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PhD Thesis

EVALUATION OF BIOLOGICAL ASPECTS OF *FRANKLINIELLA OCCIDENTALIS* PERGANDE (THYSANOPTERA: THRIPIDAE) IN RELATION TO MICROBIOME ENDOSYMBIOTIC ASSOCIATIONS

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Abstract

The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), is a widespread phytophagous thrips species in many crops worldwide, causing damage with the feeding and transmission of Tospovirus. It shows high resistance to insecticides and it is difficult to control. Obligate bacterial symbionts are widespread across many insects, where they are often confined to specialized host cells (or tissues) and are transmitted directly from the mother to her progeny and it is often difficult to assess the multiple details of the relationships. At the beginning of this Ph.D. Thesis, the introductory section offers an overview of the current knowledge on the WFT (morphology, biology and ecology) and the endosymbiotic microorganisms associated with the main pest thrips species. The experimental part of the work investigated some aspects related to the influence of endosymbionts on the biological cycle of WFT and their molecular characterization. More specifically, experimental tests on the influence of the symbionts of some aspects (oviposition, fecundity and longevity) of the life cycle of the WFT, were conducted-on laboratory populations of thrips. By using Next Generation Sequencing (NGS) technologies and bioinformatics techniques, substantial results have been obtained on the metagenomic study of the microbiome associated with WFT natural populations, collected on different crops in greenhouses and open fields in southern Italy. For all of the *F. occidentalis* natural populations that were studied, molecular characterizations based on five gene fragments have also been provided.

Riassunto

La *Frankliniella occidentalis* (Pergande) è una specie diffusa di tripidi fitofagi su molte colture in tutto il mondo, causando danni con l'alimentazione e la trasmissione di Tospovirus. Presenta un'elevata resistenza agli insetticidi ed è difficile da controllare. I simbionti batterici obbligati sono diffusi in molti insetti, dove sono spesso confinati a cellule ospiti specializzate (o tessuti) e vengono trasmessi direttamente dalla madre alla prole ed è spesso difficile determinare la complessità delle relazioni. All'inizio di questa tesi di dottorato una parte introduttiva offre una panoramica delle attuali conoscenze sul WFT (morfologia, biologia ed ecologia) e sui microrganismi endosimbiotici associati alle principali specie di tripidi parassiti. La parte sperimentale del lavoro è stata articolata attraverso la valutazione dell'influenza degli endosimbionti sul ciclo biologico del WFT e la loro caratterizzazione molecolare. In particolare, i saggi biologici sulle associazioni endosimbiotiche, ed i relativi risultati sull'influenza degli stessi sul ciclo di biologico della specie, sono stati condotti su popolazioni di tripidi di laboratorio. Grazie all'impiego di recenti metodologie molecolari, Next Generation Sequencing (NGS), e con l'uso di tests bioinformatici avanzati, sono stati ottenuti risultati sostanziali sullo studio metagenomico del microbioma associato alle popolazioni naturali del WFT, raccolte su diverse colture agrarie in serra ed in pieno campo in diverse aree del Sud Italia. Per tutte le popolazioni naturali di *F. occidentalis*, è stata fornita anche la caratterizzazione molecolare, basata su cinque frammenti genici.

Keywords

- western flower thrips
- microbe–insect interaction
- endosymbionts
- thrips-rearing
- antibiotic treatment
- molecular characterization
- 16S rRNA sequencing

Chapter 1. Introduction

1.1 Microbe-insect interactions

In recent years, the importance and prominence of studies of insect-microbiome interactions has increased. It is well known that symbiotic microorganisms can affect some important phases of the biological cycle of their hosts. However, although several species of microorganisms associated with insects have been identified, their role and mode of action, with very few exceptions, are poorly known. Insects represent a category of living beings that could adapt and survive even in various extreme environmental conditions (Misof et al., 2014). In comparison to a large number of important ecological insects, many crop pests are responsible for nearly 20% of annual production losses in the agricultural sector. (Deutsch et al., 2018). Janson et al. 2008, claim that the evolutionary success of insects is due to interactions with numerous beneficial microbes. The microorganisms permanently or transiently associated with insects are bacteria, archaea, fungi, protozoa and viruses (Figure 1) (Kaufmanm et al., 2000; Feldhaar, 2011; Hammer et al., 2017). These microorganisms can colonize the insect exoskeleton, the gut and the hemocoel, and within insect cells (Douglas, 2015). They are called “endosymbionts” when they develop inside specialized cells, and “ectosymbionts” if they live outside the host (Thompson & Simpson, 2009). Endosymbiotic microorganisms are mainly located in the digestive tract where they play a key role as modulators (both in terms of diet and ecological niches) of their host lifestyle. Different activities are linked to the gut microflora, such as: (1) to facilitate the feeding of recalcitrant substrates; (2) to compensate the nutrient-poor diet (e.g., in the case of sap-sucking insects); (3) to provide immunity and protection from predators, pathogens and parasites; (4) to mediate inter and intra-specific communication; (5) to control the success of mating and reproduction; (6) to aid digestion; (7) to supply essential amino acids, metabolic compounds and nutrients and (8) to metabolize toxins (Dillon & Dillon, 2004; Russell et al., 2014; Douglas, 2015; Arbuthnott et al., 2016; Wielkopolan and Obrêpalska-Stêplowska, 2016; Engl and Kaltenpoth, 2018). Associations with

microbes may be classified as obligated or primary and optional or secondary, depending on the degree of dependence (Baumann, 2005; Moran et al., 2008).

In 2020, Jing et al. have shown that the supply of essential nutrients is the main task of the symbionts, followed by digestion and detoxification. This implies that insects depend heavily on their gut microbiome for their survival and the basic activities of their life cycle. In recent years, studies relating to insect microbiome have intensified through new sequencing approaches that make it possible to classify most of the associated microbial clusters. All microbial components can influence the life cycle of the host and its relationships with the external environment (Janson, 2008).

Shrestha (2019) reports how climate change is affecting the pattern of insect migration, their population dynamics, and their life cycle times. In addition to overcoming climate challenges, it has also allowed some of them to expand their plant hosts-range, and influenced their behaviour and biology, thus helping them to invade and colonize different agro-climatic areas of the world. Bacterial species present in the gut of an insect may associate through mutualistic relationships, commensalism, or by developing a strong parasitic action (Dillon e Dillon, 2004). To promote microbial colonization, insects hosting huge microbial communities provide an optimal pH in their gut to create a favorable environment (Engel and Moran, 2013). Mikonranta et al. (2014), describes that in the initial symbiosis phase, insects respond by activating an immunity response against pathogenic bacteria but can selectively maintain beneficial microbes. Recent studies (Mergaert, 2018), have revealed that symbiotic antimicrobial peptides (AMP) regulate symbiotic interactions by limiting the reproduction of symbiotic bacteria, sometimes transforming them into a differentiated form and eliminating sensitive and unwanted bacteria. Several symbionts interact differently with the insect's immune system; many can successfully bypass the insect's cellular immune response, while others influence some defense mechanisms present in insects (i.e. melanization) (Thomas et al., 2011). Among the most interesting biological aspects, symbionts can affect the efficiency of virus transmission (Mcmeniman et

al., 2009; Ricci et al., 2012) or the development time (Chouaia et al., 2012) and both these activities could provide targets for potential disease control. Although the existence of processes through which the endosymbionts can bypass the immune system of the hosts is known, it is not yet clear the strategy with which the insect can distinguish the beneficial symbionts from the potentially pathogenic ones. Yuval (2017), draws attention to the changes that the bacterial genome undergoes to acclimatize to the intestinal environment. Some microbes are even able to manipulate their host insect for their survival. All microbes, individually or together, can affect the biology and fitness of insect pests, as exemplified by the processes indicated in Figure 1. Given the complexity of potential interactions, pest species should be seen as mini-ecosystems, in which the microbiome and the host interact as a system.

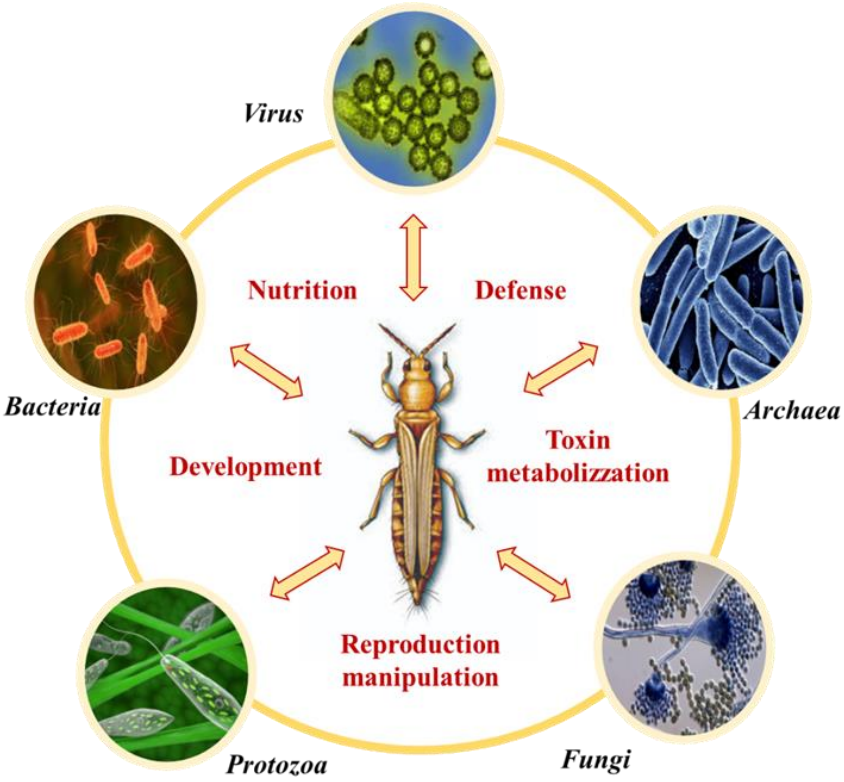


Figure 1. General representation of the relationships and complexity of the microbiome associated with pest insects.

1.2 Western Flower Thrips

1.2.1 Pest status and *host-plant* range

Frankliniella occidentalis is a highly polyphagous pest species, distributed worldwide since the ‘80s. Its invasiveness is largely attributed to the international plant trade and its resistance to insecticides. European Plant Protection Organisation (EPPO) reports this species as number 177 on the list of A2 quarantine pests. WFT also poses a potentially serious threat to the countries where it is not yet present. With a range of over 250 crop species from over 60 plant families (Table 1), it can damage numerous agricultural and ornamental crops in greenhouses and open fields (Kirk and Terry, 2003; Reitz et al., 2020). In northern Europe, it is present in greenhouse crops, such as cucumbers, peppers, chrysanthemums, Gerbera, roses, Saintpaulia and tomatoes. At the same time in southern Europe, it is extremely harmful to many crop fields, including peppers, tomatoes, strawberries, table grapes and artichokes. Southern Italy has become a dominant member of the thrips fauna associated with flowers from a wide range of wild plants (Marullo, 2002, 2003). The damage is caused by feeding from, and the oviposition on: leaves, flowers and fruits (Childers & Achor, 1995; Childers, 1997). The parts of the plant damaged by bites are also susceptible to fungal infections. As recently reported by Reitz et al. (2020), WFT is the most efficient vector of seven species of *Orthotospovirus*, including Alstroemeria necrotic streak virus, *Chrysanthemum stem necrosis orthotospovirus*, *Impatiens necrotic spot orthotospovirus*, *Groundnut ringspot orthotospovirus Tomato spotted wilt orthotospovirus*, *Tomato chlorotic spot orthotospovirus* and *Tomato zonate spot virus*. First and second larval stages can acquire these viruses by feeding off of an infected plant and can maintain them until they develop into mobile adults; the adult stages can only transmit but cannot acquire the viruses from infected plants (Moritz et al., 2004). As it is known for most harmful insects, before the invasive epidemics were recorded in the 1970s, *F. occidentalis* was also described as a sporadic parasite in its native area of western North America (Bailey, 1938; Bryan & Smith, 1956). Although

many studies on this species are available, some biological aspects remain poorly understood and must be cleared. The conspicuous studies of this invasive species has lead researchers to consider it a model organism for the study of multitrophic interactions (Reitz et al., 2020). In addition to parasitic activity on plants, WFT feeds on the eggs and immature stages of other arthropods, including mites, and can have a predatory impact (Trichilo & Leigh, 1986). Predation provides extra dietary proteins, which increase the species reproductive success (van Maanen et al., 2012).

Table 1. *Host-plant* range and other plants affected by Western Flower Thrips.

Plant name	**Family**
Allium cepa (onion)	Liliaceae
Amaranthus palmeri (Palmer amaranth)	Amaranthaceae
Arachis hypogaea (groundnut)	Fabaceae
Begonia	Begoniaceae
Beta vulgaris (beetroot)	Chenopodiaceae
Beta vulgaris var. saccharifera (sugarbeet)	Chenopodiaceae
Brassica oleracea var. capitata (cabbage)	Brassicaceae
Capsicum annuum (bell pepper)	Solanaceae
Carthamus tinctorius (safflower)	Asteraceae
Chrysanthemum indicum (chrysanthemum)	Asteraceae
Chrysanthemum morifolium (chrysanthemum)	Asteraceae
Citrus sinensis (navel orange)	Rutaceae
Citrus x paradisi (grapefruit)	Rutaceae
Cucumis melo (melon)	Cucurbitaceae
Cucumis sativus (cucumber)	Cucurbitaceae
Cucurbita maxima (giant pumpkin)	Cucurbitaceae
Cucurbita moschata (pumpkin)	Cucurbitaceae
Cucurbita pepo (marrow)	Cucurbitaceae
Cucurbitaceae (cucurbits)	Cucurbitaceae
Cyclamen	Primulaceae
Cynara cardunculus var. scolymus (globe artichoke)	Asteraceae
Dahlia	Asteraceae
Daucus carota (carrot)	Apiaceae
Dianthus caryophyllus (carnation)	Caryophyllaceae
Euphorbia pulcherrima (poinsettia)	Euphorbiaceae

Eustoma	Gentianaceae
Eustoma grandiflorum (Lisianthus (cut flower crop))	Gentianaceae
Ficus carica (common fig)	Moraceae
Fragaria ananassa (strawberry)	Rosaceae
Fuchsia	Onagraceae
Geranium (cranesbill)	Geraniaceae
Gerbera jamesonii (African daisy)	Asteraceae
Gladiolus (sword lily)	Iridaceae
Gladiolus hybrids (sword lily)	Iridaceae
Gossypium (cotton)	Malvaceae
Gypsophila (baby's breath)	Caryophyllaceae
Hibiscus (rosemallows)	Malvaceae
Impatiens (balsam)	Balsaminaceae
Kalanchoe	Crassulaceae
Lactuca sativa (lettuce)	Asteraceae
Lathyrus odoratus (sweet pea)	Fabaceae
Leucaena leucocephala (leucaena)	Fabaceae
Limonium sinuatum (sea pink)	Plumbaginaceae
Malus domestica (apple)	Rosaceae
Medicago sativa (lucerne)	Fabaceae
Mentha piperita (Peppermint)	Lamiaceae
Nicotiana tabacum (tobacco)	Solanaceae
Orchidaceae (orchids)	Orchidaceae
Origanum majorana (sweet marjoram)	Lamiaceae
Pelargonium (pelargoniums)	Geraniaceae
Petroselinum crispum (parsley)	Apiaceae
Phaseolus vulgaris (common bean)	Fabaceae
Pistacia vera (pistachio)	Anacardiaceae
Pisum sativum (pea)	Fabaceae
Prunus armeniaca (apricot)	Rosaceae
Prunus domestica (plum)	Rosaceae
Prunus persica (peach)	Rosaceae
Prunus persica var. nucipersica (nectarine)	Rosaceae
Prunus salicina (Japanese plum)	Rosaceae
Purshia tridentata (bitterbrush)	Rosaceae
Ranunculus (Buttercup)	Ranunculaceae
Raphanus raphanistrum (wild radish)	Brassicaceae
Rhododendron (Azalea)	Ericaceae
Rosa (roses)	Rosaceae
Rumex crispus (curled dock)	Polygonaceae
Saintpaulia ionantha (African violet)	Gesneriaceae
Salvia (sage)	Lamiaceae
Secale cereale (rye)	Poaceae
Sinapis arvensis (wild mustard)	Brassicaceae

Sinningia speciosa (gloxinia)	Gesneriaceae
Solanum lycopersicum (tomato)	Solanaceae
Solanum melongena (aubergine)	Solanaceae
Solanum tuberosum (potato)	Solanaceae
Sonchus (Sowthistle)	Asteraceae
Syzygium jambos (rose apple)	Myrtaceae
Trifolium (clovers)	Fabaceae
Triticum aestivum (wheat)	Poaceae
Vaccinium (blueberries)	Ericaceae
Vitis vinifera (grapevine)	Vitaceae
Zinnia	Asteraceae

1.2.2 Taxonomy

WFT is a slender insect with narrow fringed wings with an adult body length between 0.8- and 1.0-mm. Adults in field populations of this species exist in a wide range of colour forms. In spring, following the wet season, the dark form predominates; during the rest of the year, the pale form is dominant.

Female macroptera. The adult female has a more rounded abdomen with a pointed posterior and their body colour can vary from yellow to brown. Its body varies from yellow to brown, but the widespread pest strain is usually mainly dark yellow in color with brown areas medially on each tergite; antennal segments II & VI-VIII brown, III–V yellow with apices variably brown; legs mainly yellow washed with brown; fore pale wings with dark setae. Antennae 8-segmented, III-IV segments with a forked sensorium, VIII longer than VII. Its head wider than it is longer; 3 pairs of ocellar setae present, pair III longer than distance between external margins of hind ocelli, arising on anterior margins of ocellar triangle; postocular setae pair I present, pair IV longer than the distance between hind ocelli. Pronotum with 5 pairs of major setae; anteromarginal setae slightly shorter than anteroangulars, one pair of minor setae present medially between posteromarginal submedian setae. Metanotum with 2 pairs of setae at anterior margin, campaniform sensilla present. Fore wing with 2 complete rows of veinal setae. Tergites V–VIII with paired lateral ctenidia, ctenidia sometimes weakly developed on IV, on VIII anterolateral to spiracle; posteromarginal comb on VIII

complete, with short slender microtrichia arising from triangular bases. Sternites III–VII without discal setae.

Male macroptera. The adult male is smaller than the female, has a narrow abdomen with a rounded end and is pale-yellow (almost white). Tergite VIII without marginal comb; IX with median pair of dorsal setae shorter than lateral pair, posterolateral setae stout in larger males; sternites III–VII with a transverse pores plate.

Pupa. The early pseudopupa is characterized by the appearance of wing pads and by shortened, erect antennae. The late pseudopupa shows scarce mobility; adult setal patterns start forming; longer wing sheaths; antennae turned backwards. Both pupal stages are white.

Nymph. There are two nymphal instars, the first translucent and the second golden-yellow.

Eggs. Opaque, reniform and about 200 μm long.

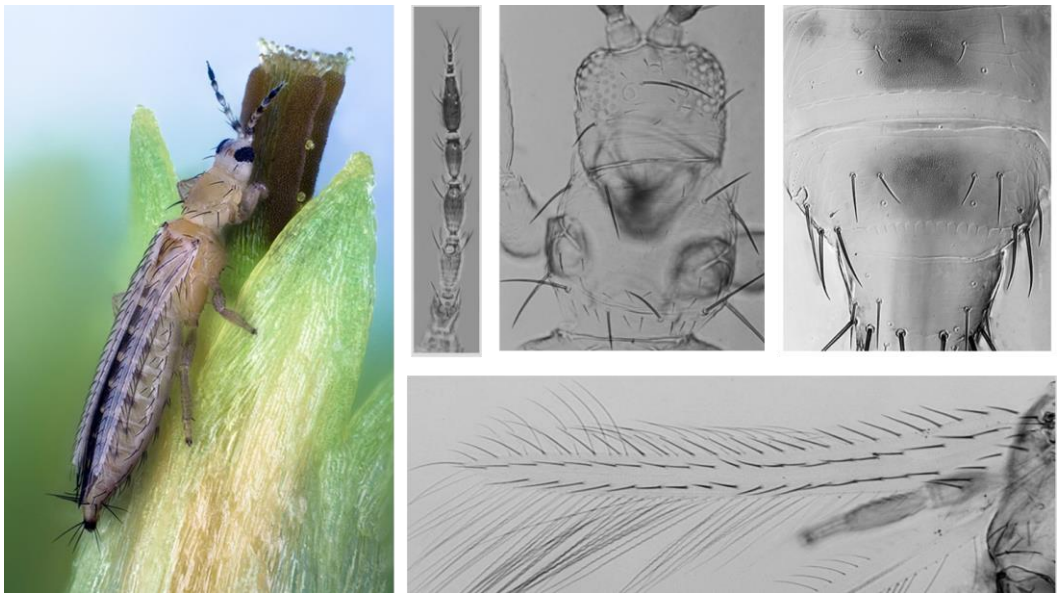


Figure 2. Adult female and main morphological characters useful for the taxonomic identification of *Frankliniella occidentalis*.

1.2.3 Biology and development

The life cycle of *F. occidentalis* is strongly influenced by climatic variables such as temperature and humidity. Western Flower Thrips exhibit an arrhenotokous reproduction as females arise from fertilized eggs and males from unfertilized eggs. Nevertheless, occasionally virgin females may produce female offspring (thelytoky) (Kumm & Moritz, 2010). The life cycle is completed in about 20 days, at average temperature values of 25°C. The mature females lay eggs in the parenchyma of leaves, flower parts and fruits, most frequently, under the epidermis of the leaves. Eggs of WFT usually hatch in about 4 days at 27°C after oviposition. The appearance of the adults follows four larval stages: the first two are active, feeding stages (first and second-instar nymph), while the pre-pupal and pupal stages do not feed. Subsequently, the pre-pupae reach the soil, where they find the protection, to complete the development. The first stage nymph emerges from the plant tissue and begins to feed almost immediately. The first change of cuticle occurs at 25°C, within 1-3 days, and in 7 days at 15°C. The second stage nymphs are much more active than the first and the development phase varies from 3 days at 27°C to 12 days at 15°C. The mature nymph progressively reduces the feeding activity, moults, and transforms in a pseudopupa. This phase is completed in one day at 27°C and four days at 15°C. Environmental conditions, such as the time of day (Holmes et al., 2012), relative humidity (Steiner, 2011), or structural complexity of the host plant (Buitenhuis & Shipp, 2008) are influential factors which indicate when to leave the host plant. Winged adults generally emerge after 2-9 days, with temperatures needing to be above a minimum of 8–10°C for growth. Optimal developmental rates occurring between 25°C and 30°C (Reitz, 2009). The development, besides the temperatures, is influenced by the photoperiod, with slower growth during shorter days (Brodsgaard, 1994). Lower or higher temperatures can significantly affect the vital and reproductive activity of WFT (Wang et al., 2014). Reitz (2009) argues that an average longevity, in laboratory conditions, ranges from 2 to 5 weeks but is probably lower in field populations.

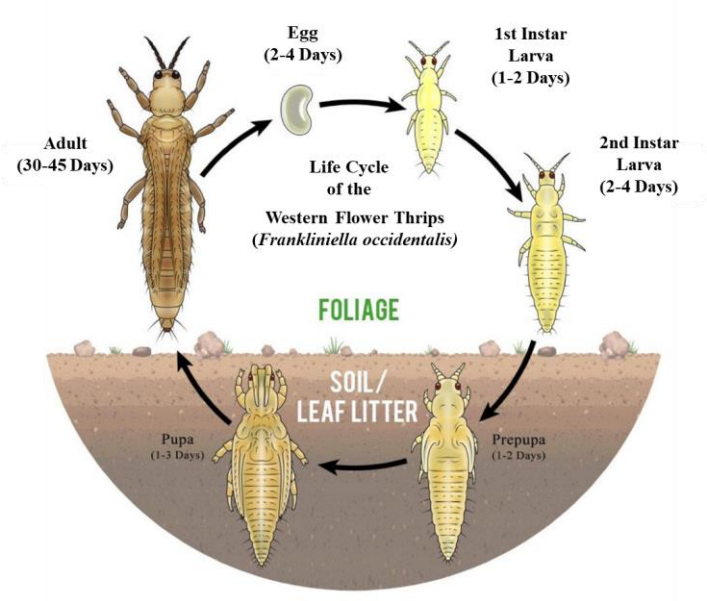


Figure 3. Schematic representation of the life cycle of *F. occidentalis* in the optimum range of humidity and temperature values.

1.2.4 WTF Endosymbiosis

Despite the progress in the formulation of increasingly effective pesticides, plant health control of the harmful thrips species is very difficult. Their success into crop colonization and exploitation is due to numerous factors, such as polyphagy, different reproduction modes but also high resistance to pesticides. In recent years, some studies have shown that even interactions with symbiotic microorganisms make them particularly successful (de Vries 2010; Chanbusarakum and Ullman 2008), even if studies of bacterial communities in thrips have received relatively low attention. Some thrips species are frequently infected by symbionts that can affect their reproduction (Nguyen et al. 2015, Kaczmarczyk et al. 2018). The first studies concerning the microbiome of *F. occidentalis* are dated back to 1989, when Ulman et al., through the use of electron microscopy evidenced the presence of bacteria in the hindgut of the species. The microorganisms observed had a rod shape and were present in all thrips samples examined, regardless of diet and age. De Vries et al. (1995) confirmed the presence of these bacteria using standard microbiological methods. A few years later, de Vries et al. (2001a) revealed, from populations

of thrips collected on chrysanthemum and cucumber plants, the presence of two types of bacteria, united in a single monophyletic group by phylogenetic analyses. The same authors found that bacteria in *F. occidentalis* were facultative symbionts and therefore potentially cultured under laboratory conditions outside their hosts. This conclusion suggests that they might not be entirely host dependent. At the larval stage, the thrips were infected by bacteria belonging to the Enterobacteriaceae family; these bacteria were associated with food uptake and formed a monophyletic group with *Escherichia coli* in phylogenetic analysis. Additional studies showed that the presence of endosymbionts in WFT is related to abundant food sources and helps it to survive in case of nutritional deficiencies (de Vries et al., 2004). In relation to transmission studies, it was found that bacteria spread among unrelated individuals through ingestion and excretion on plant surfaces, and between the mother and the offspring, when the females release the eggs into the tissues of the host plants where the micro-organisms lived (de Vries et al., 2001b). Although thrips can survive in the absence of symbiotic bacteria, de Vries et al. (2001a) stated that there is a correlation between dietary habits and host adaptation. Some bacteria species belonging to the family Enterobacteriaceae were found in WFT thrips colonies in Netherland greenhouses, but this single finding did not justify the association as universally symbiotic for all WFT natural populations. Studies have compared bacterial communities of *F. occidentalis* populations from different regions, highlighting a remarkable resemblance between the colonies; this suggests that bacterial microorganisms and their hosts share a widespread association (Chanbusarakum and Ullman, 2008). Molecular phylogeny based on the 16S rRNA gene and biochemical analysis of thrips bacteria, using API 20E Biochemical tests, suggested that two distinctive groups of microbes are present in thrips (Chanbusarakum and Ullman, 2008; Facey et al., 2015). The first phylogenetic analysis of bacteria associated with WFT populations, revealed that thrips symbionts belong to two different genera and originated from environmental microbes (Facey et al., 2015).

1.3 Scope of the Thesis

Considering the importance that the microbiome assumes in the biological cycle of pest thrips of agricultural and ornamental crops, the purpose of this work is:

i) to evaluate various biological aspects of lab populations of *F. occidentalis* (oviposition, fecundity and longevity) regarding the two main groups of associated symbionts already described in the above literature;

ii) to provide a molecular characterization of the natural populations of *F. occidentalis* collected on the main economic crops present in southern Italy in order to evaluate genetic differences;

iii) to provide a molecular characterization of microbial communities associated with WFT natural populations in different host-plants and locations.

Chapter 2. Materials and Methods

2.1 Rearing of *F. occidentalis*

Insect rearing is fundamental to carry out laboratory experiments. WFT and other thrips species are bred in the laboratory for various purposes, including the study of their biological cycle (Gaum et al., 1994; Zhang et al., 2007), the potential control with natural enemies (Blaeser et al., 2004), the toxicity of pesticides and the development of resistance (Morse et al., 1986; Thalavaisundaram et al., 2008), the study concerning associations with microorganisms (de Vries, 2001), behavioural and pheromone studies (Terry & Schneider, 1993; Kirk & Hamilton, 2004) and aspects related to virus transmission (Ullman et al., 1992). The thrips can be raised in cages of different styles and fed with different foods (Loomans & Murai, 1997). Whole plants of chrysanthemum and bean, broad beans, cotyledons (Murai & Loomans, 2001), French dwarf beans, green bean pods and bean leaves (Steiner & Goodwin, 1998) are generally used as a natural food source. Diets can also be increased with sugar solutions, pollen, honey and plant extracts powder (Loomans & Murai, 1997).

Rearing in cages (Maintenance of thrips culture)

The WFT culture was carried on bean plants under controlled conditions (26 ±3°C temperature, 80% relative humidity and 16:8 L:D). Bean plants (*Phaseolus vulgaris*) were sown in the laboratory and grown in climatic cells. Four pots of bean plants were put inside each cage. Every 6-7 days, older plants were replaced with new ones. The plant management scheme in cages is shown in figure 5. Before being discarded, the old plants were carefully checked to recover any living WFT specimen present.

Figure 4. Front view of the rearing cages containing bean plants used for mass rearing of *F. occidentalis*.

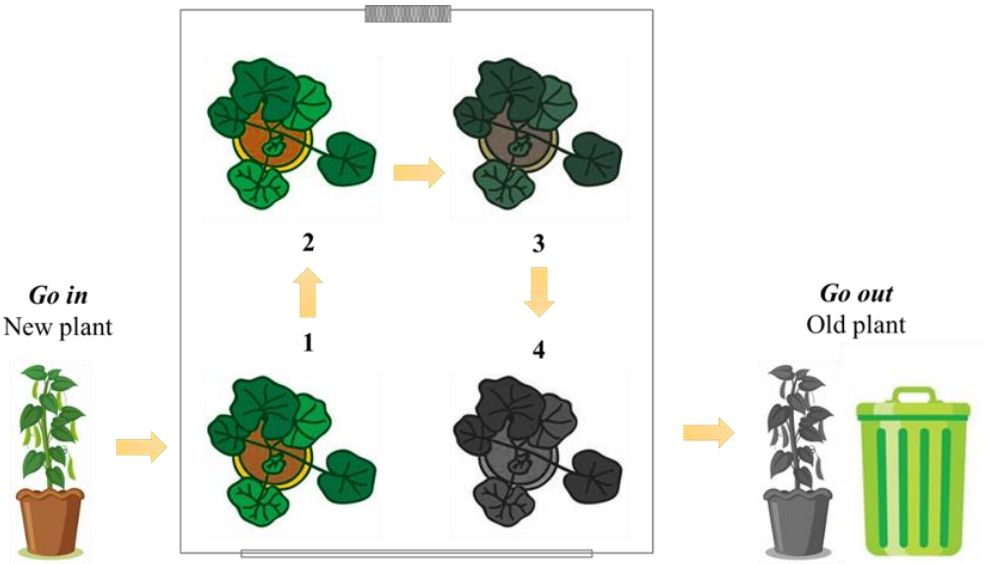


Figure 5. Demonstration scheme of the management and replacement of bean plants within *F. occidentalis* mass rearing cages.

2.2 Antibiotic treatment bioassay

The endosymbiotic bacteria bioassay using antibiotic treatment, provided two important steps. The first step is the breeding of specimens of the same characteristics. These batches were made by taking adult females from the thrips culture and allowing them to oviposit for 24 h on bean pods. The second step is the creation of aposymbiont thrips by using an antagonist antibiotic for the bacteria involved in the study.

a. Synchronised thrips rearing

In order to obtain specimens with the same characteristics (sex, age, reproduction method) to be used in biological tests, a group of virgin females, was bred in cylindrical laboratory tubes on bean pods. Considering the type of reproduction in WFT and the females born from fertilized eggs, it was necessary to proceed with the mating of virgin females and males. The coupling was performed and observed inside an arena constructed with dental wax quadrants (30 mm long, 20 mm wide, and 1.5 mm thick), from which the center was removed (5 mm diameter) using a cork drill. The arena was installed (with a slight pressure) in the middle of a microscope slide previously washed with distilled water. During the observation, the arena was covered with a glass cover slip gently pressed into the wax to form a transparent roof.

b. Rearing aposymbiotic thrips

The addition of antibiotics in the diet of the insect leads to the elimination of intestinal bacteria, thus obtaining aposymbiotic thrips for the bacterium species target under study. The antibiotics used in this study were Tetracycline and Amoxicilline dissolved in the water with a final concentration of 1 mg/ml. The antibiotic concentration used was far from producing direct lethal effects on thrips, as demonstrated in other studies on insect-bacteria associations conducted with the same technique (Baines,

1956; Douglas, 1997; Douglas, 1989; Hagen, 1966; Pierre, 1964; Stouthamer et al., 1993). Tetracycline was used to eliminate bacteria of the genus *Erwinia* (Gram +), while Amoxicillin to eliminate bacteria of the genus *Pantoae* (Gram-). For each experiment, previously obtained virgin females were placed in Murai cages (Murai and Ishii, 1982), round perspex cylinders of 10 cm in diameter. The solution containing the antibiotic was offered to the insects on a very thin layer of parafilm, at the top end of the cylinder. The thrips reached the antibiotic solution piercing the parafilm with their mouthparts or the ovipositor. B;ue dye methylene was added to the solution to check the antibiotic ingestion by all specimens used in subsequent tests. After 24 hours, all the thrips had a blue colour. The cylinders with the specimens were placed in a glass box with a lid, containing a potassium tartrate solution to maintain proper humidity levels.

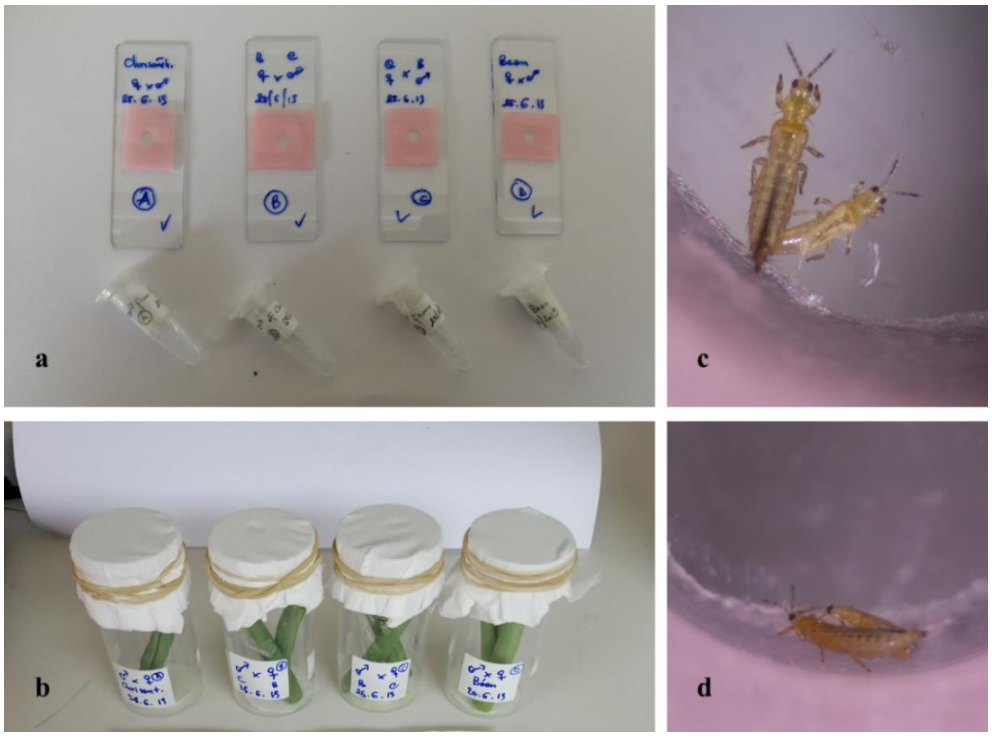


Figure 6. **a)** Mating arenas prepared from pieces of modelling wax sealed on the middle of a microscope slide made ready for copulation bioassays; **b)** cylindrical tubes for rearing of WFT females after mating with males; **c** and **d)** microscopic observation of a female and a male during copulation.

Figure 7. Rearing cages were obtained from previously fertilized female specimens of the same age to be used in the experiments.

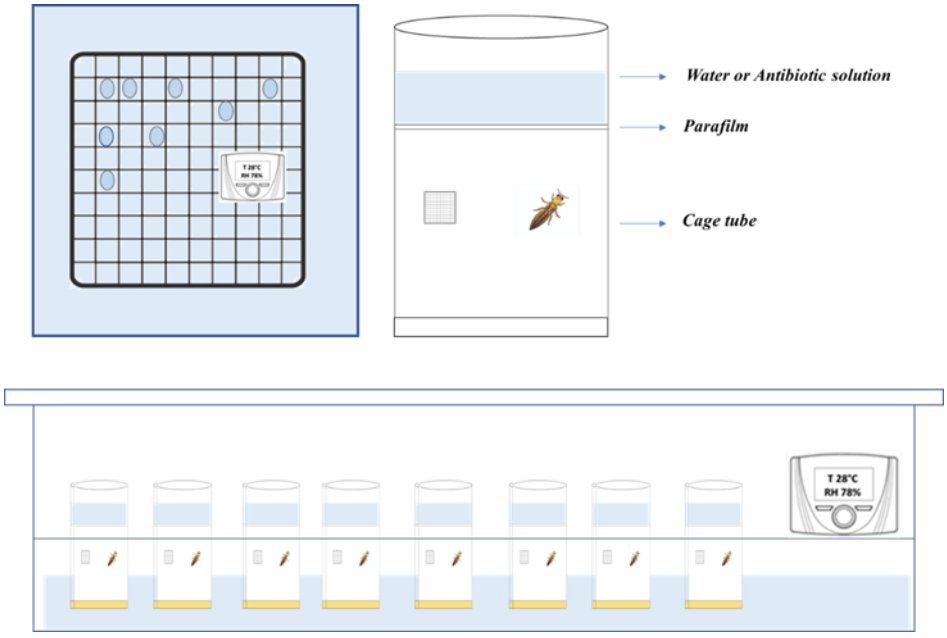


Figure 8. Murai cages used for antibiotic treatments of virgin females.

Preparation of plates with leaf discs

24-well plates were used for biological tests on oviposition and longevity. The wells were set up with a thin layer of agarose for microbiology at a concentration of 1.5% to prevent dehydration. A disc of bean leaf previously prepared and washed with distilled water, was inserted in each well. Before starting the experiments, the assembled plates were exposed to UV treatment for two hours.

2.2.1 *Oviposition test*

The oviposition test was conducted to assess the number of eggs laid by virgin females (after the antibiotic treatment) at four periodic intervals (24, 48, 72 and 96 hours). Five replicates were carried out for each antibiotic solution. In addition, the oviposition as related to antibiotic treatments was evaluated with and without the addition of pollen as food. In detail, the following tests were conducted:

- Tetracycline treatment with pollen (**Tt+P**);
- Amoxicillin treatment with pollen (**At+P**);
- Tetracycline treatment without pollen (**Tt-P**);
- Amoxicillin treatment without pollen (**At-P**);
- Control with pollen (**C+P**);
- Control without pollen (**C-P**).

The experiment began by placing six single virgin females in the well-plates (A -1,6). Every 24 hours, the six females were transferred to the next row of wells with a fresh leaf disc (B-1,6) and the 6 discs of leaves were subjected to heat treatment in a microwave oven for 30 seconds. The leaf discs were immediately observed under a microscope for egg counting. The same procedure was repeated after 48, 72 and 96 hours.

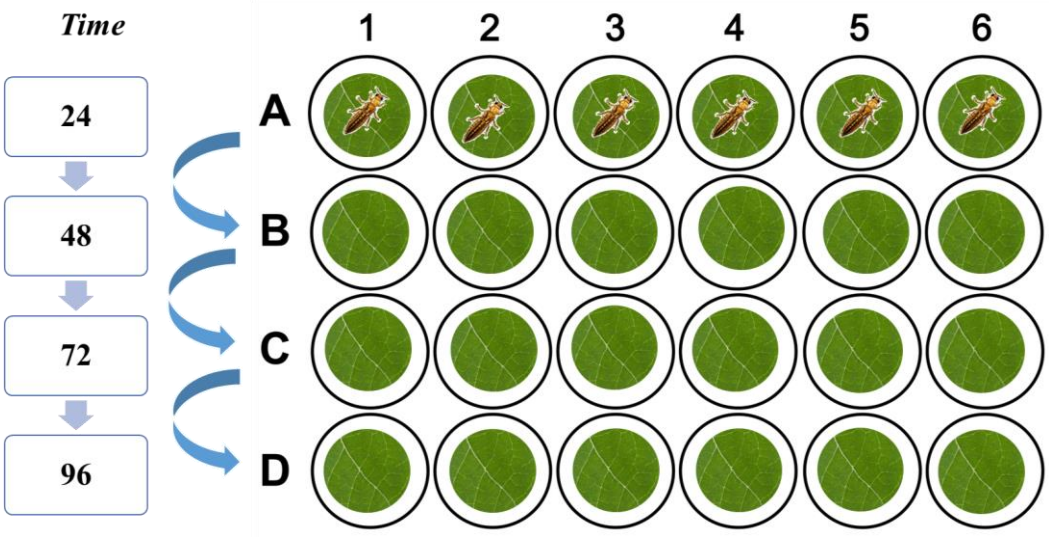


Figure 9. Graphic representation of the oviposition test carried out with 6 virgin females in a 24-well plate.

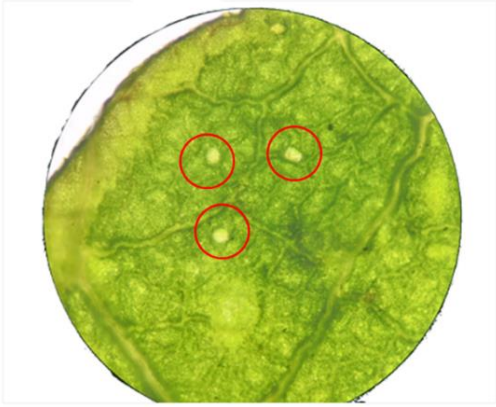


Figure 10. Photo under a microscope of a bean leaf disc exposed to oviposition after a microwave oven heat treatment. The red circles indicate the eggs of *F. occidentalis* within the foliar mesophyll.

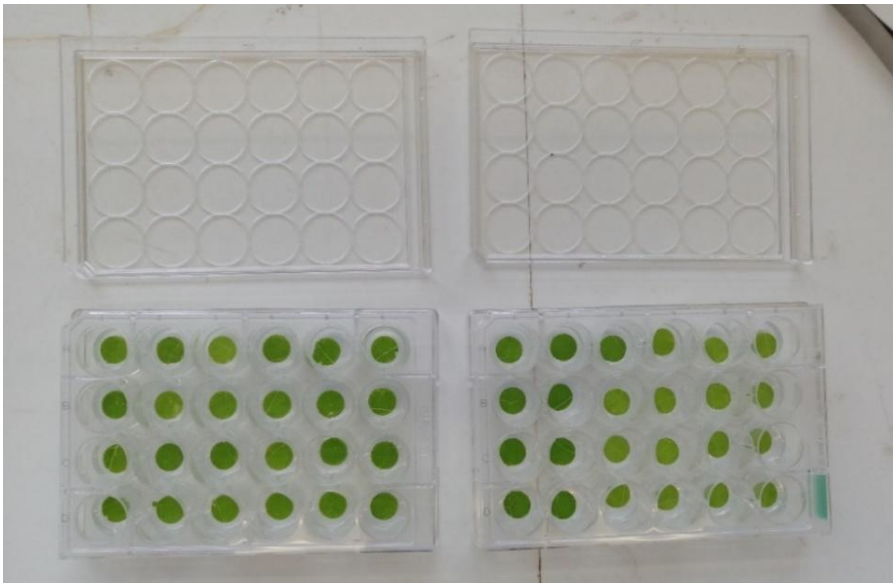


Figure 11. 24-well plates ready for oviposition tests.

2.2.2 *Fecundity test*

The fecundity of WFT in relation to antibiotic treatments was tested in five replicates in groups of fifteen virgin females. As for the ovideposition test, two treatments were evaluated for each antibiotic solution with and without pollen as a food source (**At+P**, **At-P**, **Tt+P**, **Tt-P**), plus the two controls (**C+P**, **C-P**). The experiment was carried out in cylindrical cages with a diameter of 15 cm and height of 10 cm using bean pods as a source of food and oviposition tissue. After a 24-hour interval, the 15 females were transferred to a new cylinder with fresh bean pods. In each replicate the transfer of females was done with the same interval for 4 days. The cylinders were kept under controlled conditions (28°C T, 78% RH and 16:8 L:D) and after 48 hours from the removal of the females, new specimens emerged from when the eggs were counted. The new individuals were counted precisely at intervals of 48, 72 and 96 hours.

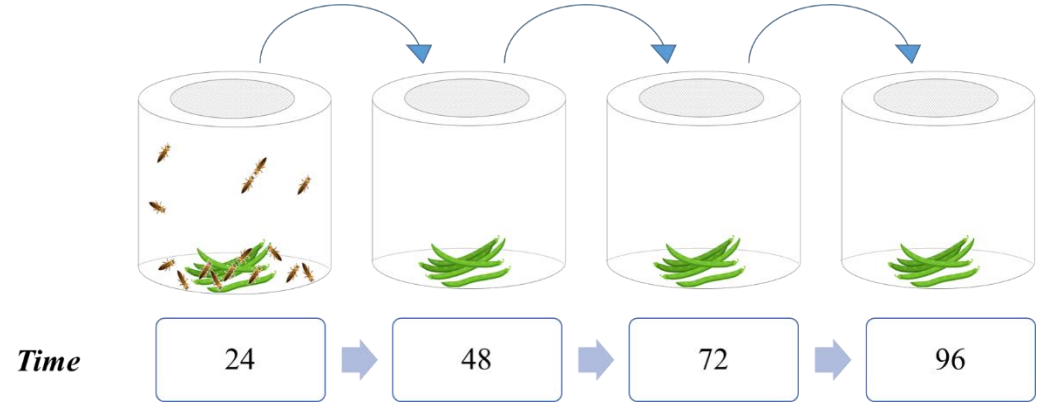


Figure 12. Graphic representation of the fecundity test conducted with a group of 15 females on bean pods in cylindrical cages.

2.2.3 *Longevity test*

In order to accurately assess the longevity of insects in relation to antibiotic treatments, the experiment was conducted repeated three times for each treatment (with antibiotics) and control (without antibiotics). Each treatment involved the use of 24 samples (virgin females) and tests were conducted in the well plates as shown in Figure 12. In addition, the longevity of *F. occidentalis* in relations to antibiotic treatments was evaluated, as was the biological aspect, with and without the addition of pollen as food (**At+P**, **At-P**, **Tt+P**, **Tt-P**, **C+P** and **C-P**). The plates were maintained throughout the test with the same parameters as the cages. The vitality control of the thrips was carried out at regular intervals of 24 hours over a period of 10 days.

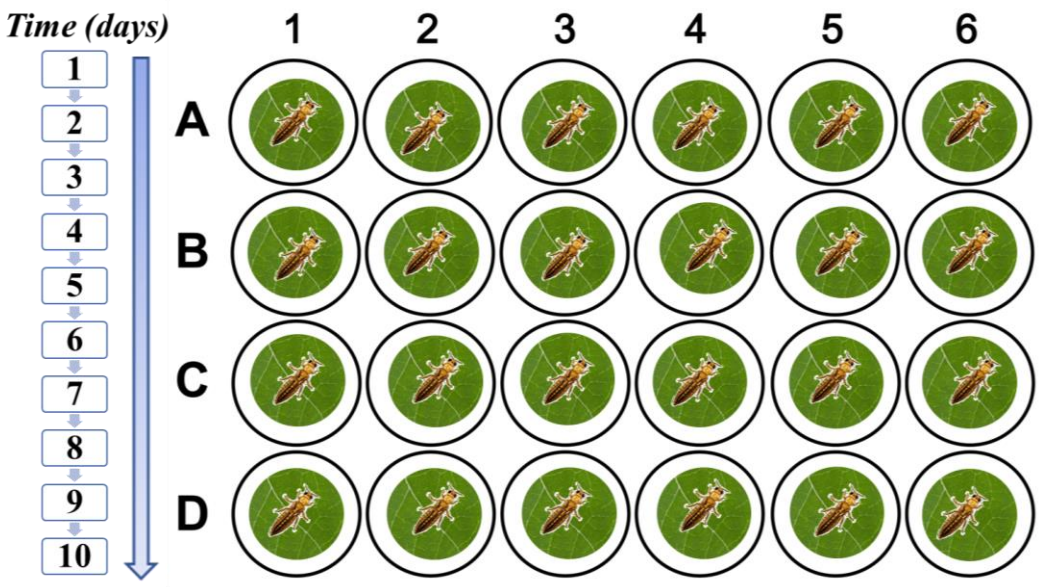


Figure 13. Graphic representation of the longevity test carried out with 24 virgin females leaf discs in a 24-well plate.

2.3 Data analysis

To assess the effects of antibiotic and pollen treatments on some of the WFT biological aspects that were investigated Unianova was performed using SPSS version 23. Post-hoc comparisons by t-test with Bonferroni correction were performed following a significant result highlighted by the Univariata analysis. The homogeneity of variances was checked through the Levene test and Microsoft Excel was used to produce graphs. All data was expressed as untransformed mean values ± standard error (SE).

2.4 Studies of natural population of *F. occidentalis*

2.4.1 Study of sites and thrips sampling

This study involves the WFT natural populations, collected on the main host plants of southern Italy (Calabria and Campania). In particular, the sampling was carried out in greenhouse crops and open fields. The direct sampling took place on strawberries, bell peppers, eggplants, chrysanthemum, rosa sp. and alfalfa plants. Samples were collected directly from the flowers of the plants and stored individually in Eppendorf tubes with absolute ethanol (96%) at -20°C. The samples were then inserted into plastic clips with descriptions. The location of the sampling was marked using a Global Positioning System (GPS) to obtain coordinates. Information about WFT samples was reported in Table 2.

Table 2. Information about *F. occidentalis* natural populations collected from different locations and host-plants.

Thrips population Code	Date of records	*Host-plants*	Location	Coordinate	Growing environment
FO1	27/05 2018	*Fragaria vesca*	Curinga (CZ)	38°49'16,8" N 16°13'16,8" E	greenhouse
FO2	09/06 2018	*Solanum melongena*	Bisignano (CS)	39°29'44.0"N 16°15'55.9"E	greenhouse
FO3	25/06 2018	*Capsicum annum*	Scafati (SA)	40°45'56.6"N 14°32'48.9"E	open field
FO4	04/06 2018	Rosa spp.	Ponticelli (NA)	40°51'44.1"N 14°20'36.6"E	greenhouse
FO6	03/08 2018	*Medicago sativa*	Squillace (CZ)	38°78'99".31 N 16°46'29".31 E	open field
FO7	18/06 2018	*Chrisantemum spp.*	Torre del Greco (NA)	40°46'51.5"N 14°23'55.4"E	greenhouse
FO8	31/06 2018	*Capsicum annum*	Angri (SA)	40°44'40.3"N 14°34'07.3"E	greenhouse
FO9	07/06 2018	*Fragaria vesca*	Bagnara Calabra (RC)	38°16'47.7"N 15°49'09.0"E	open field
FO10	18/05 2018	*Fragaria vesca*	Eboli (SA)	40°36'41.0"N 15°03'20.6"E	greenhouse

2.4.2 Morphological Identification of the target thrips species

Following the complete genomic DNA extraction processes, the samples were cleaned entirely from the Chelex solution residues with several 70% ethanol washes. The slide preparation of adult specimens of both sexes was based on the recorded methods of Mound and Marullo (1996) and Marullo (2003). The morphological identification of the species was performed directly under a stereomicroscope OLYMPUS SZX 9 using the keys of identification in Mound and Kibby (1998), Marullo (2003) and ThripsID (2020). Voucher specimens were deposited in the Department of Agriculture, Mediterranean University of Reggio Calabria, Italy.

2.5 Molecular analysis

2.5.1 DNA extraction

Rather than grinding the specimens, total genomic DNA was extracted using a Chelex–proteinase K-based non-destructive method (Walsh et al. 1991). DNA extraction was performed on individual insects, incubated in 5 µL of proteinase K (20 mg/mL) and 80 µL of 5% Chelex 100 suspension at 55 °C for 1 h. Proteinase K was then inactivated at 100 °C for 8 min. The supernatant containing the DNA was removed after centrifugation. The supernatant, containing the total DNA, was removed after centrifugation at 13000 rpm for 3 minutes, and stored at -20°C.

2.5.2 Amplification and sequencing and Phylogenetic analysis

Molecular tools have proven useful in the last decade to integrate various fields of biology, from systematics to ecology (Bikford et al., 2007). For all WFT natural populations, five genes were amplified and sequenced: the mitochondrial cytochrome c oxidase subunit I (COI), and ribosomal gene, the expansion segment D2 of the 28S ribosomal subunit (28S-D2), Internal Transcribed Spacer 2 (ITS2), Histone 3 (H3) and Elongation factor 1 alpha (EF-1α).

- *Mitochondrial fragment*

  DNA Barcoding employs partial fragments of the mitochondrial cytochrome c oxidase I (mtCOI) gene for species-level identification, and this has achieved broad acceptance as an additional method to resolve taxonomic ambiguities (Habert et al., 2003; Besansky et al., 2003). Mitochondrial genes have also been used to estimate genetic diversity below species level (Avise, 2000). Its usefulness as a quick and authentic tool for species identification is well recognized in a wide variety of insect taxa around the world (Jalali et al., 2015). Fwor Mitochondrial DNA has been widely used for molecular characterization and identification of several thrips species (Marullo et al., 2020). The mitochondrial (mt) genomes of most animals are circular DNA molecules of approximately 15–17 kb in size that encode 37 genes:13 protein-coding genes, 2 rRNA genes, and 22 tRNA genes (Boore, 1999; Wolstenholme, 1992). The complete mitochondrial genome of WFT is shown in Figure 14.

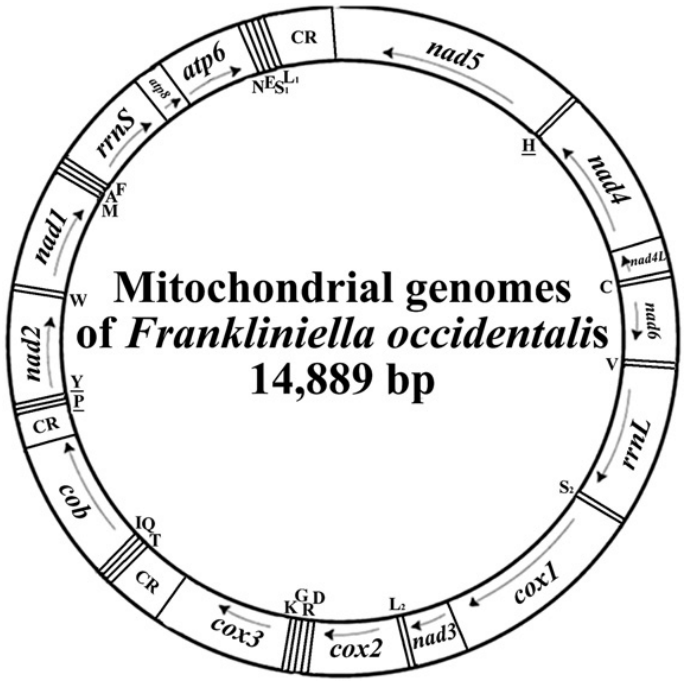


Figure 14. The gene map for mitochondrial genome of *Frankliniella occidentalis*. (From Yan et al., 2012).

- *Ribonuclear fragments*

  28S-D2 fragment represents the D2 expansion domain of the 28S subunit. This gene is highly conserved, and it is largely used for the molecular discrimination of species at genus- level (Campbell et al., 1993; 2000).

- The **ITS2** region (Internal Transcribed Space 2) represents a rapidly evolving insertion, between the domain 1 and the region codifying the LSU (Large subunit), which includes the 28S gene. The non-coding ITS2 region has been widely used to identify closely related species (Hackett et al. 2000; Wilkerson et al. 2004; Naegele et al. 2006).

- **Histone 3** (H3) is a nuclear protein-coding gene that is highly conserved as it has a vital role in packaging DNA in eukaryotic chromosomes (Hoy, 2013). In the thrips, the sequences produced from the amplification of H3 fragment, concatenated with four other molecular loci, were used to re-examine the relationship of families and sub-families, within the two sub-orders of Thysanoptera (Buckman et al., 2012).

- **Elongation factor - 1α** (EF1a) is a nuclear protein-coding gene largely used for phylogenetic studies as it has a highly conserved amino acid sequence (Cho et al., 1995).

The polymerase chain reactions (PCRs) cycle (thermocycler conditions) and primers were used to amplify a fragment of mitochondrial COI, 28S-D2, ITS2, H3 and EF-1α fragments are reported in Table 3. For gene amplifications the PCRs were performed on a Mastercycler® Nexus X2 Series thermocycler using 20 μL reaction volumes, consisting of 1 × Promega PCR buffer (containing MgCl2), 0.2 mM each of dNTP, 0.25 μM of each primer, 10 mg/mL bovine serum albumin, 1.5 units GoTaq G2 DNA polymerase (Promega Italia, Milan, Italy), and 2 μL of DNA template. The concentration of the DNA samples was determined by Nanodrop analysis (qualitative and quantitative), and the PCR products were checked on a 1.2% agarose gel stained with GelRED® (Biotium,

Fremont, CA, USA), visualized and photographed under UV light. All PCR products produced a single band and were cleaned using the ExoSAP protocol. To confirm the identity of *F. occidentalis*, Sanger sequencing was performed in both directions through the same primer pairs used for the amplification reactions. All sequences were aligned via manual trimming in BioEdit version 7.2.5 (Hall,1999) and were virtually translated into the corresponding amino acid chain to detect frame-shift mutations and stop codons using EMBOSS Transeq (Madeira et al., 2019). Edited sequences were checked against the GenBank database and BOLD using "BLASTn" (NCBI, accessed on 20 May 2020).

Table 3. Primer sequences with the relative amplification program of the COI and 28S D2, IS2, EF-1α and H3 genetic regions for molecular characterization of WFT natural populations.

Name	Sequence 5'-3'	Fragment	Source	PCR Cycle		
				T (°C)	Time	N. Cycle
LCO-1490	GGTCAACAAATCATAAAGATATTGG	COI	Simon et al, 1994	95	1'	
				94	30"	40
				48	1'30"	
HCO-2198	GTAAATATATGRTGDGCTC			72	1'	
				72	7"	
D2F	CGTGTTGCTTGATAGTGCAGC	28S D2	Campbell et al., 1993	95	5'	
				93	15"	
				52	45"	35
D2R	TTGGTCCGTGTTTCAAGACGGG			72	1'30"	
				72	7"	
ITS2F	TGTCAACTGCAGGACACATG	ITS2	Glover et al., 2010	95	3'	
				94	45"	
				55	45"	35
ITS2R	AATGCTTAAATTTAGGGGGTA			72	1'	
				72	7"	
H3NF	ATGGCTCGTACCAAGCAGAC	H3	Colgan et al., 1998	95	1'	
				94	30"	40
H3R	ATATCCTTRGGCATRATRGTGAC			48	1'30"	
				72	1'	
				72	7"	
EF1	GACAACGTTGGCTTCAACGTGAAGAACG	EF- 1α	Chen et al., 2003	95	10'	
				95	30"	
				50	40"	35
EF2	ATGTGAGCAGTGTGGCAATCCAA			72	45"	
				72	6'	

2.6 Microbiome analysis

2.6.1 16S General screening

To investigate the positivity of endosymbiotic microorganisms, a general screening amplifying the 16S rRNA fragment was carried out in all WFT populations collected in greenhouses and open fields. After DNA extraction, the Polymerase Chain Reaction was performed with primers 27F [AGAGTTTGATCMTGGCTCAG] 1513R [ACGGYTACCTTGTTACGACTT]. The thermocycler conditions were as follows: Initial denaturation at 94°C for 3 min, followed by 35 cycles at 94 °C for 45 s, 52 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 5 min.

2.6.2 Bacterial 16S rRNA gene amplification, library preparation and sequencing

Metagenomic studies are frequently performed by analysing the insect bacteria communities through the 16S ribosomal RNA gene (16S rRNA), which is approximately 1,500 bp long and contains nine variable regions interspersed between conserved regions. The preparation and sequencing of amplifier libraries are quite flexible and allow a wide range of experimental models (Head et al., 2014). In this study, the following experimental workflow was used to generate library amplicons. Variable regions of 16S rRNA are frequently used in phylogenetic classifications such as genus or species in diverse microbial populations. Which 16S rRNA region to sequence is an area of debate, and your region of interest might vary depending on things such as experimental objectives, design, and sample type. This protocol describes a method for preparing samples for sequencing the variable V3 and V4 regions of the 16S rRNA gene. This protocol can also be used for sequencing other regions with different region-specific primers. This protocol, combined with a benchtop sequencing system, on-board primary analysis, and secondary analysis using MiSeq Reporter or

BaseSpace, provides a comprehensive workflow for 16S rRNA amplicon sequencing.

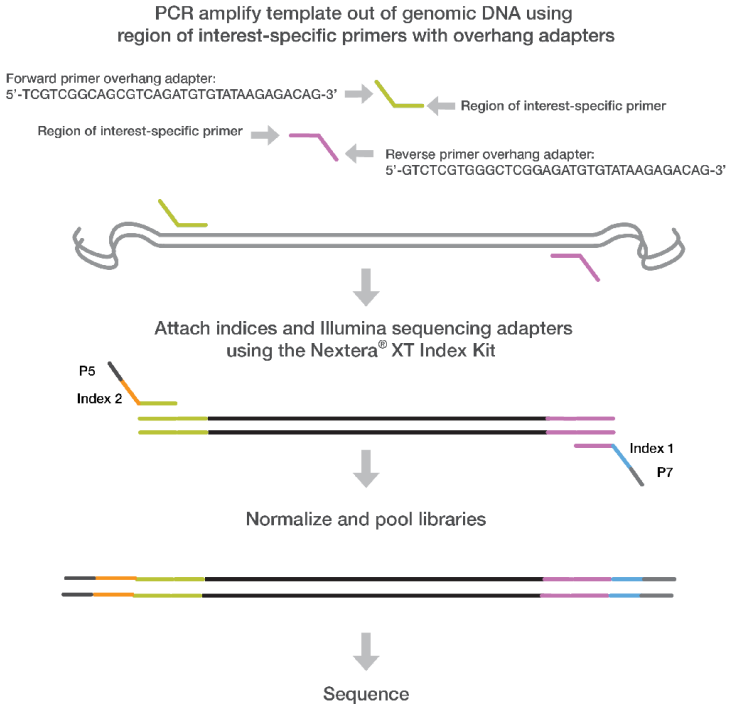


Figure 15. 16S V3 and V4 regions Library Preparation Workflow

- *1° Stage* - Amplicon PCR

  DNA of all *F. occidentalis* natural populations that showed positive to preliminary 16S screening was involved for metagenomic analyses. PCR amplicons were obtained by amplifying the 16S V3 and V4 region. PCR was carried out using 16S Amplicon PCR Forward Primer = 5' [TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG] and16S Amplicon PCR Reverse Primer = 5' [GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC] (Klindworth et al., 2013). For gene amplifications, the PCRs were performed on an Eppendorf thermocycler using 25 µl reaction volumes, consisting of 12,5 µl of 2x KAPA HiFi HotStart ReadyMix (containing $MgCl_2$), 5 µl of each primer, and 2,5 µl of Microbial DNA template. The thermocycler conditions were as follows: Initial denaturation at 95°C for 3 min, followed by 35 cycles at 98°C for 20 s, 56°C for 30 s, 72°

C for 40 s, and a final extension at 72°C for 5 min. To verify the size, the PCR products were checked on a 2% agarose gel stained with GelRED® (Biotium, Fremont, CA, USA), visualized and photographed under UV light.

- *PCR Clean-Up*

  This protocol step was performed to purify the 16S V3 and V4 amplicons away from free primers and primer-dimer species by using magnetic beads (Invitrogen). To each sample was added 20 µl of AMPure XP beads and, were shaken at 1800 rpm for 2 minutes before incubating at room temperature for 5 minutes. To remove and discard the supernatant, the plate must placed on a magnetic stand for 2 minutes or until the supernatant has cleared. Subsequently, with the Amplicon PCR plate on the magnetic stand, two washing cycle beads were carried out with 200 µl freshly prepared 80% ethanol. The beads were resuspended adding 52.5 µl of 10 mM Tris pH 8.5 to each sample. Finally, 50 µl of supernatant containing the purified PCR amplicons was removed and used for the next step.

- *2° Stage* - Index PCR
- For Index amplifications the PCRs were performed using 50 µl reaction volumes, consisting of 25 µl of 2x KAPA HiFi HotStart ReadyMix (containing $MgCl_2$), 5 µl of each index (Nextera XT Index Primer 1 (N7xx) and Nextera XT Index Primer 2 (S5xx)), 10 µl of grade water and 5 µl of resuspended PCR product DNA. The thermocycler conditions were as follows: Initial denaturation at 95°C for 3 min, followed by 8 cycles at 98°C for 20 s, 57°C for 30 s, 72° C for 40 s, and a final extension at 72°C for 5 min. To verify the size, the PCR products were checked on a 2% agarose gel stained with GelRED®, visualized and photographed under UV light, followed by the second step of purification (see PCR Clean-Up).

Table 4. List of index sequences used for Library preparation.

Index 1	(i7) Sequence	Index 2	(i5) Sequence
N701	TAAGGCGA	S501	TAGATCGC
N702	CGTACTAG	S502	CTCTCTAT
N703	AGGCAGAA	S503	TATCCTCT
N704	TCCTGAGC	S504	AGAGTAGA
N705	GGACTCCT	S505	GTAAGGAG
N706	TAGGCATG	S506	ACTGCATA
N707	CTCTCTAC	S507	AAGGAGTA
N708	CAGAGAGG	S508	CTAAGCCT
N709	GCTACGCT		
N710	CGAGGCTG		
N711	AAGAGGCA		
N712	GTAGAGGA		

- *Library Quantification, Normalization, and Pooling*

  Quantification was performed using a fluorometric quantification method (QUBIT). The DNA concentration was calculated in NM based on the size of DNA amplicons evaluated through agarose gel electrophoresis using the following formula:

$$\frac{(concentration\ in\ ng/\mu l)}{(660\ g/mol \times average\ library\ size)}\ 10^6 = concentration\ in\ mM$$

  The final concentration of the library was diluted with a Resuspension Buffer (RSB). Subsequently, 5 µl of each sample was taken to form the final pool of libraries.

- *Library Denaturing and MiSeq Sample Loading*

  The analysis protocol provides a hot denaturation of the final pool of libraries through a 0.2 N of a NaoH solution and a hybridization buffer (HT1). Each run must include a minimum of 5% PhiX to serve as an internal control for these low diversity libraries.

2.6.3 Bioinformatics and statistical analysis

To identify the bacteria community present in the WFT sample sequences, results needed to be filtered for quality, aligned into contigs, and blasted against existing bacterial sequence databases. NGS sequencers occasionally produce poor quality reads errors occur near the site of the sequencing primer and towards the end of longer sequences. If such errors are not removed by trimming, they can lead to distortions in later stages of alignment and sequence analysis, such as terminal gaps in cluster alignments. From the sequencing of 9 samples was obtained with an average of 323,216 sequences coupled with a length of 302 bp (minimum 248,343; maximum 523,156). To obtain amplicon sequence variants (ASV), the raw sequences of our dataset were trimmed by removing the bases with low quality scores, using DADA2, a trimming algorithm able to retain as much information as possible (Callan et al., 2016). ASV's with a total frequency of less than ten, and those matching with chloroplast, mitochondria and eukaryotic sequences were discarded from the data set and analysis. In addition, the filtered sequences were then assembled and subjected to another quality control with PANDASEQ, a program that assembles the paired-end reads very quickly, corrects most errors and assigns a quality score (Masella et al., 2012). Analysis of the WFT natural populations gut microbiome was performed using the framework QIIME2 2017.9. Once assembled, the filtered sequences were grouped into Operational Taxonomic Units (OUT's), using a pre-trained Silva 132 99% OTUs based naïve Bayes classifier and a confidence threshold of 0.7 using q2-feature-classifier plugin. A multiple sequence alignment was performed for all ASVs using MUSCLE (Multiple Sequence Comparison by Log-Expectation) (Madeira et al., 2019). The alignment produced was subsequently used for the construction of phylogeny through FastTree vers.2 (Price et al., 2010). The next step was to summarize the metrics of alpha diversity in the structure of microbial communities with respect to its richness (number of taxonomic groups), uniformity (distribution of group abundances. In this study, the alpha diversity analysis of amplicon sequencing data was performed to assess differences between different host-plants and locations.

The rarefaction analysis was performed with a minimum and a maximum depth of 100 and 10000, respectively using the following indices:

- Shannon: is a diversity index used in statistics for populations with an infinite number of elements. It considers both the richness in species and their evenness. The advantage of this index is that it manages to make rare species stand out;
- Chao1: estimates the absolute number of species in a sample, based on the number of rare species, as the number of species actually observed added to the ratio of singletons to doubletons;
- Simpson: measures the probability that two individuals, randomly selected within a group, belong to the same species.

Finally, Beta diversity was performed to highlight differences between microbial communities of samples and was quantified using a metric based on phylotype abundances.

Chapter 3. Results and Discussion

3.1 Bioassay and Antibiotic treatment results

Thrips rearing is particularly difficult, due to the complex relationship with the hygrothermal parameters, fundamental for the survival and the completion of the biological cycle under controlled conditions (Murai & Loomans, 1997). However, cage rearing has been maintained in optimal conditions during the study period, thus avoiding slow trials due to the lack of specimens. The statistical analysis allowed us to evaluate the biological aspects of longevity, fecundity and oviposition concerning the different antibiotic treatments. In addition to the antibiotic treatments, evaluations were conducted on the addition or absence of pollen as a food source.

3.1.1 Longevity test

All plates were observed daily at the same time, for a period of 10 days, to count live specimens in individual wells. Longevity was positively affected by pollen, while the presence of antibiotics led to a reduction. In general, all specimens fed with antibiotic solutions showed lower longevity than specimens fed with no antibiotic (mean 5.79d vs 7.16d). The presence of pollen also increases longevity in antibiotic treatments (mean 6.38d vs 5.20d). The results of the Univariate analysis evaluating the effect of antibiotic and pollen treatments are shown in Table 5.

Table 5. Univariate analysis evaluating the effect of antibiotic and pollen treatments on Longevity test in *F. occidentalis* (Levene test: F= 1,01; df = 3, 428; P=0,388).

	Source	***df***	**F**	**P**
Longevity Test	Model	4	4300,646	<0,001
	Antibiotic	1	45,81	<0,001
	Pollen	1	37,40	<0,001
	Interaction (antibiotic*pollen)	1	0,0296	0,784

Unianova highlighted the differences between specific treatments (F = 37.46 df =5,414; P<0.001; Levene test: F= 1.088; P=0.363) (Figure 16). The results showed an increase in longevity in individuals when in their respective treatments; individuals were given pollen as food. In terms of days, longevity increases by 0,85 ±0,023 in Amoxicillin treatment, 1,51 ±0,0014 in Tetracycline treatment and 1,29±0,074 in control. By performing a Bonferroni multiple comparison test we checked the significant differences among means (Figure 16). In particular, the results showed significant statistical differences between Control with pollen and all the other treatments. Also, in the paired comparison, the results showed significant statistical differences between Amoxicillin and Tetracycline treatments without pollen and all the other treatments. No significant statistical difference was detected between Amoxicillin and Tetracycline treatments with pollen and Control without pollen.

Table 6. Mean (±ES) of the longevity of *F. occidentalis* to different treatments.

	Mean	Std. Error of Mean
Amoxicillin treatment without pollen (At+P)	5,83	±0,24
Amoxicillin treatment with pollen (At-P)	6,68	±0,22
Tetracycline treatment without pollen (Tt-P)	4,57	±0,22
Tetracycline treatment with pollen (Tt+P)	6,08	±0,24
Control without pollen (C-P)	6,51	±0,24
Control with pollen (C-P)	7,8	±0,22
Total	6,25	±0,10

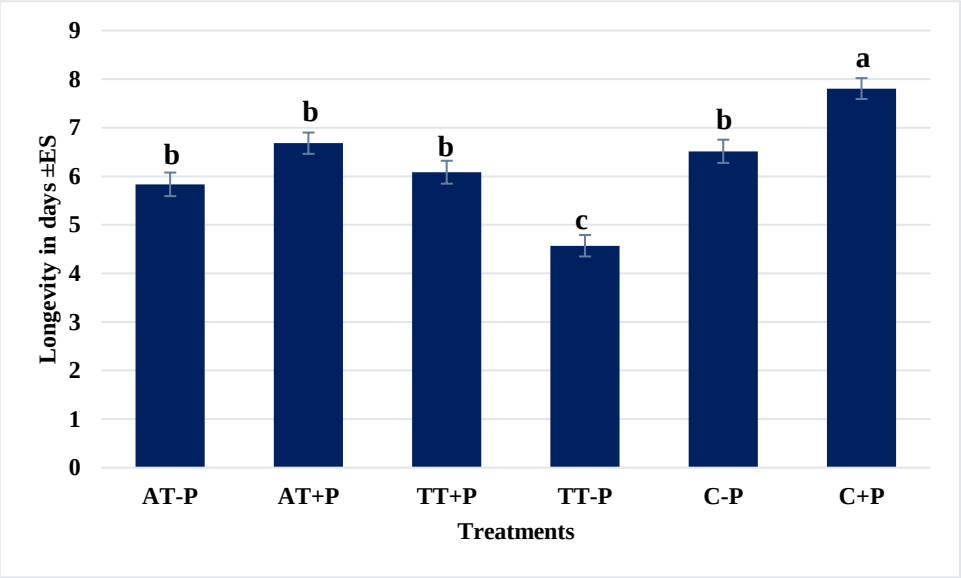


Figure 16. Mean longevity (±SE) of *F. occidentalis*, estimated over a time interval of 10 days in different antibiotic treatments (At-P, At+P, Tt-P and Tt+P) and controls (C+P and C-P). Means with different letters are significantly different ($P < 0.01$).

3.1.2 *Fecundity test*

The results of the Unianova test show that both antibiotics and pollen have an effect on fertility (P <0.001) (see Table 7). The different time intervals show influence on fertility despite the probability being above 0.05 (P=0.074). The comparison of the overall average between antibiotic treatments and controls (no antibiotics) shows that antibiotics strongly reduces fecundity (69.23 vs 33,22). The comparison of the overall average of the treatments with and without the addition of pollen shows how the presence of pollen strongly increases fertility (60.85 vs 41.60). The number of specimens that emerged from bean pods exposed to a group of 15 females is a strong indication of the assessment theory. The results showed a reduced fecundity in all groups of females previously treated with antibiotics, compared to controls. The lowest mean fecundity values of 20,13 ±1,87 and 28,20 ±2,55 were recorded in tetracycline treatments with and without pollen. The highest mean values were found in females not treated with antibiotic solutions and pollen fed. The values obtained were 90,53 ±5,246 and 47,93 ±2,804 respectively in control with pollen and control without pollen. With mean values of 45,80±2,87 and 38,73±2,68, the results obtained in amoxicillin treatment with and without pollen were placed between the observations recorded in the two tetracycline treatments and the two controls. Statistical differences between treatments are shown in Figure 17. Statistical analyses have also shown the number of larvae that have emerged in different time intervals (48, 72 and 96 hours) between the treatments (Figure 18). The data used in the construction of the histograms show three different trends. In amoxicillin and tetracycline treatments and control without pollen, a progressive reduction of the individuals born is observed. In amoxicillin treatment and control with pollen, there is an initial increase of the number of individuals born from the oviposition at 72 hours and a subsequent decrease of the number of individuals born from the bean pods exposed to the thrips after 96 hours. In the tetracycline treatments with pollen, there are similar mean values for 48 and 72-hour intervals and a decrease to 96 hours after treatment.

Table 7. Univariate analysis evaluating the effect of different antibiotics, pollen and hours treatments on fecundity test in *F. occidentalis* (Levene test: F= 11,630; df = 11, 78; P=0,36).

	Source	*Df*	F	P
Fecundity Test	Antibiotics	1	98,45	<0,001
	Pollen	1	31.62	<0,001
	Hours (48, 72, 96)	2	2,683	0,074

Table 8. Mean values of fecundity test individuals of *F. occidentalis* emerged from bean pods at different intervals (48, 72 and 96 hours) in the different antibiotic treatments.

	Hours	Mean	Std. Error of Mean
Amoxicillin treatment without pollen	48	47,40	±3,124
	72	39,20	±4,005
	96	29,60	±3,108
	Total	38,73	±2,675
Amoxicillin treatment with pollen	48	44,00	±6,325
	72	52,80	±4,454
	96	40,60	±2,561
	Total	45,80	±2,866
Tetracycline treatment without pollen	48	26,80	±2,596
	72	20,00	±2,074
	96	13,60	±1,965
	Total	20,13	±1,869
Tetracycline treatment with pollen	48	31,20	±3,353
	72	29,80	±5,181
	96	23,60	±4,707
	Total	28,20	±2,553
Control without pollen	48	52,80	±6,184
	72	48,40	±4,106
	96	42,60	±3,789
	Total	47,93	±2,804
Control with pollen	48	78,00	±6,221
	72	104,80	±9,024
	96	88,80	±8,828
	Total	90,53	±5,246
Total	48	46,70	±3,588
	72	49,17	±5,411
	96	39,80	±4,795
	Total	45,22	±2,693

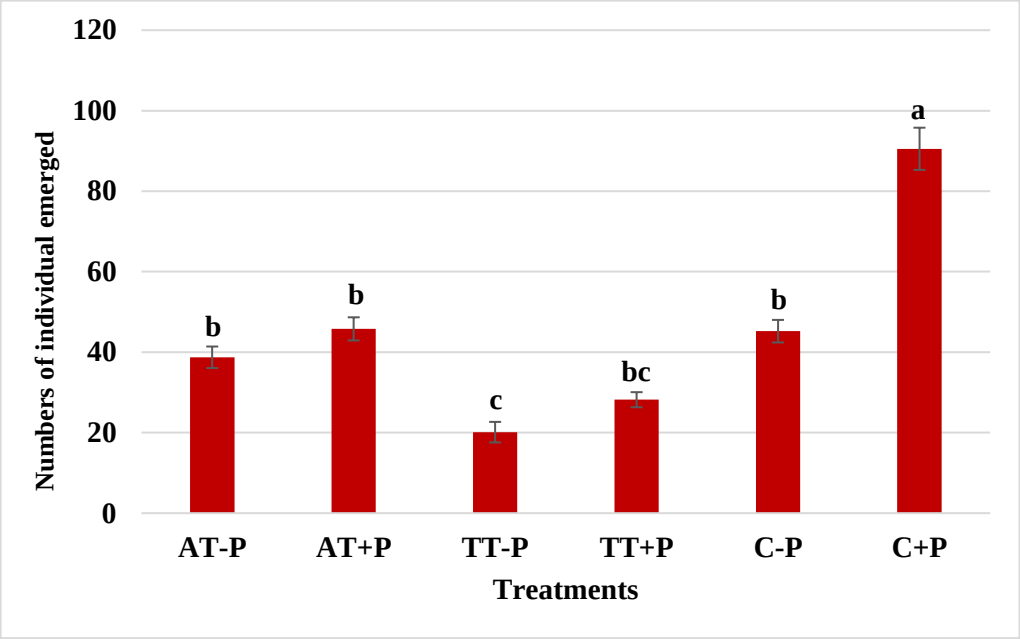


Figure 17. Total fecundity means (±SE) of *F. occidentalis*, estimated over a time interval of ten days in different antibiotic treatments (At-P, At+P, Tt-P and Tt+P) and controls (C+P and C-P). Means with different letters are significantly different (P < 0,001).

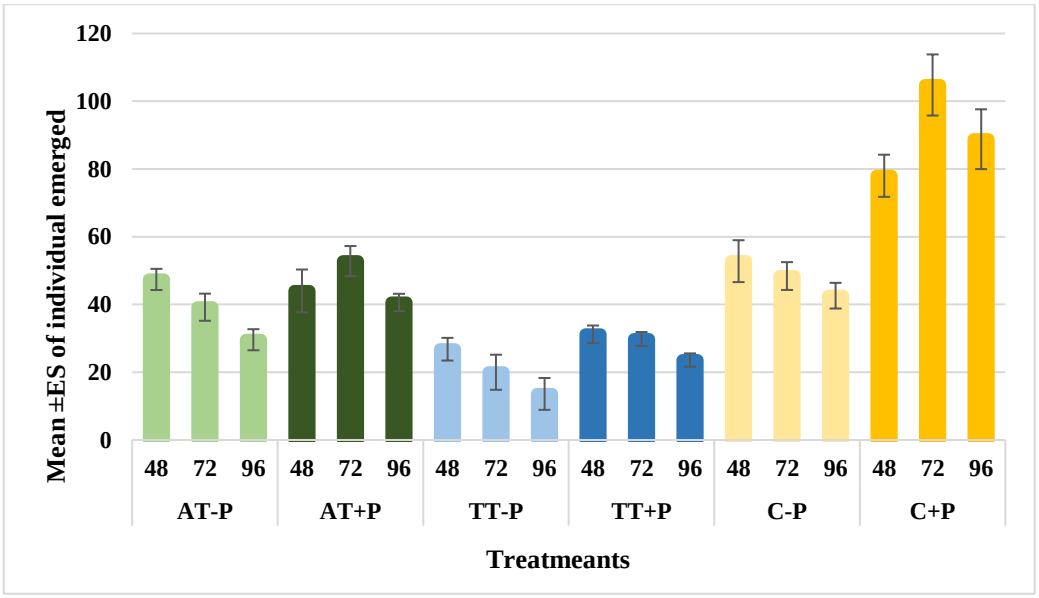


Figure 18. Mean (±SE) at different intervals and treatments on the fecundity of *F. occidentalis*.

3.1.3 *Oviposition test*

The results of the Univariate analysis showed that the different variables (antibiotic treatments, addition of pollen as a food source and oviposition times) influenced the biological aspect of oviposition in the tests performed (see Table 9).

Table 9. Univariate Analysis (UNIANOVA) evaluating the effect of different antibiotic treatment on Oviposition test in *F. occidentalis* (Univariate test: F= 21,094; df = 5,414; P<0,001).

	Source	***Df***	**F**	**P**
Oviposition test	Antibiotic	1	53,03	<0,001
	Pollen	1	39,07	<0,001
	Hours of oviposition	3	14,86	<0,001

Comparing the treatments performed with antibiotic solutions without pollen analysis of variance (ANOVA) evaluating the effect of different antibiotic treatments on oviposition test in *F. occidentalis* (F= 21,094; df = 5,414; P<0,001), the observed difference between (At-P and Tt-P) the average total value is 0.325 ±0.001. Comparing the same antibiotic treatments in which thrips were offered pollen as a food source (At+P and Tt+P), the average oviposition value obtained by difference of the mean values of the two treatments is 0.82 ±0.078. In untreated antibiotic treatments (C+P and C-P), the mean oviposition values are 1.66 ±0.149 and 2.50 ±0.201, respectively, for controls without and with the addition of pollen. Statistical differences between treatments are shown in Figure 20. Statistical analyses also made it possible to assess the comparison between treatments of the number of larvae that emerged in different time intervals (24, 48, 72 and 96 hours) (Figure 19).

Table 10. Table of mean values of oviposition individual test of *F. occidentalis* emerged from bean pods at different intervals (24, 48, 72 and 96 hours) in different antibiotic treatments.

	Hours	Mean	Std. Error of Mean
Amoxicillin treatment without pollen	24	1,37	±0,29
	48	1,10	±0,20
	72	0,87	±0,18
	96	0,77	±0,15
	Total	1,02	±0,11
Amoxicillin treatment with pollen	24	2,97	±0,52
	48	1,90	±0,38
	72	1,67	±0,33
	96	1,10	±0,20
	Total	1,91	±0,19
Control without pollen	24	2,03	±0,33
	48	1,80	±0,27
	72	1,57	±0,31
	96	1,27	±0,28
	Total	1,67	±0,15
Control with pollen	24	3,50	±0,53
	48	2,87	±0,39
	72	1,97	±0,33
	96	1,70	±0,23
	Total	2,51	±0,20
Tetracycline treatment without pollen	24	1,00	±0,25
	48	0,73	±0,13
	72	0,73	±0,20
	96	0,33	±0,11
	Total	0,70	±0,09
Tetracycline treatment with pollen	24	1,43	±0,31
	48	1,13	±0,21
	72	1,03	±0,23
	96	0,72	±0,16
	Total	1,08	±0,12
Total	24	2,05	±0,17
	48	1,59	±0,12
	72	1,30	±0,11
	96	0,98	±0,09
	Total	1,48	±0,06

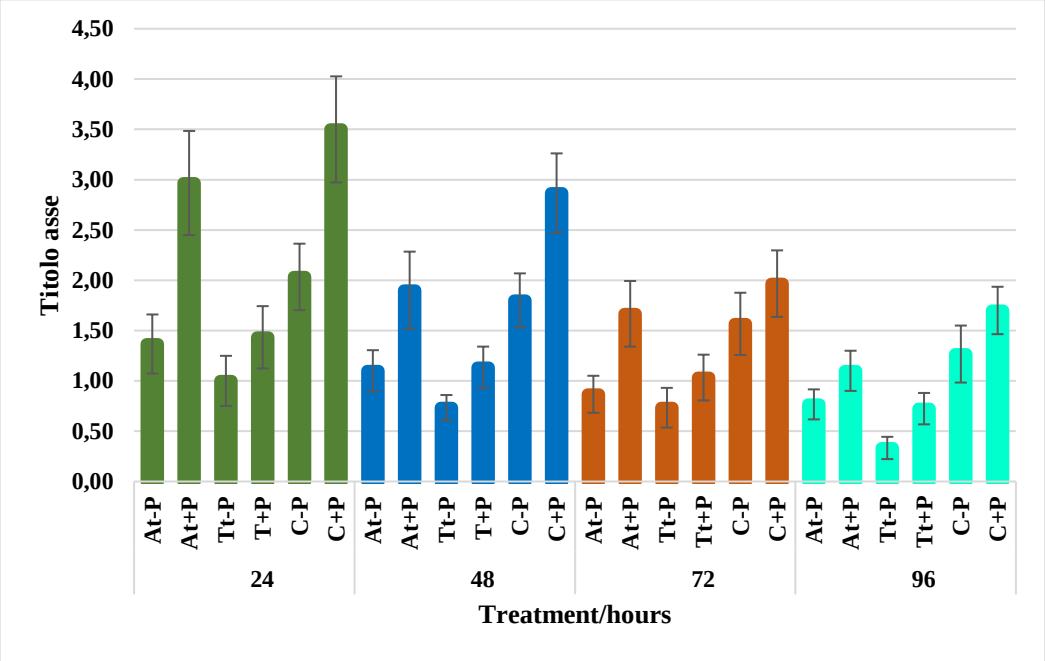


Figure 19. Mean of oviposition (±SE) of *F. occidentalis*, estimated at different interval time. Means with different letters are significantly different (P < 0,01)

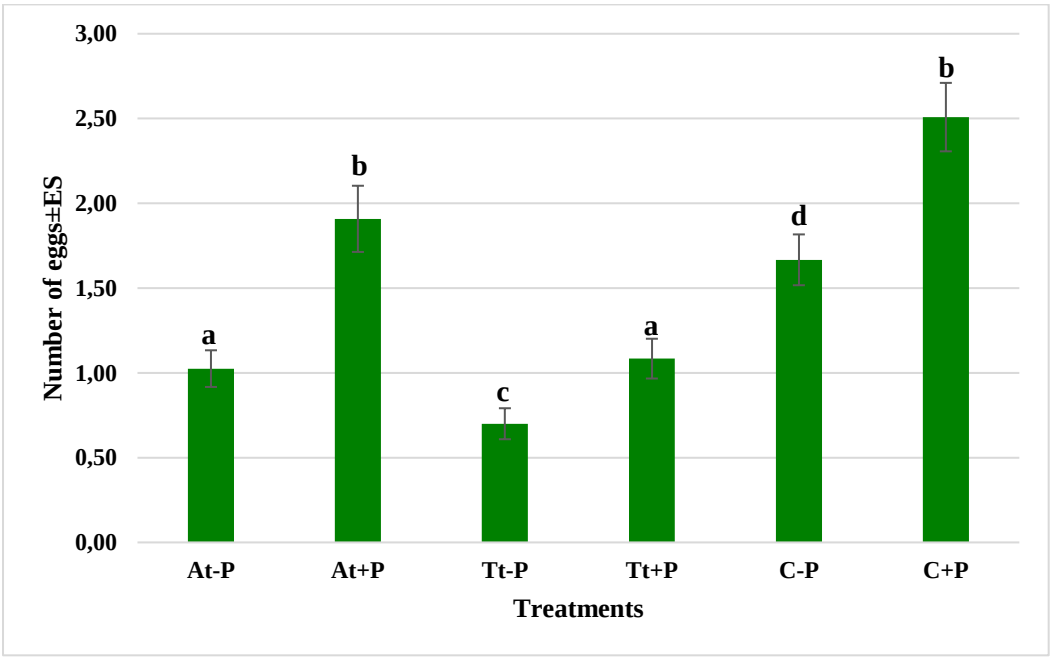


Figure 20. Total oviposition means (±SE) of *F. occidentalis*, estimated over a time interval of 1-10 days in different antibiotic treatments (At-P, At+P, Tt-P and Tt+P) and controls (C+P and C-P). Means with different letters are significantly different (P < 0,01).

3.2 Discussion

Alteration of insect-symbiont relationships through the use of antibiotics can help to clarify microbial functions that go beyond short-term nutritional value. In this study, and for the first time (in laboratory conditions) several biological parameters such as longevity, fecundity, and oviposition in *F. occidentalis* adult females were evaluated. In particular, the existing rapports between the insect and microorganisms present in their intestines were evaluated based on the effects produced from the treatments.

Considering that the target bacteria of the study are transmitted horizontally (de Vries, 2004), the work plan was carried out by trying to reproduce the normal conditions found in nature which, in turn, would permit thrips to interact with microorganisms on cultivated plants in greenhouses and open fields. Furthermore, considering the need to assess biological aspects (i.e., fecundity and oviposition) that require the involvement of females who have reached sexual maturity, it has been decided not to intervene in the possible interactions with the microorganisms in the young insect specimens. First, we obtained individuals of the same age and from fertilized eggs. The fertilization of the females has been assured by the controlled coupling of the virgin females under the microscope. The virgin females which have immediately shown a good receptivity to the virgin male, after the mating, have been employed for obtaining the progeny destined for the antibiotic treatment bioassay. The mating success, for the production of female generations (thelytoky) of WFT, has been amply observed and described by Terry and Schneider (1993).

It is crucial to compare the performance of antibiotic treated thrips with that of untreated thrips, in order to understand the impact of endosymbiotic bacteria on the biology of thrips. Therefore, trials on uninfected and infected thrips were performed. In these experiments, a significant reduction in endosymbiotic charge was achieved by offering individuals antibiotic solutions with tetracycline and amoxicillin for 24 hours. The success of the treatment was evaluated the next day when the thrips turned blue in color due to the dye (Methylene blue) added to the antibiotic solution. All treatments were evaluated with and without the addition of pollen as a food source. Numerous studies show that feeding with pollen increases the biological performance

of the insect such as oviposition (Trichilo & Leigh, 1988; Kirk, 1997), also pollen is the main food for the acquisition of endosymbionts in horizontal transmission (de Vries, 2001). All the biological aspects investigated were affected by the antibiotic treatments. However, the effects of antibiotics decreased when in addition to the antibiotic solution, the thrips were offered pollen as a food source. According to Kirk, (1984) and Van Rijn et al. (1995), thrips, like many other polyphagous insect species, have shorter longevity and higher fecundity when feeding on the combination of pollen and leaf tissue. Also, when pollen is the only available food source, oviposition is sustained, but this leads to increased mortality (Murai and Ishii, 1982). In all biological tests the thrips have been fed with plant tissues to avoid nutritional imbalances. In detail, the evaluation of the oviposition and longevity have been proved by keeping the thrips on a bean leaf disc (5 mm diameter), while the fecundity tests have been conducted by offering bean pods to thrips.

The lowest values observed in antibiotic treatments, without the addition of pollen as a food source, highlight how diet is a fundamental aspect of the acquisition and preservation of microbes within their body and inevitably influences their biological performance. Further studies have shown that aphids, deprived of mycetomic symbionts known to provide amino acids, vitamins, and/or sterols, need more time to develop, reach smaller adult size, and are unable to produce offspring (Baumann *et al.*, 1995; Douglas, 1992; Sasaki *et al.*, 1991).

In all of the biological tests conducted, the lowest observed values for ovideposition, fecundity and longevity are linked to the tetracycline treatments. In one study, conducted on larval stages of *Plutella xylostella* (Lepidoptera: Plutellidae) the effectiveness of antibiotics commonly used in the rapid removal of intestinal microbes from their hosts was evaluated. The results showed that tetracycline caused the highest mortality rate; comparisons with other antibiotics also showed that it was the most toxic antibiotic. (Lin et al., 2015). Tetracyclines are also known to inhibit several processes essential for the survival and growth of bacterial cells, most notably synthesis of bacterial proteins (Klajn, 2001, Ophardt 2003).

F. occidentalis is characterized by a high rate of fecundity. Tests conducted in this study have shown that antibiotic treatments determine a significant reduction of this important parameter. Ridley et al. (2013) showed that antibiotics added to diets could reduce the number of eggs laid by more than 40% in *Drosophila melanogaster*.

These results show that antibiotic treatments can disrupt microbial communities associated with *F. occidentalis*, with significant reductions in important parameters related to its survival and spread.

3.3 Molecular Results

DNA extraction, verified through the aid of the instrument NANODROP proved effective in all of the tested samples. The DNA concentrations for each supernatant µl were within a range from a minimum value of 37,4 ng/µl to a maximum value of 73,3 ng/µl. The mean values for the quality and purity of DNA samples were 0,23 and 0,96 for the absorbance ratio between nucleic acids and polysaccharides (260/230) and between nucleic acids and proteins (280/260). The polymerase chain reaction of *mt*-COI produced fragments of 743 bp, and, after trimming, the final alignment consisted of 623 bp. The nucleotide composition of these sequences was T(U) = 32.1%, A = 29.65%, C = 13.48%, and G = 14.77%. The average A + T content was high (71.75%), which was in agreement with values for insects in general (Simon et al., 1994; Liu, 1992). The final alignment of D2 expansion domain of the 28S subunit was 450 bp and the nucleotide compositions were T(U) = 13.33%, A = 21,78%, C = 34.89%, and G = 26%. Amplification of the ITS2 gene fragment produced 550 bp gene sequences. The final alignment was 450 bp with a nucleotide composition of T(U) = 24%, A = 21,7%, C = 28.4%, and G = 25.9%

For nuclear genes H3 and EF-1α, sequences with final lengths of 305 and 94 bp respectively were obtained. The nucleotide composition of the H3 gene fragment was T(U) = 20,98%, A = 20%, C = 33.4%, and G = 25.5%, while the EF-1α fragment had a nucleotide composition of T(U) = 25,53%, A = 21,28%, C = 34,89%, and G = 19,15%

Although the specimens were collected from several host plants and geographical areas, alignment of sequences, using BioEdit software, showed no existence of haplotypes in the *F. occidentalis* natural populations analyzed in the present study.

The field strains of *F. occidentalis* were analyzed through a maximum likelihood phylogenetic tree based on the general time-reversible model (Saitou et al., 1987) of mitochondrial genes (COI), that was constructed using MEGA version 7 software, using the general time reversible (GTR) G+ I (gamma + invariant) model, suggested

by Partition Finder version 2.1.1 The COI sequences of WFT of *Thips tabaci* (MN036460.1) was used as an outgroup.

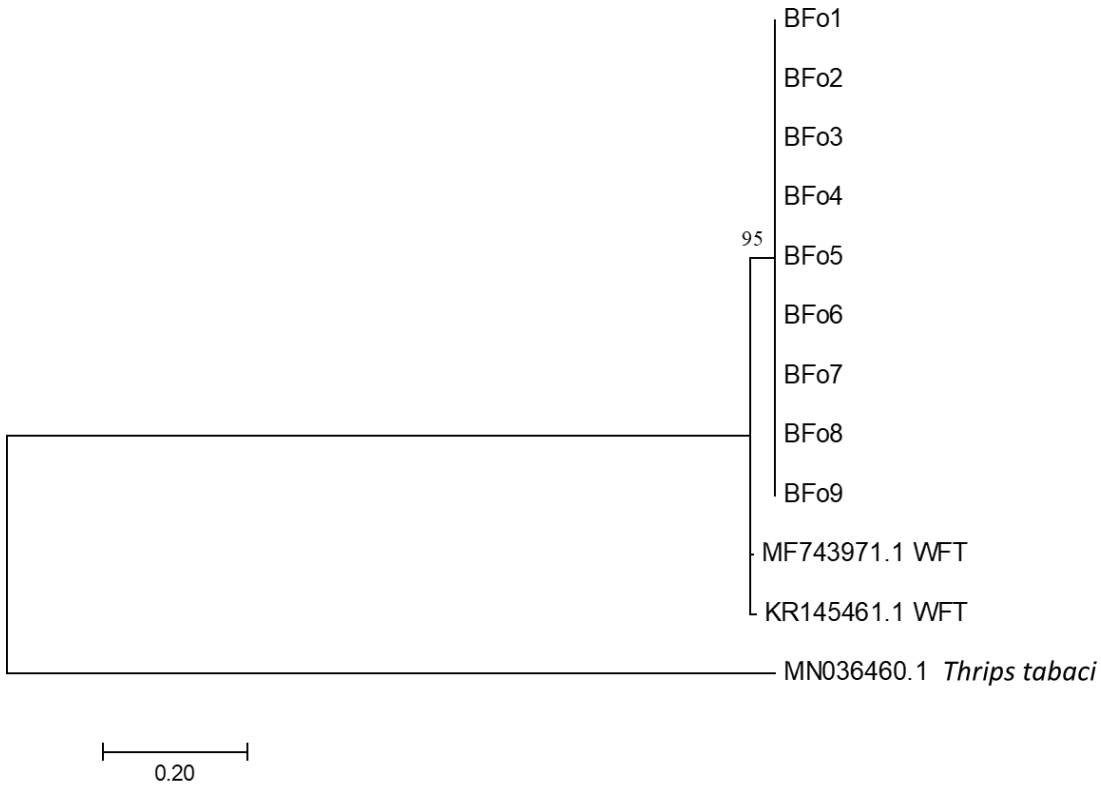


Figure 21. Bootstrap consensus tree generated using the maximum likelihood (ML) method and general time reversible (GTR) G + I (gamma + invariant) model showing the genetic differences and relationship among *F. occidentalis* natural populations obtained by *mt*-COI sequences and other WFT sequences available in GenBank. *Thrips tabaci* was used as an outgroup. Species name and GenBank accession number are shown in the figure.

3.4 Microbiome Results

The Illumina MiSeq sequencing of the V3 and V4 regions of 16S rRNA gene of *F. occidentalis* natural populations collected from nine different host-plants and locations yielded 248,343-523,156 raw reads for **BcFo*n*** (Bacterial community *Frankliniella occidentalis*) sample. Good data quality has been evaluated based on Phred scores above 30 (>Q30; error-probability > = 0.001) for over 85% of reads. The content of the G+C bases varied between 40% and 60% and the length of the filtered sequences was about 300bp. Post-processed reads from all samples were pooled for a total of 602242 reads, and from them, a total of 1475 OTUs were identified (Table 6).

Table 11. Sequencing analysis of V3 and V4 region of 16S rRNA gene of *F. occidentalis* natural populations.

Host-plants	**WFT Population code**	**Location**	**Coordinate**	**Growing environment**	**No. of reads**	**OUT's**
Fragaria vesca	**BcFo1**	Curinga (CZ)	38°49'16,8" N 16°13'16,8" E	greenhouse	85179	299
Solanum melongena	**BcFo2**	Bisignano (CS)	39°29'44.0"N 16°15'55.9"E	greenhouse	64411	124
Capsicum annum	**BcFo3**	Scafati (SA)	40°45'56.6"N 14°32'48.9"E	open field	73456	182
***Rose* spp.**	**BcFo4**	Ponticelli (NA)	40°51'44.1"N 14°20'36.6"E	greenhouse	82537	233
Medicago sativa	**BcFo5**	Squillace (CZ)	38°78'99".31 N 16°46'29".31 E	open field	69352	218
***Chrisantemum* spp.**	**BcFo6**	Torre del Greco (NA)	40°46'51.5"N 14°23'55.4"E	greenhouse	28542	37
Capsicum annum	**BcFo7**	Angri (SA)	40°44'40.3"N 14°34'07.3"E	greenhouse	66630	44
Fragaria vesca	**BcFo8**	Bagnara Calabra (RC)	38°16'47.7"N 15°49'09.0"E	open field	54695	226
Fragaria vesca	**BcFo9**	Eboli (SA)	40°36'41.0"N 15°03'20.6"E	greenhouse	57440	112

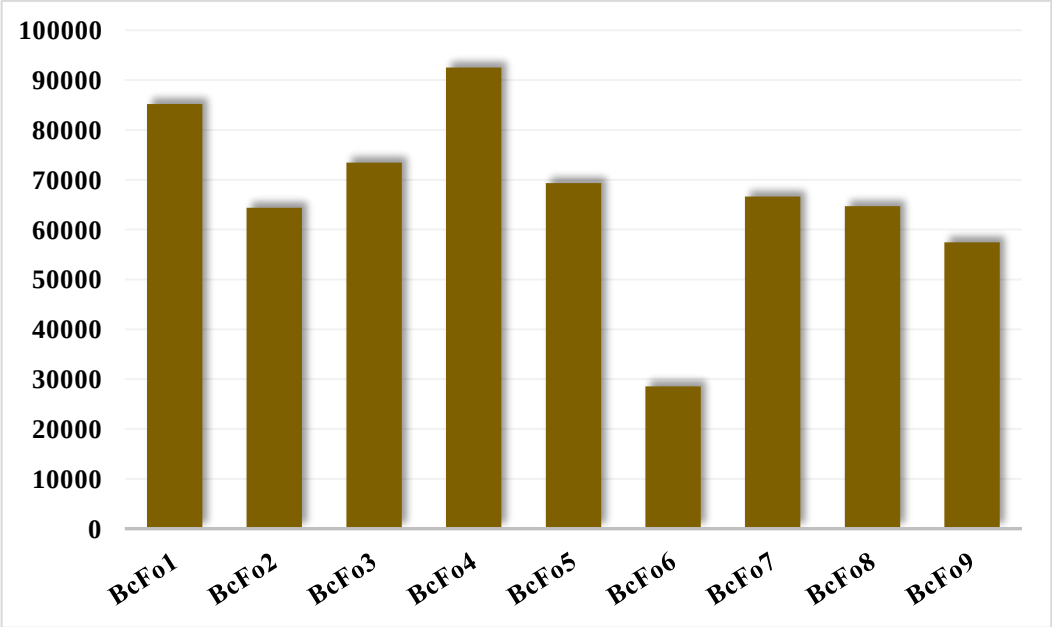


Figure 22. Reads distribution for the microbial communities of each **BcFo** (Bacterial community *Frankliniella occidentalis*) sample.

In this study, post-sequencing analyses have made it possible to identify bacterial phyla belonging to natural WFT populations. A total of 21 phyla were detected in all the analyzed samples.

The most numerous clusters are represented from phylum *Proteobacteria* with 20%, followed by *Firmicutes* with 19%, *Actinobacteria* with 16%, *Bacteroidetes* with 8% and *Cyanobacteria* with 3%.

These five phyla represent more than 66% of the total microbiome and over 98% of the microbiomes identified in the natural populations of *F. occidentalis* utllized in the study and collected from cultivated plants. About 32% of sequences remained unassigned, while 2% is further broken down into smaller clusters such as: *Acidobacteria, Chloflexi, Gemmatimonadetes, Potescibacteria, Verrucomicrobia* and others (Figure 21 and 22).

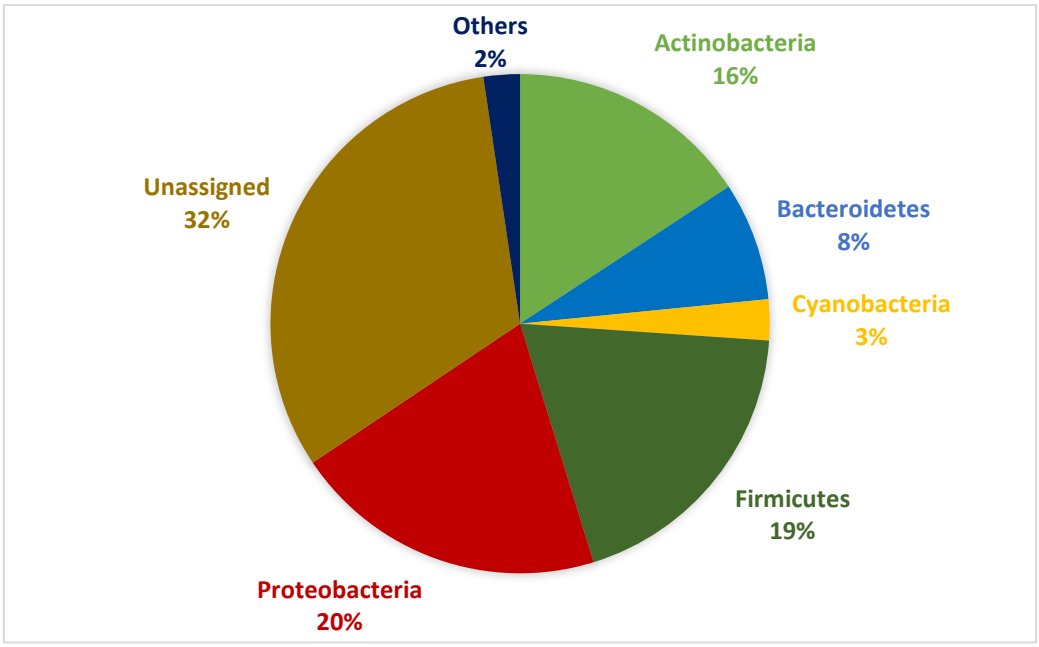


Figure 23 The relative abundance of dominant bacterial phyla represented in *F. occidentalis* samples collected from nine different host-plants and locations across southern Italy.

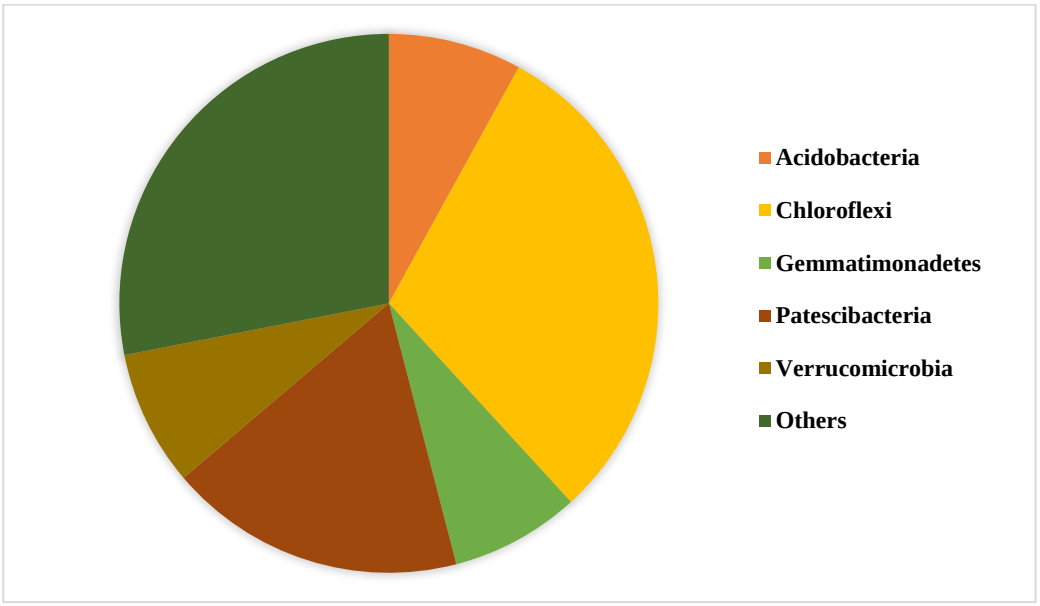
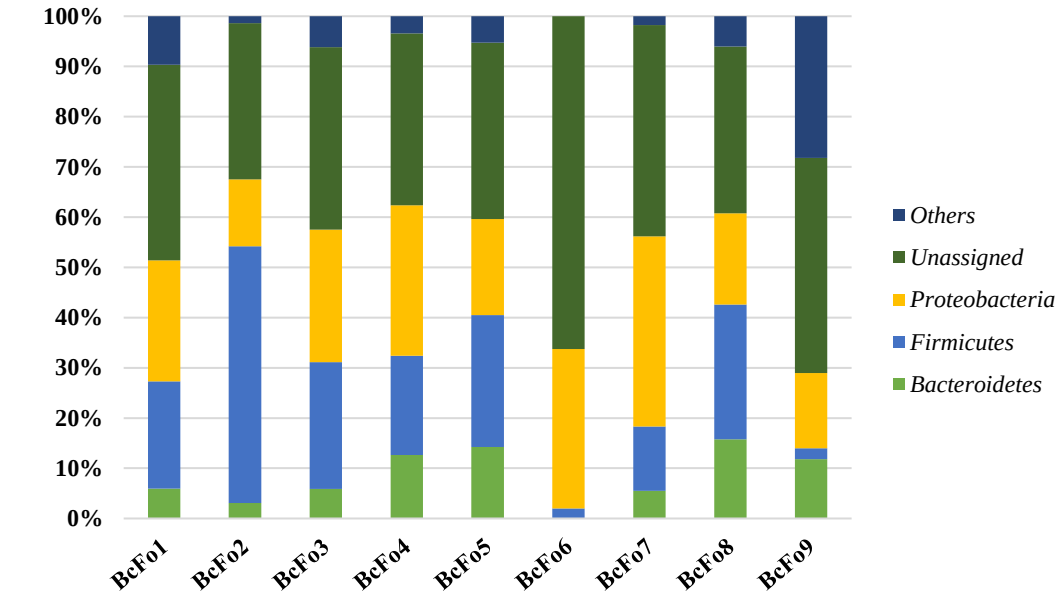
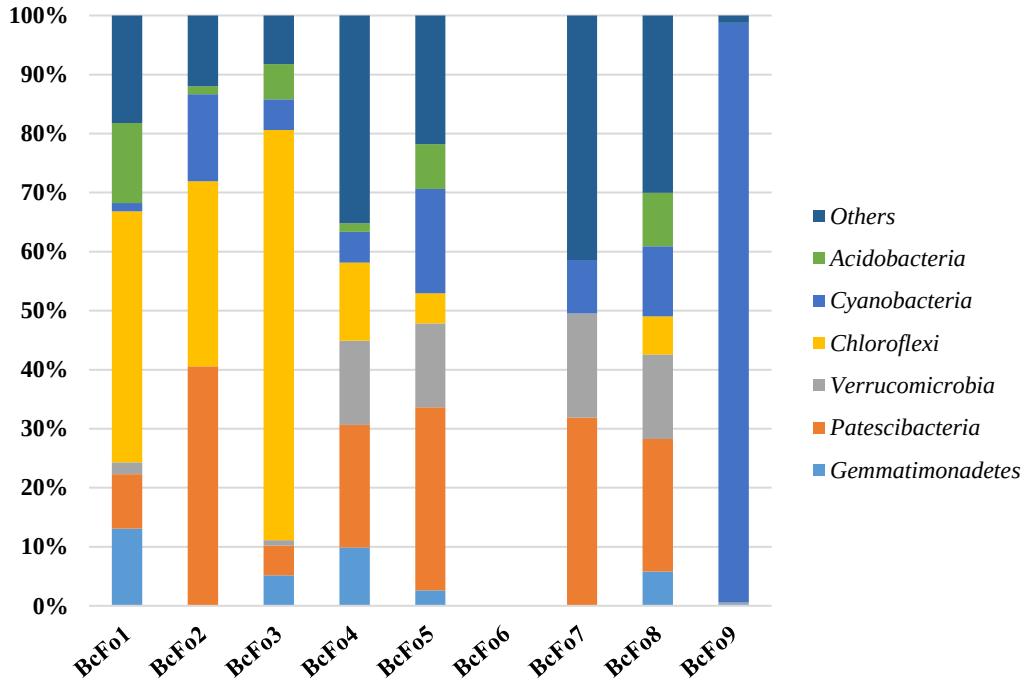


Figure 24. Breakdown of 2% (Figure 23) of other less represented phyla identified in *F. occidentalis* samples, collected from nine different host-plants and locations across southern Italy.



A



B

Figure 25 A and B. The abundance of different phyla of bacterial community of *F. occidentalis* from nine different host-plants and locations in southern Italy.

The bacterial class identified in the five major phylums and their percentages of abundance are reported in the following table:

Table 12. Abundance of the 5 Phyla most present in *F. occidentalis* and their identified and abundance bacterial classes.

Bacteria Phylum	**Abundance %**	**Bacteria Class**	**Abundance %**
Proteobacteria	20	*Gammaproteobacteria*	75
		Alphaproteobacteria	24
		Deltaproteobacteria	1
Firmicutes	19	*Negativicutes*	30
		Clostridia	24
		Bacilli	21
		Erysipelotrichia	4
		Unassigned	21
Actinobacteria	16	*Actinobacteria*	97
		Unassigned	3
Bacteroidetes	8	*Bacteroidia*	100
Cyanobacteria	3	*Oxyphotobacteria*	94
		Melainabacteria	5
		Sericytochromatia	1

The results of these analyses allowed us to identify the main genera present in the most abundant phyla of the different samples. Altogether, 219 genera have been identified. The highest value was recorded for the phylum *Proteobacteria* with 54.75 genera, followed by the phylum *Firmicutes* with 48.4 and *Actinobacteria*, *Bacteroidetes* and *Chloroflexi* with 34.9, 23.01 and 10.31 respectively.

Concerning phylum Proteobacteria, 22 genera have been identified in the three Classes (*Alphaproteobacteria, Deltaprotobacteria e Gammaproteobacteria*). The most abundant order is the *Enterobacteriales* (34%), followed by the *Pseudomonodales* (20%), *Rizhobiales* 11%, *Alteromonadales* (6%), *Oceanospirillales*, *Xsanthomonadales* and *Sphingomonadales* (4%). Other identified genera have an abundance less than 3% (Figure 26).

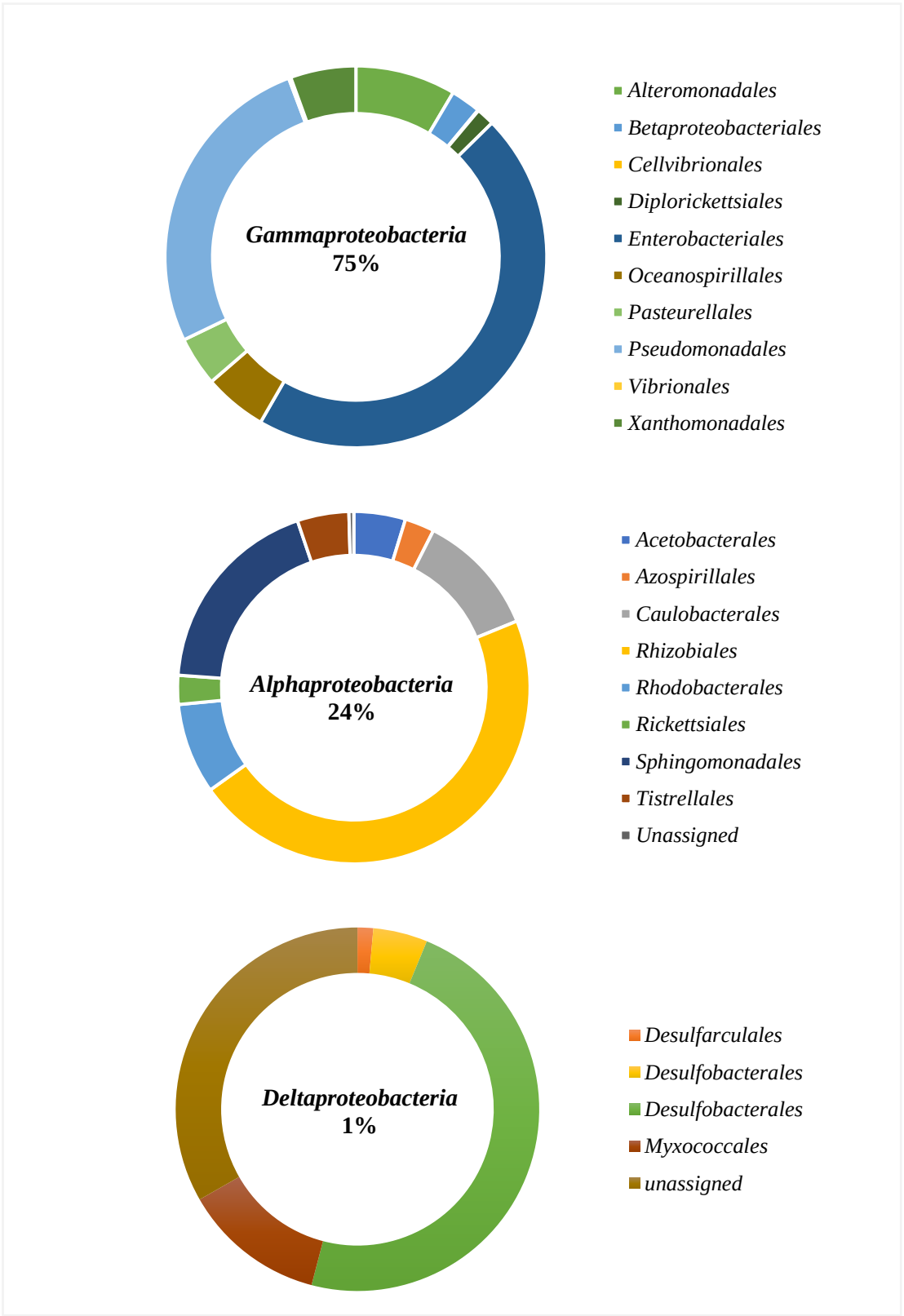


Figure 26. Breakdown of the phylum *Proteobateria* into the three classes identified (*Gammaproteobacteria*, *Alphaproteobacteria* and *Deltaproteobacteria*) and their orders.

3.5 Microbiome diversity of WFT on different host-plants and locations

Alpha analysis, conducted with the Shannon and Simpson indices, highlights the differences between bacterial communities in each WFT population collected from different host plants and locations. The analysis showed that the highest bacterial diversity was observed in WFT samples collected on *Fragaria vesca* (BcFo1) in greenhouses and in samples collected on *M. sativa* (BcFo5), both in the province of Catanzaro. The lowest diversity value was observed in bacterial communities of WFT populations collected on Chrisantemum spp. (Bcfo6) in the province of Naples.

The Chao1 diversity index detected the highest species richness in the samples harvested on strawberries (BcFo1) in the province of Catanzaro and the lowest species richness in samples on Chrisantemum spp. flowers (Bcfo6) in the province of Naples.

Beta diversity analysis was performed to evaluate the variation in bacterial composition in the different samples analyzed, through a weighted and unweighted Unifrac approach. Beta diversity considers the variations in bacterial community composition for different environments. Bacterial diversity among host-plants and locations was assessed using both the weighted and unweighted Unique Fraction Metric (UniFrac) approach. This method calculates the distances based on the fraction of branch length of the 16S rRNA phylogenetic tree shared between two bacterial communities. The phylogenetic construction of the tree, using the calculated distance as a matrix, was performed using the weighted pair method with arithmetic mean (UPGMA method). The Unifrac distance matrix was also used for generating the PCOa plots (PC1, PC2 and PC3), revealing a great diversity among bacterial communities of *F. occidentalis* from different host-plants and locations.

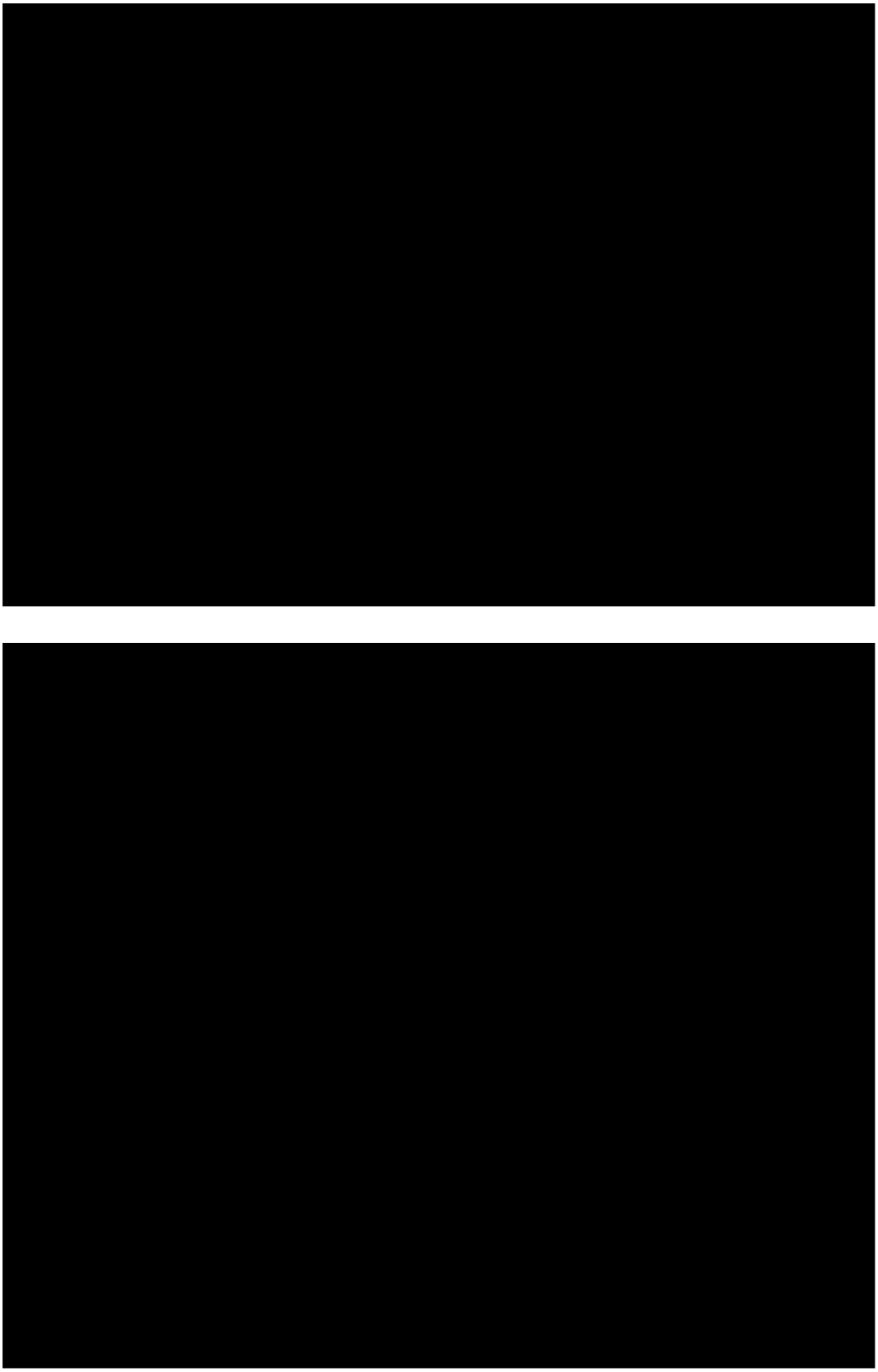


Figure 27. UPGMA PCoA plot (**a**) and tree (**b**) showing relationships between the endosymbiotic bacterial communities from *F. occidentalis* collected from 9 different *host-plants* and geographic locations and based on β-diversity metrics calculated using UniFrac.

3.6 Discussion

The molecular characterization of the natural populations of *F. occidentalis* was performed to assess the presence of polymorphisms within the species. The results showed no genetic differences in all populations analyzed, for all five genes investigated. This suggests that all of the natural populations present in southern Italy, have developed and spread from the same strain introduced in the peninsula in the early '80s. The absence of genetic differences could also be due to the prevailing parthenogenetic reproduction of the species, which strongly reduces the rearrangement of the characters and, therefore, the genetic variability, compared to the sexual reproduction.

The molecular characterization of the composition of the bacterial communities inside insects harmful to plants represents an important goal for the understanding of some aspects related to the biological cycle of the host. Though WTF has been a key-pest on many crops all over the world for more than thirty years, studies devoted to the composition of its microbiome are scarce. Nowadays, studies related to the microbiome are facilitated by new sequencing technologies (NGS), which allow performing fast and in-depth analyses on microbial compositions in different environments.

The analyses carried out in this study showed 21 different phyla of bacteria present in the bacterial communities of nine different populations of *F. occidentalis* collected on important economic crops of southern Italy.

This study revealed that the most abundant Phyla in WFT natural populations are *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes* and *Cyanobacteria*.

These results are consistent with other studies on other insect orders and species belonging to the Thysanoptera (Jones et al, 2013; Yun et al, 2014; Dickey et al., 2014; Facey et al., 2015; Powell et al., 2015; Kaczmarczyk et al., 2018).

The phylum Proteobacteria is the most abundant in the samples analyzed. Three classes have been identified, such as: *Alphaproteobacteria, Deltaproteobacteria* and *Gammaproteobacteria*. The class Gammaproteobacteria is dominated by the order *Enterobacteriales* to which the genera *Erwinia* and *Pantoae* belong. The

abundance of this order and an in-depth phylogenetic analysis of the species belonging to the two genera, have been extensively studied by Facey et al. (2015) on lab populations of *F. occidentalis.*

Some studies show that bacteria belonging to Phylum *Proteobacteria* play a key role in the synthesis of vitamins, in the detoxification from pesticides and in the degradation of complex molecules such as carbohydrates (Delalibera et al., 2005; McCutcheon & Moran, 2007; Cheng et al, 2017; Itoh et al., 2018).

Phylum *Firmiculites* is the second cluster in terms of abundance. Martinson et al. (2011), Brown et al. (2012), Chen et al. (2016) and Auer et al. (2017), in their studies on insects and other animals, recognize that the species belonging to this phylum play a role in the digestion of cellulose and hemicellulose, thanks to their ability to metabolize food resources by increasing energy conversion from their entire diet.

The phylum *Actinobacteria* represents the third most abundant cluster in the bacterial communities identified. Information about the interactions between insects and microbes in this Phylum is scarce. The bacterial species belonging to this group are thought to be able to exploit (more or less) complex food resources which enable them to adapt to various metabolic functions (Pasti & Belli, 1985; Schäfer et al., 1996). Furthermore, studies have shown that these bacteria are capable of producing secondary metabolites with antibiotic functions (Kaltenpoth, 2009).

Bacteroidetes and *Cyanobacteria* represent the fourth and fifth most abundant phylum observed in the WFT populations collected in this study.

Concerning the phylum *Bacteriodetes*, only species belonging to the order *Bacteroidia* have been identified; some studies demonstrate their endosymbiotic role in the production of enzymes (*glucanase*, *mannanase*, *xylanase*, etc.) that are involved in the degradation of complex carbohydrates (Flint et al., 2008; Dai et al., 2012).

The highest values of Phylum *Cyanobacteria* were recorded in the population collected on *Capsicum annum* (BcFo3) in the province of Salerno. Krivosheina (2008) in a study on insect feeding on cyanobacteria, associates the high presence

of these species with the abundant availability of vitamins, proteins and micro-nutrients. Moreover, in the same study, these micro-organisms are described as responsible for producing toxic substances.

According to Facey et al. (2015) *Enterobacteriales* is the main genus highlighted in this study.

Chapter 4. Conclusions and future perspectives

Several aspects of microbial interactions associated with *F. occidentalis* were evaluated in this study.

The articulation of the research activity was carried out to acquire new basic information, providing the first molecular characterization of the microbial communities present in the natural populations of the WFT in southern Italy. Moreover, through the maintenance of laboratory populations, it has been possible to evaluate some biological aspects related to the presence of the main bacterial groups reported in the bibliography.

The first aspect, related to the influence of symbiont bacteria on some aspects of the insect's biological cycle, was evaluated through biological tests with antibiotic treatments. Laboratory tests have been carried out on the fecundity, ovideposition and longevity, previously feeding the specimens with antibiotics such as Tetracycline and Amoxicillin. The same aspects were also evaluated through the addition of pollen to the diet of the populations as it is considered the main food in the horizontal transmission of bacteria in WFT. The results showed that antibiotic treatments and the related reduction of bacterial load within the gut of the thrips, can strongly influence both the reproductive potential and the survival of the species, at least in laboratory conditions.

The second step of the work was carried out with the help of molecular biology techniques. The molecular studies allow both the rapid acquisition of information related to the target species (genetic polymorphisms), and the in-depth identification of microbial species associated with it.

The collection of field populations from different host plants and different sites was aimed at providing information on the genetic variability of the species. The results have shown the absence of polymorphisms in the different WFT natural populations, which have all shown to be identical in the different gene fragments investigated.

In this study, molecular characterization of the microbiome from natural populations of *F. occidentalis* was performed for the first time. The results confirmed the abundant presence of bacterial species belonging to the phylum *Proteobacteria*, as reported in

studies conducted on laboratory populations of WFT. The NGS sequencing and bioinformatic analysis detected the presence of 21 phyla, showing the abundance and diversity of microbial communities in *F. occidentalis*.

These results confirm the important role those symbiotic microorganisms play in the biological cycle of WFT and their contribution to a significant enrichment of the basic knowledge of the species. This information can be considered important to initiate further studies on microbial interactions that affect the physiological processes of thrips. Further advanced studies could provide useful knowledge for sustainable control strategies as an alternative to the use of chemical control.

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References

Arbuthnott D, Levin TC and Promislow DEL (2016). The impacts of *Wolbachia* and the microbiome on mate choice in *Drosophila melanogaster*. *J. Evol. Biol.* **29**: 461–468.

Auer L, Lazuka A, Sillam-Dussès D, Miambi E, O'Donohue M, Hernandez-Raquet G (2017) Uncovering the potential of termite gut microbiome for lignocellulose bioconversion in anaerobic batch bioreactors. *Front. Microbiol.* **8**:2623.

Avise JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, MA, pp. 447.

Bailey SF (1938) *Thrips of economic importance in California*. Circ. 346, Agric. Exp. Stn., Coll. Agric., Univ. Calif., Berkeley, pp. 78.

Baines S (1956) The role of the symbiotic bacteria in the nutrition of *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol.* **33**:533-541.

Baumann P, Baumann L, Lai CY, Rouhbakhsh D, Moran NA, Clark MA (1995) Genetics, Physiology and Evolutionary Relationships of the Genus *Buchnera*: Intracellular Symbionts of Aphids. *Annu. Rev. Microbiol.* **49**:55–94.

Besansky N, Severson DW, Ferdig MT (2003) DNA barcoding of parasites and invertebrate disease vectors: what you don't know can hurt you. *Trends. Parasitol.* **19**: 545–546.

Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I (2007) Cryptic species as a window on diversity and conservation. *Trends Ecol. Evol.* **22**:148-55.

Blaeser P, Sengonca C, Zegula T (2004) The potential use of different predatory bug species in the biological control of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *J. Pest Sci.* **77**: 211-219.

Boore JL (1999) Animal mitochondrial genomes. *Nucleic Acids Res.*, **27**:1767–1780.

Brodsgaard HF (1994) Effect of photoperiod on the bionomics of *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae). *J. Appl. Entomol.* **117**:498–507.

Brown SD, Lamed R, Morag E, Borovok I, Shoham Y, Klingeman DM, Johnson CM, Yang Z, Land ML, Utturkar SM, Keller M (2012) Draft genome sequences

for *Clostridium thermocellum* wild-type strain YS and derived cellulose adhesion-defective mutant strain AD2. *J. Bacteriol*. **194**:3290–3291.

Bryan DE & Smith RF (1956) The *Frankliniella occidentalis* (Pergande) Complex in California (Thysanoptera: Thripidae). *Univ. Calif. Publ. Entomol*. **10**:359-410.

Buckman RS, Mound LA, Whiting MF (2013) Phylogeny of thrips (Insecta: Thysanoptera) based on five molecular loci. *Syst. Entomol*. **38**:123–133.

Buitenhuis R & Shipp JL (2008) Influence of plant species and plant growth stage on *Frankliniella occidentalis* pupation behaviour in greenhouse ornamentals. *J. Appl. Entomol.* **132**: 86–88.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. methods*. **13**:581.

Campbell BC, Stefen-Campbell JD, Werren JH (1993) Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. *Insect Mol. Biol.* **2**: 225–237.

Campbell BC, Heraty JY, Rasplus K Chan, Steffen-Campbell JD, Babcock C (2000) Molecular systematics of the Chalcidoidea, using 28S-D2 rDNA. 59-73 In: *Hymenoptera - Evolution, Biodiversity and Biological Control*. Edit by: Austin, A.D. and M. Dowton, CSIRO Publishing, Collingwood, Australia, pp. 468.

Chanbusarakum L & Ullman D (2008) Characterization of bacterial symbionts in *Frankliniella occidentalis* (Pergande), Western flower thrips. *J. Invertebr Pathol*. **99**:318–325.

Chen B, Butlin RK, Harbach RE (2003) Molecular phylogenetics of the Oriental members of the Myzomyia series of *Anopheles* subgenus Cellia (Diptera: Culicidae) inferred from nuclear and mitochondrial DNA sequences. *Syst. Entomol.* **28**: 57–69.

Chen B, Teh BS, Sun C, Hu S, Lu X, Boland W, Shao Y (2016) Biodiversity and activity of the gut microbiota across the life history of the insect herbivore *Spodoptera littoralis*. *Sci. Rep*. **6**:29505.

Cheng D, Guo Z, Riegler M, Xi Z, Liang G, Xu Y (2017) Gut symbiont enhances insecticide resistance in a significant pest, the oriental fruit fly *Bactrocera dorsalis (*Hendel). *Microbiome* **5**: 13.

Childers CC (1997) Feeding and oviposition injuries to plants. In: *Thrips as crop pests*. Edited by: T. Lewis. CAB International, Wallingford. pp 505-537.

Childers CC & Achor DS (1995) Thrips Feeding and Oviposition Injuries to Economic Plants, Subsequent Damage and Host Responses to Infestation. In *Thrips Biology and Management*, Edit by: Parker, B.L., Skinner, M., Lewis, T., Plenum Press: New York, NY, USA. pp. 31–52.

Cho S, Mitchell A, Regier JC, Mitter C, Poole RW, Friedlander TP, Zhao S (1995) A highly conserved nuclear gene for low-level phylogenetics: *elongation factor-1 alpha* recovers morphology-based tree for heliothine moths. *Mol. Biol. Evol.* **12:** 650-656.

Chouaia B, Rossi P, Epis S, Mosca M, Ricci I, Damiani C, Ulissi U, Crotti E, Daffonchio D, Bandi C, Favia G (2012) Delayed larval development in *Anopheles mosquitoes* deprived of Asaia bacterial symbionts. *BMC Microbiol.* **12**: S2.

Colgan DJ, McLauchalan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Aust. J. Zool.*, **46**: 419-437.

Dai X, Zhu Y, Luo Y, Song L, Liu D, Liu L, Chen F, Wang M, Li J, Zeng X, Dong Z (2012) Metagenomic insights into the fibrolytic microbiome in yak rumen. *PloS one*. **7**: e40430.

de Vries E J, Jacobs G, Breeuwer J A J (1995) Symbiotic bacteria of the western flower thrips, *Frankliniella occidentalis*. *Proc. Sec. Exper. & Appl. Entomol. (NEV)* **6**: 87-92.

de Vries EJ, Breeuwer JAJ, Jacobs G, Mollema C (2001a) The association of western flower thrips, *Frankliniella occidentalis*, with a near *Erwinia* species gut bacteria: transient or permanent? *J. Invertebr. Pathol*. **77**:120-128.

de Vries EJ, Jacobs G. Breeuwer JAJ (2001b) Growth and transmission of gut bacteria in the Western Flower Thrips, *Frankliniella occidentalis*. *J. Invertebr. Pathol.* **77**:129-137.

de Vries EJ (2010) *Symbiosis of thrips and gut bacteria.* [PhD Thesis] Institute for Biodiversity and Ecosystem Dynamics (IBED).

de Vries EJ, Jacobs G, Sabelis MW, Menken SBJ, Breeuwer JAJ (2004) Diet-dependent effects of gut bacteria on their insect host: the symbiosis of *Erwinia* sp. and western flower thrips. *P. Roy. Soc. B-Biol. Sci.* **271**: 2171-2178.

Delalibera I Jr, Handelsman JO, Raffa KF (2005) Contrasts in cellulolytic activities of gut microorganisms between the wood borer, *Saperda vestita* (Coleoptera: Cerambycidae), and the bark beetles, *Ips pini* and *Dendroctonus frontalis* (Coleoptera: Curculionidae). *Environ. Entomol.* **34**:541–7.

Deutsch CA, Tewksbury JJ, Tigchelaar M, Battisti DS, Merrill SC, Huey R, Naylor RL (2018). Increase in crop losses to insect pests in a warming climate. *Science* **361:** 916–919.

Dickey AM, Trease AJ, Jara-Cavieres A, Kumar V, Christenson MK, Potluri LP, Morgan JK, Shatters RG Jr, Mckenzie CL, Davis PH, Osborne LS (2014) Estimating bacterial diversity in *Scirtothrips dorsalis* (Thysanoptera: Thripidae) via next generation sequencing. *Fla. Entomol*. **97**:362-366.

Dillon RJ & Dillon VM (2004) The Gut Bacteria of Insects: Nonpathogenic Interactions. *Annu. Rev. Entomol.* **49**: 71–92.

Douglas AE (1989) Mycetocyte symbiosis in insects. *Biol. Rev*. **64**:409-434.

Douglas AE (1992) Requirements of pea aphids (*Acyrtosiphon pisum*) for their symbiotic bacteria. *Entomol. Exp. Appl*. **65**:195-198.

Douglas AE (1997) Provenance, experience and plant utilisation by the polyphagous aphid, *Aphis fabae*. *Entomol. Exp. Appl.* **83**:161-170.

Douglas AE (2015) Multiorganismal insects: diversity and function of resident microorganisms. *Annu. Rev. Entomol*. **60**:17–34.

Engel P & Moran NA (2013) The gut microbiota of insects - diversity in structure and function. *FEMS Microbiol. Rev.* **37:** 699–735.

Engl T & Kaltenpoth M (2018) Influence of microbial symbionts on insect pheromones. *Nat. Prod. Rep*. **35**: 386–397.

Facey P D, Meric G, Hitchings M D, Pachebat JA, Hegarty MJ, Chen X, Morgan LVA, Hoeppner JE, Whitten MMA, Kirk WDJ, Dyson PJ, Sheppard SK, Del Sol R (2015) Draft genomes, phylogenetic reconstruction and comparative genomics of two novel cohabiting bacterial symbionts isolated from *Frankliniella occidentalis*. *Genome Biol. Evol.* **7**: 2188-2202.

Feldhaar H (2011) Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecol. Entomol*. **36**: 533-543.

Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA (2008) Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat. Rev. Microbiol*. **6**:121.

Gaum WG, Giliomee JH, Pringle KL (1994) Life history and life tables of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), on English cucumbers. *Bull. Entomol. Res.* **84:** 219-224.

Glover RH, Collins DW, Walsh K, Boonham N (2010) Assessment of loci for DNA barcoding in the genus Thrips (Thysanoptera: Thripidae). *Mol. Ecol. Resour*. **10:** 51–59.

Hackett BJ, Gimnig J, Guelbeogo W, Costantini C, Koekemoer LL, Coetzee M, Collins FH, Besansky NJ (2000) Ribosomal DNA internal transcribed spacer (ITS2) sequences differentiate *Anopheles funestus* and *An. Rivulorum* and uncover a cryptic taxon. *Insect Mol. Biol.* **9**: 369 –374.

Hagen K S (1966) Dependence of the olive fly, *Dacus oleae*, larvae on symbiosis with *Pseudomonas savastanoi* for the utilization of olive. *Nature* **209**:423-424.

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41:** 95–98.

Hammer TJ, Janzen DH, Hallwachs W, Jaffe SP, Fierer N (2017) Caterpillars lack a resident gut microbiome. *PNAS USA* **114**: 9641–9646.

Head SR, Komori HK, LaMere, SA, Whisenant T, Van Nieuwerburgh F, Salomon, DR, Ordoukhanian P (2014) Library construction for next-generation sequencing: overviews and challenges. *BioTechniques*, **56**:61–passim.

Hebert PDN, Cywinska A, Ball S L, de Waard J R (2003) Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B.Biol. Sci.* **270**: 313–321.

Holmes ND, Bennison JA, Maulden KA, Kirk WDJ (2012) The pupation behaviour of the Western flower thrips, *Frankliniella occidentalis* (Pergande). *Acta Phytopathol. Hung.* **47**:87–96.

Hoy MA (2013). Genetic Systems, Genome Evolution, and Genetic Control of Embryonic Development in Insects *Insect Molecular Genetics (Third Edition)* Elsevier Science pp. 104-166.

Itoh H, Tago K, Hayatsu M, Kikuchi Y (2018) Detoxifying symbiosis: microbe-mediated detoxification of phytotoxins and pesticides in insects. *Nat. Prod. Rep.* **35**:434–54.

Jalali S, Ojha R, Venkatesan T (2015) DNA Barcoding for Identification of Agriculturally Important Insects. In *New Horizons in Insect Science: Towards Sustainable Pest Management*, Edit by: Chakravarthy A, Springer, New Delhi, pp. 13-23.

Janson EM, Stireman JO, Singer MS, Abbot P (2008) Phytophagous insect-microbe mutualisms and adaptive evolutionary diversification. *Evolution* **62:** 997–1012.

Jing TZ, Qi FH, Wang ZY (2020) Most dominant roles of insect gut bacteria: digestion, detoxification, or essential nutrient provision? *Microbiome* **8**:38.

Jones RT, Sanchez LG, Fierer N (2013) A cross-taxon analysis of insect-associated bacterial diversity. *PLoS one* **8**:e61218.

Kaczmarczyk A, Kucharczyk H, Kucharczyk M, Kapusta P, Sell J, Zielińska S (2018) First insight into microbiome profile of fungivorous thrips *Hoplothrips carpathicus* (Insecta: Thysanoptera) at different developmental stages: molecular evidence of Wolbachia endosymbiosis. *Sci. Rep.* **8**:14376.

Kaltenpoth M (2009) Actinobacteria as mutualists: general healthcare for insects? *Trends Microbiol.* **17**: 529–35.

Kaufman MG, Walker ED, Odelson DA, Klug MJ (2000) Microbial community ecology & insect nutrition. *Am. Entomol*. **46**: 173–185.

Kirk WDJ (1997a) Feeding. In: *Thrips as Crop Pest*. Edited by: T. Lewis. CAB International, Wallingford, UK pp. 119-174.

Kirk WDJ (1997b) Distribution, abundance and population dynamics. In: *Thrips as Crop Pest*. Edited by: T. Lewis. CAB International, New York, USA pp. 217-258.

Kirk WDJ & Hamilton JGC (2004) Evidence for a male-produced sex pheromone in the western flower thrips *Frankliniella occidentalis*. *J. Chem. Ecol.* **30:** 167-174.

Kirk WDJ & Terry LI (2003) The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). *Agric. For. Entomol.* **5**: 301-310.

Klajn R (2001) Tetracycline: Chemistry and Chemical Biology of Tetracyclines. http://www.chm.bris.ac.uk/motm/tetracycline/tetracycline.htm. (accessed on 13 December 2020).

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **41**:1.

Krivosheina MG (2008) On insect feeding on cyanobacteria. *Paleontol. J.* **42**:596–9.

Kumm S & Moritz G (2010) Life-cycle variation, including female production by virgin females in *Frankliniella occidentalis* (Thysanoptera: Thripidae). *J. Appl. Entomol.* **134**:491–97.

Lin XL, Kang ZW, Pan QJ, Liu TX (2015). Evaluation of five antibiotics on larval gut bacterial diversity of *Plutella xylostella* (Lepidoptera:Plutellidae). *Insect Sci.* **22**:619–628.

Liu H & Beckenbach AT (1992) Evolution of the mitochondrial cytochrome oxidase II gene among 10 orders of insects. *Mol. Phylogenet. Evol*. **1:** 41–52.

Loomans AJM & Murai T (1997) Culturing thrips and parasitoids. In: *Thrips as crop pests*. Edited by: T. Lewis. CAB International, Wallingford, UK. pp 477-503

Madeira F, Park YM, Lee J, Buso N, Gur T, Madhusoodanan N, Basutkar P, Tivey ARN, Potter SC, Finn RD, Lopez R (2019) The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res*. **47**: W636–W641.

Martinson VG, Danforth BN, Minckley RL, Rueppell O, Tingek S, Moran NA (2011) A simple and distinctive microbiota associated with honey bees and bumble bees. *Mol. Ecol*. **20**:619–28.

Marullo R (2002) Impact of an introduced pest thrips on the indigenous natural history and agricultural systems. In: *Thrips and Tospoviruses*. Proceedings of the 7th International Symposium on Thysanoptera, Marullo R. & Mound L. Eds., ANIC, Canberra, AU: 285-288.

Marullo, R. (2003) *Conoscere i Tisanotteri. Guida al Riconoscimento delle Specie Dannose alle Colture Agrarie*. Edagricole, Bologna, Italy. pp. 75.

Marullo R, Mercati F, Vono G (2020) DNA Barcoding: A reliable method for the identification of thrips species (Thysanoptera, Thripidae) collected on sticky traps in onion fields. *Insects* **11**: 489.

McCutcheon JP & Moran NA (2007) Parallel genomic evolution and metabolic interdependence in an ancient symbiosis. *Proc. Natl. Acad. Sci. U.S.A.* **104**:19392–7.

McMeniman CJ, Lane RV, Cass BN, Fong AW, Sidhu M, Wang YF, O'Neill SL (2009) Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science* **323:** 141–144.

Mergaert P. (2018). Role of antimicrobial peptides in controlling symbiotic bacterial populations. *Nat. Prod. Rep.* **35:** 336–356.

Mikonranta L, Mappes J, Kaukoniitty M, Freitak D (2014) Insect immunity: oral exposure to a bacterial pathogen elicits free radical response and protects from a recurring infection. *Front. Zool.* **11**:23.

Misof B, Liu S, Meusemann K, Peters RS, Donath A, Mayer C, Frandsen PB, Ware J, Flouri T, Beutel RG, Niejuis O, Petersen M, Izquierdo-Carrasco F, Wappler T, Rust J, Aberer AJ, Aspock U, Bartel D, Blanke A, Berger S, Bohm A, Buckley TR, Calcott B, Chen J, Friedrich F, Fukui M, Fujita M, Greve C, Grobe P, Gu S, Huang Y, Jermiin LS, Kawahara AY, Krogman L, Kubiak, M, Lanfear R, Letsch H, LiY, Li Z, Li J, Lu, H, Machida R, Mashimo Y, Kapli P, McKenna DD, Meng G, Nakagaki Y, Navarrete-Heredia JL, Ott M, Ou Y, Pass G, Podsialowski, Pohl H, von Reumont BM, Schutte K, Sekiya K, Shimizu S, Slipinski A, Stamakis A, Song W, Su X, Szucsich NU, Tan M, Tan X, Tang M, Tang J, Timelhalter G, Tomizuka S, Trautwein M, Tong X, Uchifune T, Walzl MG, Wiegmann BM, Wilbrandt J, Wipfler B, Wong TKF, Wu Q, Wu G, Xie Y, Yang S, Yang Q, Yeates DK, Yoshizawa K, Zhang Q, Zhang R, Zhang W, Zhang Y, Zhao J, Zhou C, Zhou L, Ziesmann T, Zou S, Li Y, Xu X, Zhang Y, Yang H, Wang J, Wang J, Kjer KM (2014) Phylogenomics resolves the timing and pattern of insect evolution. *Science* **346**:763–767.

Moritz G, Kumm S, Mound LA (2004) Tospovirus transmission depends on thrips ontogeny. *Virus Res.* **100**: 143-149.

Morse JG, Bellows TS, Iwata Y (1986) Technique for evaluating residual toxicity of pesticides to motile insects. *J. Econ. Entomol.* **79:** 281-283.

Mound LA & Kibby G (1998) *Thysanoptera: An Identification Guide*; CABI International: Wallingford, UK, pp. 70.

Mound LA & Marullo R (1996) The Thrips of Central and South America: An Introduction. *Mem. Entomol. Int.* **6:**1–488.

Murai T & Ishii T (1982) Simple rearing methods for flower thrips (Thysanoptera: Thripidae) on pollens (in Japanese). *Jpn. J. Appl. Entomol. Zool.* **26**:149-154.

Murai T & Loomans AJM (2001) Evaluation of an improved method for mass-rearing of thrips and a thrips parasitoid. *Entomol. Exp. Appl.* **101:** 281-289.

Naegele MP, Da Costa PI, Da Rosa JA (2006) Polymorphism of the ITS-2 region of the ribosomal DNA of the Triatominae *Rhodnius domesticus, R. pictipes, R. prolixus* and *R. stali*. *Med. Vet. Entomol.* **20**:353–357.

National Center for Biotechnology Information (NCBI) Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. (1988). Available online: https://www.ncbi.nlm.nih.gov/

Nguyen DT, Spooner-Hart RN, Riegler M (2015) Polyploidy versus endosymbionts in obligately thelytokous thrips. *BMC Evol. Biol.* **15:** 23.

Ophardt CE (2003) Introduction to Drug Action: Other Antibiotics. http://www.elmhurst.edu/~chm/vchembook/654antibiotic.html (accessed on 26 October 2020).

Pasti MB & Belli ML (1985) Cellulolytic activity of actinomycetes isolated from termites (Termitidae) gut. *FEMS Microbiol. Lett.* **26**:107–12.

Pierre LL (1964) Uricase activity of isolated symbionts and the aposymbiotic fat body of a cockroach. *Nature* **201**:54-55.

Powell CM, Montiel AL, Beddingfield B, Hanson JD, Bextine BR (2015) Comparison of Bacterial Communities of Flower Thrips (*Frankliniella tritici* 1) and Potato Psyllid (*Bactericera cockerelli* 2). *Southwest. Entomol*. **40**:765–74.

Price MN, Dehal PS, Arkin AP (2010) FastTree 2- Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE*, **5**(3): e9490.

Reitz SR (2009) Biology and ecology of the western flower thrips (Thysanoptera: Thripidae): the making of a pest. *Florida Entomol*. **92**:7–13.

Reitz SR, Gao Y, Kirk WD, Hoddle MS, Leiss KA, Funderburk JE (2020) Invasion biology, ecology, and management of western flower thrips. *Annu. Rev. Entomol.* **65:** 17–37.

Ricci I, Damiani C, Capone A, DeFreece C, Rossi P, Favia G. (2012) Mosquito/microbiota interactions: from complex relationships to biotechnological perspectives. *Curr. Opin. Microbiol.* **15:** 278–284.

Rotenberg D & Whitfield AE (2018) Molecular interactions between tospoviruses and thrips vectors. *Curr. Opin. Virol.* **33**:191–97.

Russell CW, Poliakov A, Haribal M, Jander G, Van Wijk KJ, Douglas AE (2014) Matching the supply of bacterial nutrients to the nutritional demand of the animal host. *Proc. Biol. Sci*. **281**:1163.

Saitou N & Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4:** 406–425.

Sasaki T, Hayashi H, Ishikawa H (1991) Growth and reproduction of the symbiotic and, aposymbiotic pea aphids, *Acyrtosiphon pisum*, maintained on artificial diets. *J. Insect Physiol*. **37**:749-756.

Schäfer A, Konrad R, Kuhnigk T, Kämpfer P, Hertel H, König H (1996) Hemicellulose-degrading bacteria and yeasts from the termite gut. *J. Appl. Microbiol.* **80:**471–8.

Shrestha S (2019). Effects of climate change in agricultural insect pest. *Acta Sci. Agric.* **3**:74–80.

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

Steiner MY & Goodwin S (1998). Methods for collecting and rearing thrips (Thysanoptera) and their natural enemies. *Australian J. Entomol*. **37**:101-106.

Steiner MY, Spohr LJ, Goodwin S (2011) Relative humidity controls pupation success and dropping behaviour of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Aust. J. Entomol.* **50**:179–86.

Steiner MY, Spohr LJ, Goodwin, S. (2011) Relative humidity controls pupation success and dropping behaviour of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Australian Journal of Entomology* 50, 179-186.

Stouthamer R, Breeuwer JAJ, Luck RF, Werren JH (1993) Molecular identification of micro-organisms associated with parthenogenesis. *Nature* **361**:66-68.

Terry I & Schneider M (1993) Copulatory behaviour and mating frequency of the western flower thrips, *Frankliniella occidentalis* (Insecta: Thysanoptera). *Zoology (IJPAZ)* **4**: 339-354.

Thalavaisundaram S, Herron GA, Clift AD, Rose H (2008) Pyrethroid resistance in *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and implications for its management in Australia. *Aust. J. Entomol.* **47:** 64-69.

Thomas P, Kenny N, Eyles D, Moreira LA, O'Neill SL, Asgari S (2011) Infection with the wMel and wMelPop strains of *Wolbachia* leads to higher levels of melanization in the hemolymph of *Drosophila melanogaster*, *Drosophila simulans* and *Aedes aegypti*. *Dev. Comp. Immunol.* **35**:360–365.

Thompson SNN & Simpson SJJ (2009) Nutrition. In *Encyclopedia of Insects*. Edit by: H. Resh and R. T. Cardé, Cambridge, M: Academic Press, pp. 715–720.

ThripsID. Available online: www.thrips.net (accessed on 27 April 2020).

Trichilo PJ & Leigh TF (1986) Predation on spidermite eggs by the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), an opportunist in a cotton agroecosystem. *Environ. Entomol.* **15**:821–25.

Ullman DE, Cho JJ, Mau RFL, Westcot DM, Custer DM (1992) A midgut barrier to tomato spotted wilt virus acquisition by adult western flower thrips. *Phytopathology* **82**: 1333-1342.

Ullman DE, Westcott DM, Hunter WB, Mau RFL (1989) Internal anatomy and morphology of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) with special reference to interactions between thrips and tomato spotted wilt virus. *Int. J. Insect Morphol. Embryol*. **18**:289-310.

van Maanen R, Broufas G, Oveja MF, Sabelis MW, Janssen A (2012) Intraguild predation among plant pests: western flower thrips larvae feed on whitefly crawlers. *Biocontrol* **57**:533–39.

Van Rijn PCJ, Mollema C, Steenhuis-Broers GM (1995) Comparative life-history studies of *Frankliniella occidentalis* and *Thrips tabaci* (Thysanoptera: Thripidae) on cucumber. *Bull. Entomol. Res*. **85**:285-297.

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* **10**:506–513.

Wang JC, Zhang B, Li HG, Wang JP, Zheng CY (2014) Effects of exposure to high temperature on *Frankliniella occidentalis* (Thysanoptera: Thripidae), under arrhenotoky and sexual reproduction conditions. *Florida Entomol*. **97**:504–10

Wielkopolan B & Obrêpalska-Stêplowska A (2016) Three-way interaction among plants, bacteria, and coleopteran insects. *Planta* **244**: 313–332.

Wilkerson RC, Reinert JF, Li C (2004) Ribosomal DNA ITS2 sequences differentiate six species in the *Anopheles crucians* Complex (Diptera: Culicidae). *J. Med. Entomol*. **41**:392–401

Wolstenholme DR (1992) Animal mitochondrial DNA: structure and evolution. In *Mitochondrial Genomes. International Review of Cytology*. Edited by: Wolstenholme D. R., Jeon K. W. Academic Press, New York, pp. 173–216.

Yan D, Tang Y, Xue X, Wang M, Liu F, Fan J (2012) The complete mitochondrial genome sequence of the western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae) contains triplicate putative control regions. *Gene*, **506:** 117–124.

Yun JH, Roh SW, Whon TW, Jung MJ, Kim MS, Park DS, Yoon C, Nam YD, Kim YJ, Choi JH, Kim JY (2014) Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Appl. Environ. Microbiol.* **80**:5254–64.

Yuval B (2017) Symbiosis: gut bacteria manipulate host behaviour. *Curr. Biol.* **27**: R746–R747.

Zhang ZJ, Wu QJ, Li XF, Zhang YJ, Xu BY, Zhu GR (2007) Life history of western flower thrips, *Frankliniella occidentalis* (Thysanoptera., Thripidae), on five different vegetable leaves. *J. Appl. Entomol.* **131:** 347-354.

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