Downloaded from UvA-DARE, the institutional repository of the University of Amsterdam (UvA) http://dare.uva.nl/document/161378

File ID 161378

Filename Chapter 1: Introduction

SOURCE (OR PART OF THE FOLLOWING SOURCE):

Type Dissertation

Title Symbiosis of thrips and gut bacteria

Author E.J. de Vries Faculty Faculty of Science

Year 2010 Pages 173

ISBN 978 90 76894 85 0

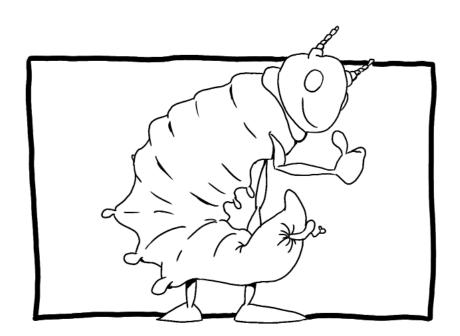
FULL BIBLIOGRAPHIC DETAILS:

http://dare.uva.nl/record/328135

Copyright

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other then for strictly personal, individual use.

Egbert J. de Vries



SYMBIOSIS

Origin of the symbiosis concept

Ever since Anton de Bary (1879) introduced the notion of *symbiosis* in biology, it has been controversial among scientists. Its importance in explaining species interactions and the role it plays in the evolution of species is intuitively understood. But reaching a common definition of symbiosis has been difficult. In this introduction, problems and possible solutions to define symbiosis are discussed, and an outline is given of the example of symbiosis that is studied in this thesis; the gut bacteria of thrips.

De Bary was a plant pathologist. In his book 'Die Erscheinung der Symbiose' (1879), he describes symbiosis as 'the living together of two different species'. Based on his primary field of interest, associations leading to a parasitic outcome were explicitly part of his concept of symbiosis. This view has been much debated among parasitologists. The idea that parasites that are living most or all of their lives within the body of their host, may be part of the group of symbionts, is still not generally accepted. For some researchers, symbiosis implies that both partners gain equal benefit from the association, or at least no damaging effect should be experienced by any of the two partners. Paul Buchner, the most cited insect microbiologist, states in his book 'Endosymbiosis of Animals with Plant Micro-organisms' (1965): "parasites mistaken for symbionts...". He draws a clear distinction between symbionts and parasites. The problem with this approach to symbiosis is that most associations are not always mutualistic or always parasitic. And not all mutualistic interactions are symbiotic, meaning they are not all of a stable and long-lasting nature. The concept of mutualism and the concept of symbiosis should not be blurred.

Mutualism

Commensalism and mutualism are concepts that were introduced to biology in the same period as symbiosis. The Belgian biologist Pierre van Beneden suggests in his book 'Les Commensaux et les parasites' (1875) that mutualists should be added to the already existing concepts of commensalists and parasites. According to Van Beneden, mutualists are organisms of different species that mutually provide each other service. This phraseology adds to the suggestion that political and social circumstances of that time have had an impact on the work and publications of De Bary and Van Beneden (Boucher *et al.*, 1982). In the 1880s, there was an increasing attention for the growing poverty among the working class, which led to the rise of the socialist movement. In this movement, communes and collectives of individuals with mutual support to each other were central themes.

This common language can still be found today, for example in the Mutualité in Belgium, an establishment where people can secure their savings and receive loans under reasonable conditions. In the last decades of the nineteenth century the famous communauté in Paris, where people lived together and shared their possessions, was flourishing. Communautarism is a political ideology that has survived till today, for example in the Blairite/Clintonian Third Way ideology of the nineties. Boucher *et al.* (1982) even suggests that this political background may explain the unpopularity of concepts such as symbiosis and mutualism among American and European scientists during the pre-WWII and post-WWII years, when communism ruled a large part of our planet. During the last three decades

symbiosis has regained its position in research and is seen as an important factor driving ecological an evolutionary change (Douglas, 1995; Keddy, 1990; Margulis, 1993).

Today, the generally accepted view on mutualism makes it difficult to maintain the assumed equation of symbiosis to mutualism. Mutualism, in which both partners gain benefit from interacting, is not restricted to situations where two different species live close together for a long time. For example, pollinating insects have mutualistic associations with the plants they pollinate. Given the level of specificity of the particular insect species, this can be one or many different plant species. None of these plants are visited for a long period of time. Seed dispersal by animals is another clear-cut example of mutualism without a symbiotic interaction. The symbiotic status of cleaning birds on rhinoceroses, cleaning fish, and of ants weeding aphids could be questioned. Their mutualism, however, is not.

To benefit from the interaction

The mutualistic outcome of an association is not static. More and more scientists adopt the view that the outcome of the interaction of two different species is depending on the conditions under which the association takes place (Bronstein, 1994). Depending on the diet of the host, the density of symbionts or another ecological value or environmental situation, the outcome of the interaction can be mutualistic, parasitic or neutral. Such a conditional outcome of a species interaction is described in this thesis, where the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), may benefit from the bacterium *Erwinia* species, depending on the food substrate for thrips and symbiont. Variable outcomes of species interaction have been described in other insect symbiotic systems (Kaufman *et al.*, 1989).

The mutual benefit of the host and symbiont is often just assumed, without proper experimental confirmation. The outcome of the interaction in different situations, and on both partners, is hardly ever studied. The endosymbiotic bacteria of Homoptera, living in specific tissue near the insects' gut, were assumed to be in a mutualistic symbiosis with their host for a long period of time before anybody studied the exact benefit the symbiosis gives to the symbiont (Douglas, 1995). For many other mutualistic interactions, the exact benefit of the interaction to the symbiont has never been established (Douglas and Smith, 1989; Werren and O'Neill, 1997). It is difficult to determine the effects of interactions on the fitness of microorganisms, especially when it concerns obligate symbionts, because they cannot survive and be studied outside the host. These symbionts were labelled 'domesticated' (Werren and O'Neill, 1997) or may even be called 'slaves' (Maynard-Smith and Szathmáry, 1995). To draw an analogy with drug dealers and addicts; both benefit from the interaction, but you may wonder whether the addict would not be better off without the interaction as such (Law and Dieckmann, 1998). The most extreme cases of 'domesticated' symbionts are the mitochondria and chloroplasts (Margulis, 1993).

Generally it is assumed that long-lasting associations will become commensalistic or even mutually beneficial in the long run, particularly if transmission of the symbiont is vertical. Vertical transmission is the direct transfer of symbionts from a mother to her offspring. Long-lasting associations would lead to adaptation and to co-evolution, which is enhanced by vertical transmission because that diminishes invasion of new symbiont types. Model studies based on this assumption have shown that mutualism may evolve from such interactions (Maynard-Smith and Szathmáry, 1995, and references therein; Yamamura, 1993). On

the other hand, a number of intimate, long-lasting symbiotic associations have been found which are parasitic to the host. For example, obligate endosymbiotic *Wolbachia* symbionts are parasitic to many arthropods (Stouthamer *et al.*, 1999; Werren *et al.*, 1995). This parasite is vertically transmitted and causes changes in the reproductive system of the insect, such as cytoplasmic incompatibility or sex ratio distortion.

Additionally, there are well-known examples of horizontal transmission of symbionts that nevertheless have developed into symbiotic interactions. Horizontal transmission is the transfer of symbionts to the new host via the food source. For example, the gut bacteria studied in this thesis are horizontally transmitted to thrips, but still can benefit the host. The possibility of development to mutualism in the case of horizontal transmission was predicted recently in theoretical papers (Genkai-Kato and Yamamura, 1999; Van Baalen and Jansen, 2001).

Invasion of the symbiont

The problem with defining symbiosis is not only the controversy whether or not to include parasitic associations. It also has to do with the broadness of the concept (Douglas and Smith, 1989; Pirozynski and Hawkworth, 1988). The definition of De Bary (1879) – the living together of two species – is not sufficiently clear because in an ecosystem, all species are living together. De Bary's definition is powerful in its inclusiveness but weak in its precision. But it has been difficult to come up with a limitation of the definition. It is possible to restrain symbiosis to cases where the two partners are unequal in size and generation time, to clearly define a host and a symbiont living in or on the host. Many forms of symbiosis would fit that definition, but some would not. Mycchorizal fungi are often larger than their host, and most of their body is living completely outside the host. Yet they are in symbiosis with their host plants (Harley and Smith, 1983). To solve this problem of definition it is best to assume that the symbiont invades the host, and is completely or partially attached to or present inside the host. This will make it clear which species is the symbiont and which is the host. Attachment to the gut of the host is included in this definition.

Duration of symbiosis

Another difficulty with the definition of De Bary (1879) is the minimum period of time of two different species living together before the interaction can be called symbiotic. It is clear that a brief meeting between two specimens of different species is not to be called symbiotic. But how long is the threshold time for an association to become symbiotic? A complete generation time? That would exclude all cases of horizontal transmission, where the host in each generation has to pick up the symbionts from the environment. The best approach is to include in our definition of symbiosis that associations should last for a large part of the generation time of both partners.

Novel metabolic capabilities

In her book on symbiosis, Angela Douglas ('Symbiotic interactions' 1994) takes the following perspective. Symbiosis is the group of associations where one of the partners or both have acquired a novel metabolic capability from the other partner. The advantages of this

definition is, that it still includes all kinds of interactions in the range of parasitism to mutualism, and at the same time excludes all kind of short term interactions such as predation, pollination, consumption and transport. The challenge of this definition is that in many symbiotic associations such a novel metabolic capability has not (yet) been elucidated. In many cases, the symbiosis is not purely physiological, which may be suggested by the concept of metabolic capacity. Perhaps the concept should be broadened to the acquisition of novel *ecological* capabilities. Furthermore, the concept seems to focus on the host and not so much on the symbiont.

There are many examples of metabolic capabilities that were acquired due to symbiosis. This could be the production of essential vitamins, such as the mycetomic bacteria of Homoptera (Baumann *et al.*, 1995; Douglas, 1995), or cellulose digestion in xylophagous insect species (Breznak, 1982; Treves and Martin, 1994). Another example is the protection against diseases or toxic agents, for example the gut bacteria of the grasshopper *Schistocerca gregaria* (Dillon *et al.*, 2000). Nitrogen fixation is occurring in different symbiotic systems, such as mycorrhiza in plants (Harley and Smith, 1983), and specialised bacteria in termites and xylophagous bugs (Bridges, 1981; Potrikus and Breznak, 1977). The crucial question concerning this definition of symbiosis is to select the particular novel metabolic capability that the symbiont would offer to the host and vice versa. Additionally, this metabolic capability may depend on the life stage of host or symbiont, on the symbiont numbers or another ecological factor.

Symbiosis, the definition used in this thesis

Symbiosis is an association between two different species, a host and a symbiont, in which the symbiont(s) invade(s) the host, which lasts for a considerable part of the generation time of both partners, and in which one or both of the partners acquire a novel ecological capability from the other partner. This definition is not far from the one used by Douglas (1994), and also not far from Zook (1998) and Dillon and Dillon (2004).

In many cases, the symbiont is living inside the host, in its body fluids or in specialised cells, like the aforementioned mycetomes. These kinds of symbionts are called endosymbionts, to distinguish them from the free-living symbionts that reside on the host or in the gut system of the host. To the latter group of symbionts belong the *Erwinia* gut bacteria that are described in this thesis. A major difference between the two groups of symbionts is that endosymbionts are obligatory symbiotic and not likely to survive outside the host.

GUT BACTERIA

Animal guts are commonly inhabited by bacteria. Vertebrates often possess a large and diverse microflora, which offer different novel metabolic capabilities. Insect species also contain gut bacteria, but the composition of their microflora tends to be less diverse. For most animal species, very little is known about the composition of the gut microflora, the way microorganisms invade the gut system, or the exact nature of the interaction with the host. The presence of bacteria in the gut of animals not necessarily implies a form of symbiosis. It may also be just a temporary phenomenon and reflect the composition of the diet (Cazemier *et al.*, 1997; Thibout *et al.*, 1995).

While most insect species have only extra-cellular gut symbionts, some species possess in addition intracellular endosymbionts. These endosymbionts are present in specialised organs near the gut (mycetome), in the fat body close to the gut, or in the gut epithelium (Baumann and Moran, 1997; Buchner, 1965; Campbell, 1990; Koch, 1967). Examples of insects with endosymbionts are aphids and other homopteran species, mosquitoes and tsetse flies, and cockroaches. Insects with symbiotic gut bacteria have been described in species belonging to nearly all orders of the Class Insecta (Campbell, 1990; Dillon and Dillon, 2004; Douglas and Beard, 1996). Table 1.1 gives an overview of insect species in which gut bacteria have been studied.

The insect gut

Insect guts are not as stable as the specialised host organs used by endosymbionts. This requires more adaptability from their gut symbionts. The gut system of insects can be subdivided into different structures, which may offer different conditions to microorganisms. It generally consists of a foregut, midgut, and hindgut. Specialised organs are connected, such as the salivary glands (foregut), and the caeca and Malpighian tubules (midgut to hindgut). For an overview of functions in relation to gut bacteria see Bignell (1983). This thesis describes the symbiotic bacteria in the western flower thrips hindgut. The hindgut is the part of the gut system of insects where symbiotic bacteria usually are found (Bignell, 1983; Campbell, 1990).

The gut environment is influenced by several factors. One important factor is the insect growth cycle. The number of life stages and their duration varies between insect species, and the extent of peritrophic membrane change during a moult is also variable. This membrane may function as a shield against microorganisms invading into the haemocoel, but its secretion and disruption during a moult may also provide a chance for microbes to colonise the gut by adhering to the epithelium (Bignell, 1983). The colonisation success of bacteria in the gut is also affected by chemical conditions and food availability. The most important chemical factors are pH levels and oxygen pressure.

pH level

The pH level is rather constant in one part of the gut system during one insect life stage, but probably differs between various parts of the gut. Whether the gut parts will be more basic or acidic varies from species to species. For example, the midgut of mosquitoes has a very high pH level (10.5; Dadd, 1975), and that of houseflies is very low (3.5; Greenberg, 1968). Such extreme levels require adaptations in microorganisms. It was also found that in some insect species the pH level in the same part of the gut changes depending on the life cycle (Bignell, 1983). This may imply that bacteria are able to pass the gut system to the hindgut in one part of the life cycle but not in another, because the midgut has obtained toxic pH levels in the next life stage.

Oxygen pressure

Oxygen concentrations are variable in the gut system, particularly in situations where fermentation is intensive. Parts of the gut lumen may become micro-aerophilic or even anaer-

Thongaram et al., 2005

First proctodeal segment of

the hindgut

Alkaliphiles, several types, many Clostridiae or Bacillae

Stingle et al., 2005

Internal symbiont of flagellate protists in the hindgut

Endomicrobia

TABLE 1.1 - Bacteria associated with the insect gut system. This is an overview of various insect species and their bacterial symbiont species, on basis of recent literature sources. Pathogenic gut colonisers were not taken into consideration. For an overview of Wolbachia infected insects we refer to review articles (Stouthamer et al., 1999; Werren, 1995).

Insect species	Bacteria species	Location	Reference
ORDO Thysanura			
Firebrat (Thermobia domestica)	Symbionts	Gut lumen	Treves and Martin, 1994
ORDO Collembola			
Folsomia candida	Various Enterobacteriaceae	Gut lumen	Thimm <i>et al.</i> , 1998
ORDO Orthoptera			
Common house cricket (Acheta domestica)	Various Enterobacteriaceae	Hindgut lumen	Ulrich et al., 1981; Kaufmann et al.,
			1989; Domingo et al., 1998
Migratory grasshopper (Melanoplus	Various Enterobacteriaceae	Gut lumen	Bucher and Stephens, 1959;
sanguinipes)			Mead et al., 1988
Migratory locust (Locusta migratoria)	Enterobacter cloaca	Gut lumen	Colgan, 1987
Desert locust (Schistocerca gregaria)	Pantoea agglomerans	Gut lumen	Hunt and Charnley, 1981;
			Dillon et al., 2000
	Various Enterobacteriaceae and		Stevenson, 1966
	Clostridiae		
ORDO Isoptera			
Termites - several species, such as Mastoter-	Enterobacteriaceae, Spiro-	Hindgut lumen, paunch	Grassé and Noirot, 1959; Schultz and
mes darwiniensis, Recticulitermes flavipes,	chaetae, Streptococcus sp.,		Breznak, 1978; Bignell et al., 1980;
Nastitulitermes exitosus, Speculitermes sp.	Actinomycetae		Breznak, 1982; Czolij <i>et al.</i> , 1985; Ohkuma and Kudo, 1996; Brauman <i>et</i>
			al., 2001; Schmitt-Wagner et al., 2003
	Flavobacteriaceae	Mycetome	Bandi <i>et al.</i> , 1995

TABLE 1.1 – Continued

Insect species	Bacteria species	Location	Reference
ORDO Dictyoptera			
Cockroaches – several species, such as Perip-	Enterobacteriaceae, Clostridiae,	Gut lumen, hindgut	Bracke et al., 1979; Cruden and
laneta Americana, Eublaberus posticus,	Methanospirillum, Chryseo-		Markovetz, 1984; Gijzen et al., 1994;
Blatella germanica	bacterium indolgenens		Dugas et al., 2001
	Flavobacterium	Fat body cells	Bandi <i>et al.</i> , 1994
ORDO Homoptera			
Planthopper (Nilaparvata lugens)	Pseudomonas sp., Erwinia	Gut lumen	Watanabe et al., 1996, 1997
	ananas, Erwinia herbicola		
Cicads (Euscelis sp., Halochora communis)	Endosymbiont	Mycetome	Douglas, 1988; Chang and Musgrave, 1972
	Symbionts	Gut lumen	Chang and Musgrave, 1972
Psyllids	Endosymbionts	Mycetomes	Spaulding and Von Dohlen, 1998;
			Thao et al., 2000b; Subandiyah et al.,
			2000
	Secondary symbionts	Secondary mycetocytes	Fukastsu and Nikoh, 1998; Thao et
			<i>al.</i> , 2000a
Mealy bugs (Pseudococcidae)	γ -Proteobacteriaceae	Mycetome	Munson et al., 1992; Fukatsu and
			Nikoh, 2000a
	β-Proteobacteriaceae	Mycetome	Thao <i>et al.</i> , 2002
Aphids - several species such as Aphis fabae,	Buchnera aphidicola	Mycetome	Houk and Griffiths, 1980; Douglas,
Schizaphis graminum, Myzus persicae,			1989; Munson et al., 1991; Baumann
Acyrtosiphon pisum, Macrosiphum avenae, Colopha kansugei			et al., 1995
	Secondary symbionts	Mycetome	Ishikawa, 1981; Chen and Purcell, 1997; Fukatsu et al., 2000b
	Bemisia type symbiont (T-type) U-shaped symbiont	Secondary mycetocytes Secondary mycetocytes; sheat cells, hemolymph	Darby and Douglas, 2003 Tsuchida <i>et al.</i> , 2005

	Hamiltonella defensa, Regiella insecticola, Serratia symbiotica	Haemocoel, sheet cells, secondary bacteriocytes	Moran <i>et al.</i> , 2005
	Enterobacteriaceae	Gut lumen	Harada <i>et al.</i> , 1996; Haynes <i>et al.</i> , 2003
White flies – species such as Bemisia tabaci	Endosymbionts	Mycetome	Campbell et al., 1992; Zchori-Fein and Brown, 2002
ORDO Heteroptera	Arsenphonus sp.	<i>:</i>	Thao and Baumann, 2004
Lice species (Cimex sp. Stenotus sp.)	Endosymbionts	Gut epithelium	Chang, 1974
Cotton fleahopper (Pseudotomescelis seriatus)	Endosymbiont	Mycetome	Grisham et al., 1987
Reduvidid bugs (Rhodnius prolixus,	Rhodococcus rhodnii,	Haemocoel	Goodchild, 1955; Baines, 1956; Hill
Triatoma infestans)	Actinomycetae		et al., 1976; Hypsa, 1993; Beard et al., 2002
	Arsenophanus triatominarum	Haemocoel	Hypsa and Dale, 1997
Pentatomid bug (Nezara viridula)	Enterobacteriaceae, incl. <i>Pantoea</i> sp.	Gut lumen	Ragsdale <i>et al.</i> , 1979; Hirose <i>et al.</i> , 2006
Black bug (Pyrrhocoris apterus) ORDO Thysanoptera	Hafnia sp., Streptococcus sp.	Gut lumen	Haas and König, 1987
Caudothrips buffai	Endosymbiont	Mycetome	Bournier, 1961
Western flower thrips (Frankliniella	Enterobacteriaceae, Pantoea	Gut lumen	Ullman et al., 1989; De Vries et al.,
occidentalis)	agglomerans		1995
Tobacco thrips (Frankliniella fusca)	Enterobacteriaceae, Pantoea	Gut lumen	Wells et al., 2002; Gitaitis et al., 2003
	ananatis, Pantoea agglomerans		
ORDO Neuroptera			
Ant lions (Myrmeleon mobilis)	γ-Protobacteria	Gut system	Dunn and Stab, 2005
ORDO Phthiraptera			
Pigs louse (Haematopinus suis)	Endosymbionts	Mycetome	Bewig and Schwartz, 1956
ORDO Coleoptera			
Grass grub (Costelytra zeelandica)	Symbionts	Hindgut lumen	Hoyt and Osborne, 1971; Bauchop and Clarke, 1975

TABLE 1.1 – Continued

Insect species	Bacteria species	Location	Reference
[ORDO Coleoptera]			
Oryctes nasicornis	Methanogens	Hindgut lumen	Bayon, 1980
Mealworms (Tenebrionidae; Tenebrio molitor)	Endosymbionts	Gut lumen	Genta et al., 2006
Furniture beetle (Anobium bipunctatum)	Endosymbionts	Mycetome	Behrenz and Technau, 1959
Long-horned beetles (Anoplophora	α-Proteobacteriaceae	Gut lumen	Delalibera et al., 2005
glabripennis, Saperda vestita)			
Bark beetles (Ips paraconfusus, Scolytus	Enterobacteriaceae, Bacillus	Gut lumen	Moore, 1972; Brand et al., 1975;
scolytus, Phloeosinus armatus)	sp., Pseudomonas sp., Coryne-		Burges et al., 1979; Bridges, 1981
Passalid beetle (Odontotaenius disjunctus)	Endosymbionts	Hindgut	Nardi <i>et al.</i> , 2006
Scarab beetles (Pachnoda ephippiata)	Symbionts	Midgut and hindgut	Egert et al., 2003
	Promicromonospora pachnodae	Hindgut	Cazemier et al., 2003
Weevils (Sitophilus zeamais, Sitophilus oryzae)	Endosymbionts	Mycetome	Musgrave, 1964; Nardon and Grenier,
			1988; Campbell et al., 1992; Heddi et
			al., 1999; Lefevre et al., 2004
ORDO Hymenoptera			
Carpenter ant (Camponotus sp.)	Endosymbiont, Blochmannia	Mycetome	Schröder et al., 1996; Sauer et al.,
	floridanus		2000; Degnan et al., 2004
Tetraponera binghami	Flavobacteriaceae	Gut lumen	Van Borm <i>et al.</i> , 2002
Red imported fire ant (Solenopsis invicta)	Lactococcus, Staphylococcus	Gut lumen	Peloquin and Greenberg, 2003
Honey bee (Apis mellifera)	Enterobacteriaceae, Gram-	Gut lumen	Gilliam and Valentine, 1974; Gilliam
	positive cocci		1997
European beewolf (Philantus triangulum) ORDO Lepidoptera	Streptomyces sp.	Antennae	Kaltenpoth et al., 2005
Wax moth (Galleria mellonella)	Pseudomonas sp., Strep- tococcus faecalis	Gut lumen	Jarosz, 1979; Gilliam and Lohrenz, 1983
Douglas fir tussock moth (Orygia sp.)	Bacillus sp.	Gut lumen	Andrews and Spence, 1980

Enterobacteriacea, Strep- Midgut lumen Charpenter et al., 1978	Gut lumen Kaneko et al., 1995	γ -Proteobacteria incl. $Pantoea$, Midgut Broderick <i>et al.</i> , 2004 low G+C gram-positive bacteria	Whole gut Sittenfeld et al., 2002	Gut lumen Takahashi et al., 1995	Gut lumen Takatsuka and Kunimi, 2000 Gut lumen Thibout <i>et al.</i> , 1995	Gut lumen Klug and Kotarski, 1980	Midgut lumen Demaio et al., 1996; Straif et al., 1998	Pseudomonas melophtera Haemocoel Baerwald and Boush, 1968; Howard et al., 1985	Enterobacter agglomerans Gut lumen MacCollem et al., 1992; Lauzon et al., 2000	Enterobacteriaceae, Pseudo- Gut lumen Hagen, 1966; Drew et al., 1983; monas sp. Lloyd et al., 1986; Fitt and O'Brien, 1995	Enterobacteriaceae, Pseudomo-Gut lumen as p., Xanthomonas sp., Staphas p., Enterobacter sp., Enterobacter sp., Enterobacter sp., Klebsiella sp. Enterobacter al., 1972; Tsiropoulos, 1976; Iverson et al., 1984; Daser and Brandl, 1992; sp., Klebsiella sp. Z002; Behar et al., 2005	Enterobacteriaceae Gut lumen Kadavy <i>et al.</i> , 1999 Erwinia herbicola, Pseudomonas Gut lumen Cole <i>et al.</i> , 1990
Turnip moth (Scotia segetum)	Diamondback moth (Plutella xylostella)	Gypsy moth (Lymantria dispar)	Saturniidae (Automeris zugana)	Mulberry pyralid (Glyophades pyloales)	Oriental tea tortrix (Homona magnanima) Hyponomeutidae (Acrolepsis assectella) ORDO Diptera	Crane fly (Tipula abdominalis)	Mosquito species (Culex, Aedes, Anopheles, and Psorophora sp.)	Apple maggot fly (Rhagoletis pomonella)		Olive fruit fly (Dacus oleae)	True fruit flies – several species such as Anastrepha sp., Tetanops mypaeformis, Bactrocera tryoni, Ceratitis capitata	Oil fly (Helaeomyia petrolei) Carrot root fly (Psila rosae)

TABLE 1.1 – Continued

Insect species	Bacteria species	Location	Reference
[ORDO Diptera]			
Tsetse flies (Glossina sp.)	Endosymbionts, Wiggleswor-	Gut epithelium	Nogge, 1978; O'Neill et al., 1993;
	thia glossinidia		Aksoy et al., 1995; Akman et al.,
			2002
	Secondary symbiont, Sodalis	Gut epithelium	Dale and Maudlin, 1999; Chen et al.,
	glossinidis		1999
Onion maggot fly (Delia antigua)	Pseudomonas sp., Enterobac-	Gut lumen?	Hough et al., 1981; Marshall and
	teriaceae		Eymann, 1981
Blow flies – several species	Enterobacteriaceae	Gut lumen	Greenberg, 1968
Common house fly (Musca domestica)	Enterobacteriaceae	Gut lumen	Greenberg, 1968; Espinoza-Fuentes
			and Terra 1987; Zurek <i>et al.</i> , 2000

obic. This has been studied in termites, where the hindgut has an extension to digest cellulose (paunch). The termite paunch has an area where the oxygen level drops back to zero, to which some symbionts are adapted (Brune *et al.*, 1995). Termites and their gut bacteria represent a specialised example of symbiosis, but gut bacteria have to cope with variation in oxygen levels in other gut systems as well. Facultative anaerobic microorganisms, such as γ -Proteobacteria, could have a selective advantage in such environments.

Insect food

The type and amounts of food consumed by the insect host is another variable that influences the symbiosis. Most gut bacteria are heterotrophs, and will be dependent on the feeding habits of the host for their food supply. The difference between residing in the gut of a generalist or specialist insect species is obvious: the diet of a generalist is such that the symbiont is provided with a larger variety of nutrients.

Insects harbouring endosymbionts usually live on relatively poor diets. The plant juice sucking Homoptera all have various kinds of mycetomes, with either yeasts or bacteria or both (Baumann *et al.*, 1995; Buchner, 1965). Xylophagous beetles and termites, living on wood, have developed special symbiotic associations to improve cellulose digestion and nitrogen recycling (Breznak, 1982; Buchner, 1965). Mycetomes have also been found in blood-sucking insects (Buchner, 1965, and references therein). All of these diets are known to be lacking one or more essential nutrients. However, some species living on richer diets, and some generalist species, also have associations with bacteria. For example, cockroaches have specialised symbiont-bearing cells in their fat body (Cruden and Markovetz, 1984; Koch, 1967). Some of the phytophagous orthopteran species have gut bacteria (Bucher and Stephens, 1959; Dillon *et al.*, 2000; Stevenson, 1966; Ulrich *et al.*, 1981). Some insects seem to be able to use the gut bacteria as food source (Drew *et al.*, 1983, Greenberg, 1968; Lemos and Terra, 1991).

Symbiont transmission in insects

Endosymbionts are maternally inherited (Werren and O'Neill, 1997). These symbionts leave their specialised tissue, migrate through the body of the mother towards the ovaries and are included in the eggs (Buchner, 1965; Koch 1967). Vertical transmission is efficient, resulting in nearly 100% infection of the offspring, and secures the transmission of one particular type of bacteria, the symbiont species. Insects depending on extracellular gut symbionts have to develop other mechanisms of transmission. Various methods to secure some form of vertical transmission of extra cellular bacteria have been found, for example the smearing of bacteria on the egg when it is deposited. Female *Dacus oleae* flies have a specific bacterial depot in their abdomen near the anus to be able to smear the eggs (Petri, 1904). Buchner (1920) showed that wood-eating anobiid beetles actually ate the eggs' shell, which was infected with symbionts. Consequently, the larvae remain sterile when the outside of the eggs is sterilised and they hatch in a sterile environment. This was shown by Wigglesworth (1936), with *Triatoma* and *Rhodnius* blood-sucking heteropteran bugs. In cockroach larvae, the gut microflora is maintained because they eat their own excrements after every moult (Koch, 1967).

Horizontal transmission routes of gut bacteria are likely to be less reliable than vertical transmission of symbionts. Invasion of another type of microorganism is than possible.

Western flower thrips gut bacteria, described in this thesis, use horizontal transmission. This means that bacteria have to invade the gut of every new generation of thrips; thereby passing through parts of the gut in which they do not reside permanently (they only stay in the hindgut). Apart from less favourable chemical conditions in these other gut parts; they also have to compete with other, non-symbiotic invaders. Mechanisms that enable the thrips to acquire the right type of symbionts, for example thrips' feeding preferences, are described in this thesis. Food uptake from places where other thrips have been feeding, and have deposited their gut bacteria, will enhance uptake of the symbiont type of bacteria.

WESTERN FLOWER THRIPS AND *ERWINIA* SPEC. GUT BACTERIA

The symbiosis between western flower thrips (*F. occidentalis*) and *Erwinia* spec gut bacteria is the central topic of this thesis. With electronmicroscopy it was found that thrips have bacteria in their hindgut (Ullman *et al.*, 1989). We confirmed the presence of these bacteria using standard microbiological methods (De Vries *et al.*, 1995). Following up on those results, we have studied the gut bacteria in various strains of western flower thrips and the effect of these gut bacteria on thrips. In one of the chapters of his thesis, the gut microbiology of a second thrips species, the onion thrips (*Thrips tabaci* Lindeman), is presented. Both thrips species have been studied extensively over the past 50 years, because of their status as pest insect in greenhouse and open field agriculture.

Western flower thrips

Western Flower Thrips is a small (2 mm long) polyphagous species with cryptic habits, which makes it an invasive insect (Morse and Hoddle, 2006). Thrips are a major pest of both vegetables and ornamental crops. Field studies on thrips report them to feed on leaves, flowers, and pollen, of many plant species in different taxa, but also feeding on fungi and on eggs of arthropods, including cannibalism, was reported (Bryan and Smith, 1956; Lewis, 1997; and references therein). The studies on thrips nutritional requirements suggest that thrips adults survive best on a mixed diet of leaves and pollen. However, thrips is able to feed and reproduce on leaves alone (Van Rijn *et al.*, 1995). Pollen in the diet increases thrips reproduction (Kirk, 1997a; Van Rijn *et al.*, 1995). The versatility of thrips regarding nutrition has interested many researchers, and the possible role of bacteria was hypothesised before (Mollema *et al.*, 1995).

Since the 1980s, thrips has expanded from its original habitat in the western part of the United States to a global distribution. It has been found in Europe (Mantel and De Vrie, 1986; Zur Strassen, 1986), in South Africa (Giliomee, 1989), in Israel, in Japan, and in New Zealand (Martin and Workman, 1994). To explain the spread of thrips across the world, we have to assume that it is able to survive on plants without pollen or nectar, and that seedlings of plants have been carrying thrips to other regions of the world (Frey, 1993, Vierbergen 1995, Morse and Hoddle 2006). Thrips has become one of the major pest insect species in the world (Lewis, 1997). Apart from direct damage due to feeding on plants, thrips is also the major vector of tospoviruses, like tomato spotted wilt virus (TSWV) (see Whitfield *et al.*, 2006; and references therein). This virus ranks among the most devastating plant viruses (Goldbach and Peters, 1994).

The microbiology of Thysanoptera has not been widely studied before this thesis. Apart from the *Erwinia* bacterial symbionts in western flower thrips, there are studies documenting the transfer of *Pantoea ananatis* in the guts of tobacco thrips *Frankliniella fusca* (Gitaitis *et al.*, 2003; Wells *et al.*, 2002), and of bacteria in mycetomes of *Caudothrips buffai* (Bournier 1961). *Caudothrips buffai* is a mycophagous insect outside the large thrips family of Thripidae. Bournier did not find mycetomes in any of the species of Thripidae that she studied.

The existence of biotypes of Western flower thrips has been suggested several times in the literature. Thrips were found to vary in insecticide resistance (Immaraju *et al.*, 1992; Brødsgaard, 1994), transmission efficiency of tomato spotted wilt virus (Van de Wetering, 1999), and response to plant resistance (De Kogel, 1997; De Kogel *et al.*, 1997a; Soria and Mollema, 1994). It is not known whether variation exists in the gut bacteria of thrips and whether this possible variation leads to biotype formation.

See for more backgroud information on thrips biology Annex 1.

Erwinia spec.

Erwinia spec. gut bacteria in thrips belong to the bacterial family of Enterobacteriaceae (De Vries et al., 2001a). This type of gut bacteria will be called Erwinia spec throughout this thesis because there is no species name for them yet. Based on biochemical and phylogenetical data the bacteria are very similar to one particular member of the Erwiniae, namely Enterobacter agglomerans, which was renamed Pantoea agglomerans (Gavini et al., 1989). The reason that both the genus names Enterobacter and Erwinia were used in parallel for such a long time was that different groups of microbiologists used different names (phytopathologists and clinical microbiologists).

Further background information on the *Erwinia* group of bacteria is given in Annex 2. The central issue of this thesis is the nature the symbiosis of *Erwinia* spec gut bacteria and western flower thrips. How is the symbiosis created and maintained? Does the symbiosis enhance thrips pest status and/or adaptability to new (resistant) host plants?

OUTLINE OF THIS THESIS

In 1993, the technology foundation STW, part of the Dutch science foundation (NWO), launched a large multidimensional project to study adaptability in thrips. Resistant cultivars of cucumber and sweet pepper had been found in another, earlier study. The central issue of this project was: is it worthwhile to start using these cultivars on a commercial scale or will thrips adapt quickly to the new cultivars, making all investments futile? The project focussed on three aspects of adaptability. De Kogel (1997) studied life history of various thrips populations on different resistant and susceptible cucumber cultivars. Van de Wetering (1999) studied the variation in virus transmission of different thrips populations on various cultivars. And De Vries, in the present thesis, studied the effect of symbiosis with gut bacteria in different thrips populations and on different host plants. Cucumber and chrysanthemum are the host plants that have been used in the present study.

The first step was to study the presence of bacteria in different life stages and generations of thrips. Then, we looked at the influence of gut bacteria on the fitness of thrips, the way

thrips acquire gut bacteria, and the variation of gut bacteria within and between thrips populations. Once that has been elucidated, we are able to look at the possible effect of gut bacteria on adaptability of thrips to resistant cultivars. The presence of bacteria in the hindgut of thrips was shown before with electronmicroscopical studies (Ullman *et al.*, 1989) and bacteriological studies (De Vries *et al.*, 1995). Gut bacteria were present in high numbers in all thrips individuals. They were identified as Enterobacteriaceae, possibly an *Erwinia* species, on the basis of API20E biochemical tests (Chapter 2). The first larval instar thrips have only a few bacteria, but the number of bacteria rapidly increases and reaches a peak of about 10⁵ per thrips in the second instar. Pupal thrips have again fewer bacteria but the adults regain bacteria to the same number as second instar larvae (Chapter 3). The bacteria are passed on to the next generation of thrips via the plant being the food source, presumably because other thrips deposit bacteria on the leaves with their saliva or faeces (Chapter 3).

Thrips larvae without bacteria needed a longer time to develop on leaves of cucumber or bean than thrips larvae with bacteria. Adult female thrips laid fewer eggs on these leaves when there are no bacteria present. Interestingly, this effect was reversed when pollen was added to the diet of thrips, because then the oviposition was higher and the development to adults faster in thrips without gut bacteria (CHAPTER 4). The thrips had to pick up bacteria from the leaves, and hence it would stimulate bacterial uptake if thrips would preferably feed from sites on the leaves where other thrips have been feeding. Indeed, thrips preferred feeding on sites where other thrips had been feeding before, to feeding on clean sites (CHAPTER 5).

The variation of bacteria among different thrips was studied for a number of populations. We used cultures of different populations and isofemale lines of thrips from these different populations. However, the variation in gut bacteria between thrips populations was as large as the variation within one population between thrips individuals, so no host plant effect was found (Chapter 6). The presence of bacteria in another thrips species than *F. occidentalis*, namely the onion thrips *T. tabaci*, was also studied (Chapter 7). Onion thrips had gut bacteria just as western flower thrips. These gut bacteria were also identified as *Pantoea agglomerans*, but belonged to another clade (16S rDNA data). Finally, we also found that gut bacteria have no effect on the transmission of TSWV virus by thrips (Chapter 8). It was decided not to study possible effects of gut bacteria on adaptability of thrips to different resistant cultivars, because there did not seem to be any host plant influence on the composition of the gut flora.

These different aspects of thrips and *Erwinia* spec symbiosis are descibed in detail in the following seven chapters. In Chapter 9 we will come to conclusions and make suggestions for further research, including the possibilities for using this as a model for symbiosis and for developing thrips control methods using the gut bacteria.

Acknowledgements

The author wishes to thank the following people for their contribution to the introduction. Hans Breeuwer, Andrew Weeks, Paul van Rijn, Maurice Sabelis, and Steph Menken are thanked for comments on earlier versions of the text. Willem Jan de Kogel, Fennet van de Wetering, and Gerrit Jacobs contributed partly to the information and discussion used in this text. The author was supported with a grant from the technology foundation (STW) of the Netherlands Organisation for Scientific Research (NWO).

ANNEX 1 - WESTERN FLOWER THRIPS

Thrips was the name Linnaeus gave to four species of small, slender insects, hiding themselves in leaves and flowers (1758). This genus was soon promoted to be an order, and the name Thysanoptera was introduced by Halliday in 1836. 'Thysanoptera' describes the appearance of the insects' wings, in Greek, 'thysanos' means brush and 'ptera' means wings. The insects have brush-type wings that enable them to travel considerable distances by floating. Thrips has always been used as the name for this group of insects, as well as for a single specimen. There are more than 5000 thrips species described at present, but 8000 is probably a more accurate estimate of the number of extant species (Gaston and Mound, 1983). The order is subdivided into two suborders, Tubilifera and Terebrantia. The difference between these suborders was confirmed with molecular phylogenetic analysis of 18S rDNA (Crespi *et al.*, 1996).

Thrips taxonomy

There are eight different families within the order Thysanoptera (Mound *et al.*, 1980). The families Phlaeothripidae and Thripidae are feeding on crops, and are by far the most speciose (Table 1.2). Two genera within the family Thripidae are the largest within the whole order and contain the most important crop pests. For that reason, thrips from these two genera, *Thrips* and *Frankliniella*, have been most intensively studied (Mound, 1997). Two species, *Frankliniella occidentalis* and *Thrips tabaci* are discussed in this thesis.

The common name of the thrips species that plays a major role in this thesis, western flower thrips (*F. occidentalis*), reflects the traditional division between leaf thrips and flower thrips. This division has lost its significance long ago. Many thrips species are able to survive and proliferate on both leaves and flowers. In fact, western flower thrips is very polyphagous and will feed on many food sources, such as leaf tissue, flower tissue, pollen, and nectar. Fungi can be an additional food source for thrips species. Western flower thrips is also capable of predating on other small arthropods, for instance mite eggs (Pallini, 1999; Pickett *et al.*, 1988; Trichilo and Leigh, 1986), and even cannibalism has been shown (Kirk, 1997a).

Western flower thrips

Western flower thrips adults are between 1.2 and 1.9 mm in length. Males are usually smaller than females and the female abdomen is larger. The colour of this thrips species is variable, but mostly brown. Black variants have been reported (Martin and Workman, 1994), but others assume that this is another *Frankliniella* species (G. Vierbergen, pers. comm.). Identification of *Frankliniella* at the species level is laborious. Misidentification often occurs, which leads to wrong conclusions on the pest status of a particular species (Mound and Teulon, 1995). Surface structures and the number and position of hairs (setae) on head and thorax are characteristics used for identification of western flower thrips. The thrips usually hide inside leaf crevices or inside the flowers of their food plants. The presence of thrips is nevertheless observed quickly because thrips feeding on plant material results in visible damage, in the form of large, silver-coloured spots. Sudden changes in air pressure (for example the arrival of thunder in the field) can lead to a change in thrips behaviour. Then

TABLE 1.2 – Thysanoptera family-group names and numbers of species. From Mound (1997).

Suborders	Families	Subfamilies	No. species
Terebrantia	Uzelothripidae	-	1
	Merothripidae	-	15
	Aelothripidae	Aeolothripinae	210
		Melanthripinae	50
	Adiheterothripidae	-	5
	Fauriellidae	-	4
	Heterothripidae	-	70
	Thripidae	Thripinae	1400*
		Panchaetothripinae	120*
		Dendrothripinae	70*
		Sericothripinae	120*
Tubulifera	Phlaeothripidae	Phlaeothripinae	2500*
		Idolothripinae	600

^{*} indicates presence of pest species.

the thrips are more visible and walk on the outside parts of leaves and flowers. For that reason, thrips have received the nickname 'thunderbugs' in Dutch 'onweersbeestjes'.

Distribution and migration of thrips

Frankliniella occidentalis was given the English name western flower thrips because it originated from the western part of North America. In a study published in 1956, Bryan and Smith reported the presence of this thrips type all over the Western part of the USA, from maritime to alpine regions. According to their study this thrips species is extremely variable in colour and other morphological characteristics and has a very broad range of host plants belonging to practically every important plant family. The dispersal of this thrips species to other parts of the USA and the rest of the world has taken place in a very short period of time, presumably between 1975 and 1995. Apart from solid recordings of western flower thrips in New Zealand since 1920 (Martin and Workman, 1994), the species has never been reported anywhere outside the western part of North America before 1970. The 'newer invasion' of western flower thrips in New Zealand in 1992, was found to have changed the behaviour and pest status of the insect species in that country, which leads to the question whether western flower thrips has changed during the last 20 years (Gillings et al., 1995).

Thrips dispersal is linked to developments in modern agriculture. Global introduction of greenhouses for growing crops, the rise of international trade, and the growth of agricultural business, are all factors that have played a role in the rapid dispersal of thrips and its rapid rise on the list of major pest insect species. Important dates in thrips dispersal history are the introduction of the species in Europe, presumably in 1984 (Brødsgaard, 1989; Helyer and Brobyn, 1992; Mantel and De Vrie, 1988; Zur Strassen, 1986). It was introduced in Asia in 1987 and in South Africa in the same year (Giliomee, 1989). Thrips dispersal is obviously linked to agricultural trade (Frey, 1993; Lewis, 1997; Vierbergen, 1995). The presence of

thrips inside small seedlings is difficult to detect because the thrips are small and hide. It is very difficult to correctly identify the species because the transported thrips are often larvae.

The western flower thrips are here to stay. In subtropical countries they have appeared in field crops and in countries with a moderate climate thrips are exchanged between greenhouses in the summer. Due to the dispersal of western flower thrips during the last 25 years, and its increasing pest status, research on this thrips species has shown a considerable increase (Mound, 1997).

Thrips life history

Thrips are hemimetabolic and have five life stages before becoming adults. After the egg phase, two larval stages, a prepupal, and a pupal stage can be distinguished. Thrips eggs are laid inside the tissue of leaves or petals and develop inside tissue until the eggs hatch (Moritz, 1997). Eggs that are kept outside the leaves dry out and never develop into larvae (personal observations). The eggs have a very thin scale and after 1 day the insects' eyes are visible through the egg shell. The division between first and second instar larvae is difficult because these stages look similar. The two larval stages are usually distinguished using the non-feeding moulting phase in between the two stages as a criterium. The feeding and nonfeeding stages can be observed because the abdomen turns green when thrips are feeding on leaves. At the end of the second larval stage, the larvae become more mobile and often leave the leaf on which they have developed before starting pupation. Thrips pupae and prepupae are relatively immobile and often stay in the soil. These life stages can be distinguished because the antennae become shorter in the prepupal stage and bent backwards in the pupal stage. The wings start to develop in the pupal stage. Only in the adult phase it is possible to discriminate between males and females (Kirk, 1997b). The generation time is dependent on temperature, humidity and period of daylight (Van Rijn et al., 1995).

Mating and reproduction

Thrips mating takes place within a few hours after eclosion. The number of mating partners is usually small (Terry, 1997). *Frankliniella occidentalis* males can be aggressive and fight for females (Terry and Dyreson, 1996). The thrips (mostly males) tend to aggregate in flowers (De Kogel *et al.*, 1997b; Matteson and Terry, 1992; Terry and Dyreson, 1996). After mating, adult female thrips start laying eggs about 1.8 days after eclosion from the pupal stage (Van Rijn *et al.*, 1995). The second and third days of oviposition are the most productive days. Oviposition can continue for more than 3 weeks under optimal conditions (Van Rijn *et al.*, 1995).

All thysanopteran species are haplo-diploid, with males being haploid and females being diploid. Parthenogenesis was found in a number of thrips species (Mound, 1997). *Thrips tabaci*, the onion thrips, one of the thrips species used for this thesis, has a very skewed sex ratio: Male onion thrips are rare. Western flower thrips also produces a sex ratio biased towards females. Little is known about the mechanisms underlying these sex ratios. One explanation is the possibility that females influence the sex ratio of their offspring, which was found in other haplo-diploid taxa such as spider mites (Nagelkerke, 1996). Another possible explanation is the parasitic symbiont *Wolbachia*. *Wolbachia* has been found in many arthropod species, including thrips (Moritz, 1997; Werren *et al.*, 1995). Influence on the sex

ratio of haplo-diploid spider mites by *Wolbachia* was found before (Vala *et al.*, 2003). We searched for *Wolbachia* in both onion thrips and western flower thrips but were unable to detect the presence of this bacterial parasite in our cultures of thrips (data not shown).

Feeding

The thrips penetrate the epidermis of the interveinal leaf tissue with their stylet during feeding. After a series of test probes, thrips make a few short and directed probes that create a large pool of leaking parenchymous cells (Harrewijn *et al.*, 1996). Thrips excrete saliva (Kirk, 1997a), and then the actual feeding starts when the thrips probe the leaf for a longer period of time. This feeding method results in large scars, visible on the leaf with the naked eye. Usually, feeding sites are close together, but when they are feeding on resistant types of cucumber the thrips are more mobile and the feeding sites will be scattered over the whole leaf (Mollema *et al.*, 1995).

Pollen is another suitable food source for *F. occidentalis*. Thrips, like many other polyphagous insect species, have shorter longevity and higher fecundity when feeding on the combination of pollen and leave tissue (Kirk, 1984; Van Rijn *et al.*, 1995). When pollen are the only food source available, oviposition is sustained, but this does lead to higher mortality, as shown in artificial cages with only pollen and water (Murai and Ishii, 1982). The nutritional value of leaf tissue and pollen is different. The nitrogen concentration in pollen is higher than in leaves (Kirk, 1997a; and references therein). The thrips attack pollen in the same way as they feed on leaf or petal tissue; a series of short probes followed by prolonged probing (Kirk, 1997a). Thrips are also acting as pollinators (Endress, 1994; Kirk, 1988).

Feeding behaviour of thrips is dependent on their life stage. Larvae stay on the leaf where they hatched, but adults move around in plants. Adults tend to move to the flowers, presumably to feed on pollen or to find a mate (Pickett *et al.*, 1988). Within a plant, the thrips tend to prefer shoot tips and younger leaves, probably because these contain the highest concentrations of amino acids (De Kogel, 1997; Theunissen and Legutowska, 1991).

Tospovirus transmission

Thrips are an important vector of plant pathogens, such as fungi, bacteria, and viruses, which are carried around from plant to plant on the outside of the thrips body or in the thrips gut. Studies on transmission of pathogens by western flower thrips concern *Fusarium* in corn (Farrar and Davis, 1991) and bacterial wilt in corn (Elliot and Poos, 1940). Western flower thrips and onion thrips are the most important insect species able to transmit viruses of the genus *Tospovirus* via their saliva (Ullman *et al.*, 1997; Wijkamp, 1995). Tosposviruses, in particular tomato spotted wilt virus (TSWV), belong to the world's most important plant viruses in terms of damage to agricultural crops. TSWV has a huge effect on many crop plants, and a successful method for TSWV control has not yet been established.

Tospovirus are transmitted via the saliva of thrips. Young thrips larvae acquire the virus by feeding on infected plant material. The older the larva, the less likely it is that it will get infected (Van de Wetering *et al.*, 1996). Apparently, a mechanism develops within the older larva that prevents infection (Nagata, 1999). Infection takes place when virus particles pass the midgut barrier. The presence of virus inside the insect body can be detected using ELISA tests with polyclonal antiserum against TSWV nucleocapsid protein (De Àvila *et al.*, 1990;

Resende *et al.*, 1991). Within 24 h after ingestion, the virus particles in the midgut epithelium start replicating. They are distributed through the insect body, presumably via ligaments that connect the midgut with the salivary glands (Nagata, 1999). Salivary glands become infected with virus particles 48 h after the ingestion of virus. Once the virus has reached a significant level in the salivary glands, the thrips starts infecting its plant food source with virus. Usually, virus transmission can start in the second instar larval stage (Wijkamp and Peters, 1993). The fact that TSWV is able to multiply inside the insect vector without causing any damage to the thrips is an important factor in TSWV virulence (Ullman *et al.*, 1997). Gut bacteria pass the midgut of first instar larvae of thrips in order to establish a symbiosis in the hindgut. The possible interference or non-interference of TSWV particles and gut bacteria is studied in this thesis. By comparing bacteria-free and infected thrips, we have not found any evidence for influence of gut bacteria on tospovirus acquisition.

Control of thrips

Frankliniella occidentalis and T. tabaci are among the most serious insect pests in greenhouse crops. Frankliniella occidentalis, which has developed into a worldwide pest in only 10 years time, has been studied intensively to find effective control methods. Although biological control of thrips is possible, chemical control still prevails in practice (Lewis, 1997). Most effective in killing western flower thrips are organophosphates and malathion (Helyer and Brobyn, 1992). The use of these pesticides in horticulture and greenhouse agriculture has increased dramatically since thrips has given rise to pest outbreaks. However, large differences in thrips susceptibility to insecticides exist (McDonald, 1995). The frequent treatment against F. occidentalis with pesticides such as carbamates, organophosphates, and pyrethroids, has led to diminishing efficacy and resistant thrips populations (Brødsgaard, 1994; Immaraju et al., 1992; Robb, 1989). The mechanism behind resistance in western flower thrips was studied in a few cases (Liu et al., 1992, Zhao et al., 1994).

Biological control of thrips is successfully applied in greenhouses on certain crops. There are a large number of different predators of *F. occidentalis* and *T. tabaci* (Sabelis and Van Rijn, 1997; and references herein). Nowadays, biological control of thrips is common practice in greenhouse crops such as cucumber (Jacobsen, 1997), but the use of pesticides to combat thrips is still widespread in many field crops and in horticulture (Lewis, 1997). New ways of biological control, such as entomopathogenic fungi and nematodes, are currently studied. We have speculated that control of thrips could also be done by targeting their gut symbionts. The efficacy of this alternative method will be discussed in the general discussion of this thesis.

Host plant resistance

Variation in plant resistance for thrips was found in a number of different important crops. For example, damage caused by thrips is highly variable among cotton varieties (Leigh, 1995). The variation in *T. tabaci* damage in cabbage and onion was the incentive for breeding programmes (Coudriet *et al.*, 1979; Sutton *et al.*, 1983). Cultivars of cucumber, resistant to *F. occidentalis*, were described earlier (Mollema *et al.*, 1995). Large variation in thrips' effects on chrysanthemum cultivars was also found (De Jager, 1995), and resistance was found in rose (Gaum *et al.*, 1994), tomato (Kumar *et al.*, 1995) and pepper (Fery and Schalke, 1991).

ANNEX 2 - ERWINIA BACTERIA

Bacterial taxonomy is not an easy subject (Roselló-Mora and Amman, 2001). Bacteria have relatively few phenotypic characteristics and phenotypic plasticity is common. The biological species concept, based on interbreeding, is obviously not applicable to this group of asexually reproducing organisms that are able to exchange genes between completely unrelated specimens using conjugation mechanisms. No wonder that bacterial taxonomy is a tricky business. This also applies to assigning species names to bacterial strains belonging to the family of Enterobacteriaceae, which contains many bacteria that cause disease in man (*Yersinia pestis*, *Salmonella typhimurium*) and its food plants (the plant pathogens are within the Erwiniae division). Enterobacteriaceae is a large bacterial family within the group of gamma Proteobacteria. They are gram-negative, non-spore forming, and motile rods, with peritrichous flagellae, which are mostly heterotrophic, opportunistic, and are able to survive in aerobic and anaerobic circumstances.

The bacterial strains studied in detail in this thesis have a long history. They have changed names several times, and their phylogenetic position remains ambiguous. We have therefore decided to use 'Erwinia spec.' to describe these bacteria throughout this thesis. In this part of the introduction, I will give some background information about its taxonomy. Beijerinck (1888) was probably the first to describe isolated batches of bacteria that are closely related to the gut symbiotic bacteria of western flower thrips studied in this thesis. He described rod shaped, motile bacteria with colonies of a slightly brownish colour, which he had isolated from red clover (Beijerinck, 1888; Ewing and Fife, 1972). Because of the shape of the colonies, perfectly round just like *Proteus agglomerans*, he decided to use the suffix 'agglomerans', combined with Bacillus as genus name to Bacillus agglomerans. Later, in the beginning of the 20th century, Düggeli (1904) isolated similar bacteria from plants and called them Bacterium herbicola aureum, referring to their place of origin (plants/grasses) and to the yellow colour of the colonies. Although Beijerinck and Rant (1905) were quick to point out that these two bacterial types were probably similar, the herbicola/agglomerans divide has existed since these two publications (Ewing and Fife, 1972; and references therein).

The use of the genus names Erwinia and Enterobacter has a similar dichotomous history, and is caused by the different worlds of plant pathologists and clinical microbiologists. Wilson and others produced the genus name Erwinia in 1920 during the heroic attempt of the American Society of Microbiology to categorise and rename all existing bacterial strains into species. Erwin F. Smith was a distinguished American phytobacteriologist (Hauben et al., 1998). The Erwinia genus of Enterobacteriaceae would refer to all phytopathogenic species in the family. Like all other Enterobacteriaceae they are gram-negative motile rods, but their optimal growth temperature is often lower (Bergey's manual, 1974). Soon, researchers started to realise that there were in fact many subgroups among these phytopathogenic bacteria. Dye (1964) describes four subgroups, which have been used by other researchers as well: the 'herbicola' group, the 'carotovera' group, the 'amylovora' group and a rest group. The 'carotovera' group had been named Pectobacterium already in 1945 (Waldee), because of its pectolytic capacity, but this name is only recently become used on a regular basis. Within the 'amylovora' and rest group, probably two genera can be distinguished: Erwinia and Brenneria, based on 16S rDNA data (Hauben et al., 1998).

The most difficult group of the Erwiniae has always been the herbicola/agglomerans group. The resemblance between *Erwinia herbicola* (name introduced by Dye [1964]), and related species, on the one hand, and other bacterial species such as *Citrobacter* and *Klebsiella* species on the other hand, was recognised throughout the twentieth century (Ewing and Fife, 1972; and references therein). The coexistence of different names can be explained by the fact that there was hardly any contact between plant pathologists and clinical microbiologists. *Erwinia herbicola* is the name used by phytopathologists but clinical microbiologists use *Enterobacter*. Although the phytopathological status of *Erwinia herbicola* remains uncertain, this bacterium is known to benefit from lesions and disease spots in plants.

The 'agglomerans' bacterium has been found in organic tissue and the gut system of both vertebrates and invertebrates. A small outbreak of patients infected with agglomerans type of bacteria in the sixties of the last century led to increased attention to this species and the creation of the species name Enterobacter agglomerans (Hauben et al., 1998). Enterobacter is a genus name that was established long after its first species were found. Its type species, Enterobacter cloaca, was first isolated in 1890 and it was immediately recognised that this was not one of the Aerobacter species (Beijerinck) although it closely resembled them (Bergey's manual, 1974). The species names Cloaca cloacae and Bacterium cloacae were used. Uncertainty about the taxonomic position of this species and of related species such as 'aerogenes' existed since than. In 1960, the genus name Enterobacter was introduced (Hormaeche and Edwards). Because of the resemblance in biochemical and morphological characteristics, Ewing and Fife (1972) decided to rename the existing agglomerans bacterium to Enterobacter agglomerans, a name which has been used for these bacteria ever since. They decided that, although in doubt about the real position of agglomerans, introducing a new genus name would not be necessary taking into account the huge similarities with other Enterobacter species.

Obviously this kind of reasoning could also work out exactly to its opposite. The *E. herbicola/E. agglomerans* problem and the overwhelming evidence from DNA data (hybridisation, sequencing) clearly point to the conclusion that *agglomerans* and *herbicola* are no *Enterobacter*. They are most probably also no *Erwinia*. In 1989, the appropriate decision was taken to create a new genus, *Pantoea*, and to rename the species *Pantoea agglomerans* (Gavini *et al.*, 1989). *Pantoea* is derived from Greek (pantoios) and means from many places, indicating the widespread, numerous places where the bacterium has been found. *Agglomerans* was chosen as a species name, and not *herbicola*, because it was the first name used for these bacteria, by Beijerinck (1888). This bacterial species is related to a few other *Pantoea* species (Hauben *et al.*, 1998). The future of the genus name *Enterobacter* is uncertain. DNA studies show that the remaining species in this genus are scattered among the Enterobacteriaceae and probably belong to different genera (Hauben *et al.*, 1998).

Many strains of bacteria have been named *E. agglomerans* during the last 30 years. This was due to the fact that the species is probably highly proliferated, but also to the fact that standard biochemical identification methods such as the API test often lead to this species. In fact, API and related tests resulting in *E. agglomerans* are dubious (G. Mergaert, pers. comm.). Mergaert and his group have done many studies on different strains, which were biochemically typified as *E. agglomerans* (Lind and Uring, 1986; Mergaert *et al.*, 1984; and references therein), but concluded that a large variation exists between these strains and that only part of them were really *E. agglomerans*. This is particularly relevant to insect – sym-

Chapter 1

biont interactions, because many researchers have identified 'symbionts' as *E. agglomerans* (Table 1.1). Meanwhile, the new name *P. agglomerans* still has to get accustomed for the bacterial species that has worn so many names already.