are created, called ecosystems, are real arranged entities with a set of general, functional and structural attributes, that make them recognizable, analysable, and predictable (Kormondy, 2012). Ecosystems consist of all coexisting organisms and the abiotic components with which they interact (Dunson and Travis, 1991; Muoghalu, 2009), and their analysis helps to understand the factors that regulate the quantities and flows of matter and energy (Chapin et al., 2011).

Many fluxes within ecosystems, such as the absorption of minerals by plants, decomposition of dead organic materials by soil microorganisms, the consumption of plants by herbivores, and the feeding of herbivores by predators, involve biotic components. The latter are sensitive to environmental and biological factors regulating the population dynamics and species relations in communities (Morin, 2009; Delić and Fišer, 2019). According to Hagen (1992), to understand the numerous processes occurring in an ecosystem it is necessary to consider the transfer of materials among plant, animal and microbe species, so the feeding relationships among organisms, defined as trophic interactions, become essential to be investigated. In this respect, Elton (2001) defined each living cellular species as a connection in a food chain able to explain the movement of matter, starting from plants that take energy and transfer it to animals, and to end with decomposers (DeAngelis et al., 1989; Morin, 2009; Price et al., 2011). A food chain is a group of organisms connected by consumption and hierarchically organized into trophic levels (Morin, 2009; Price et al., 2011). Trophic levels can be defined as groups of the communities with similar feeding habits (Morin, 2009); in fact, in a plant-based trophic system, for example, plants are at the first trophic level, herbivores represent the second, primary carnivores consist of the third, secondary carnivores that eat mainly primary carnivores are at the fourth, etc. (Lindeman, 1942; Odum, 1959). Although food chains are an easy way to conceptualize the trophic dynamics of an ecosystem, they are simplifications for the many species that eat more than one type of food (Price et al., 2011).

In actual situations, the real energy and matter movements that occur in all ecosystems are complex food webs (Kormondy, 1969; Gange and Brown, 2009). Many organisms feed on prey from more than one trophic level (Polis, 1991), originating the so-called “multitrophic interactions” (Kormondy, 1969; Tscharntke and Hawkins, 2002). To follow these transfers across the complex multitrophic interactions it is essential to know the impact of each link in the chain on the nutrition of each organism in the ecosystem, even if it is challenging to assign most species to a single trophic level (Morin, 2009). This phenomenon is determined mainly by the presence of omnivores that, feeding on different resources, create links with a large variety of organisms belonging to numerous trophic levels and complicate the regulation of energy and nutrient flow among ecosystems (Polis and Strong, 1996).

In this regard, various trophic systems exhibit some combination of bottom-up and top-down controls (Polis, 1999; Allison, 2006). It is known that the availability of food at the base of the food chain can limit the production of upper trophic levels across bottom-up controls (Allison, 2006). Contrarily, predators controlling their prey's abundance exert top-down control on food webs (Allison, 2006). So, the ecological characteristics of organisms are the results of their evolution and the competitive interactions that separate species into communities where they successfully grow, survive, and reproduce (Vrba and Gould, 1986). Ecosystem dynamics also depend on the processes that govern plant, animal, and microbial links and abundances, which establish which species are present and the food sources they need (Chesson, 2013). Multitrophic interactions between three or more species can be uni- or bidirectional with positive, negative, or neutral links (Polis and Strong, 1996; Delić and Fišer, 2019). These complex links also refer to competition, predation, herbivory, mutualism, commensalism, and parasitism (Morin, 2009; Delić and Fišer, 2019). Interactions with negative demographic trends are predation, parasitism, herbivory, and competition (Delić and Fišer, 2019). They occur when a predator, parasite, or a more robust competitor species causes population decline of the prey, host, or a weaker competitor, respectively (Delić and Fišer, 2019). Contrarily, interactions that result in positive demographic trends are unidirectional commensalism, where only one species has a positive effect from the other, and bidirectional mutualism, where the relationship between a pair of species shows benefits obtained from each other (Morin, 2009). Of course, in this context it is important to understand also how surrounding abiotic characteristics can affect the communities of organisms and alter the available space for the various niches (Harpole et al., 2016). For example, environmental changes consisting in increasing atmospheric CO_2 levels, by influencing the population's abundance, community composition, and physiology of organisms (Sala et al., 2000), cause alterations to the biotic links (e.g. predation, parasitism, and pollination) that are the base of ecosystem services on which human wellbeing is dependent (Dobson et al., 2006). Another type of environmental change derives from the addition of nutrients which, facilitating the growth of certain producers, induces their domination on others, which then tend to disappear (Hautier et al., 2009). Differently, niche differences in an ecosystem involve processes that permit the coexistence of different species, and cause an increasing amount of biomass produced, which can be transferred to various trophic levels (Eisenhauer et al., 2019). So, niche differences cause positive effects in food webs characterized by richness on ecosystem species and functions and provision of ecosystem services (Hodapp et al., 2016).

1.1.1 Dynamics of aquatic ecosystems

In aquatic ecosystems, multitrophic interactions derive from nutrients cycles and feeding links within a community of organisms and develop from very simple food chains to highly complex food webs (Jacobsen and Dangles, 2017; Kwak and Park, 2020). Both matter cycle processes and food webs are important to explain the complex structure of an aquatic ecosystem. In particular, the formers regard the quantity and quality of sources of primary production in these systems (Jacobsen and Dangles, 2017), while the latter refer to food consumption and multitrophic interactions among consumers (Jacobsen and Dangles, 2017). However, it is known that the most important biological interaction in the aquatic food web derive from the joint involvement of both mechanisms in the food chain-mediated movement of nutrients process (Carrillo et al., 2006). In particular, the sources of primary production constitute a network from which the energy and matter movement starts, going from the preys up to the predator species and organisms of higher trophic levels (Kwak and Park, 2020). Moreover, these sources are essential elements that can transfer nutrients within different organism groups, also arranged in intermediate positions between the trophic levels of the food chain (Kwak and Park, 2020), and connect different food webs in an unique structure (Jacobsen and Dangles, 2017).

Resources can be autochthonous/internal, such as algae, cyanobacteria, mosses, and aquatic vascular plants, and allochthonous/external, like organic matter that derive from living or decomposed terrestrial plants (Thorp and Rogers, 2010). They can be influenced by environment, so it is crucial to consider the impact of human disturbance on surrounding elements of food web structures and functions. For example, human activities influence the cycling of nutrients, changing the niche differences, the number of species in freshwater ecosystems and their relative abundance (Statistics Canada, 2013; Jacobsen and Dangles, 2017). Also, the temperature and hydrochemical characteristics of waters could influence the growth and development of organism communities (Yin et al., 2021). In fact, from the experiment performed by Yermolaeva et al. (2021) on zooplankton, it was found that the richness and abundance of species are closely related to the pH of water and concentration of mineral compounds (e. g. phosphates and nitrates), which can negatively or positively influence the abundance of zooplankton, respectively.

To understand multitrophic interactions in aquatic environments, another important aspect to consider is the sensory system, as this is an essential source of information for many organisms (Ferrari et al., 2010). Particularly, the predator-prey interactions, which can regulate the species composition in the ecosystem (Carrillo et al., 2006) rely on a complex of olfactory mechanisms which make them each rewarded when they gain an information advantage over the other (Ferrari et al., 2010). In this regard, early detection is the best way to get an advantage: predators can win when catch unaware prey, while preys can win when across specific mechanism, they are able to escape detection by predators (Ferrari et al., 2010). Even in aquatic ecosystems, multitrophic interactions regard a lot of trophic levels consisting of producers, herbivores (consumers of algae or plant tissue), detritivores (that eat dead organic matter), invertivores (predators on other invertebrates), and higher carnivore/predator levels (Kwak and Park, 2020). However, most aquatic organisms are defined generalists or omnivores (e.g., suspension feeders and planktivores) (Kwak and Park, 2020), so some categories in trophic levels overlap and it becomes difficult to distinguish each link between distinct species in a big food web.

1.1.2 The terrestrial ecosystems and their mechanisms

In terrestrial ecosystems, plants use solar radiations to reduce CO_2 to organic compounds, most of which can directly move into the soil, where they are decomposed by bacteria and fungi (Bot and Benites, 2005; Gleixner, 2013). If decomposition does not occur, significant deposits of dead organic matter would sequester the nutrients needed to support plant development (Chapin et al., 2011). In this context, animals (especially herbivores) transfer energy and materials and can regulate the quantity and activities of plants and soil microbes (Urabe et al., 2002). So, the essential biotic components of multitrophic interactions in a terrestrial ecosystem are plants, animals, and decomposers (Chapin et al., 2011), while the most important abiotic elements with which they interact are water and atmosphere, which provide carbon and nitrogen, and soil, which supplies support, storage, and other resources required by organisms (Edwards, 1984).

In terrestrial ecosystems, the distribution of resources across multitrophic interactions can be described as a pyramid of biomass, with more matter in plants and progressively less in the higher trophic links (Hatton et al., 2015). It is similar to the energy pyramid, in which the production at each trophic level depends on the production at the previous one and on the trophic efficiency with which the production of the prey is converted into that of the consumers (Chapin et al., 2011). An example, in which the progressive decrease of the nutritional resources available in the following trophic levels is marked, concerns the forests, where the dominant plants produce a large percentage of woody biomass inedible for many herbivores (Chapin et al., 2011). Conversely, in grasslands, producers generate fewer woody portions and more biomass available for herbivores, increasing the number of higher trophic levels (Chapin et al., 2011).

The influence of human activities on abiotic traits of terrestrial ecosystems are important for the distribution of nutrients and energy among trophic levels of a food chain (Statistics Canada, 2013; Schrum et al., 2016). For example, deforestation, land degradation, and other human disturbances decreased the natural forest cover over thousands of years (Kaplan et al., 2009; Gaillard et al., 2010; Simmons, 2019), with important negative consequences on species richness and interactions (Statistics Canada, 2013). The activity of all living organisms is also strongly influenced by temperature, capable of altering life cycles and behaviours (Peñuelas and Filella, 2001). In plants, the high temperatures can affect the photosynthesis and deficit of vapour pressure, while in microbes it can speed up the decomposition ability of organic matter (Valladares et al., 2005).

Moreover, especially in low-nutrient environments and by stimulating mechanisms of natural defence, plants can often limit the amount of available resources to herbivores (e.g. ruminants and insects), which need plant biomass with at least a 1% nitrogen concentration (Chapin et al., 2011). This can be based on ecological metabolism of both carbon, originating organic compounds containing no nitrogen, like resins, tannins and essential oils that deter feeding by most herbivores (Coley et al., 1985), and nitrogen, creating compounds, such as alkaloids, that are toxic to generalist herbivores (Chapin et al., 2011).

As it has been shown by recent studies, also predator-prey relationships can influence the structure of multitrophic interactions in terrestrial ecosystems, as upon detection of the predator cues prey can modify their behaviour to decrease predation risk (Catalán et al., 2021). Such changes can include moving away whenever possible and reduction of feeding activities (Weissburg et al., 2014; Buchanan et al., 2017; Gaynor et al., 2019; Hettyey et al., 2019), resulting in unsuitability of the prey (Peacor et al., 2020).

Like in the case of Breviglieri et al. (2017), who demonstrated that avian predators indirectly altered the structure of many invertebrate communities, various studies have shown that when a generalist predator exerts negative consequences on several prey, the abundance of various other species could increase (Hammill et al., 2015). Therefore, it is crucial to understand how specifically a predator can directly and indirectly affect the several species simultaneously, changing consequently the complex structure of multitrophic interactions.

1.2 Multitrophic interactions in agroecosystems

With the intensification of agriculture over the years, the natural ecosystems in the world have undergone a profound change (Matson et al., 1997), as the great diversity of fauna and flora in the original biological systems has been transformed into a restricted set of crops, pests, and weeds (Conway, 1986). Precisely for this reason it is no longer opportune to speak of ecosystems, but of agroecosystems. The agroecosystem is an ecosystem intentionally altered by human beings to acquire goods and services with various objectives (Gliessman, 1990; Dalgaard et al., 2003). Like the ecological systems they replace, agroecosystems are the locations where dynamic interactions occur between biological component and physical environment (Gliessman, 1990; Dalgaard et al., 2003), and they also include relationships between socio-economic and ecological processes (Conway, 1986). So, the basic ecological processes persist, such as competition, herbivory and predation among species and trophic levels, but they appear overlaid and controlled by the agricultural processes of cultivation, pest control, harvesting and marketing (Conway, 1986).

Farmers preserve the productive function of the agroecosystems by a constant investment on external inputs and by managing the available resources to replenish the materials extracted across harvest (Díez Sanjuán et al., 2019). Aboveground (AG) and belowground (BG), the flow of materials must keep a certain level of energy efficiency, because the rate of mining of resources cannot exceed the rate of their regeneration (Tello et al., 2016; Díez Sanjuán et al., 2019). Several deficits and surpluses of nutrients in the long term can involve both soil nutrient mining, with a reduction of cropland efficiency, and a loss of productivity, with pollution consequences, respectively (Díez Sanjuán et al., 2019).

As modified terrestrial ecosystems, agroecosystems are based on plants that are the central component of many food webs and complex multitrophic interactions. Plants fix in the soil across roots, which allow them to obtain resources like water and mineral nutrients needed by their aerial part for carbon assimilation. In this way they can develop complex food webs both above and below the ground involving symbionts, pollinators, pathogens, pests and their natural enemies (Ohgushi, 2005; Shikano, 2017; Jacobsen and Raguso, 2018).

1.2.1 Aboveground interactions

Plants and pests create the most dominant and intricate relationships in terrestrial biological systems (Strong et al., 1984). Behavioural decisions of herbivore insects to recognize and accept a plant species as host, for feeding and oviposition, are mainly based on contact-sensory information (Knolhoff and Heckel, 2014). On the other hand, plants have developed various physical and chemical defence mechanisms to resist pest infestation. The mechanisms can include morphological structures, like cuticle, trichomes and adhesive glands on the leaf surface, that inhibit the movement or reduce the colonization of aerial tissues of plants by pest; but also, secondary compounds, called allelochemicals, that may deter and modify the growth and development of the feeding herbivores, increasing their mortality (Schoonhoven et al., 2005).

Like pest develop on plants, many other arthropods have evolved to attack and consume herbivore insects (Gamborena, 2009). This type of organisms, named natural enemies of pests, are mainly represented by predators and parasitoids, and may contribute to the structure of communities, regulating pest infestations (Landis et al., 2000; Holland et al., 2016). They can be influenced by the host plants and their hosts or preys (Hare, 2002) in many ways: directly, across the level of predation, survival and reproduction of natural enemies, and indirectly, across the distribution, abundance, and vulnerability of the pests (Tscharntke and Hawkins, 2002). In particular, the nutritional composition of the plants can affect parasitoids, reducing or improving the quality of their insect herbivore hosts, and consequently influencing the growth and development of the emerging parasitoids (Poelman et al., 2014). An example is the study carried out by Weber et al. (2020) on the parasitoid species *Asecodes parviclava* (Thompson), different genotypes of the wild strawberry (*Fragaria vesca* L.) and the host of the parasitoid, the strawberry leaf beetle (*Galerucella tenella* L.), which showed that the performance of the parasitoid are strongly influenced by the plant genotype. Parasitoids are insects whose adults are free-living, whereas larvae develop in or on the body of other insects, that die due to parasitism (Godfray, 1994). So, unlike predators, which may feed on several preys, the resources for parasitoid survival are restricted and enclosed into a single host (Harvey et al., 2013). Catching pest victims by predators can also be directly affected by plants, due to the presence of physical (e.g. trichomes, acting as barriers) and visual and volatile cues (e.g. compounds produced by plants as a result of pest infestation) (Vet and Dicke, 1992; Turlings et al., 1998; Dicke, 1999; Riddick and Simmons, 2019).

Another source of change in plant traits (e.g resistance to herbivores) and pest – natural enemy relationships is represented by pathogens and in particular by the relationships between plants, fungi and insects. For instance, a research conducted by Faeth and Bultman (2002) showed that mycotoxins produced by grass endophytes not only negatively affect pests, but also consistently influence insect parasitoids.

In the past years, in order to better manage pests in agriculture, the focus was more placed on the resistance of host plants and biological control techniques. Therefore, interest has grown in understanding the trophic interactions between plants, pest and natural enemies and their related effects (Tscharntke and Hawkins, 2002). In this context, the concept of multitrophic interactions indicates that developed plant characteristics can improve the success of natural enemies as mortality agents of pests (Bottrell et al., 1998).

However, it is necessary to remember that food webs are very complex: there are predators, parasitoids (hyperparasitoids) and pathogens of natural enemies (Rosenheim, 1998) that can constitute another trophic level of an agroecosystem. If these organisms are present, can cause a significant negative effect in crops, due to their capacity to reduce the populations of predators and parasitoids of pests.

1.2.2 Relations between soil organisms and microorganisms

Belowground (BG) components of food webs highlight that soil ecosystems form the basis of almost all terrestrial life (Scheu and Setälä, 2002). Soil communities are complex due to the multitude of species occurring and the prevalence of generalist feeders and omnivores, which may exert strong regulation of other trophic levels in ways not predicted by cascading trophic interactions (Pace et al., 1999). For this reason, it is complicated to ascribe decomposer organisms to certain trophic levels and individuate the trophic cascades, although top-down control appears to be widespread (Scheu and Setälä, 2002). Some examples can be represented by fungivores, nematodes, and detritivores that under specific conditions are controlled by predators, and by the interactions between protozoa and nematodes and bacteria that regulate the acquisition of chemical nutrients by crops (Griffiths, 1994). However, the complex nature of soils has major implications for predator–prey interactions. Norton (1994), in fact, has demonstrated that one of the most diverse soil organism groups, the oribatid mites, may have developed such effective defence mechanisms that adults now live in a predator free space.

With the deepening of the roots in the soil, plants are involved in the trophic dynamics of the BG food webs of the agroecosystems. The resources provided by plants not only consist of above ground (AG) residues, as also BG products comprise a mix of resources (e.g. dead roots, cells lost by growing roots, and a variety of liquid compounds exuded by roots) (Trolldenier et al., 1987; Lynch, 1990) which involve many microbial and animal activities. Therefore, even if the rhizosphere is surely the site where decomposer organisms and plants interact most intensively, they also relate outside the rhizosphere across the plant resources produced above ground, the decomposition of plant litter and the nutrients mineralized belowground by decomposers.

In the rhizosphere, since mineral nutrients are also essential products for many bacteria and fungi, plants and decomposers may compete across consumption of these abiotic resources. A well-known example is related to carbon: if this element is abundant, microorganisms result stronger competitors for nutrients, causing plants to suffer from minerals deficiency (Harte and Kinzig, 1993; Schmidt et al., 1997).

Despite the existence of various negative interactions, the relationship between plants and decomposer organisms in the rhizosphere is usually beneficial, and the most common example is the plant–mycorrhiza mutualism, which mitigate the competition for nutrients (Ingham and Molina, 1991). Among the other trophic interactions, there are those with rhizobia, mutualistic bacteria of the rhizosphere, antagonistic microorganisms of root pathogens, and microbi-detritivores which indirectly modify plant growth by changes in soil structure and by hormone-like effects (Stephens and Davoren, 1997; Boyle et al., 1997; Muscolo et al., 1999).

In addition, other links between plants and soil organisms or microorganisms can regard plant seed dispersal by soil animals (Willems and Huijsmans, 1994), and the damage due to root herbivores and pathogens like nematodes (Strong et al., 1996; Zunke and Perry, 1997).

In conclusion, since primary production by plants is regulated by the availability of soil resources and the decomposer community also relies on this type of source, not only the direct top-down force regulated by organisms is relevant, but also the indirect interactions based on abiotic resources (nutrients) result crucial for understanding the functioning of these food webs.

1.2.3 Aboveground – Belowground links

Currently, there is an increasing interest to link interactions between plants, organisms in the soil and insect communities aboveground. Recent research indicated that soil organisms can be a key factor influencing interactions between plants and insects in the AG compartment (Heinen et al., 2018).

The relationships between BG and AG components are mediated by plants, involve many different species, feeding guilds and phyla and can be of antagonistic, synergistic, or neutral nature for the members of the various communities that are associated with a specific plant species (Bezemer et al., 2005; Poveda et al., 2005). These relationships involve many plant species and link AG herbivores and pathogens with BG herbivores and other soil organisms (e.g. nematodes, pathogens and mutualistic microorganisms).

Regarding the herbivores, BG ones facilitate feeding by AG ones, as it was observed by Moran and Whitham (1990) and Masters (1995) on aphids living on the same plant, contrary to AG herbivores which often reduce performance of those BG on the roots. Many times, feeding by BG herbivores have negative consequences on roots, which may trigger drought stress responses (Masters et al., 1993) The latter can be characterized by the increasing quantity of primary metabolites (e.g. sugars and amino acids) in the phloem of the aerial part of plants, where sap-sucking insects feed, improving their performance. On the contrary, it has been observed that the trophic activity of AG herbivores can reduce the photosynthetic potential of the plant and consequently the distribution of resources to the roots and their accessibility for BG herbivores. Also, in studies conducted by Soler et al. (2009) and Erb et al. (2009), AG-BG interactions were analysed. In the first, where roots were infested by larvae of the cabbage root fly *Delia radicum* (L.), the shoot and leaves of *Brassica nigra* (L.) crops showed a higher concentration of glucosinolates, which are chemical defence compounds normally present in the *Brassicaceae* family, and a low level of infestation by AG herbivores. In the second, the presence of *Diabrotica virgifera* (LeConte) larvae on roots of maize induced resistance to caterpillars in the aboveground portion of plants, increasing the rate of glycosides.

Regarding the other higher trophic levels, it was found that the herbivores can indirectly affect other organisms, such as pollinators and natural enemies of shoot and root in the food webs (Masters et al., 2001; Johnson et al., 2011). The effects regard the modifications referred also to the amount and nature of volatile organic compounds (VOCs) of host plants stimulated by herbivores. An example could be that of some parasitoids that use volatiles as signals to identify plants with mycorrhizal roots or without BG herbivores (Rasmann and

Turlings, 2007; Soler et al., 2007). It was also noted that parasitoids in search of AG hosts avoid *B. nigra* plants infested with large root fly larvae (Soler et al., 2007).

As for the rhizosphere, it was noted that AG herbivores can affect mutualistic BG microorganisms, while the colonization of plant roots with beneficial soil bacteria [e.g. plant-growth promoting rhizobacteria (PGPR)] can stimulate the induced systemic resistance (ISR) to AG pathogens (Zehnder et al., 1999; Pieterse et al., 2001). However, although several mycorrhized host plant species show better development rates, these microorganisms can also improve AG herbivore performance (Kempel et al., 2010), inducing an increased susceptibility of plants.

1.3 Whiteflies: problematic pests and vectors of viruses in agriculture

Nowadays, one of the most pressing global environmental troubles are invasive alien organisms, as they often displace indigenous species, thus altering ecological communities and negatively affecting biodiversity, agricultural pest management, and human wellbeing (Reitz, 2007). Agroecosystems, with their low diversity and expected continuous high food production, are particularly sensitive to biological invasions (Paini et al., 2016).

In this context, whiteflies are considered important pests on a global scale, as they attack a wide range of agricultural crops in almost all cultivated areas of the world (Mound and Halsey, 1978; Gerling, 1990).

All whiteflies have a mostly temperature-regulated life cycle ranging from 2-3 weeks to several months (Gerling, 1990). It includes six life stages characterized by: egg, four nymphal stages and winged adult stage (Walker et al., 2010). The former hatches into crawlers, the only immature mobile stage, which once fixed, transforms into sessile second stages with dysfunctional legs (Inbar and Gerling, 2008). After two more moults, adults develop within the cuticle of the fourth stage, sometimes referred to as the pupal stage (Kedar et al., 2014), emerging winged (~ 1–2 mm) and living up to several weeks (Byrne and Bellows, 1991).

Whiteflies are phloem feeders so, using the mouthparts suitable for sucking and an adapted digestive system, they extract sufficient protein-building amino acids from the sap to assist body development, while producing a large amount of excrement rich in sugars (Martin et al., 2000). The excreta, called honeydew, may help the growth of sooty mould on affected plants; thus, when they are large, whitefly infestations cause a strong loss of sap and consequently a large production of sooty mould, which interferes with photosynthesis. Furthermore, ants are attracted to the honeydew, influencing with their presence the activity of natural enemies of the pests (Martin et al., 2000). However, one of the biggest problems caused by whiteflies is the ability of some species to transmit viral plant diseases, where these viruses can cause a variety of symptoms in crops (Bedford et al., 1994) and often even their death (Inbar and Gerling, 2008).

Over time, whiteflies have received a lot of attention due to several polyphagous species, including the sweet potato whitefly, *Bemisia tabaci* (Gennadius), and the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Gerling, 1990; Oliveira et al., 2001).

Bemisia tabaci is a global pest that causes significant losses to a wide variety of agricultural products. This whitefly was first described by Gennadius (1889) after being collected in Greece on tobacco plants. After several decades of research on the naming of *B. tabaci*, nowadays it is increasingly evident that this entity is a complex of morphologically indistinguishable species (Dinsdale et al., 2010; Tay et al., 2012). In this regard, several researchers used a variety of naming conventions including biotype, genetic groups, and species to identify whitefly variants overall. Among the latter, the main ones are the whitefly of the Middle East Asia Minor 1 (MEAM1), formerly known as biotype B or *Bemisia argentifolii* (Bellows and Perring), and the Mediterranean whitefly (MED), formerly known as biotype Q and now known to be the original *B. tabaci* (Dinsdale et al., 2010; Boykin, 2014; Cuthbertson and Vänninen, 2015).

Trialeurodes vaporariorum, known as the greenhouse whitefly, is the most economically important species of its genus (Russell, 1948). It was first described by Westwood in 1856, after being collected on tomatoes in greenhouses throughout Europe (Quaintance and Banks, 1900; Cockerell, 1902), although according to Cockerell (1902) this whitefly is supposed to have originated in Brazil. It is now distributed worldwide too (CABI, 2022), due to the expansion of its host plants range.

1.3.1 Whiteflies as protagonists in multitrophic interactions with plants, pathogens and natural enemies

1.3.1.1 Whiteflies on host plants

Whiteflies can affect the biochemistry, physiology, anatomy, and development of infested plants. Although compared to other feeding guilds, sap-sucking insects cause minimal mechanical damage to plants, while they influence various plant traits. *Bemisia tabaci*, for example, can alter stomata-regulated gas exchange, transpiration and photosynthesis rates, as well as the chlorophyll content, as it has been discovered in tomato leaves by Buntin et al. (1993), but also in cotton, to which it is able to cause the accumulation of soluble sugars in the infested leaves (Lin et al., 2000).

The presence of whiteflies can regulate the expression of plant defense genes, resulting in an altered profile of secondary metabolites as discovered by Kempema et al. (2007) through studies conducted on *Arabidopsis thaliana* (L.) infested with whitefly nymphs. Similar results were also found by Quintana-Camargo et al. (2015) in tomato plants infested with *T. vaporariorum*. Furthermore, whitefly infestation has been reported to activate gene expression in salicylic acid (SA) signaling pathways and repressed gene expression in jasmonic acid (JA) signaling pathways (Zarate et al., 2007).

Another important aspect, noted by Xu et al. (2011) is that the invasive species MEAM1 and MED of the *B. tabaci* species complex in China can adapt to more numerous hosts of different plant families than the indigenous whitefly competitors (Xu et al., 2011), due to their activity in detoxification. Peng et al. (2013) also demonstrated that *B. tabaci* MEAM1 has a greater ability to detoxify high concentrations of phenolics than *T. vaporariorum*, which contributes to a significant advantage for MEAM1 in maintaining high levels of fitness on plants with induced resistance.

1.3.1.2 How can whiteflies relate to each other and to parallel trophic levels?

Although, there are 1556 accepted species names in 161 known whitefly genera worldwide (Martin and Mound, 2007), the two species *B. tabaci* and *T. vaporariorum* are the ones causing most of the damage (Nauen et al., 2014) especially on horticultural crops (Perring et al., 2018) at global level and can also relate to each other.

Tsueda et al. (2014) noted that in Japan, when both species occur on tomatoes in the greenhouse, *T. vaporariorum* select upper leaflets, while *B. tabaci* settle on those of the middle part of plants. This type of choice is related to certain volatiles emitted by the tomato leaflets in their respective positions and reflects in the number of eggs laid by each species. Other research carried out by Zhang et al. (2011) in China have shown that *B. tabaci* is able to produce up to nearly five times the progeny as *T. vaporariorum*, developing faster, living longer, and having a higher female:male sex ratio than *T. vaporariorum*.

Chen et al. (2015) found that these two species influence each other across their impact on the tomato plants. Prior *B. tabaci* infestation can improve development, longevity, and fecundity of *T. vaporariorum*; on the contrary, prior *T. vaporariorum* infestation does not impact the development of *B. tabaci*. Therefore, the feeding of *B. tabaci* can decrease the plant defenses, while that of *T. vaporariorum* does not.

Whiteflies interact with other parallel trophic levels involving both direct and plant-mediated mechanisms. For example, in the study by Inbar et al. (1999b), on *B. tabaci*-infested cabbages first-stage larvae of the cabbage moth, *Trichoplusia ni* (Hübner), were more frequent on the adaxial side of the whitefly-free leaves and the duration of their development was prolonged. The juvenile stages of *T. ni* are strongly affected, probably because the whitefly can prevent the access to leaf tissue and induce plant resistance (Inbar et al., 1999b). The presence of *B. tabaci* can also negatively influence the preference and performance of some leaf miners (Diptera: Agromyzidae) on various plants, as noted by Zhang et al. (2005). These relationships, however, are asymmetrical, since leaf miners have shown to have no significant effects on *B. tabaci* (Inbar et al., 1999a).

With reference to whitefly-mite links, plant responses induced by mites can negatively influence the density of *B. tabaci* (Agrawal et al., 2000). For example, the broad mite, *Polyphagotarsonemus latus* (Banks), a polyphagous pest widely distributed in vegetable crops along with whiteflies, is attracted to whiteflies (Fan and Petitt, 1998), reacting to whitefly wax particles as the main olfactory attractants (Soroker et al., 2003). Mites benefit across passive dispersal between hosts and sites, while whiteflies can directly compete with mites through their influence on shared plants (Agrawal et al., 2000). Thus, the results of interspecific interactions between whiteflies and other parallel trophic levels depend on several factors including colonization time, suitability and characteristics of the plant, and the density and feeding habits of other herbivores (Agrawal et al., 2000).

1.3.1.3 Whiteflies controlled by natural enemies

A thorough understanding of the role of natural enemies within the agroecosystems is needed to comprehend the evolutionary processes and forces that regulate multitrophic interactions (Price et al., 1980). Natural enemies of whiteflies include a wide variety of predators, parasitoids, and entomopathogenic fungi (Perring et al., 2018).

Among predators, Deligeorgidis et al. (2005) found that *Coccinella septempunctata* (L.), a ladybird beetle, provides good control of *T. vaporariorum* on tomato, although it prefers *B. tabaci*. In addition, *Delphastus catalinae* (Horn), another ladybird beetle which especially consumes *B. tabaci* eggs, is the most used for controlling whiteflies on various ornamental crops (Perring et al., 2018). Moreover, among predatory mites, *Amblyseius swirskii* (Athias-Henriot) and *Euseius scutalis* (Athias-Henriot) appear to be potential biological control agents against *B. tabaci* due to their high intrinsic rates of increase (Nomikou et al., 2001). In vegetable protected crops, successful biological control of *B. tabaci* includes the inoculative and augmentative release of *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) (Lambert et al., 2005). Predation on *B. tabaci* and *T. vaporariorum* often can be compromised where *M. pygmaeus* and *N. tenuis* coexist on tomato plants, but it can be improved when the parasitoid *Eretmocerus mundus* (Mercet) is included in the mix (Moreno-Ripoll et al., 2012). So, the combined use of the parasitoid and predators can give better results than the use of a single natural enemy, especially when whitefly infestations are very high in greenhouses (Gabarra et al., 2006).

The majority of whiteflies parasitoids are from the genera *Encarsia* Foerster and *Eretmocerus* Haldeman (Aphelinidae) (Perring et al., 2018); in fact, *Encarsia formosa* (Gahan) and *Eretmocerus eremicus* (Rose and Zolnierowicz) are the most widely used insects for biological control of protected vegetable crops (Perring et al., 2018). On some plants, the whiteflies reproduce so rapidly that parasitoids cannot follow them (Hoddle et al., 1997); therefore, the mixed presence of two parasitoids is particularly useful for controlling the infestations of *B. tabaci* and *T. vaporariorum*, especially when they both live together in the same greenhouse (Stansly and Natwick, 2009).

Entomopathogenic fungi are natural enemies easy to apply to control whiteflies, although good coverage is required on the lower foliar surfaces where pest reside (Perring et al., 2018). Entomopathogenic fungi produce basically no risk to human health, and most studies show that they are quite innocuous to other natural enemies (Vestergaard et al., 2003; Zimmermann, 2008). Entomopathogenic fungi like *Beauveria bassiana* (Balsamo-Crivelli) and *Isaria fumosorosea* (Wize) act on second and third instar nymphs of whiteflies (Perring et al., 2018); while *Lecanicillium lecanii* (Zare and Gams) showed to have a high pathogenicity to all developmental stages of *B. tabaci*, involving adhesion of spores to the insect cuticle, germination, penetration, and internal colonization culminating in host death (Wang et al., 2007).

Plant traits have a key role in multitrophic interaction between pest and natural enemy. In the studies carried out by Romeis and Zebitz (1997) on the parasitoid *E. formosa* and in those by Blackmer and Cross (2001) on *E. eremicus*, it was observed that these natural enemies were attracted from a distance to leaf coloration. Moreover, it was shown that exposure of bean leaves to adult *B. tabaci* induces the emission of a blend of volatile compounds that differ from those emitted from noninfested bean plants and attract the females of *E. formosa* (Birkett et al., 2003). Varying degrees of leaf pubescence affect both prey and predators: experiments on the effects of tomentosity showed that on hairy cotton leaves *D. catalinae* collects honeydew, without the consumption of whitefly adults (Guershon and

Gerling, 1999). In addition, leaf pubescence can deter parasitoids, limiting their locomotion and host-finding abilities (Inbar and Gerling, 2008).

Plant chemical components can affect natural enemies both directly and through the quality of the whiteflies and their acquired secondary metabolites (Inbar and Gerling, 2008). For example, phytoseiid mites can feed, survive, and reproduce on pollen, leaves, and *B. tabaci* honeydew (Nomikou et al., 2003a), while *E. formosa* prefers *B. tabaci* developing on fertilized plants than on unfertilized ones (Bentz et al., 1996).

However, the presence of natural enemies may also affect plant selection by whiteflies, as in the research by Nomikou et al. (2003b) demonstrating how whitefly adults avoid cucumber plants with predatory phytoseiid mites (Nomikou et al., 2003b).

1.3.1.4 Whiteflies as vectors of phytopathogenic microorganisms

What influences the epidemiology of plant virus diseases concerns the virus species, strain and isolate, the host range, the ability of the vector to spread the virus, and abiotic environmental conditions (Navas-Castillo and Fiallo-Olivé, 2017). Viruses in their native environments rarely cause harmful diseases, but new evolutionarily recent virus – host – vector – environment interactions have emerged due to human activities and climate change (Navas-Castillo and Fiallo-Olivé, 2017; Trebicki, 2020). An example is the increase of cassava cultivation in Africa, introduced by America, with the consequent emergence of devastating virus diseases, such as Cassava Mosaic Disease (CMD), caused by more than nine species of native African bipartite begomoviruses (family *Geminiviridae*) (Fauquet and Fargette, 1990), and the Cassava Brown Streak Disease (CBSD), caused by ipomoviruses, such as Cassava Brown Streak Virus (CBSV) and Ugandan Cassava Brown Streak Virus (UCBSV) (Patil et al., 2015).

Plant traits, like the physiological stage, are responsible for susceptibility to vector infestation and multiplication and viral infection, in fact young plants are more susceptible to both the vector and the virus.

Bemisia tabaci and other whiteflies, such as *Trialeurodes abutilonea* (Haldeman), *Trialeurodes ricini* (Misra) and *T. vaporariorum*, are known natural vectors of several plant viruses (Jones, 2003; Polston et al., 2014). The transmission of the virus and the direct forms of damage to plants, such as the induction of phytotoxic symptoms of silvering, arise from the feeding of the whitefly within the vascular system of the plants through the piercing-sucking mouth apparatus (Andreason et al., 2020).

On the basis of the duration of the infectivity, after the acquisition by the vector and the movement of the virus inside it, the viruses can be grouped as circulative or non-circulative (Whitfield et al., 2015). The non-persistent and semi-persistent types of transmission are both non-circulative, in fact the interactions of the virus with its vector insect are characterized by the localization and retention of the virus within the stylets of the vector or the foregut before transmission (Andreason et al., 2020). Instead, the types of persistent-circulative and persistent-propagative transmission are both circulative, in fact they are characterized by the acquisition of the virus in the vector hemolymph across the filter chamber, midgut, or hindgut and circulation of the virus to the salivary glands for subsequent transmission (Fiallo-Olivé et al., 2020; Andreason et al., 2020). In addition, viruses with propagative capacity, are also able to replicate within the vector (Fiallo-Olivé et al., 2020).

B. tabaci is known for its persistent circulative transmission capacity of begomovirus, but also non-circulative transmission of viruses belonging to the genera *Crinivirus* (family *Closteroviridae*), *Ipomovius* (family *Potyviridae*), *Carlavirus* (family *Betaflexiviridae*), and *Torradovirus* (family *Secoviridae*) (Jones, 2003; Polston et al., 2014; Whitfield et al., 2015; Iwaki et al., 1982; Wisler et al., 1998; Verbeek et al., 2014). The way in which plant viruses are transmitted is relevant in this area of research, as it could determine whether there is an interaction between endosymbionts, their metabolites and the virus transmitted by insects (Andreason et al., 2020).

1.3.1.4.1 The role of whitefly endosymbionts

Sap-sucking insects, including whiteflies, feed on plant diets across the evolutionary incorporation of endosymbiotic bacteria (Baumann, 2005). Obligate intracellular bacteria in whiteflies directly facilitated their host's adaptation to nutritionally limited phloem diets, which are poor in essential amino acids and rich in carbohydrates (Byrne and Miller, 1990).

In these pests, the maternally inherited primary (obligate) endosymbiont is *Candidatus* Portiera aleyrodidarum (Thao and Baumann), needed for the suitability and survival of the insect (Thao and Baumann, 2004). In fact, across its presence, whiteflies are able to find both essential amino acids and missing carotenoids (Sloan and Moran, 2012). Furthermore, due to the very close and long-lasting link, Portiera has not been cultured till now, because it depends on metabolism of its host (Baumann, 2005). Similarly, as showed in several studies that involve the use of antibiotics, whiteflies cannot survive without Portiera and, in addition, they lay a small number of endosymbiont-free eggs, unable to hatch (Zhang et al., 2015; Zhao et al., 2020).

In addition to primary endosymbionts, maternally inherited secondary (facultative) endosymbionts are often found in sap-feeders hosts, as in the case of *B. tabaci* which is able to host highly diverse communities of endosymbionts (Gueguen et al., 2010). The seven genera of secondary endosymbiotic bacteria associated with this whitefly are: *Hamiltonella* (Zchori-Fein and Brown, 2002), *Rickettsia* (Gottlieb et al., 2006), *Fritchea* (Everett et al., 2005), *Wolbachia*

(Nirgianaki et al., 2003), *Arsenophonus* (Zchori-Fein and Brown, 2002), *Cardinium* (Weeks et al., 2003) and *Hemipteriphilus* (Bing et al., 2013). They are not necessary for the host survival but have various roles in the fitness and efficiency of virus transmission by whiteflies (Morin et al., 1999). For example, *Hamiltonella* provides general fitness benefits, can enhance the virus transmission properties of its host, mitigate the adverse effects that result from Tomato Yellow Leaf Curl Virus (TYLCV) infection (Kliot and Ghanim, 2013; Su et al., 2013), and it can be also involved in the production of molecules that suppress the defense in tomato plants by jasmonic acid pathways (Su et al., 2015). Studies on *Arsenophonus*, on the other hand, show how this can reduce virus acquisition and retention (Ghosh et al., 2018).

1.3.1.4.2 Weeds as hosts for whiteflies

The environment has significant impact on the successful development of a virus epidemic (Navas-Castillo and Fiallo-Olivé, 2017). In this regard, wild plants can host viruses in addition to their vectors, acting as reservoirs (Srinivasan et al., 2012). These are annual or perennial weeds that may not have been harvested in the previous season or present among crops (Håkansson, 1982). However, the effects of these primary sources of infection are highly dependent on the host range of the vectors and viruses involved, but also on the likelihood that enough efficient vectors will acquire the virus, become infectious and inoculate it to healthy plants (Navas-Castillo and Fiallo-Olivé, 2017).

Thus, wild plants play a crucial role in the spread of many plant viruses, acting as sources of inoculum and bridging harvest seasons (Alvarez and Srinivasan, 2005; Arli-Sokmen et al., 2005; Wisler and Norris, 2005), often showing few or no symptoms (Wisler and Norris, 2005).

B. tabaci has many important weed hosts (Cook, 1986), as was found in the case of Smith et al. (2015) who found that *Amaranthus* *retroflexus* (L.) is also a TYLCV host. It is also important to remember that the weeds present in the fields, if not recognized as hosts can act as a support and refuge for the natural enemies of sap-sucking insects, regulating pest populations and decreasing the viruliferous part of the vector population (Villegas et al., 2013).

1.4 *State of knowledge and need for this research*

1.4.1 *Knowledge gap*

In an agricultural context, where a multitude of intricate and complex relationships occur among organisms and surrounding environment, all elements involved in the system interact in important ways and cannot be considered individually. Thus, only by using a holistic way of thinking it is possible to grasp the peculiarities of these complex mechanisms of ecological interrelation, which include also the important multitrophic interactions influencing both the strength of predators (top-down) and resources (bottom-up) against pest (Tscharntke and Hawkins, 2002), but also the impact that higher trophic levels could have on food production.

Due to the diversity of plant damage they cause, research on whiteflies has varied widely in the recent years. In fact, many studies focus on resolving crop losses due to one or more kind of damage, mainly obtaining results applicable on a local scale, but whose validity may be also extended on a larger scale for growers around the world (Perring et al., 2018). In this regard, there is a gap of knowledge in the ways each organism involved in the system affect the others, and particularly in how whiteflies interact with other trophic levels of their food webs. Therefore, identifying all trophic levels involved (vector, virus, endosymbionts, host plant and natural enemy) and understanding the nature of their intricate multitrophic relationships could offer new and useful information for developing new strategies to manage whiteflies and their related viruses in agriculture.

Specifically, in addition to the lack of updates on the distribution of the *B. tabaci* species complex in Sicily, there are no recent studies aimed at evaluating the effect of *B. tabaci* on specific morphological and physiological traits of the host plants, especially those ones belonging to the family *Solanaceae*. Also, in spite of the numerous studies existing on biology and predatory activity of zoophytophagous mirid bugs, there is a knowledge gap in the way they can influence the health of host plants in the presence of their prey. Furthermore, and last but not least, there is a paucity of studies on the transmission capacity of some important viruses by *T. vaporariorum*, but also on how efficiency in virus transmission changes depending on the whitefly vector species and the cultivated or wild plant considered.

1.4.2 Research questions

To improve eco-compatible control strategies against whiteflies and their related virus problems on crops, research has been carried out to highlight some aspects of the complex relationships linking these pests with members of some of the trophic levels being part of their food webs, e.g.: plants, predators and viruses. With the aim of bridging the identified knowledge gap, the present study has been based on laboratory experiments that analyze the impact of each single organism involved in the abovementioned multitrophic interactions, answering the following research questions:

1. How is the *B. tabaci* species complex with its relative endosymbionts distributed and, if a predominance exists, which and where are localized the dominant species in Sicily?
2. How does *B. tabaci* influences morphological and physiological traits of its host plants and how this is related to the kind and level of damage to vegetable crops?

3. What consequences, in terms of morphological and physiological traits, may derive to the host plants by the combined presence of *B. tabaci* and one of its mirid bugs predators? And what the consequence can be on the health status of a vegetable crop?
4. Is the whitefly *T. vaporariorum* an efficient vector in transmitting begomoviruses between cultivated plants?
5. Do weeds always act as inoculum sources in the transmission of begomoviruses?

In order to achieve the objectives mentioned above, the present work focuses on multitrophic interactions with some solanaceous, but also both cultivated and spontaneous cucurbits, as host plants, *B. tabaci* MED and *T. vaporariorum* as pest species, *M. pygmaeus* as predator species and Tomato Leaf Curl New Delhi Virus (ToLCNDV) as a phytopathogenic microorganism.

2 Geographic distribution of *Bemisia tabaci* species in Sicily and patterns in facultative endosymbiont community composition

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2.1 *Abstract*

Bemisia tabaci is a group of cryptic and morphologically indistinguishable species able to cause severe damage to many agricultural and ornamental plants. Among the various species of this complex, the Mediterranean (MED) and Middle East Asia Minor 1 (MEAM1) species stand out as they are highly polyphagous, invasive, easily in acquiring resistance to many insecticides and reproduce quickly. Considering how (1) the geographic distribution of *B. tabaci* is changing with the ongoing global climate change and (2) no knowledge is presently available on phylogenetics of whitefly endosymbionts in Sicily, a population survey was conducted in the most important horticultural production areas of the island to assess the species composition within the *B. tabaci* complex, including their endosymbiont community and their geographic distribution. Our results show that the MEAM1 species and two mitochondrial variants of *B. tabaci* MED are present in pure or mixed populations, the MED Q1 presents the highest level of genetic variability within the MED populations in Sicily, having been found almost across the island and, MED Q2 was nearly exclusively detected in the Ragusa province. MEAM1 individuals were rare and exclusively detected in two localities in the Trapani province. The survey on endosymbiont community revealed the existence of a species specific composition, showing the lowest endosymbiont diversity in MED Q2 populations, typically characterized by only *Rickettsia*. Moreover, except for Portiera in MEAM1, no sequence variation was found within any endosymbiont sequence. Co-infection patterns of different endosymbiont species are discussed in the context of their needs for host cell metabolites. The present study defines an updated distribution map of cryptic species and phylogenetic groups of the *B. tabaci* complex and the first status on the endosymbiont community in Sicily; but it also represents the first report of whitefly

endosymbionts sequences not only from Sicily but from Italy as a whole.

2.2 Keywords

Whitefly, Italy, taxonomy, population, survey, Aleyrodidae

2.3 Introduction

Bemisia tabaci is a global sap-sucking insect pest causing severe damage to many agricultural crops and ornamental plant species (De Barro et al., 2011). The taxonomy of *B. tabaci* is an ongoing area of research, with the current consensus being that it represents a cryptic species complex consisting of over 40 morphologically indistinguishable species (Dinsdale et al., 2010; Tay et al., 2012). These species differ in life table parameters, geographic distribution, fecundity, and host range and preference, but also in insecticide resistance and virus transmission efficiency (Bedford et al., 1994; Brown et al., 1995; Milenovic et al., 2019). Originally identified as biotypes, today as cryptic species (De Barro et al., 2011), these whiteflies are in the ongoing process of speciation as they over time evolve various adaptive traits as a response to their environment (Chu et al., 2012; Watanabe et al., 2019). Most commonly, *B. tabaci* species are discriminated on the basis of mitochondrial cytochrome oxidase subunit 1 (COI) sequence variability (Boykin and De Barro, 2014; De Barro et al., 2011; Dinsdale et al., 2010). Of many *B. tabaci* cryptic species, the Mediterranean (MED) and Middle East-Asia Minor 1 (MEAM1) species stand out as fast reproducing, very invasive, highly polyphagous, globally distributed and resistant to many groups of once very effective insecticides (Health, 2013). Additionally, the MED species shows a remarkable intraspecific variability and several mitochondrial variants have been identified, out of which the most common were designated as Q1, Q2, Q3 and ASL (Tsagkarakou et al., 2007; Chu et al., 2008; Gueguen et al., 2010).

The geographic distribution of different *B. tabaci* species is changing with the ongoing global climate change, aided by fast movement of plant and consequently insect material around the globe. This results in invasions of some species to new environments and displacements of others (Aregbesola et al., 2019). Although MED and MEAM1 can coexist in the same environment, during the last decade a continuous increase of MED infestations, resulting in the displacement of the MEAM1 species, has occurred in greenhouses, in open field crops, and in weed species (Bosco et al., 2006; Simón et al., 2007; Parrella et al., 2012).

In Italy, following the introduction of new begomoviruses, such as Tomato Yellow Leaf Curl Virus (TYLCV), Tomato Yellow Leaf Curl Sardinia Virus (TYLCSV), and Tomato Leaf Curl New Delhi Virus (ToLCNDV) (Rapisarda and Tropea Garzia, 2002; Davino et al., 2006; Bertin et al., 2018), *B. tabaci* species complex and its associated virus problems have gained greater importance. To better understand dynamics of *B. tabaci* species, various monitoring activities have been carried out in Italy during recent years, in areas where both the vector and virus species were noted and, in those ones, where the presence of *B. tabaci* was only occasional and no virus epidemics had ever occurred (Bertin et al., 2018; Bertin et al., 2021). In Central Italy (Lazio region), zucchini plants infected with ToLCNDV and infested with both single and mixed populations of MED and MEAM1 were found (Bertin et al., 2018). In plants exclusively infested by the MED species in Lazio, the prevalent haplotype was MED Q2 (Bertin et al., 2018), as reported also by Parrella et al. (2014) in Campania and by Bertin et al. (2021) in two areas of Sicily, confirming how the spread of this haplotype is favored by high temperatures typical of Southern Italy.

Species of *B. tabaci* complex are also characterized by a high diversity of bacterial endosymbionts (Brumin et al., 2019). Whiteflies live in obligatory symbiosis with *Candidatus* Portiera aleyrodidarum, a bacterium that synthetizes essential amino acids and carotenoids which are scarce in the phloem sap of the host plant (Santos-Garcia et al., 2012; Santos-Garcia et al., 2015). Additionally, whitefly can, but not necessarily do, harbor additional, facultative endosymbiotic bacteria from the genera *Arsenophonus*, *Cardinium*, *Fritschea*, *Hamiltonella*, *Hemipteriphilus*, *Rickettsia*, and *Wolbachia* (Kanakala and Ghanim, 2019). Their roles are considerably less explored compared to the obligatory endosymbiont and range from vitamin biosynthesis to potentially parasitic relationship. It has been hypothesized that they provide fitness benefits in a context dependent manner and that their diversity is at least partially responsible for the great adaptability and global success of *B. tabaci* whiteflies (Milenovic et al., 2022a). As such, whitefly endosymbionts are important modifiers of whitefly biology and together form one ecological unit, whitefly holobiont. Phylogenetic analysis of whitefly endosymbionts revealed that their taxonomy is sometimes non congruent with the taxonomy of their host, indicating a horizontal transfer of endosymbiont species between different whitefly species, and possibly other insects (Kanakala and Ghanim, 2019). The diversity of endosymbiont community has been extensively explored in some regions of the world (e.g., Israel), others are severely under sampled. In Italy, there are no published phylogenetic studies of whitefly endosymbionts to date.

Considering how geographic distribution of the *B. tabaci* species is changing, it necessitates continuous monitoring, which combined with the fact that there is no knowledge on phylogenetics of whitefly endosymbionts in Sicily (nor for the rest of Italy), a dedicated population survey has been realized in the most important Sicilian vegetable production areas. The aim was to assess the species composition within the *B. tabaci* complex, including their endosymbiont community and their geographical distribution.

2.4 *Materials and methods*

2.4.1 *Sample collection*

The study was conducted from April 2021 to November 2022. Samples of *B. tabaci* were collected by a mouth aspirator (John W. Hock Company, Gainesville, FL, United States) at 25 sites in Sicily on vegetable crops grown in both greenhouse and open field conditions, as well as on weeds (Table 1). The whitefly individuals were then stored in 99% ethanol at 4 °C until the laboratory analysis. Each sample consisted of 3-70 specimens collected on diverse host plants and at distinct localities.

Table 1 - Sampling Sicilian localities [town (province)], geographic coordinates (latitude, longitude) and host plants from which the samples were collected.

Locality	**Geographic coordinates**	**Date of sampling**	**Host plant**
Vittoria (RG)	36.97134, 14.424505	13/04/2021	*Solanum melongena* (L.)
Vittoria (RG)	36.905165, 14.439656	13/04/2021	*Solanum lycopersicum* (L.)
Vittoria (RG)	36.905593, 14.482508	05/05/2021	*Solanum melongena* (L.)
Vittoria (RG)	36.957562, 14.407787	05/05/2021	*Solanum melongena* (L.)
Vittoria (RG)	36.983418, 14.431998	05/05/2021	*Capsicum annuum* (L.)
Ragusa (RG)	36.831278, 14.495398	12/05/2021	*Capsicum annuum* (L.)
Ragusa (RG)	36.828487, 14.479026	12/05/2021	*Solanum melongena* (L.)
Santa Croce Camerina (RG)	36.811414, 14.47895	12/05/2021	*Solanum melongena* (L.)
Marina di Ragusa (RG)	36.788314, 14.533365	12/05/2021	*Capsicum annuum* (L.)
Ragusa (RG)	36.798317, 14.588599	12/05/2021	*Solanum melongena* (L.)
Scicli (RG)	36.760296, 14.669167	12/05/2021	*Cucumis sativus* (L.)
Santa Maria del Focallo (RG)	36.697643, 14.970866	17/05/2021	*Conyza* spp. (Less)
Ispica (RG)	36.771684, 14.985896	17/05/2021	*Capsicum annuum* (L.)

Ispica (RG)	36.771554, 14.979323	17/05/2021	*Cucumis melo* (L.)
Gela (CL)	37.127696, 14.143832	17/06/2021	*Solanum lycopersicum* (L.)
Gela (CL)	37.127665, 14.143848	17/06/2021	*Solanum nigrum* (L.)
Gela (CL)	37.126001, 14.142878	17/06/2021	*Citrullus lanatus* (Thunb.) Matsum. & Nakai
Sortino (SR)	37.122843, 14.968177	17/06/2021	*Capparis spinosa* (L.)
Licata (AG)	37.121502, 13.877564	17/06/2021	*Cucumis melo* (L.)
Campobello di Mazara (TP)	37.639937, 12.727814	18/06/2021	*Sonchus asper* (L.) and *Solanum melongena* (L.)
Marsala (TP)	37.78784, 12.457519	18/06/2021	*Phaseolus vulgaris* (L.)
Paceco (TP)	37.989567, 12.546775	18/06/2021	*Cucumis melo* (L.)
Vittoria (RG)	36.967932, 14.479791	28/06/2021	*Solanum melongena* (L.)
Caltanissetta (CL)	37.435092, 14.050988	22/09/2021	*Solanum melongena* (L.)
Licata (AG)	37.1155656, 13.8640772	10/11/2021	*Solanum lycopersicum* (L.), *Solanum melongena* (L.) and *Cucumis sativus* (L.)

2.4.2 *Molecular identification of* B. tabaci *species and its endosymbionts*

Whiteflies were individually removed from ethanol, briefly dried and DNA was extracted according to the protocol described in Milenovic et al. (2022b). Ten randomly selected, unsexed individuals were selected from each location (or less if <10 individuals were available) and DNA was extracted. Individual whiteflies were placed in 1.5 ml centrifuge tube containing 25 µl of lysis buffer (10 mM Tris-HCl pH 8.0, 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% Tween-20, 60 µg/ml Proteinase K) and homogenized using plastic pestle and vortex mixer. Crude lysates were spun down for 30s at 10,000 x g and transferred to a 96-well plate. Plates were incubated in a thermocycler for 50 min at 54°C for the optimal proteinase K activity, followed by 20 min at 80°C to heat inactivate proteinase K enzyme. Heated lid was set to 90°C. After cooling on ice, samples were diluted by adding 180 µl of PCRgrade H_2O to each well. Separate master mixes were prepared using Q5® Hot Start High-Fidelity 2X Master Mix (New England Biolabs, MA, United States) and a primer pair for whitefly COX1 gene, and each of the endosymbionts as described in Milenovic et al. (2022b). To avoid human error in setting up over 1,000 PCR reactions, Eppendorf epMotion 5073C (Eppendorf, Hamburg, Germany) automated pipetting system was programmed to distribute master mix and add DNA sample to each well. PCR conditions were as previously described. Following the PCR reaction, 5µl of the reaction were used for gel electrophoresis using E-Gel™ 96 Agarose Gels, 2% (Thermo Fisher Scientific, Waltham, Massachusetts, United States) with 12 min runtime. Images of the gels were taken using Syngene InGenius LHR2 Gel Imaging System (Syngene Cambridge, United Kingdom) with 400ms exposure, aperture set to 2, and zoom adjusted to fill the frame. Images were processed using E-Editor 2.0 Software (Thermo Fisher Scientific, Waltham, Massachusetts, United States).

Positive and inconclusive samples were identified visually based on the expected PCR product size and sent to Macrogen Europe BV (Amsterdam, Netherlands) for sanger sequencing in both forward and reverse direction. Raw sequencing reads were manually trimmed, and error corrected using Sequencher 5.4.6 software (Gene Codes, Ann Arbor, Michigan, United States). Unique sequences were identified, and BLAST search was performed at NCBI website using default settings.

B. tabaci identity was determined by alignment to the mtCOI reference dataset (Boykin et al., 2017) followed by phylogenetic tree construction as previously described in Milenovic et al. (2022b).

Geographic distribution of *B. tabaci* species and their endosymbionts were visualized using QGIS 3.28.1 software. Base map is based on “ESRI Terrain” map provided by the QuickMapServices 0.19.32 QGIS plugin.

2.5 Results

The DNA was extracted from 210 whitefly individuals, out of which 204 produced a sequence of sufficient quality to determine the species. Results of the whitefly population survey show presence of two *B. tabaci* cryptic species, MED and MEAM1, with the MED representing over 92% of the analyzed individuals. Within MED species, two major phylogenetic groups were detected, previously described as Q1 and Q2. All Q2 sequences in this study were identical to each other and identical to the NCBI GenBank accession MH205753.1. Similarly, MEAM1 sequences showed uniformity and identity with the previously described accession KR559508.1. Q1 sequences on the other hand were represented by eight unique sequences with various levels of similarity. These Q1 subgroups were arbitrarily numbered in the present study. Phylogenetic analysis reveals Q1_4 group as the most distant from other Q1 subgroups, while the others group into two branches with one branch consisting of closely related Q1_8, Q1_5, Q1_1, and Q1_7 and the other of closely related Q1_9, Q1_6, Q1_10. A total of 3 samples failed at various stages of the analysis, hence their identity could not be determined, and 3 samples had sequence long enough to be determined to belong to MED Q1 group, but too short to differentiate between eight different Q1 subgroups detected in the present study. A breakdown of number of samples detected for each group, as well as the best GenBank BLAST hit is presented in the Table 2.

Geographically, the detected species and phylogenetic groups show a non-random distribution (Figure 1). MEAM1 samples were exclusively detected at the two localities in the Trapani province, while MED Q2 is almost exclusively detected in the Ragusa province, with only a few Q2 samples detected in the neighboring areas. Q1 groups were detected across the island, with Q1_4 detections limited to the Ragusa province.

Table 2 - Top BLAST hit sequences for ten detected whitefly phylogenetic groups from this study, and their identity.

Group	**GenBank ID**	**Identity**
Q1_1	LN614545.1	Identical
Q2	MH205753.1	Identical
MEAM1	KR559508.1	Identical
Q1_4	HE863759.1	Identical
Q1_5	MH205752.1	Identical
Q1_6	MW604192.1	Identical
Q1_7	KX954191.1	Identical
Q1_8	MH205752.1	1nt difference
Q1_9	MW604168.1	Identical
Q1_10	KX954191.1	1nt difference

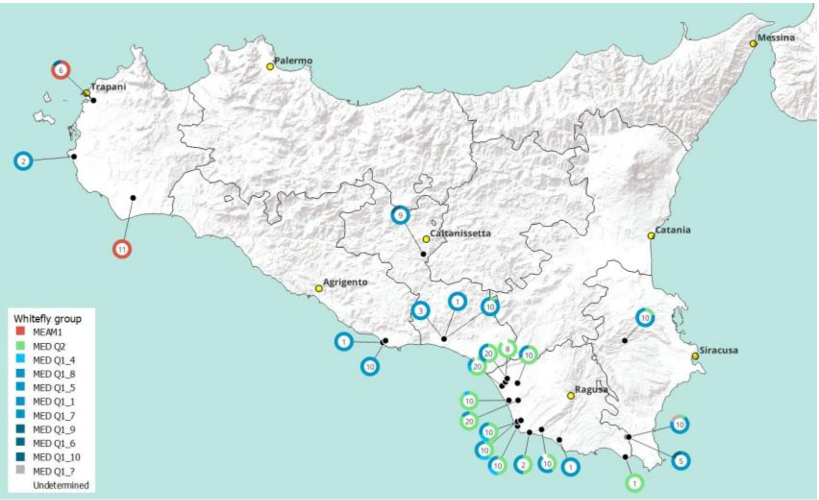


Figure 1 - Geographic distribution of whitefly phylogenetic groups across sampling locations visualized using QGIS 3.28.1 software. Each black point represents one sampling location. The number in the callout shows the number of analyzed samples, and the colors surrounding the number represent the proportion of different

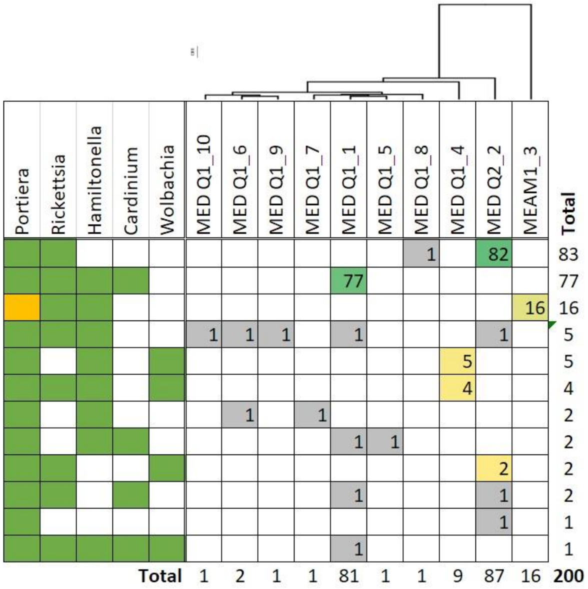
phylogenetic groups at each location. Groups that differ only by a single nucleotide are shown with the same color.

Whitefly endosymbiont composition was successfully determined for 200 individuals, or 98% of those with identified whitefly group. Individuals for which whitefly species was undetermined, or if any of the detected endosymbionts (by PCR product gel electrophoresis) failed to produce a sequence, were excluded from the analysis. A failure in sequencing might mean poor DNA quality or contamination, both of which could result in an overall biased result of endosymbiont composition. All detected endosymbionts showed identical sequences, with the exception of Portiera in MEAM1 whiteflies which showed a single nucleotide difference from Portiera sequences from MED whiteflies. Best NCBI GenBank BLAST hits for endosymbiont sequences are presented in the Table 3. Figure 2 showing endosymbiont composition across whitefly phylogenetic groups reveals a striking uniformity in endosymbiont composition and a clear link between endosymbiont composition and whitefly identity. As Portiera is expectedly present in all individuals, our study focuses on the facultative endosymbionts. MEAM1 whiteflies are characterized by harboring *Rickettsia* and *Hamiltonella* (16/16 samples), while MED Q2 primarily harbors only *Rickettsia* (82/87 samples). Additionally, three specimens of MED Q2, harbored additional *Hamiltonella* or *Cardinium*, or lacked *Rickettsia*. Two MED Q2 individuals harbored *Wolbachia* in addition to *Rickettsia*. All nine MED Q1_4 individuals harbored *Wolbachia* and *Hamiltonella*, while 4/9 also harbored *Rickettsia*. This was the only Q1 population to harbor *Wolbachia*. Most Q1 samples belong to the group here named Q1_1, out of which 77/81 harbored *Rickettsia*, *Hamiltonella* and *Cardinium*. This subgroup was in fact the only one to harbor *Cardinium*. Single whitefly individuals missing either *Rickettsia*, *Hamiltonella*, or *Cardinium* were detected, as well an additional individual harboring four endosymbionts (*Rickettsia*, *Hamiltonella*, *Cardinium* and *Wolbachia*). The other Q1 subgroups had only one or two individuals, making any conclusions, or even their existence, hard to confidently claim. Endosymbiont composition of these individuals is nevertheless presented in the Figure 2. Endosymbionts *Fritschea* and *Hemipteriphilus* were not detected in the present study.

Arsenophonus proved difficult to amplify in the present study using previously described and apparently commonly used primers. Efforts to amplify *Arsenophonus* resulted in secondary bands on the electrophoresis gel, and attempts to sequence the main product resulted in either extremely unreliable base calls or produced Portiera sequence. The issue was not resolved by varying primer annealing temperature. As a part of the troubleshooting process, we attempted to use strain-specific primers, designed for novel *Arsenophonus* strain described recently by Milenovic et al. (2022b), on a subset of 30 samples of the localities 3, 4, and 5 from the Table 1, which included nine Q1_1 individuals, one Q1_4, and 20 Q2 individuals. The results of this test reveal *Arsenophonus* presence only in Q2 samples, with the presence confirmed in 19/20 samples.

Table 3 - Top BLAST hit sequences for detected whitefly endosymbionts from this study, and their identity.

Endosymbiont	**GenBank ID**	**Identity**
Portiera MEAM1	CP007563.1	Identical
Portiera MED	CP016304.1	Identical
Hamiltonella	CP016303.1	Identical
Rickettsia	CP016305.1	Identical
Wolbachia	CP016430.1	3nt difference
Cardinium	MH908678.1	Identical



Portiera	Rickettsia	Hamiltonella	Cardinium	Wolbachia	MED Q1_10	MED Q1_6	MED Q1_9	MED Q1_7	MED Q1_1	MED Q1_5	MED Q1_8	MED Q1_4	MED Q2_2	MEAM1_3	**Total**
🟩	🟩										1		82		83
🟩	🟩	🟩	🟩						77						77
🟨	🟩	🟩												16	16
🟩	🟩	🟩			1	1	1		1				1		5
🟩		🟩		🟩								5			5
🟩	🟩	🟩		🟩								4			4
🟩		🟩				1		1							2
🟩		🟩	🟩						1	1					2
🟩	🟩			🟩									2		2
🟩	🟩		🟩						1				1		2
🟩													1		1
🟩	🟩	🟩	🟩	🟩					1						1
				Total	1	2	1	1	81	1	1	9	87	16	**200**

Figure 2 - Endosymbiont composition of each of the detected whitefly phylogenetic groups. Green boxes on the left side signify the presence of the corresponding endosymbiont, while the yellow box signifies presence, but the sequence is distinct from the rest in the same column. Boxes with numbers show the number of detected individual whiteflies with the corresponding whitefly-endosymbiont combination, colored from high to low using a green-yellow-gray gradient. Right and below the matrix are the sums per endosymbiont combination and whitefly group, respectively. Above the matrix is a maximum likelihood phylogenetic tree of the whitefly phylogenetic groups.

2.6 Discussion

The main horticultural production areas in Sicily are in the south and southeast where the greenhouse cultivation of tomato, eggplant and pepper crops dominates, while the provinces of Caltanissetta, Agrigento and Trapani are important production areas of melon, watermelon and certain *Solanaceae* crops. In these areas, viral diseases are often responsible for serious reductions in crop yields, and among the viruses present, those transmitted by whiteflies on cucurbits and *Solanaceae* are the most common (Rapisarda and Tropea Garzia, 2002; Davino et al., 2006).

Over the years, it has been observed that the viral outbreaks in Sicily are closely correlated with the distribution of *B. tabaci* (Bosco and Caciagli, 1998). Furthermore, incidence of high densities of this pest on crops have been linked to gradual shift in its populations especially where *B. tabaci* MEAM1 was displaced by *B. tabaci* MED (Bertin et al., 2021). Previous studies have shown that in Italy the MEAM1 species of this whitefly is present at lower levels, while the MED species is gradually adapting to outdoor conditions and frequently spreads from protected to field crops, confirming that the geographical and genetic status of *B. tabaci* populations continues to change rapidly (Bosco and Caciagli, 1998; Bertin et al., 2018).

Our results show how two *B. tabaci* MED mitochondrial variants (Q1 and Q2) as well as the MEAM1 species are present in single or mixed populations among whitefly samples collected in some of the main cultivated areas in Sicily. Moreover, presence of MED Q2 primarily in the intense greenhouse production area in the Ragusa province support the findings of previous studies conducted in southern Italy and other Mediterranean countries, according to which MED Q2 variant is better able to adapt to the high summer temperatures that characterize greenhouses (Tsagkarakou et al., 2007; Parrella et al., 2014; Bertin et al., 2021).

The present survey also confirms that MED Q1 presents the highest level of genetic variability within the MED populations of Sicily, as MED Q2 specimens belonged to only one phylogenetic group, as indicated in several studies (Chu et al., 2006; De Barro and Ahmed, 2011; Chu et al., 2012; Gauthier et al., 2014; Barboza et al., 2019). This level of genetic differentiation, which differs between the two mitochondrial groups, may depend on the fact that MED Q1 was originally present in the western Mediterranean countries, whereas MED Q2 has infested this area more recently (Gauthier et al., 2014;

Bertin et al., 2018). Alternatively, it is possible that multiple Q1 variants were introduced to the region. The low number (one or two individuals, Figure 2) of detected individuals for most MED Q1 groups might represent a PCR or sequencing error, therefore, more samples of these populations are needed to definitively claim their presence. In the case of here labelled MED Q1_1 and MED Q1_4 however, the confidence is high due to much larger number of detected individuals, greater sequence differences as also reflected in distances visible from the phylogenetic tree, and distinct endosymbiont composition between the two groups. The present study did not establish any clear link between whitefly biotype and host plant species.

Study of the endosymbiont composition revealed how each whitefly group harbors a distinct set of endosymbionts, with only a few exceptions. Lowest endosymbiont diversity was observed in the Q2 population which was characterized typically by only harboring *Rickettsia* of the facultative endosymbionts. In fact, *Rickettsia* was the most detected endosymbiont presents in over 90% of all analyzed whitefly individuals, which might mean that the bacteria *Rickettsia* provides fitness benefits in the environmental conditions, regardless of the whitefly genetic background. Previous studies have shown how *Rickettsia* can affect the adaptive capacity of *B. tabaci* (Brumin et al., 2011) and manipulate the defense patterns of its host plants (Shi et al., 2021). The effects of *Rickettsia* endosymbiont on the fitness of *B. tabaci* are however far less clear, with different studies reporting both strong benefits and no measurable effects (Cass et al., 2015; Bockoven et al., 2020). At the same time, *Rickettsia* is known for its dynamic changes in frequency in whitefly populations over time (Bockoven et al., 2020). The group Q1_4 was the only one where absence of *Rickettsia* was detected with high confidence. Curiously, this population was the only one with apparently fixed presence of previously unreported strain of *Wolbachia*. This supports the previous observations of rare *Rickettsia-Wolbachia* coinfection, for which the working hypothesis is the fact that both bacteria are dependent on and would compete for the NAD+ from the host cell (Zchori-Fein et al., 2014; Opatovsky et al., 2018). With the exception of Portiera in MEAM1, no sequence variation was found within any endosymbiont sequences. This uniformity not necessarily expected as several strains of facultative endosymbionts are known to exist. At the first glance, the uniformity might have resulted from horizontal transfer of endosymbionts between whitefly phylogenetic groups. This is supported by the previously shown evidence for the possibility of horizontal transfer (Milenovic et al., 2022a), as well as by the presence of mixed whitefly populations at the same locality. However, this is not possible to claim with certainty and strong natural selection for the most beneficial or the most infective strains is equally likely based solely on the present data. Presence of *Cardinium* only in Q1_1 population, in coinfection with *Hamiltonella* goes against the previously posed hypothesis that these two endosymbionts are in competition, with *Cardinium* displacing *Hamiltonella* (Zhao et al., 2018). The distinct endosymbiont communities coincide with differences in whitefly phylogeny, which makes it impossible to hypothesize if observed differences in infestation patterns are resulting from whitefly genetic background or its endosymbionts, which further corroborates the whitefly holobiont paradigm, which calls for treating whiteflies and their endosymbionts as one ecological unit (Milenovic et al., 2022a).

The present study defines a new map of the distribution of cryptic species and phylogenetic groups of the *B. tabaci* complex and the first state on the endosymbiont community in Sicily, where the strong presence of MED species causes several concerns due to its high insecticide resistance and efficient virus transmission (Sánchez-Campos et al., 1999; Pan et al., 2012; Bertin et al., 2021). Much is still unknown about the nature of the symbiosis between whiteflies and their facultative endosymbiont community, but much remains to be investigated. This research adds to the body of knowledge about their geographic distribution, coinfection patterns, and association status with different whitefly species/phylogenetic groups for up to now unsampled region. The present study is the first to obtain sequences of whitefly endosymbionts not only from Sicily, but from Italy as a whole.

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3 *Bemisia tabaci* (Hemiptera: Aleyrodidae): what relationships with and morpho-physiological effects on the plants it develops on?

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3.1 Simple summary

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) has a cosmopolitan distribution, and it is a feared pest of many agricultural crops. It is a complex of numerous genetically differentiated species, most of which may rapidly acquire insecticide resistance, consequently making their control problematic. This study aims to improve knowledge on the direct damage of this pest, as well as its impact on the main traits of vegetable crops. Overall, the results confirm how different host plants display variable susceptibility to *B. tabaci* infestation and explain trophic links between plant and pest forecasting plant growth and development under *B. tabaci* presence.

3.2 Abstract

Although many crops have developed several adaptation mechanisms that allow them to defend against limiting factors, some biotic and abiotic stresses may cause reversible or irreversible changes in plants. Among the biotic stresses, the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is probably one of the main important pests that negatively affect several vegetable crops that are grown in greenhouses. The present study evaluated its impact on the morphology and physiology of two solanaceous plants, i.e., tomato (*Solanum lycopersicum* L.) and eggplant (*S. melongena* L.), under laboratory conditions. The results showed that, for tomatoes, plant height, shoot dry weight, leaf area, and indirect chlorophyll content were strongly reduced in infested plants, compared to the uninfested control, by 39.36%, 32.37%, 61.01%, and 37.85%, respectively. The same has been shown for eggplant, although the reduction percentages of plant height, root dry weight, and indirect chlorophyll content were less marked (i.e., 16.15%, 31.65%, and 11.39%, respectively). These results could represent interesting information for a better understanding of the *B. tabaci* influence on plant growth, as well as

for the development of management strategies to successfully control its infestations in a cropping system.

3.3 Keywords

Whitefly, vegetable crops, plant morphology, plant physiology, trophic interactions

3.4 Introduction

The economic importance of the plant family *Solanaceae* has been extensively discussed (Hawkes, 1999), as well as the role these plants have had in the progress of traditional cultures and civilizations (Barboza et al., 2016). Among them, tomato (*Solanum lycopersicum* L.) and eggplant (*S. melongena* L.) are very common vegetable crops, widely spread worldwide and known for their culinary, medicinal, and ornamental uses (Daunay et al., 2006; Barboza et al., 2016).

Plants interact with the environment, and any unfavorable conditions may impose stress and reduce their growth and development (Giordano et al., 2021). Although plants have developed several adaptation mechanisms that allow them to defend against limiting factors, biotic and abiotic stresses may generate reversible or irreversible changes in their morphology and physiology; in cultivated plants, this may lead to losses in crop production and yield (Žanić et al., 2018).

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera, Aleyrodidae) is a species complex with a worldwide distribution, considered a serious pest of many agricultural crops (Ying et al., 2003). Throughout its life cycle, it feeds on the leaves, underside of the host plants, causing direct and indirect damages by piercing leaves, sucking sap, and producing honeydew (on which sooty mould develop), as well as altering the growth, photosynthesis, and chemical and phenological processes (Nabity et al., 2009; Boopathi et al., 2015), in addition to transmitting various plant viruses (more than 350) that cause serious diseases (Polston et al., 2014). Indeed, *B. tabaci* consists of numerous genetically and biologically different cryptic species, more or less invasive, frequently with a different impact on many economically important crops (Ying et al., 2003; Li et al., 2014).

Control of *B. tabaci* is problematic because of the numerous generations it annually develops and its ability to rapidly acquire resistance to insecticides (Wang et al., 2017). Among various applicable control strategies, the use of tolerant varieties of the host plants is one of the cornerstones for the management of this pest (Hasanuzzaman et al., 2016). Many studies on the food preferences of *B. tabaci* and host suitability among different plant varieties have been carried out over the last years (Bernays et al., 1999; Naranjo, 2001; Shah and Liu, 2013; Hasanuzzaman et al., 2016; Žanić et al., 2018). Other research focused on the effect of plant development (e.g., plant age and size) on whitefly infestation and reproductive activity (Rodríguez-Álvarez et al., 2017). However, the combination of physiological and morphological changes, induced by *B. tabaci*, to the infested host plants is still poorly investigated. In the present study, the impact of the *B. tabaci* (MED) species, Q2 subclade, on some of the less investigated traits (e.g., indirect chlorophyll content, root dry weight, etc.) of whole eggplant and tomato plants was evaluated, in order to provide basic knowledge in the frame of research aimed at forecasting the growth and development of these plants under pest pressure.

3.5 Materials and methods

The study was carried out at the laboratories of the Applied Entomology section of Di3A (Department of Agriculture, Food and Environment), University of Catania, in the period December 2020–June 2021.

To assess the impact of *B. tabaci* on the host plants growth, fourteen young tomato plants (*Solanum lycopersicum* L. cv. Dovizio) and fourteen eggplant plants (*S. melongena* L. cv. Gloria), with six fully expanded leaves, were used in the test. Experimental plants were grown from seeds germinated and raised in polystyrene planting tray in the nursery. Then, the seedlings were individually transplanted into black plastic pots (10 cm x 10 cm x 12 cm), using a professional potting soil specific for vegetable sowing, and maintained under controlled environment at the laboratory (T = 25 ± 2°C; R.H. 65 ± 5% and photoperiod of 10L:14D h) throughout the experiment. Each potted plant was then confined in a netted cage (Length x Width x Height: 25 cm x 25 cm x 70 cm), representing a replication. Four weeks after transplanting, seven of each plant species were artificially infested, collecting ten pairs of newly emerged whitefly adults (<24 h old) from the insectarium and releasing them on the floor, as well as in the center of each cage. The whitefly adults were allowed to lay eggs for five days, before being removed from the cages by a mouth aspirator (John W. Hock Company, Gainesville, FL, United States); to ensure that oviposition had occurred, the number of eggs laid was counted on three leaves/plant, using a stereomicroscope (Olympus Optical Co., Ltd., Tokyo, JP, Japan, SZX-ILLK200). All plants were watered twice a week.

The *Bemisia tabaci* adults used in the experiment were originally collected in September 2020 from an eggplant crop grown under greenhouse in south-eastern Sicily (province of Ragusa, 36.97134 lat.; 14.424505 long.). The specimens were maintained on eggplant plants, reared in laboratory, under controlled environmental conditions (25 ± 2°C, RH 65 ± 5%, and a photoperiod of 14L:10D h). Before running the test, the species identity of *B. tabaci* has been genetically attained on about 30 whiteflies, collected from the rearing described above. To this aim, the total DNA was extracted from single individuals, following the method described by Walsh et al. (1991) and De Barro and Driver (1997). The mitochondrial cytochrome oxidase I (mt COI) gene (about 710 bp) was amplified using universal primers LCO1490 and HCO2198 (Folmer et al., 1994; Gautam et al., 2020; Song et al., 2021). For each sample, the 10 µL reaction volume contained 5 µL of FailSafe™ 2X PreMixes buffers (Lucigen, Middleton, WI, United States), 3.75 µL of DNA, 0.25 µL of taq polymerase, and 0.5 µL of each forward and reverse primer. The PCR was performed with initial denaturation at 96°C for 5 min, followed by 35 cycles, each consisting of denaturation for 45 s at 96°C, annealing for 60 s at 45°C, with final extension for one minute at 72°C, followed by final extension for 10 min, at 72°C. PCR-amplified products (10 µL) were visualized with 0.9% agar-gel electrophoresis (5 µL), and products with the target fragment were selected for sequencing. Successfully amplified DNA (5 µL) was purified and sequenced by BMR genomics. Identity of *B. tabaci* MED were based on more than 99% of the sequence similarity, obtained by NCBI blast comparison.

To assess the effects of *B. tabaci* on *S. lycopersicum* and *S. melongena* growth, the height of the plants, indirect chlorophyll content, fresh and dry plant biomass (roots and shoots), and leaf area were measured at the end of the experiment, when whitefly adults of the first generation were detected inside the cages (i.e., after about 28 days form the insect’s release). Plant height, expressed in centimeters, was measured with a ruler; the fresh weight of the plant was expressed in grams, cutting and weighting shoots and roots with a high precision balance (ORMA BC 1000, Orma srl, Milan, IT, Italy; resolution 0.1 g). Regarding dry weights, the biomass was oven-dried (Thermo Fisher Scientific™, Langenselbold, DE, Germany Heratherm OGS100) at 65°C, until a constant weight was reached in three days and, finally, weighed and expressed in grams, as well. The indirect chlorophyll content measurements were performed using a Soil Plant Analysis Development (SPAD-502, Minolta, Sakai (Osaka), Japan) chlorophyll meter on three leaves per plant, which were at the principal growth stage 1 leaf development, according to the BBCH scale (Meier, 1997). The obtained values, expressed in SPAD unit, proportionally reflects the amount of chlorophyll present in the leaf (Ling et al., 2011). The plant leaf area, expressed in cm^2, was determined by ImageJ software (Wayne Rasband—Services Branch, National Institute of Mental Health, Bethesda, MD, United States), which processed the photos shot by a digital camera (48-megapixel).

3.5.1 Data analysis

The impact of the whitefly on the host plants development was expressed as the percentage reduction of the values of the parameters considered, which were calculated as follows:

$$\% \text{ decrease} = \frac{\text{Uninfested plants value-Infested plants value}}{\text{Uninfested plants value}} \text{ x } 100$$

The data, related to the different plant's parameters selected, were subjected to analysis of variance (ANOVA), and mean comparisons were performed according to the Fisher's LSD test. Statistics were carried out by using the program Statistica (StatSoft, TIBCO Software Inc., Tulsa, OK, USA).

3.6 Results

Molecular analysis identified the species used in the experiment as *B. tabaci* MED, Q2 subclade, confirming the results obtained by Parrella et al. (2014), who asserted this as the most widespread species living on solanaceous in the Mediterranean area.

After five days from the release of the adults, the mean number of eggs laid on the lower surface of each of the three selected leaves were 46.11 ± 10.08 (average: 2.2 $eggs/cm^2$) and 51.33 ± 4.45 (average: 1.8 $eggs/cm^2$) for tomato and eggplant, respectively, confirming that an equivalent oviposition level occurred in both the host plant species (Figure 1).

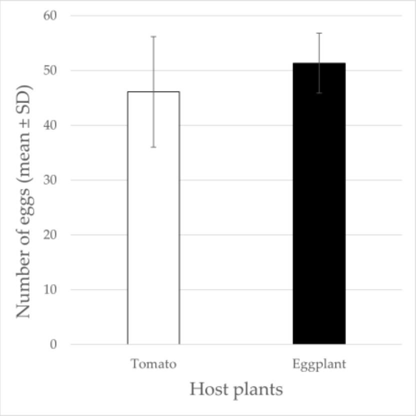
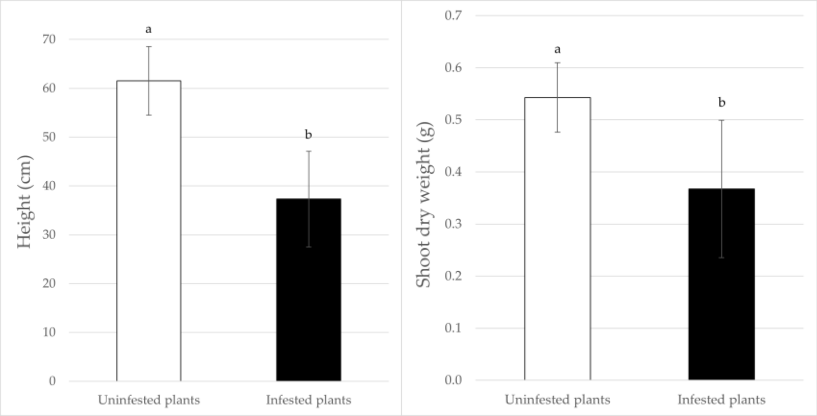


Figure 1 - Mean number of eggs laid on the lower surface of each of the three selected leaves per plant.

For tomatoes, the heights of the plants were significantly higher in the control ones (F_6 = 27.40; $p < 0.01$), with averages of 61.51 ± 9.80 and 37.30 ± 7.03 cm in the uninfested and infested ones, respectively (Figure 2a), with a reduction of 39.36%. Comparing the shoots dry weight of infested and uninfested plants, the data show a statistically different effect, too (F_6 = 7.58; $p < 0.05$) (Figure 2b), with a significant biomass reduction, in the presence of *B. tabaci*, equal to 32.37%. Similarly, the presence of the whitefly caused a reduction in the plant leaf area of 61.01%, with mean values that are statistically different, when infested and non-infested plants are compared (F_6 = 8.10; $p < 0.05$) (Figure 3a).

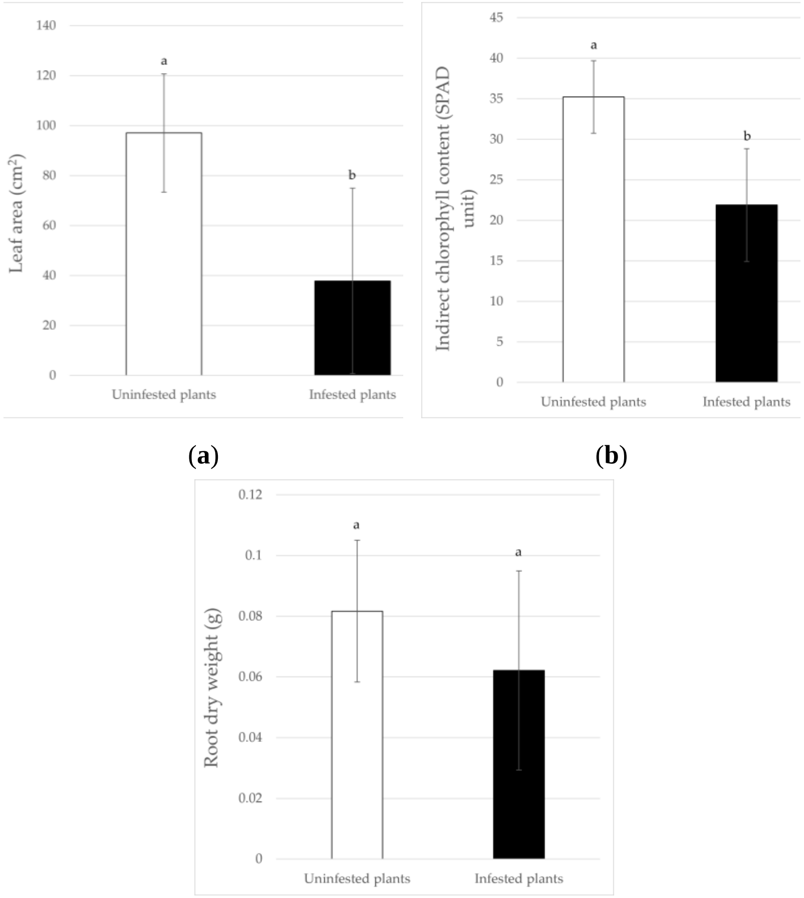


(**a**) (**b**)

Figure 2 - Incidence of *B. tabaci* infestation on the height (**a**) and shoot dry weight (**b**) of tomato plants. Different letters indicate statistically significant differences at $p < 0.01$ (**a**) and $p < 0.05$ (**b**).

As expected, the SPAD values followed the same trend: in fact, the indirect chlorophyll content was significantly influenced ($F_6 = 61.48$; $p < 0.01$) by whitefly infestation that, overall, caused a 37.85% reduction (Figure 3b). By contrast, the root dry weight was not significantly affected by the presence of the whitefly (Figure 3c).

For eggplant, the results showed that plant height, root dry weight, and indirect chlorophyll content were significantly different between the treatments. In infested plants, the height (28.53 ± 2.22 cm) and root dry weight (0.08 ± 0.02 g) were significantly lower ($F_6 = 36$; $p < 0.01$ and $F_6 = 30.24$; $p < 0.01$) than in uninfested plants, with reduction percentages of 16.55% and 31.65%, respectively (Figures 4a and 5c).

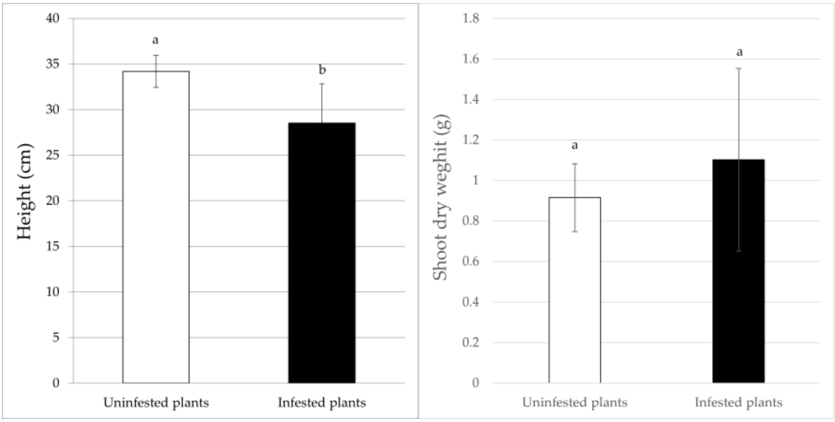


(**a**) (**b**)

(**c**)

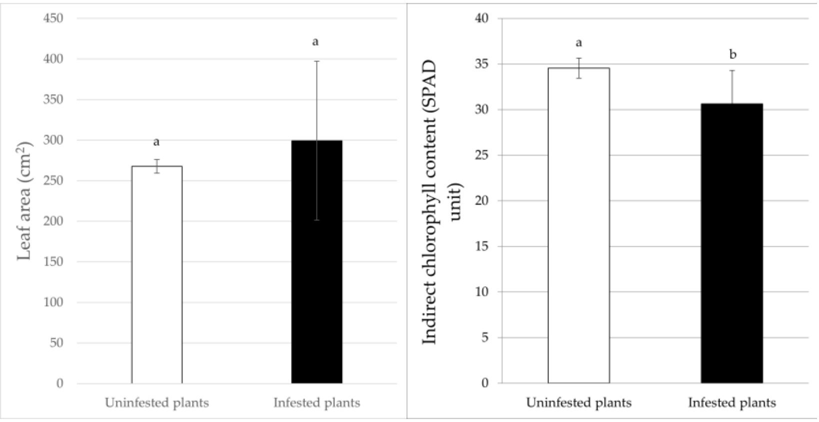
Figure 3 - Effects of *B. tabaci* infestation on the leaf area (**a**), indirect chlorophyll content (**b**), and root dry weight (**c**) of tomato plants. Different letters indicate statistically significant differences at $p < 0.05$ (**a**) and $p < 0.01$ (**b**).

Similarly, the indirect chlorophyll content values followed the same trend; in fact, the whitefly infestation significantly reduced this parameter ($F_6 = 14.66$; $p < 0.01$) by 11.39%, with an average of 30.61 ± 3.67 SPAD units in the infested plants (Figure 5b). By contrast, the leaf area data and shoot dry weight were not significantly affected by the presence of the whitefly (Figures 4b and 5a).

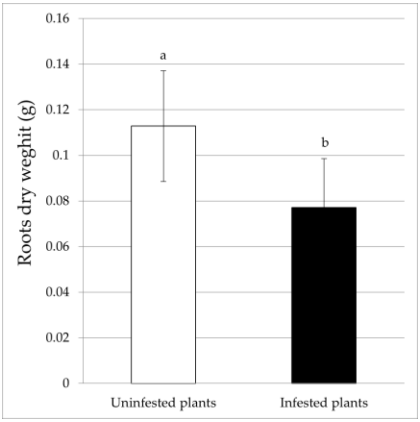


(**a**) (**b**)

Figure 4 - Incidence of *B. tabaci* infestation on the height (**a**) and shoot dry weight (**b**) of eggplant plants. Different letters indicate statistically significant differences at $p < 0.01$.



(**a**) (**b**)



(**c**)

Figure 5 - Effects of *B. tabaci* infestation on the leaf area (**a**), indirect chlorophyll content (**b**), and root dry weight (**c**) of eggplant plants. Different letters indicate statistical differences at $p < 0.01$.

3.7 Discussion

It is well-known that leaves represent the major organ for solar radiation interception and photosynthetic sources in plants; in fact, plant growth and physiological processes controlling yield and dry matter production highly depend on their health and activity.

B. tabaci is one of the most harmful insect pests, due to its direct and indirect injures to plants, which affect the yield and quality of products (Nabity et al., 2009; Boopathi et al., 2015; Padilha et al., 2021).

The present research shows how *B. tabaci* MED infestation has a significant impact on those parameters less investigated, so far (e.g., indirect chlorophyll content, root dry weight, etc.), of whole eggplant and tomato plants. In fact, previous works, dealing with *B. tabaci* MED, only consider physiological (e.g., gas exchange and chlorophyll fluorescence) and biochemical (enzymes, phenols, and flavonoids, only on leaves) aspects, but not the combined effects of whitefly infestation on plant morphology (Buntin et al., 1993; de Lima Toledo et al., 2021). In regard to the height of the two solanaceous plants, our results are in line with the findings by Islam et al. (2010), who reported that some plant-growth parameters of three eggplant varieties were negatively affected by *B. tabaci*, with a maximum reduction percentage of plant height equal to 20.6% in the “Dafeng” variety. According to Li et al. (2013), infestation by *B. tabaci* in the Middle East–Asia Minor 1 (MEAM1) species significantly inhibited the growth of tobacco plants, with plant height reductions of 28.5% and 32.7%, noted at 14 and 20 days after the start of infestation, respectively, compared to the uninfested control plants.

In particular, in our test, the shoot dry weight in tomato plants was reduced by the presence of the whitefly. Comparable findings were observed in tobacco leaves whose dry weight was significantly reduced after infestation by *B. tabaci* MEAM1 (Li et al., 2013).

Moreover, we recorded how the indirect chlorophyll content values were greater in uninfested tomato and eggplant plants, when compared with the infested ones. Buntin et al. (1993) described that feeding activity, by both *B. tabaci* nymphs and adults, reduces leaf chlorophyll content and negatively affects the rates of leaf transpiration and photosynthesis in tomato plants. Sap suction, by the whitefly, induces chlorosis and increases stomatal resistance, which is negatively associated with photosynthetic rates, because leaf stomatal resistance indicates closure of stomata and limited gas exchange (Islam et al., 2010). Contextually, the eggs being laid on the lower surface of the leaves by the whitefly females significantly decrease the stomatal conductance, because they cover the stomata and block their access to light and carbon dioxide (Chand et al., 2018). Similar results were also observed in other studies, in which *B. tabaci* infestation reduced cotton foliar photosynthetic rates (Yee et al., 1996; Lin et al., 1999; Lin et al., 2000).

In our experiment, the leaf area in tomato plants was statistically different between the infested and uninfested control. As to this aspect, Chand et al. (2018) reported how eggs deposition by *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae) on cassava (*Manihot esculenta* Crantz) reduces the effective area of leaves, lowering the overall productivity. By contrast with what has been reported on eggplant by Islam and Shunxiang (2009), who states that the whitefly presence lowered the effective leaf area (although no mention is provided on the density of whitefly), in our study, the leaf area and shoot dry weight of eggplant were not affected by the presence of the insect. This can be explained by assuming that, in our experiment, the lack of significant effects on both these parameters could be related to the low density of the whitefly. Indeed, the density of 2.2 eggs/cm^2, recorded on the smaller leaves of tomatoes, was enough to negatively affect its leaf area and shoot dry weight, compared to what we noted on eggplant, where an average density of 1.8 eggs/cm^2 did not affect these parameters. It is, in fact, well-known that leaf size and shape can vary from plant-to-plant, as well as among varieties, and this may differently affect the tolerance to whitefly infestation among different host plant species (Peeters et al., 2007; Caldwell et al., 2016; Hasanuzzaman et al., 2016; Žanić et al., 2018; Schutze et al., 2022).

3.8 Conclusions

The present research points out that one single generation of *B. tabaci* moderate infestation can lead to an important impact on both the morphology and physiology of tomato and eggplant plants. The overall results from this work are useful to describe the trophic relations between plants and *B. tabaci* MED. However, further investigations are needed to analyze the preferences and behavior of the whitefly on host plants, as well as identifying the possible role played by volatile organic compounds and nutritional quality of the selected plant varieties in influencing the activity of this insect.

3.9 Declarations

3.9.1 Author contributions

Conceptualization, C.R., A.F., A.C.B. and P.S.; methodology, A.F., A.C.B., P.S., G.E.M.C. and C.R.; formal analysis, G.L., A.F. and P.S.; investigation, A.F. and P.S.; writing—original draft preparation, A.F., C.R. and A.C.B.; writing—review and editing, C.R., A.F., P.S. and G.E.M.C. All authors have read and agreed to the published version of the manuscript.

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3.9.3 Institutional review board statement

Not applicable.

3.9.4 Informed consent statement

Not applicable.

3.9.5 Data availability statement

The data presented in this study are available on request from the corresponding author.

3.9.6 Conflicts of interest

The authors declare no conflict of interest.

4 Can *Macrolophus pygmaeus* (Hemiptera: Miridae) mitigate the damage caused to plants by *Bemisia tabaci* (Hemiptera: Aleyrodidae)?

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4.1 Simple summary

The whitefly *Bemisia tabaci* is an invasive pest that causes extensive damage to many vegetable crops and ornamental plants. To control this pest, the release of natural enemies has become increasingly important as an ecologically safe and effective method of biological control. Some species in the family Miridae are effective at controlling whitefly populations, but because they feed on both insect prey and plant tissue, their overall effect on plant performance is not well understood.

In this study, the impact of the mirid predator *Macrolophus pygmaeus* on the morphological and physiological traits of *Solanum melongena* in the presence of *Bemisia tabaci* was evaluated. Overall, the results show how the presence of this natural enemy mitigates the damage caused by whitefly infestations. They also help to clarify the multitrophic relationships between plant, pest, and natural enemy, enabling the prediction of plant development in the presence of both pest and predator.

4.2 Abstract

Nowadays, in protected vegetable crops, pest management based mainly on biological control represents the most sustainable alternative to pesticide use. The cotton whitefly, *Bemisia tabaci*, is one of the key pests that negatively impact the yield and quality of such crops in many agricultural systems. The predatory bug *Macrolophus pygmaeus* is one of the main natural enemies of the whitefly and is widely used for its control. However, the mirid can sometimes behave as a pest itself, causing damage to crops. In this study, we investigated the impact of *M. pygmaeus* as a plant feeder, by analyzing the combined impact of the whitefly pest and the predator bug on the morphology and physiology of potted eggplants under laboratory conditions. Our results showed no statistical differences between the

heights of plants infested by the whitefly or by both insects compared with noninfested control plants. However, indirect chlorophyll content, photosynthetic performance, leaf area, and shoot dry weight were all greatly reduced in plants infested only by *B. tabaci*, compared with those infested by both pest and predator or with noninfested control plants. Contrarily, root area and dry weight values were more reduced in plants exposed to both of the insect species, compared with those infested only by the whitefly or compared with noninfested control plants, where the latter showed the highest values. These results show how the predator can significantly reduce the negative effects of *B. tabaci* infestation, limiting the damage it causes to host plants, though the effect of the mirid bug on the underground parts of the eggplant remains unclear. This information might be useful for a better understanding of the role that *M. pygmaeus* plays in plant growth, as well as for the development of management strategies to successfully control infestations by *B. tabaci* in cropping environments.

4.3 Keywords

Whitefly, predator, zoophytophagy, trophic interactions, plant morphology, plant physiology

4.4 Introduction

Multitrophic interactions, understood as relationships between organisms across different trophic levels of a food web (Tscharntke and Hawkins, 2002; Gange and Brown, 2009), are gaining growing interest in ecological studies. Especially in the agricultural sector, which is increasingly oriented to achieve a reduction in chemical inputs, complex interactions that involve the binding of different organisms, living both above and below the ground, with the cultivated plants are now recognized (Ohgushi, 2005; Shikano, 2017; Jacobsen and Raguso, 2018). Improving our understanding of these relationships may help in planning a rational management of the populations involved, and it may lead to a reduction in pest infestations and their negative effects (Jaber and Araj, 2018; Zytynska and Meyer, 2019; Schifani et al., 2020).

Bemisia tabaci (Gennadius) is a notable insect pest that affects vegetable crops and many ornamental plants. It causes damage directly by the piercing of leaves, the suction of sap, and the production of honeydew on which sooty molds develop (Gerling, 1990) and also indirectly through its ability to transmit phytopathogenic viruses to numerous host plants (Martin et al., 2000). Over 100 virus species belonging to the Begomovirus, Carlavirus, Crinivirus, Ipomovirus, and Torradovirus groups are presently known to be transmitted by *B. tabaci* (Navas-Castillo et al., 2011; Fiallo-Olivé et al., 2020), causing worldwide economic damage, the value of which is difficult to estimate. For a long time, *B. tabaci* was considered as a single species, but variability among its populations has led scholars to consider *B. tabaci* as a complex of more than 40 species that are morphologically indistinguishable but that differ in their biology (host range, development performance, suitable environmental conditions, virus transmission capability, etc.) and geographic distribution (De Barro et al., 2011; Liu et al., 2012; Lee et al., 2013; Boykin et al., 2017).

Numerous studies have examined the interactions of *B. tabaci* with other components of its food web and how these influence the population levels of the insect, as well as the negative impact of the insect on the host plants. For instance, regarding the interactions of *B. tabaci* with other pests, Tan et al. (2014) showed how the infestation of tomato plants by the green peach aphid *Myzus persicae* (Sulzer) had a negative impact on *B. tabaci* development, indicating that the latter clearly prefers to settle on plant leaves not infested by the aphid. A deeper interaction between these two hemipteran species has also been demonstrated by the effect of preinfesting tomato plants with *M.*

persicae on the feeding dynamics of *B. tabaci* and on the acquisition and transmission mechanisms of TYLCV (tomato yellow leaf curl virus) by this vector—showing a clear and significant influence of aphid preinfestation on the tomato–whitefly–TYLCV system (Tan et al., 2017).

At a higher trophic level, whitefly feeding induces plant defence responses, which affect more-complex interactions with natural enemies. Thus, in the whiteflies–aphids system on tomato, a preinfestation with *B. tabaci* MEAM1 impacts the predation ability of the ladybird *Propylea japonica* (Thunberg) on *M. persicae* (Yang et al., 2017). At the top of the feeding pyramid, interactions between whiteflies and natural enemies can lead to cases of intraguild predation, which can lower their effectiveness. For instance, the overall predation on the whitefly was reduced when the two mirid bugs, *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* Reuter, occurred together on the same plant (Moreno-Ripoll et al., 2014). On the other hand, when feeding on plants, the two zoophytophagous mirids stimulate plants to release volatile organic compounds (VOCs), which repel pests, such as *B. tabaci* and *Frankliniella occidentalis* Pergande, but attract whitefly parasitoids, such as *Encarsia formosa* Gahan (Pérez-Hedo et al., 2022).

The complex interactions between plants and phytophagous insects, from which physiological, morphological, or behavioral plasticity derives in both hosts and herbivores, have been the focus of numerous studies (Ashra and Nair, 2022). For *B. tabaci*, interactions with plants are extremely important, and they are based on the attractiveness of plants, which is communicated to whiteflies by both visual and (to a lesser extent) biochemical cues. The nutrient composition of host plants therefore impacts whitefly performance, such that nutrient changes or stresses in plants affect nutrition in whiteflies (Dinant and Kehr, 2013). In turn, whitefly feeding alters the physiology and morphology of plants, causing changes in physiological (e.g., gas exchange, chlorophyll fluorescence, indirect chlorophyll content), biochemical (e.g., enzymes, phenols, flavonoids), or morphological (e.g., plant height, leaf area, shoot dry weight, root dry weight) parameters (Buntin et al., 1993; Yee et al., 1996; Lin et al., 1999; Lin et al., 2000; Islam et al., 2009; Islam et al., 2010; Li et al., 2013; Chand et al., 2018; de Lima Toledo et al., 2021). Most of these phenomena remain poorly understood and therefore need to be more deeply investigated in order to improve sustainable whitefly management.

In one previous work, the impact of *B. tabaci* MED on eggplants and tomatoes was investigated. This study considered the principal morphological and physiological traits (e.g., plant height, dry plant biomass, chlorophyll content, etc.) (Farina et al., 2022) and found that eggplant and tomato plants infested by the whitefly showed strong and significant reductions in height, shoot dry weight, leaf area, and indirect chlorophyll content, though with different levels of intensity among the two plant species. Starting from the above results, and in order to widen our knowledge of multitrophic interaction mechanisms related to the impact of whiteflies on plant biology, a further trophic level in the analysis was added.

In the Mediterranean basin, *M. pygmaeus* spontaneously colonizes tomato crops when pesticide applications are reduced (Urbaneja et al., 2012; De Backer et al., 2014). However, as a zoophytophagous insect, it can also feed on the mesophyll of leaves, the tissues of stems, inflorescences, and fruits (Wheeler, 2001; Ingegno et al., 2011); the suitability of this predator for establishment also varies depending on the species of the host plant and the part of the plant on which the predator lives (Sanchez et al., 2018). Because of this, and bearing in mind the considerable diffusion of this mirid bug in horticultural areas of the Mediterranean basin, we sought to investigate the effects on the morphology and physiology of eggplants through the combined action of *B. tabaci* and *M. pygmaeus*, and we evaluated the modifications to various morphological and physiological parameters of host plants following infestation by *B. tabaci* MED and by a combined presence of this whitefly with its predator *M. pygmaeus*, compared with totally noninfested plants.

4.5 Materials and methods

4.5.1 Insects and plants

A colony of *Bemisia tabaci* was collected from an eggplant crop grown under greenhouse conditions in southeast Sicily (Vittoria, province of Ragusa, 36.97134 lat.; 14.424505 lon.). The specimens were then transferred and maintained on eggplant plants in the laboratory under controlled environmental conditions (T = 25 ± 2°C, RH = 65 ± 5%, and photoperiod of 14L:10D h).

Macrolophus pygmaeus individuals came from commercial sources (MIRICAL®; Koppert Biological Systems, S.L., Águilas, Murcia, Spain). These were maintained in the laboratory, under the same environmental conditions as those for *B. tabaci,* on eggplants infested by the whitefly. These were also fed every 3 days with eggs of *Ephestia kuehniella* Zeller (Koppert B.V., Berkel en Rodenrijs, BE, The Netherlands).

Host plants (*Solanum melongena* L. cv. Gloria) were grown from seeds germinated and raised in polystyrene planting trays in a nursery. The seedlings were then individually transplanted into black plastic pots (10 cm x 10 cm x 12 cm), using a professional potting soil specific for vegetable sowing, and maintained under controlled environmental conditions in the laboratory (T = 25 ± 2°C; R.H. = 65 ± 5%, and photoperiod of 14L:10D h) throughout the experiment.

4.5.2 Experimental design and sampling

The study was carried out at the laboratories of the Applied Entomology section, Department of Agriculture, Food and Environment (Di3A), University of Catania, Italy, in the period

October 2021–January 2022.

The species identity of *B. tabaci* was genetically attained on about 30 whiteflies collected from the rearing described above, before running the test. Using molecular biological methods (Walsh et al., 1991; De Barro and Driver, 1997), all tested individuals were identified as *B. tabaci* MED, Q2 subclade.

The impact of *B. tabaci* and *M. pygmaeus* on the host plants was assessed on a total of 36 eggplant plants with 6 fully expanded leaves. The trial was set up using a completely randomized design with 12 replicates under each of the following three evaluated conditions (hereafter treatments): noninfested control plants (C); plants infested by *B. tabaci* (PIB); and plants infested by *B. tabaci* where *M. pygmaeus* was also released (PIBM). In order to infest the plants representing the PIB and the PIBM treatments, 4 weeks after transplanting groups of three plants were isolated in netted cages (L x W x H: 60 cm x 60 cm x 60 cm), and 60 unsexed newly emerged (<24 h old) whitefly adults (i.e., 20/plant) collected from the insectary were released on the floor in the center of each cage. The whitefly adults were allowed to lay eggs for 3 days before being removed from the cages by a mouth aspirator (JohnW. Hock Company, Gainesville, FL, USA).

Next, to assess whether oviposition had occurred, the number of eggs laid was counted on three leaves of each plant, using a stereomicroscope (Olympus Optical Co., Ltd., Tokyo, Japan, SZX-ILLK200). To verify the progress of the infestation, the nymphs fixed on the lower surface of each of the three previously selected leaves were checked 2 weeks after removal of the adults. In line with procedures described in the literature (Calvo and Urbaneja, 2004; Alomar et al., 2006), 3 weeks after oviposition by *B. tabaci*, 24 unsexed newly emerged *M. pygmaeus* adults (<24 h old) were released on the floor in the center of each cage to test the “pest + zoophytophagous predator” condition (PIBM) (i.e., 8/plant). The mirid adults were allowed to lay eggs for 6 days (Margaritopoulos et al., 2003) before being removed from the cages. The monitoring of nymphs' emergence started 8 days after their release (Moerkens et al., 2017) and continued daily for 5 weeks. All newly emerged specimens were also fed with eggs of *E. kuehniella*, glued on a paper strip and provided every 3 days. By following the method described by Sanchez et al. (2021), these specimens were counted visually on all entire eggplant plants and were removed from the cages using the mouth aspirator at the end of the experiment (i.e., after about 40 days from the introduction of *M. pygmaeus*). All plants were watered twice a week.

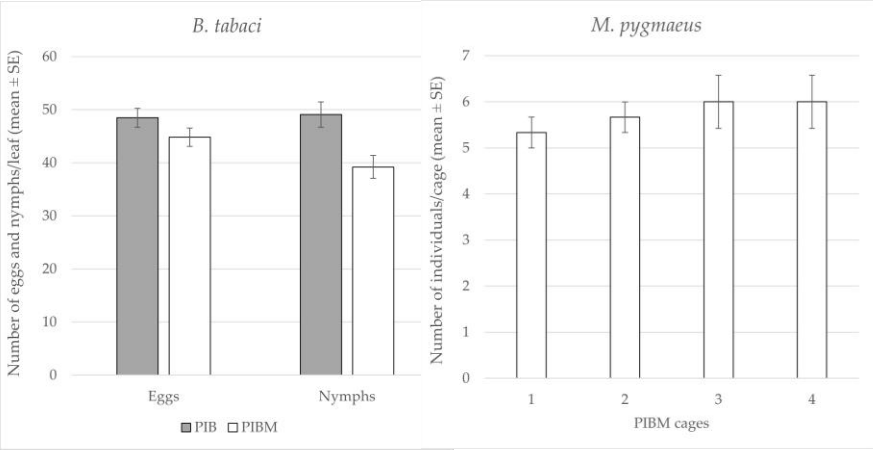
To assess the combined effects of both insects on *S. melongena* development, the height of the plants (PH), the indirect chlorophyll content (ICC), the chlorophyll fluorescence (CF), the dry plant biomass (roots and shoots: RDW and SDW), and the leaf and root areas (LA and RA) were all measured at the end of the experiment. Plant height, expressed in centimeters (cm), was measured from stem base to apex (Bresch et al., 2014) with a ruler. To obtain values for RDW and SDW, expressed in grams, the fresh hypogeal and epigeal biomass was ovendried (Thermo Fisher Scientific, Langenselbold, Germany, Heratherm OGS100) at 65°C for 3 days and finally weighed with a high-precision balance (ORMA BC 1000, Orma srl, Milan, Italy; resolution 0.1 g). To calculate the amount of chlorophyll present in the leaf (Ling et al., 2011); ICC measurements were taken using a Soil Plant Analysis Development (SPAD-502, Minolta, Sakai, Osaka, Japan) chlorophyll meter on three leaves per plant, which were at principal growth stage 1, according to the BBCH scale (Meier, 1997). To measure plant stress, the CF data were collected using an OS1-FL Chlorophyll Fluorometer (Opti-Sciences Inc., Hudson, NH, USA). Initially, a leaf in the middle third of each plant was dark-adapted for at least 20 min with detachable leaf clips. Next, the device emitted a saturation pulse through a beam of light, which was read by the device when reflected (Schutze et al., 2022). The parameter considered in our experiment was F_V/F_M, which is the ratio of the variable to the maximum fluorescence after dark-adaptation, which provides information on the functioning of photosystem II (PSII), representing the maximum quantum yield of PSII (Ibaraki and Murakami, 2006). In other words, it is a sensitive indicator of plant photosynthetic performance that enables the comparison of plant samples of the same known dark-adapted state using a normalized ratio (Fernández-Marín et al., 2019). The LA and RA of plants were expressed in square centimeters (cm^2) and determined by ImageJ software (Wayne Rasband—Services Branch, National Institute of Mental Health, Bethesda, MD, USA), which processed the pictures taken by a digital camera (Nikon D850 45.4 megapixel). To monitor the stress state of the plants, ICC and CF were performed monthly for the duration of the test (Schutze et al., 2022).

4.5.3 Data analysis

Data of parameters relating to the responses of different plants to the three tested conditions were analyzed using a one-way ANOVA. Where significant differences were detected, the means were separated by Tukey's HSD test ($p < 0.05$). Statistical analysis was carried out using the program Statistica (StatSoft, TIBCO Software Inc., Tulsa, OK, USA).

4.6 Results

The mean number of eggs laid by the whitefly adults on the lower surface of each of the three selected leaves per plant after 3 days of exposition was 46.6 ± 6.94 (average: 2.2 eggs/cm^2). Further, 17 days after the adults were released, the average number of nymphs was 44.1 ± 9.98 (mean: 2.1 nymphs/cm^2). These results confirmed that oviposition and the progress of infestation evenly occurred in all plants under both PIB and PIBM conditions (Figure 1a). The average total density of *M. pygmaeus*, expressed as the number of specimens per plant 40 days after release, was 5.7 ± 0.7 insects/plant (Figure 1b). About 2 weeks after the release of *M. pygmaeus*, its first-instar nymphs appeared, and these became adults by the end of the experiment.



(**a**) (**b**)

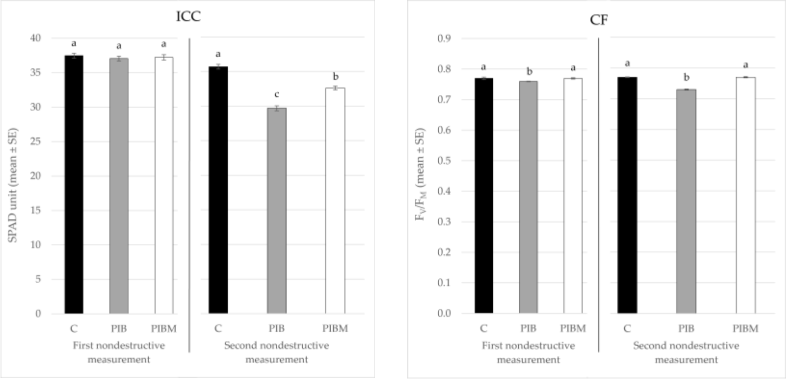
Figure 1 - Mean number (±SE) of (**a**) *B. tabaci* eggs laid and nymphs fixed on the lower surface of each of the three selected leaves per plant under both PIB and PIBM conditions and (**b**) *M. pygmaeus* specimens per PIBM cage.

The stress state of plants during the test was indicated by the first nondestructive measurement taken 1 month after the beginning of the experiment. This showed no statistical differences in the ICC values (Table 1) between C, PIB, and PIBM modalities ($F_{2,33} = 0.32$; $p < 0.727$) (Figure 2a). In contrast, CF values recorded in the same period (Table 1) showed significant differences between PIB and the other two conditions, C and PIBM ($F_{2,33} = 4.89$; $p < 0.0138$) (Figure 2b). Starting from the second measurement carried out 2 months after the beginning of the test, the ICC parameter was influenced by the presence of the two insects (Table 1), revealing statistical differences among the three conditions ($F_{2,33} = 86.31$; $p < 0.001$) (Figure 2a). However, with respect to the CF parameter (Table 1), in the second nondestructive measurement, the statistical differences found in the first measurement were maintained ($F_{2,33} = 91.9$; $p < 0.001$), with the lowest mean values recorded in plants infested by *B. tabaci* (Figure 2b).

Table 1. Stress state of plants recorded during the first and second nondestructive measurements, as indicated by the calculation of the amount of chlorophyll present in the leaf (ICC) and by calculation of the plant's photosynthetic performance (CF).

Treatment	1st ICC (SPAD unit ± SE)	2nd ICC (SPAD unit ± SE)	1st CF (F_V/F_M ± SE)	2nd CF (F_V/F_M ± SE)
C	37.48 ± 0.36 a	35.68 ± 0.34 a	0.77 ± 0.004 a	0.77 ± 0.001 a
PIB	37.07 ± 0.33 a	29.68 ± 0.35 c	0.76 ± 0.001 b	0.73 ± 0.002 b
PIBM	37.23 ± 0.39 a	32.61 ± 0.27 b	0.77 ± 0.002 a	0.77 ± 0.002 a
F; df; *p*	0.32; 2, 33; < 0.727	86.31; 2, 33; < 0.001	4.89; 2, 33; < 0.0138	91.9; 2, 33; < 0.001

ANOVA parameters are reported for each test condition. Within each column, data followed by different letters are significantly different ($p < 0.05$, Tukey's HSD test).



(**a**) (**b**)

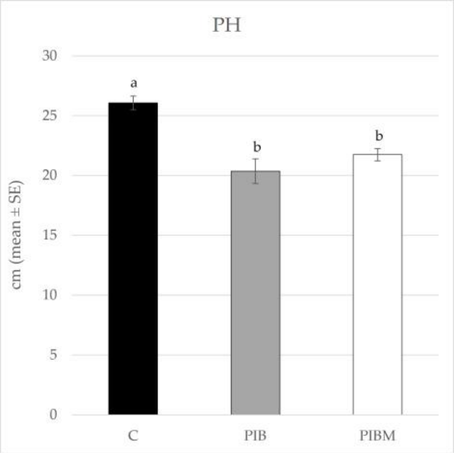
Figure 2 - Mean values (±SE) of (**a**) the indirect chlorophyll content (ICC) and (**b**) the chlorophyll fluorescence (CF) detected in the first and second nondestructive measurements performed on noninfested control plants (C), eggplants infested by *B. tabaci* (PIB), and host plants in the presence of both *B. tabaci* and *M. pygmaeus* (PIBM). Different letters indicate statistically significant differences at $p < 0.05$.

At the end of the experiment, the values for all considered plant physiology and morphology parameters showed statistical differences between the conditions examined, as reported in Table 2. Overall, plant height (PH) was negatively affected ($F_{2,33} = 16.297$; $p < 0.001$) by the presence of both the insect species, with higher mean values recorded in the noninfested plants (Figure 3a).

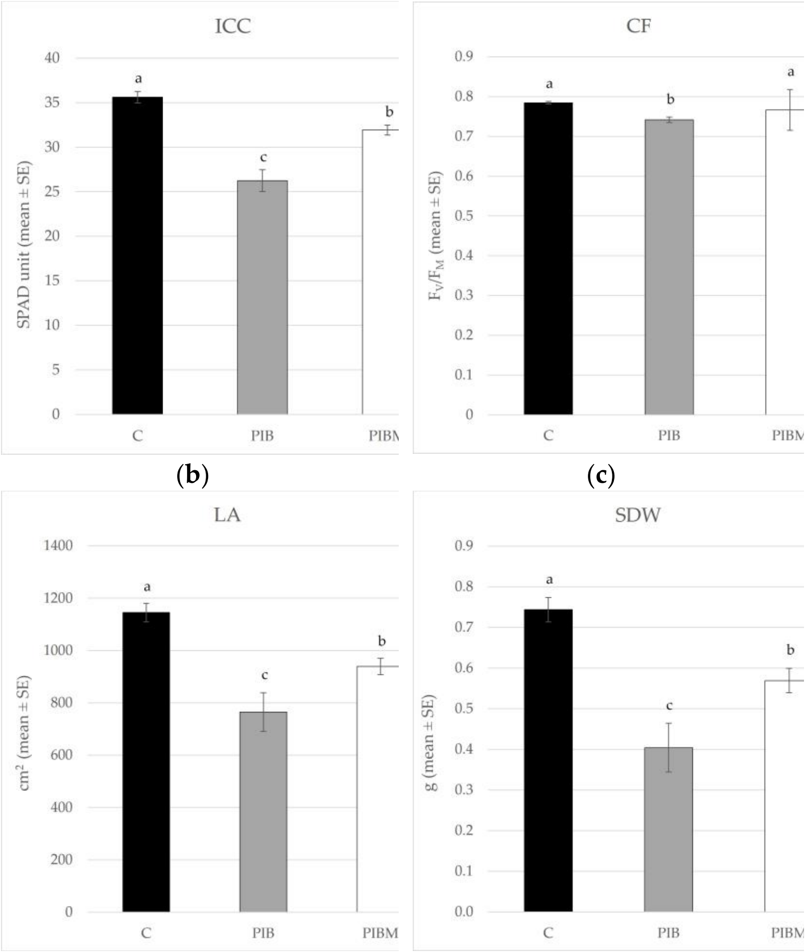
Table 2. Impact of the biological activity of *Bemisia tabaci* (PIB), either alone or in association with *Macrolophus pygmaeus* (PIBM), on the main morphological and physiological parameters of eggplants. (PH—plant height; ICC—indirect chlorophyll content; CF—chlorophyll fluorescence; LA—leaf area; RA—root area; SDW—dry shoot biomass; RDW—dry root biomass).

Treatment	**PH (cm ± SE)**	**ICC (SPAD unit ± SE)**	**CF (F_V/F_M ± SE)**	**LA (cm^2 ± SE)**	**SDW (g ± SE)**	**RA (cm^2 ± SE)**	**RDW (g ± SE)**
C	26.07 ± 0.57 a	35.60 ± 0.64 a	0.78 ± 0.003 a	1141.49 ± 35.67 a	0.74 ± 0.03 a	214.90 ± 13.69 a	0.13 ± 0.01 a
PIB	20.35 ± 1.03 b	26.23 ± 1.23 c	0.74 ± 0.007 b	765.17 ± 74.21 c	0.40 ± 0.06 c	178.91 ± 11.38 b	0.11 ± 0.01 b
PIBM	21.73± 0.51 b	31.91 ± 0.56 b	0.77 ± 0.051 a	939.17 ± 31.81 b	0.57 ± 0.03 b	149.83 ± 9.73 b	0.08 ± 0.001 b
F; df; *p*	16.297; 2, 33; < 0.001	29.728; 2, 33; < 0.001	16.159; 2, 33; < 0.001	13.45; 2, 33; < 0.001	17.12; 2, 33; < 0.001	7.74; 2, 33; < 0.0017	6.42; 2, 33; < 0.0044

ANOVA parameters are reported for each test condition. Within each column, data followed by different letters are significantly different ($p < 0.05$, Tukey's HSD test).

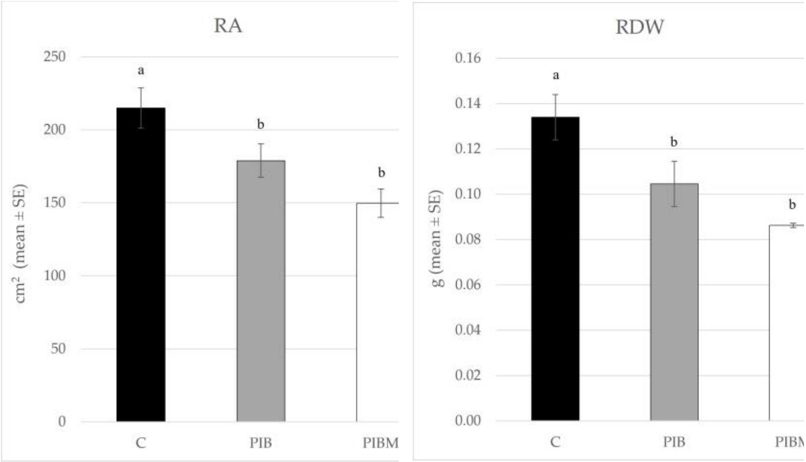


(**a**)


ICC
SPAD unit (mean ± SE)
40
35
30
25
20
15
10
5
0
a
c
b
C
PIB
PIBM
CF
F_V/F_M (mean ± SE)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
a
b
a
C
PIB
PIBM
LA
cm² (mean ± SE)
1400
1200
1000
800
600
400
200
0
a
c
b
C
PIB
PIBM
SDW
g (mean ± SE)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
a
c
b
C
PIB
PIBM

(**b**) (**c**)

(**d**) (**e**)



(**f**) (**g**)

Figure 3 - Mean values (±SE) of (**a**) plant height (PH), (**b**) indirect chlorophyll content (ICC), (**c**) chlorophyll fluorescence (CF), (**d**) leaf area (LA), (**e**) shoot dry weight (SDW), (**f**) root area (RA), and (**g**) root dry weight (RDW) of noninfested control plants (C), host plants infested by *B. tabaci* (PIB), and eggplants in the presence of both whitefly and *M. pygmaeus* (PIBM). Different letters indicate statistically significant differences at $p < 0.01$.

The release of *B. tabaci* (PIB) caused a clear detrimental effect on indirect chlorophyll content (ICC) in the leaves of infested plants, which was statistically different when compared with the other two tested modalities ($F_{2,33} = 29.728$; $p < 0.001$); indeed, in the presence of the predators (PIBM), the plants exhibited a slightly higher chlorophyll content, though it was still less than that of the noninfested plants (C) (Figure 3b).

Similarly, the CF values indicating the plant's photosynthetic performances followed a broadly similar trend; in this case, a data analysis revealed a statistically significant decrease in the CF values ($F_{2,33} = 16.159$; $p < 0.001$) of the PIB treatment in comparison with PIBM treatment (i.e., when the predator was released in the cage) that was not statistically different from the noninfested plants of the C condition (Figure 3c).

Even in the case of the leaf area (LA) of plants, there was a statistical difference between the three tested conditions ($F_{2,33} = 13.45$; $p < 0.001$); in particular, the lowest mean values of the PIB condition suggested that the presence of the predator limited the damage caused by the whitefly (Figure 3d).

Similarly, in the case of the SDW parameter, the analysis revealed a statistical difference among plants under the three conditions ($F_{2,33} = 17.12$; $p < 0.001$) (Figure 3e), where those of the PIB condition, in the absence of *M. pygmaeus*, once again exhibited the lowest values.

As was the case with plant height, the root area and root dry weight (i.e., RA and RDW) were also negatively influenced by the presence of both insect species. In particular, the highest mean values were recorded in the noninfested plants, with statistically significant differences in comparison with the plants of the other two treatments ($F_{2,33} = 7.74$; $p < 0.0017$ and $F_{2,33} = 6.42$; $p < 0.0044$—for RA and RDW, respectively) (Figure 3f,g).

4.7 Discussion

Integrated pest management aims to guide agriculture strategies by controlling arthropod infestations through the optimal selection of host plants (Magagnoli et al., 2018) and management of the activity of natural enemies (Stout et al., 2002). In this context, the responses of host plants to the presence of pests have often been studied, but it remains unclear how plants respond to the zoophytophagy of predatory omnivorous insects (Pappas et al., 2015).

The present research indicates that the mirid predator *M. pygmaeus* exerts a significant influence on some physiological and morphological traits of *S. melongena* (e.g., indirect chlorophyll content, chlorophyll fluorescence, root area and dry weight, etc.) that have been poorly investigated so far.

It is well known that infestation by *B. tabaci* affects the quantity and quality of yields in many varieties of vegetable crops (Perdikis and Lykouressis, 2004). The means by which whitefly infestations exert a negative effect on horticultural species (eggplant and tomato) have also been confirmed in a recent study (Farina et al., 2022). The findings reported here show that the presence of *M. pygmaeus* reduces the negative effects of the whitefly on plants (Bresch et al., 2014; Sanchez et al., 2018), resulting in significantly higher values of indirect chlorophyll content, chlorophyll fluorescence, leaf area, and shoot dry weight in the PIBM condition compared with the PIB. In this regard, according to Pappas et al. (2015), zoophytophagous hemipterans, such as *Orius insidiosus* (Say) and pentatomids, feed on plants mainly to acquire water from the xylem and also potentially to obtain nutrients from the mesophyll or pollen, most likely causing only some minor cell wounding on leaves. Feeding by mirid bugs therefore depends on the plant sap and not only on prey. This explains the greater suitability of the eggplant compared with other vegetable crops, which results in a relatively longer survival of the predator even in the absence of prey (Perdikis and Lykouressis, 2004). However, it is also known that plant sap may influence the taste of prey individuals, making them either more or less attractive and desirable to the predators, so that eggplants might generate better prey than other vegetable crops do (Rasdi et al., 2009). Furthermore, the presence of the eggs of *E. kuehniella*, another optimal food source for *M. pygmaeus*, may have helped the predator to successfully establish and increase a stable population (Sylla et al., 2016).

However, as observed by Bresch et al. (2014) in a study of tomato and tobacco plants infested by *Trialeurodes vaporariuorum* (Westwood), the mirid predator *M. pygmaeus* cannot always significantly reduce the negative impact of the pest. Indeed, the results of the present study reveal that *M. pygmaeus* was unable to limit the effect of *B. tabaci* on PH and on the characteristics of the roots (i.e., RA and RDW) that were not significantly different from those obtained in the PIB condition.

Insect pests can trigger the production of deterrents or toxic secondary metabolites that reduce the suitability of plant tissues for further insect colonization and may compromise the ability of the plant to activate certain resistance-related pathways (Usha Rani and Jyothsna, 2010; War et al., 2011a; War et al., 2011b). This could be the case with *B. tabaci*, which induces the activation of salicylic acid (SA) (Silva et al., 2021; Dias et al., 2022), a phytohormone able to suppress the activation of jasmonic acid (JA) (Stout et al., 2002; Zarate et al., 2007). However, the latter phytohormone can be induced by mirid insect activities (e.g., oviposition by adult females and feeding by *M. pygmaeus* nymphs) (Pappas et al., 2015) and functions in the mediation of plant responses. Specifically, the root stimulation of JA responses, following shoot damage, is completely dependent on hormone translocation (Schulze et al., 2019); thus, the phloem transport system is crucial to allocate resources among plant tissues and organs and move the jasmonates, which accumulate in vascular bundles after wounding (Stenzel et al., 2003; Wasternack and Hause, 2013; Chauvin et al., 2013).

Our findings may be seen in line with the results obtained by Schulze et al. (2019), who reported that even if, following wounding, shoot-produced jasmonates on *Arabidopsis thaliana* (L.) Heynh. move downward into the root through the phloem, the wounding on shoots is not always able to trigger the expression of JA marker genes in roots of all genotypes. Because JA may produce a local and nonsystemic effect (Zhang et al., 2018) and because our eggplants (PIBM) were exposed to *M. pygmaeus* for mating and oviposition for only a few days, it is probable that compounds related to plant defense were not transferred up to the roots during the experimental period. As indicated by Zhang et al. (2018), a longer exposure of host plants to mirids, or exposure to a greater density, could result in more-evident differences in phytohormone concentrations in various parts of plants, with consequent increases in root area and root dry weight, as was the case in our roots.

Although our results offer a preliminary insight into multitrophic interactions and mechanisms among host plant, pest, and predator, the specific response of whole eggplants to the presence of *M. pygmaeus* remains unclear. The wound-induced responses of plants are often modified by the perception of herbivore-specific elicitors, and this may be the case for zoophytophagous omnivores, especially within the Miridae, as they produce many different salivary enzymes (Hori et al., 2000; Castañé et al., 2011). Further investigations are needed to more deeply study how phytophagy by *M. pygmaeus* directly and indirectly affects host plants and whether *B. tabaci* is able to suppress or resist plant defenses stimulated by the predator.

4.8 Declarations

4.8.1 Author contributions

Conceptualization, C.R., A.F. and P.S.; methodology, A.F., P.S., G.E.M.C. and C.R.; formal analysis, A.F. and P.S.; investigation, A.F.; writing—original draft preparation, A.F. and C.R.; writing—review and editing, C.R., A.F., P.S. and G.E.M.C. All authors have read and agreed to the published version of the manuscript.

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4.8.3 Institutional review board statement

Not applicable.

4.8.4 Informed consent statement

Not applicable.

4.8.5 Data availability statement

The data presented in this study are available on request from the corresponding author.

4.8.6 Conflicts of interest

The authors declare no conflict of interest.

5 Tomato leaf curl New Delhi virus Spain strain is not transmitted by *Trialeurodes vaporariorum* and is inefficiently transmitted by *Bemisia tabaci* Mediterranean between zucchini and the wild cucurbit *Ecballium elaterium*

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5.1 Abstract

Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus (genus *Begomovirus*, family *Geminiviridae*) persistently transmitted, as all other begomoviruses, by whiteflies (Hemiptera: Aleyrodidae) of the *Bemisia tabaci* cryptic species complex. The virus, originally from the Indian subcontinent, was recently introduced in the Mediterranean basin, where it is currently a major concern for protected and open field horticulture. The Mediterranean ToLCNDV isolates belong to a novel strain named “Spain” (ToLCNDV-ES) which infect zucchini and other cucurbit crops but is poorly adapted to tomato. Recently, it has been reported that another whitefly, *Trialeurodes vaporariorum,* is able to transmit an isolate of ToLCNDV which infects chayote plants, a cucurbit. The present work aimed to clarify some aspects of the whitefly transmission of ToLCNDV-ES. It was shown that *T. vaporariorum* is not able to transmit ToLCNDV-ES between zucchini plants. Also, *Ecballium elaterium* may not act as a relevant reservoir for this virus strain in the Mediterranean basin, as *B. tabaci* MED, the most prevalent species of the complex in the region, is not an efficient vector of this begomovirus between cultivated zucchini and wild *E. elaterium* plants.

5.2 Keywords

Begomovirus, *Geminiviridae*, tomato leaf curl New Delhi virus, transmission, whiteflies, *Bemisia tabaci*, *Trialeurodes vaporariorum*, cucurbits, *Ecballium elaterium,* wild plants

5.3 Introduction

A high number of emergent diseases affecting vegetable, root and fiber crops worldwide are caused by viruses transmitted by sap-sucking insects, mainly hemipterans. Most of these viruses belong to the genus *Begomovirus* (family *Geminiviridae*), the largest genus in the entire virosphere, with 409 recognized species (Fiallo-Olivé et al., 2021). Begomoviruses are present in both the Old and New World. Indeed, the two major phylogenetic groups within the genus are related to geographical origin: Old World (OW) and New World (NW) begomoviruses. One of the most damaging and widespread OW begomoviruses is tomato leaf curl New Delhi virus (ToLCNDV), initially found infecting tomato (*Solanum lycopersicum* L.) crops in India (Padidam et al., 1995) and currently being a major concern to horticulture in many regions of the world (Moriones et al., 2017; Zaidi et al., 2017). In addition to important solanaceous (eggplant, pepper, potato and tomato) and cucurbit (cucumber, melon, pumpkin and zucchini) crops, ToLCNDV has been shown to infect a wide host range of dicot plants (Zaidi et al., 2017). ToLCNDV is a typical bipartite OW begomovirus whose genome is composed of two circular ssDNA components (DNA-A and DNA-B) separately encapsidated within twinned (geminated) quasi-icosahedral virions (Fiallo-Olivé et al., 2021). Symptoms caused by typical Indian ToLCNDV isolates in tomato include stunting, leaf curling and deformation, interveinal yellowing and yield losses.

ToLCNDV was limited to the Indian subcontinent and other Asian countries until the season 2012/2013, when it was first detected in the Mediterranean basin infecting zucchini (*Cucurbita pepo* L.) crops in the southeastern provinces of continental Spain, Murcia and Almería (Juárez et al., 2014). Symptoms observed in infected zucchini plants included curling, vein swelling, and severe mosaic in young leaves, short internodes, and fruit skin roughness (Juárez et al., 2014). In the following years, the virus caused serious epidemic outbreaks in cucurbit crops in this area, both in greenhouses and open fields (Fortes et al., 2016; Juárez et al., 2019). ToLCNDV was soon detected infecting zucchini crops in other countries in southern Europe and north Africa including Tunisia (Mnari-Hattab et al., 2015), Italy (Panno et al., 2016), Morocco (Sifres Cuerda et al., 2018), Algeria

(Kheireddine et al., 2019) and Greece (Orfanidou et al., 2019). The genetic analysis of the viral isolates present in Spain, closely related to those characterized in other Mediterranean countries, showed that they all belong to a novel strain, named "Spain strain” (ToLCNDV-ES) (Fortes et al., 2016). Although ToLCNDV-ES isolates have been detected infecting tomato plants in Spain in the field (Ruiz et al., 2015, 2017; Juárez et al., 2019;), these infections appeared to be anecdotal. Furthermore, agroinoculation experiments have confirmed that isolates of this strain are very efficient in infecting cucurbits but seem to be poorly adapted to infect tomato (Fortes et al., 2016).

Several wild plant species have been reported to be naturally infected by ToLCNDV-ES in Spain, the cucurbit *Ecballium elaterium* (L.) A.Rich., the solanaceous *Datura stramonium* L. and *Solanum nigrum* L. and the compositae *Sonchus oleraceus* L. (Juárez et al., 2019), the most commonly found being *E. elaterium*. This suggests a relevant role of this wild cucurbit in ToLCNDV epidemiology in the Mediterranean basin by acting as a source of inoculum. ToLCNDV, as all begomoviruses, is transmitted in a persistent manner by whiteflies (Hemiptera: Aleyrodidae) of the *Bemisia tabaci* cryptic species complex (Fiallo-Olivé et al., 2020). Surprisingly, it has been reported that another whitefly species, *Trialeurodes vaporariorum* (Westwood), the greenhouse whitefly, was able to transmit a ToLCNDV isolate found infecting chayote [*Sechium edule* (Jacq.) Sw.], a cucurbit, in India (Sangeetha et al., 2018).

In the absence of direct control measures, management of plant virus diseases relies on a profound knowledge of the interactions established between the virus, the host plant and the environment. In the case of viruses transmitted by a biological vector, e.g. insects, the complexity of the system increases and the role of vectors take on a special importance. In line with this, the present work aimed to assess some aspects of ToLCNDV-ES transmission by whiteflies. A first objective was to determine whether ToLCNDV-ES is transmissible by the whitefly *T. vaporariorum*. Secondly, considering that the wild cucurbit *E. elaterium* is frequently found infected by ToLCNDV-ES in Spain, the transmissibility of this virus between this virus reservoir and zucchini plants by *B. tabaci* Mediterranean (MED) species was also evaluated.

5.4 Materials and methods

5.4.1 Virus isolate and plant agroinoculation

Dimeric infectious clones for DNA-A and DNA-B genomic components of ToLCNDV-ES, isolate RV1 (E. Fiallo-Olivé and J. Navas-Castillo, in preparation) were used in *Agrobacterium tumefaciens*-mediated inoculation (agroinoculation). For agroinoculation assays, liquid cultures of *A. tumefaciens* strain C58C1 harboring each dimeric construct were added at 1:1000 to YEP liquid media containing kanamycin and rifampicin and grown at 28ºC for 2 d. Cultures were centrifuged at room temperature and then resuspended in 10 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) (pH 5.6), 10 mM $MgCl_2$ and 150 M acetosyringone to a final OD_{600} of 1.0. Equal volumes of DNA-A and DNA-B cultures were mixed before stem puncture inoculation of zucchini cv. Milenio or *E. elaterium* plants.

5.4.2 Virus detection

At 30 d post-inoculation (dpi), apical leaves were used for tissue blots of petiole cross sections (tissue printing) performed on positively charged nylon membranes (Roche Diagnostics, Mannheim, Germany) and hybridization using digoxigenin-labelled DNA probes specific to DNA-A and DNA-B of ToLCNDV-ES prepared by polymerase chain reaction according to the DIG-labelling detection kit (Roche Diagnostics, Mannheim, Germany) as previously described (Fortes et al., 2016). Hybridization analysis of tissue blots was performed at high stringency conditions (hybridization at 65ºC followed by washing steps with 0.1x saline sodium citrate, 0.1 sodium dodecyl sulfate at 65ºC). Hybridization signals were detected on X-ray film after treatment with CDP-Star (Roche Diagnostics, Mannheim, Germany) and developed following a conventional photographic process.

5.4.3 Whitefly transmission

Nonviruliferous *B. tabaci* Mediterranean (MED, formerly biotype Q) and *T. vaporariorum* populations were reared on *Solanum muricatum* Aiton cv. Sweet Long and *Nicotiana glauca* Graham plants, respectively, in insect-proof BugDorm cages (MegaView Science, Taichung, Taiwan). Unsexed adult whiteflies were collected with an aspirator and transferred to clip-cages (50 insects per clip-cage) on zucchini cv. Milenio or *E. elaterium* plants infected with ToLCNDV-ES isolate RV1.

Whitefly-inoculated plants were maintained in BugDorm cages inside a growth chamber (25°C during the day and 18°C at night, 70% relative humidity, with a 16-h photoperiod at 250 µmol s^{-1} m^{-2} photosynthetically active radiation). Whiteflies were given an acquisition-access period (AAP) of 48 h, after which the clip-cages containing the whiteflies were transferred (within the same BugDorm cages) onto healthy zucchini or *E. elaterium* plants at the two-leaf stage. After an inoculation-access period (IAP) of 48 h, clip-cages were removed, and plants were sprayed with insecticides (Confidor 20 LS, 20% imidacloprid and Atominal 10 EC, pyriproxyfen 10%). Four source plant – target plant – whitefly species combinations were assayed as follows: (i) zucchini – zucchini (n=20) – *T. vaporariorum*, (ii) zucchini – zucchini (n=20) – *B. tabaci* MED, (iii) zucchini – *E. elaterium* (n=10) – *B. tabaci* MED, and (iv) *E. elaterium* – zucchini (n=10) – *B. tabaci* MED. Two independent experiments were carried out per combination. Plants were examined daily for the appearance of symptoms. At 30 dpi, apical leaves were used for tissue printing and molecular hybridization using digoxigenin-labelled DNA probes specific to the ToLCNDV-ES DNA-A and DNA-B components as described above.

5.5 Results and discussion

5.5.1 ToLCNDV-ES is not transmitted by T. vaporariorum *between zucchini plants*

In the transmission assays with *T. vaporariorum* using ToLCNDV-ES-agroinfected zucchini plants as source of inoculum and target plants, none of the 40 inoculated zucchini plants (two independent experiments with 20 plants each) resulted infected by the virus (Table 1). However, when *B. tabaci* MED was used, 13 and 11 out of 20 target zucchini plants resulted infected in two independent experiments, respectively (Table 1). Infection was revealed by the expression of typical viral symptoms and confirmed by molecular hybridization using DNA-A and DNA-B probes (Figure 1A). These results are in disagreement with the data reported by Sangeetha et al. (2018) who found successful transmission of a ToLCNDV isolate from chayote in India. It could be argued that the differences observed between that report and the current work might be due to differences between virus isolates, *T. vaporariorum* genotypes or even experimental conditions. On this regard, it is worth to mention that the experiments carried out with ToLCNDV-ES used a higher number of whitefly adults per inoculated plant (50 vs. 20) and higher AAP/IAP (48/48 h vs. 4/24 h) than the experiment reported for ToLCNDV-chayote. In addition to this, the fact that transmission by *T. vaporariorum* was reported to occur only in the experiment carried out with an AAP of 4 h but not in those of 1, 8, 12 or 24 h, compromises the reliability of the reported data. Although *T. vaporariorum* is known to be a vector of some criniviruses (genus *Crinivirus*, family *Closteroviridae*) and torradoviruses (genus *Torradovirus*, family *Secoviridae*) (Fiallo-Olivé et al., 2020), the confirmation of a begomovirus being transmitted by this whitefly awaits further investigation.

5.5.2 *ToLCNDV-ES is not efficiently transmitted by* B. tabaci *MED between zucchini and* E. elaterium

In the transmission assays with *B. tabaci* MED using ToLCNDV-ES-agroinfected zucchini plants as source of inoculum and *E. elaterium* as target plants, only one out of the 20 *E. elaterium* plants used in two independent experiments resulted infected, as revealed by the absence of symptoms at 30 dpi and negative molecular hybridization results (Table 1) (Figure 1B). The frequent presence of ToLCNDV-ES-infected *E. elaterium* plants observed in the Spanish provinces of Murcia (Juárez et al., 2019) and Málaga (our unpublished results) contrasts with the low transmission rate from zucchini to this wild cucurbit using *B. tabaci* MED, the most prevalent species of the complex in the region. On the other hand, none of the 20 zucchini plants inoculated with the same whitefly vector starting from infected *E. elaterium* plants in two independent experiments resulted infected (Table 1) (Figure 1C). This is reminiscent of the finding that pepper (*Capsicum annuum* L.) behaves as a dead-end for another begomovirus, tomato yellow leaf curl virus (TYLCV), in Spain (Morilla et al., 2005). Thus, TYLCV could not be transmitted from infected pepper plants, most likely because of the low viral titers present, suggesting that this crop acts as a severe bottleneck in the epidemiological cycle of this begomovirus. As it was suggested for the system TYLCV/pepper (Morilla et al., 2005), it cannot completely be ruled out that *E. elaterium* may serve as a low-efficient reservoir when vector populations are extremely high.

In summary, our findings have called into question the results of ToLCNDV transmission by whiteflies other than members of the *B. tabaci* complex (Sangeetha et al., 2018) and provides insight into the questionable role of *E. elaterium*, a wild cucurbit plant, in ToLCNDV epidemics in the Mediterranean basin. These findings are a good starting point to advance our understanding of ToLCNDV epidemiology, thus helping to develop control strategies to fight the harmful disease that this virus causes to important crops.

Table 1. Transmission experiments of tomato leaf curl New Delhi virus Spain strain between zucchini cv. Milenio and *Ecballium elaterium* plants by *Trialeurodes vaporariorum* and *Bemisia tabaci* MED.

ToLCNDV-ES-infected source plant	Target plant	Whitefly species	No. infected plants/ no. inoculated plants*	
			Exp. 1	Exp. 2
Zucchini	Zucchini	*T. vaporariorum*	0/20	0/20
Zucchini	Zucchini	*B. tabaci* MED	13/20	11/20
Zucchini	*E. elaterium*	*B. tabaci* MED	1/10	0/10
E. elaterium	Zucchini	*B. tabaci* MED	0/10	0/10

*Infection was determined by tissue-printing and molecular hybridization with digoxigenin-labelled ToLCNDV DNA-A and DNA-B probes. Blots from Exp. 1 of each source plant – target plant – whitefly species combination are shown in Figure 1.

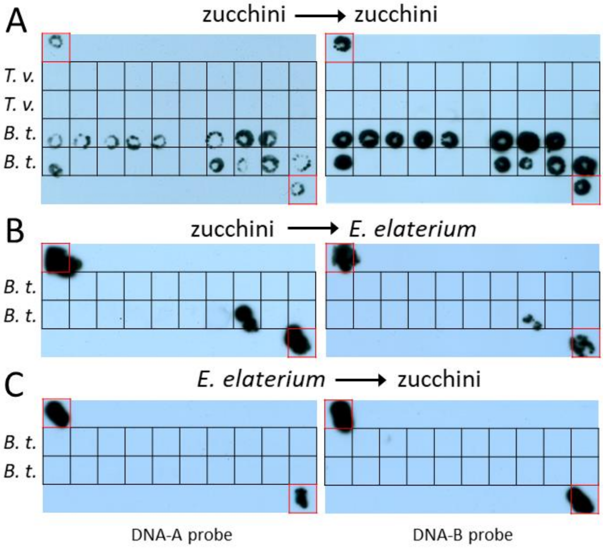

Figure 1. Agroinoculation experiments with tomato leaf curl New Delhi virus Spain strain using different source plant – target plant – whitefly species combinations. ToLCNDV (DNA-A and DNA-B) was detected by molecular hybridization after tissue printing on nylon membranes using specific digoxigenin-labelled DNA probes for each viral component. (**A**) Prints of zucchini plants inoculated using *Trialeurodes vaporariorum* (*T. v.*) or *B. tabaci* MED (*B. t.*) as a vector and infected zucchini plants as virus source. (**B**) Prints of *Ecballium elaterium* plants using *B. t.* as a vector and infected zucchini plants as virus source. (**C**) Prints of zucchini plants using *B. t.* as a vector and infected *E. elaterium* plants as virus source. Blots from one of the two independent experiments (Exp. 1 in Table 1) carried out for each source plant – target plant – whitefly species combination are shown. Prints of ToLCNDV-infected zucchini plants included as positive controls are outlined in red.

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6 Conclusion

Nowadays, the research focus in agriculture is increasingly shifting from the study of basic systems and their single components to approaches that consider all complex interactions linking organisms coexisting in an agroecosystem. Multitrophic interactions concern the dynamics of multiple trophic levels of food webs, in which plants are strongly linked with pathogens, pests and their natural enemies in a complex system that involves direct and indirect interaction mechanisms. It is well known that plants influence the behavior of both pests and their natural enemies in various ways, how relationships between two species are often mediated by a third one, and how the surrounding environment in which organisms live within an agroecosystem affects their multitrophic interactions. But it is really complicated to understand how these associations occur. Starting from these considerations, the interaction of whiteflies with other components of their food web as well as their negative impact on the host plants has been the main target of this study and is described in some detail throughout the earlier chapters of this thesis.

Starting from chapter 2, focusing on the species composition of the *B. tabaci* complex, comprising their endosymbiont community and its geographic distribution in Sicily, this study confirms the continuous and predominant increase of MED species presence, resulting in the almost disappearance of the MEAM1 species in greenhouses, open field crops, and wild plant species in areas of the island where an intensive horticultural production is realised and high inputs applied. In particular, the prevalence of the MED species in the southeastern area of the island is evident, with the highest level of genetic variability within the Q1 mitochondrial variant, in contrast to the MEAM 1 species, which is more present in the western area. The present survey also shows that there is an extreme uniformity in endosymbiont composition and a clear link between endosymbiont composition and whitefly identity.

Chapter 3, by investigating some aspects of interactions between *B. tabaci* MED and plant, confirmed that the impact of whitefly feeding on eggplant and tomato alters both the main morphological and physiological traits of the plants. Specifically, eggplant and tomato plants infested by *B. tabaci* MED showed a strong and significant reduction in height, shoot dry weight, leaf area and indirect chlorophyll content, although with different intensities between the two plant species. While chapter 3 focuses on the whitefly – vegetable crops relationships, highlighting that *B. tabaci* MED infestation can lead to relevant negative effects on both the morphology and physiology of vegetable plants, the aim of chapter 4 is to combine the above-mentioned individual components, i.e. host plant and pest, creating an integrated system to understand how plant, pest and natural enemy interact. So, by adding a third trophic level to the study, the mechanisms of multitrophic interactions developing around *B. tabaci* on plants was deepened. Specifically, through the analysis of host plant responses to the zoophytophagy of a predatory omnivorous insect, it was shown how the mirid predator *M. pygmaeus*, in the presence of its prey, has a significant positive influence on some physiological and morphological traits of *S. melongena*, which have been poorly investigated so far, limiting damage due to *B. tabaci*. However, additional studies are needed to understand the possible role played by secondary metabolites of plants in affecting both the behavior and preferences of this whitefly and also the activity of the mirid bug insect.

Taking into account the ToLCNDV – whiteflies pathosystem, with a virus considered as another important individual element added to the complex system considered, chapter 5 provides important information about the role of both whiteflies as vectors and wild plants as virus host plants, involved in host plant – pest – phytopathogenic microorganism interactions, which constitute a real critical problem in the Mediterranean basin. In this context, *T. vaporariorum* and *E. elaterium* cannot be considered a threat or a risk to cultivated zucchini plants, as *T. vaporariorum* was not able to transmit the begomovirus between zucchini plants, and *B. tabaci* MED was not able to transmit the virus from cultivated zucchini plants to wild *E. elaterium* plants and vice versa.

Many information deriving from this study may be of importance, for applying a rational control of whiteflies and their related virus problems. Firstly, by knowing the striking difference in biological traits of the various known species of the *B. tabaci* complex, having an updated status on geographic distribution and endosymbiont community of these species in Sicily may improve possibilities to apply rational control measures of their infestations, with special reference to biological control. This can also be improved through a better description of the relations between plants and *B. tabaci* MED as well as a better understanding of *M. pygmaeus* effects on plant development. Finally, this study could be an interesting starting point on which base research may be deepened to improve environmentally friendly control strategies against whiteflies and their related virus problems on crops.

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