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# nine



## General conclusions and summary

Egbert J. de Vries

## BIOTYPES OF THRIPS

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), has been the subject of extensive studies since it became a major agricultural pest in the developed world. The insect was formerly known as a flower and leaf-inhabiting plant feeder with a broad range of host plants in the western part of the USA and Canada (Bryan and Smith, 1956). Since the 1980s, it became a serious pest insect on many different agricultural crops in Western Europe, directly so by damaging leaves and indirectly by vectoring a large group of harmful plant viruses (e.g., tospoviruses). Therefore, several Dutch research groups devoted their research efforts to thrips in an attempt to answer the question what characteristics enabled this species to acquire a pest status so suddenly.

In 1992, the University of Amsterdam, Wageningen Agricultural University, and the Centre of Plant Breeding and Reproduction Research in Wageningen started a collaborative project on variability in western flower thrips with respect to its association with host plants. This thesis is the last one produced in a series of three theses on (1) variation in performance on susceptible and resistant host plants (De Kogel, 1997), (2) variation in thrips' ability to transfer plant viruses (Van de Wetering, 1999), and (3) variation in the interaction with symbiotic bacteria (this thesis). Next to this Dutch collaborative project, other projects on thrips variability were started elsewhere. These projects described variation in thrips resistance to insecticides (Brødsgaard, 1994; Imaraju *et al.*, 1992). Immaraju *et al.* (1992) studied western flower thrips in California, i.e., the area of origin of the thrips before it became a world-wide pest. Variation in thrips is thus expected to be highest in California, with possibly only few thrips variants having spread from the Californian region and developed into a pest insect. In these aforementioned papers, the researchers studied variation in ecological traits but not the associated genetic variation.

Jürg Frey of the Swiss Federal Research Station for Horticulture started a project assessing genetic variation within and among thrips populations. Frey and Brunner (2004) described genetic variation between western flower thrips populations, but the genetic variation that was found did not correlate with variation in ecological characteristics such as host plant association. The same authors did report genetic evidence for the 'occurrence of host races' in another thrips species, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) (Brunner *et al.*, 2004). Gillings *et al.* (1995) performed genetic studies on New Zealand populations of western flower thrips but found no evidence for genetically different strains of thrips. A similar result was found in a large EU-funded project on genetic variation in western flower thrips (Mound *et al.*, 1998).

For our research project, a number of thrips populations were obtained from research groups from all corners of the world (USA, Japan, Europe, New Zealand, and Israel). These populations were used to set up iso-female lines. Iso-female lines stem from one mother and were initially propagated by mother-son matings. The populations and their iso-female lines were used to study variation in the use of host plants (including plants that are susceptible and plants that are resistant to thrips feeding), the ability to transfer plant viruses, and the association with symbiotic gut bacteria. Kogel (1997) found variation between populations in their response to susceptible and resistant cucumber plants. All thrips populations were underperforming on resistant host plants but performance levels differed. However, variation among iso-female lines of one population was as high as variation among iso-female lines from different populations.

Van de Wetering (1999) studied the transmission efficiency of the TSWV tospovirus of the aforementioned populations of western flower thrips. She found large differences in transmission efficiency between thrips populations, ranging from 18 to 72%, and suggested that thrips biotypes that differ in virus transmission exist. She did not study the variation between iso-female lines of the same thrips population. Thus, the possibility remains that variation among iso-female lines of one population is as high as variation between populations.

In my study, I used the same populations and iso-female lines to assess variation in the presence and composition of symbiotic gut bacteria (chapter 8). These bacteria could play a role in host plant adaptation of thrips, and hence promote biotype formation and offer an explanation for the polyphagy of this thrips species. All populations and iso-female lines appeared to contain gut bacteria, and their number, typology, and growth in different life stages of the thrips is very similar. Genetic studies of bacteria from different thrips individuals out of several iso-female lines from a series of populations revealed that genetic variation between gut bacteria from the same iso-female line can be as large as between iso-female lines from different thrips populations.

One may wonder whether the success of thrips invasions over the last 30 years in various parts of the world, has been caused by thrips adaptation to so many different crops. It is important to note that western flower thrips was already a polyphagous insect before it became a worldwide pest (Bryan and Smith, 1956). It is still not known how the earliest pest populations originated, via a bottleneck and / or by acquiring one or more biological characteristics that are directly related to the pest status such as decomposition of plant toxic compounds. The hypothesis that the thrips' universal pest status merely arose through increased international trade (and the subsequent increased ecological opportunity in the likely absence of natural enemies), is perhaps more plausible than the hypothesis that the pest status was caused by the development of new and better adapted thrips strains. Next to feeding on parenchyma, thrips already had the ability to feed on pollen from various plant species or to be a carnivore on other insects and mites (Lewis, 1997; and references therein).

In this project, I compared western flower thrips with onion thrips, *T. tabaci* Lindeman (Thysanoptera; Thripidae). Onion thrips is an agricultural pest just like western flower thrips. Contrary to western flower thrips, it is indigenous in Western Europe and has been a pest in open field crops and greenhouse crops for decades. Variation between populations of onion thrips seems to be much larger than variation between populations of western flower thrips. Onion thrips populations vary more in the transmission efficiency of plant viruses (Van de Wetering, 1999; Wijkamp, 1995) than western flower thrips. Evidence for genetic variation between strains of onion thrips is stronger than for strains of western flower thrips, possibly because of differences in their history as a pest insect in Western Europe (Brunner *et al.*, 2004; Frey and Frey, 2004; Toda *et al.*, 2007). Whether onion thrips populations really belong to different biotypes needs to be assessed by further studies. Next to the study of biotypes in thrips species, this thesis reports on the characterisation of symbiotic gut bacteria and their effects on thrips fitness.

## SYMBIOTIC BACTERIA

Given that thrips contain bacteria in their gut system (De Vries *et al* 1995; Ullman *et al.*, 1989), I investigated how bacteria affect thrips fitness. Gut bacteria can have a positive

effect on their host (e.g., detoxification of secondary plant compounds, provision of vitamins, amino acids or other important compounds – see Dillon and Dillon, 2004) and because of their higher rate of evolutionary change, they might have been instrumental in the success of thrips. To assess if thrips benefit from gut bacteria, the effects of the microorganisms on thrips feeding behaviour and performance was studied.

Many insects carry microorganisms in their digestive system and several have them even inside specific tissues (Buchner, 1965; Koch, 1967). Research on the nature of insect-endosymbiont interactions, the ecological importance of the symbiosis, the evolutionary impact of the symbiosis, and the possible effects on agriculture, has been carried out in the last 40 years. Some endosymbionts have been fully characterised at the genomic level (e.g., *Buchnera aphidicola*, the symbiont of aphid species; Shigenobu *et al.*, 2000).

Less research has been devoted to gut-inhabiting microorganisms of insects. Although the number of insect species, in which microorganisms regularly occur in the gut system, is much larger than the aforementioned cases of endosymbiosis (Campbell, 1990; Cazemier *et al.*, 1997; Dillon and Dillon, 2004), scientist took less interest in them. Their importance for insect evolution and ecology was thought to be lower than that of endosymbionts. Only in a few cases were studied, such as the hindgut inhabiting microflora of cockroaches (Cruden and Markovetz, 1987). Since bacteria present on or in the food will inevitably be taken up during feeding, their presence inside the insect gut system is in itself no proof of any interaction between bacterium and insect. These bacteria may be there as a byproduct of the feeding process or the insect may use the bacteria as food source. To prove an insect-bacteria symbiotic interaction requires much more than the mere detection of bacteria in the gut.

Western flower thrips is a suitable species to study the interaction between gut bacteria and insects. Thrips can feed on a large number of host plant species and can use various plant parts during its life cycle. Larvae can live and feed inside flowers or on the leaves of plants, and adults visit flowers for mating and leaves for oviposition. Western flower thrips is an opportunistic species which readily colonises new habitats in the temperate and subtropical regions (Morse and Hoddle, 2006). It is able to survive on non-plant food, such as fungi or arthropod prey. All the aforementioned food sources of thrips will have different sets of bacterial species on their surface, so it is likely that thrips ingest many different types of bacteria during their life-time.

## THRIPS AND GUT BACTERIA

The first result of the work described in this thesis is that thrips guts are inhabited by a single group of bacteria, and that bacteria enter thrips in an early stage of its larval development (De Vries *et al.*, 1995, 2001a, chapter 2). The bacteria belong to the genus *Erwinia* in the family of the Enterobacteriaceae. Different species names have been used for this type of bacteria such as *Enterobacter agglomerans*, *Erwinia herbicola*, and *Pantoea agglomerans*. In this thesis, they are referred to as *Erwinia* spec. All thrips populations studied in my research project, regardless of whether they were kept in the laboratory for years, received from another research lab, or freshly collected from the field, contained this same type of bacteria in the guts of second instar larvae (chapter 8). We describe the growth of these gut bacteria in the thrips during the different life stages (De Vries *et al.*, 2001b, chapter 3) and found that they reach highest prevalence and highest numbers per thrips in second instar larvae.

## General conclusions and summary

The second result of the work described in this thesis concerns the effect of gut bacteria on thrips development (De Vries *et al.*, 2004, chapter 4). Thrips larvae with bacteria develop faster on a diet of leaves only than thrips without bacteria. Furthermore, with bacteria in their gut thrips females lay more eggs than without bacteria. This improved performance of thrips with bacteria indicates why thrips have a strong cohabitation with *Erwinia spec* bacteria. We did not study how living inside or outside the thrips affects the growth of the bacteria (*Erwinia spec* are able to grow on leaves as well). However, given the increase in numbers of bacteria inside the larvae, we speculate that they are also positively affected by the association with the host, i.e., the interaction is mutualistic. We also did not study the biological mechanism behind the thrips-bacterium interaction that leads to this beneficial effect on thrips.

The third result of the work described in my thesis is that the symbiotic effects between thrips and gut bacteria are food-source dependent (De Vries *et al.*, 2004, chapter 4). When thrips are feeding on pollen, or pollen and leaves, they experience no positive effects of the presence of gut bacteria. In fact, larvae feeding on pollen and leaves develop faster without *Erwinia spec* gut bacteria than with bacteria. This could be due to the fact that the bacteria, no longer making a positive contribution, now only confer a cost to the insect (food competition). I speculate that the bacteria provide the thrips with one or more nutritional elements derived from the plant food, which thrips cannot (or poorly) obtain themselves when they feed on leaves. The same compound (or compounds) may be directly available from pollen, as the positive effects of bacteria are absent when pollen is part of the diet of the thrips. Alternatively, bacteria detoxify secondary plant compounds. Further research is needed to assess which benefits are acquired and which costs are incurred by thrips and their gut bacteria.

Western flower thrips are not the only insect species with *Erwinia spec* gut bacteria. In fact, a number of insect species was found to contain these bacteria in their gut, for example true fruit flies (*Rhagoletis*, Lauzon *et al.*, 2000), termites (Breznak and Brune, 1994), and locusts (*Schistocerca gregaria*, Dillon *et al.*, 2000). I found that the distantly related onion thrips, *T. tabaci*, also have *Erwinia spec* gut bacteria (De Vries *et al.*, 2008, chapter 6), but the variation in type and prevalence of these bacteria between various onion thrips populations is much larger than in western flower thrips. Recently it was found that the broad-headed bug, *Riportus clavatus*, has improved fitness when *Burkholderia* bacteria are present in the crypts of its posterior midgut; the bug has to acquire these bacteria each generation from the soil in which the eggs hatch (Kikuchi *et al.*, 2007). This largely parallels with the ecology of western flower thrips and *Erwinia spec*.

The effects of *Erwinia spec* were found to differ from species to species. The fact that all insect species studied are pests can be a research bias, as these species tend to get more attention from scientists. I intend to compare *Erwinia* bacteria collected from different insect species to see whether the genetic variation profile in the bacteria varies by species of insect host. This will give an indication whether the association of *Erwinia* bacteria with insects is randomly renewed over and over again or resulted from particular evolutionary events and was maintained since then.

Many insect species have *Erwinia spec* bacteria in their gut system and the prevalence of these bacteria in western flower thrips is high. It is easy to pick up bacteria from the food source, and we have found that thrips feeding behaviour, search behaviour, and preference for sites that have been visited by other thrips enhance uptake of the bacteria (De Vries *et al.*, 2006, chapter 5). *Erwinia spec* are always the very bacteria they pick up, and these soon

become the dominant bacterium in the insect gut. At what point the interaction between thrips and gut bacteria has become symbiotic, and when and how this has led to a beneficial situation for the thrips is not yet clear. All populations of western flower thrips that we studied have the same bacteria, but they were all populations on greenhouse crops. Perhaps natural populations of thrips have more variation in prevalence and type of gut bacteria, and populations outside greenhouses may not exhibit the same interaction with bacteria as the greenhouse populations. Our results do not rule out the possibility that western flower thrips became a pest insect because of a change in composition of gut bacteria. That would require studying indigenous thrips populations in the western part of the USA. It is however questionable whether it is possible to still find such populations. This is because there are many possibilities of migration and exchange between greenhouse and field populations, also in the USA. This may have led to genetic exchange to the extent that all wild thrips populations by now have changed into pest populations.

## IMPORTANCE OF THIS THESIS FOR AGRICULTURE

Agro-businesses have a vested interest in thrips research because thrips is a ravenous herbivore and it transmits plant viruses and in particular tospoviruses, which cause a lot of damage to plants. In the Netherlands, several research projects were set up by Wageningen University and other universities in collaboration with agricultural companies to study thrips. The work in this thesis was partly steered by a sounding board composed of representatives from various seed producing companies, including S&G Seeds, Rijk Zwaan, Nunhems Zaden, and PTG. This sounding board was set up not only to facilitate direct exchange of information between university researchers and agricultural companies, but also to allow companies to influence the research agenda in order to follow the relevance of the work for the agricultural sector. The question is therefore what relevance the results of our project have had or will have for agricultural practice.

The fact that thrips variation between populations may well be as large as the variation within one population, suggests that it may not be necessary to screen a wide range of thrips populations when one wants to make sure that newly developed resistant cultivars of agricultural crops or techniques and agents to control thrips in greenhouses are really effective against western flower thrips. Variation in sensitivity to resistance seems to be organised within populations with little additional variation observed among populations. However, a note of caution is needed here: only a small subset of the populations of the species has been analysed in this study and thrips populations may have had and may still have enough opportunity for gene exchange due to international trade and plant shipments.

The presence of gut bacteria in thrips and the possible mutualistic interaction between these bacteria and thrips has positive fitness effects on the pest status of thrips. Thrips have a shorter life cycle and reproduce better on plant leaves when they have bacteria in their gut. This means that thrips population growth is higher in the presence of gut bacteria and that thrips can therefore be a more severe pest in greenhouses. Furthermore, they will also have better chances of survival on seedlings of crops that are shipped to greenhouses, because thrips with gut bacteria are better in surviving on leaves (in the absence of flowers/pollen) than thrips without bacteria. The survival of thrips on seedlings that are traded between farmers and seed companies enhances the chances of global migration of the species (Frey, 1993).

This could lead us to the conclusion that removing the bacteria from the thrips could make a contribution to thrips control in agriculture. The removal of bacteria is possible with antibiotics or by breeding larvae in an environment where bacteria are not present (see chapters 2 and 3). However, we have found that thrips quickly regain the appropriate bacteria once they are back again on the host plants (De Vries *et al.*, 2001b, see chapter 3). Eradication of bacteria from thrips with antibiotics and maintaining bacteria-free thrips therefore does not seem to be a viable - let alone a cost-effective - approach to control thrips. Instead, the tight relation between thrips and bacteria and the strong ability of bacteria to invade thrips could be used for other purposes. Recently, researchers have been able to manipulate insect micro-organisms to introduce genes whose products (e.g., a *Bacillus* toxin) function in the control of pest insects (Hussender and Grace, 2005; Kuzina *et al.*, 2002; Watanabe *et al.*, 2000). The thrips - *Erwinia* association seems a good model for such an application. *Erwinia* spec bacteria survive outside as well as inside thrips and are easy to culture. Being also a member of the Enterobacteriaceae, there seem to be ample opportunities for genetic modification of *Erwinia* spec, just like the *Enterobacter* species manipulated by the aforementioned researchers.

What could be studied with the use of genetic modification of *Erwinia* spec bacteria in thrips, is its effect on the epidemiology of tospoviruses. Our studies show that the presence or absence of gut bacteria does not influence the transmission efficiency of tospoviruses by the thrips (chapter 7). Virus is taken up in a larval stage when the gut flora has not fully developed yet and the site of uptake is probably not the same as the site where bacteria are found. But when bacteria would be able to produce and release anti-viral agents, and these were absorbed by the thrips, this could perhaps reduce virus transmission efficiency. This speculative model deserves further analysis and experimentation, as well as other bacteria related features that can be transferred to thrips.

## SYMBIOSIS

The species that I have studied, i.e. western flower thrips and *Erwinia* spec, have a close association for a large part of the thrips life cycle. All western flower thrips appear to be infected with *Erwinia* spec. *Erwinia* spec have an effect on important fitness characters of thrips such as feeding preference, generation time, and reproduction. Both symbiotic species are able to survive apart from each other, although it is perhaps impossible to maintain a bacteria-free thrips population. The symbiotic relation is mutualistic, commensalistic, or parasitic, depending on thrips' life stage and food source.

There are research papers providing good examples of symbiotic interactions that shift between being mutualistic/commensalistic and being parasitic depending on external or internal factors (coffee plant and predatory mites: Ferreira, 2007; gut bacteria of crickets: Kaufman *et al.*, 1989). The symbiosis theory was described by Bronstein (1994) and Hochberg *et al.* (2000). This phenomenon deserves more study. To determine when and how symbiosis becomes mutualistic or parasitic requires studying a broad range of situations for that particular relation. Often, research is limited to a few populations of the host species and one or a few standard environmental conditions, and does not cover all life stages of the host. The thrips example shows that this may lead to both an underestimation as well as an overestimation of the nature of the particular symbiotic interaction. Some mutualistic inter-

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actions may be overlooked because the conditions for mutualism were not part of the study, and in other cases the mutualistic interaction is not as general as thought initially, but is only present under the restricted conditions of that study.

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