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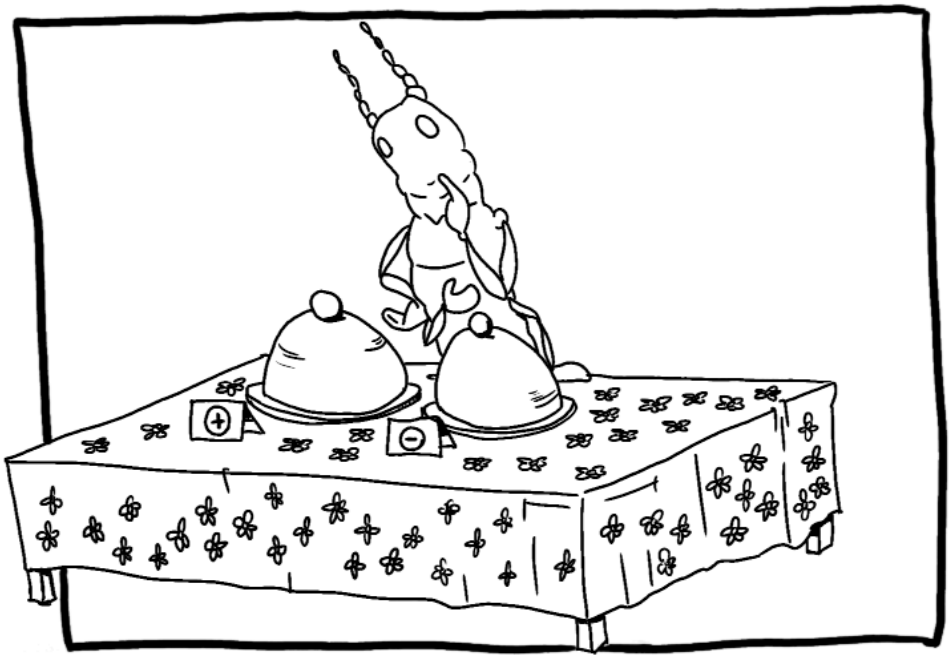
Western flower thrips preference for thrips-damaged leaves over fresh leaves enables uptake of symbiotic gut bacteria

E. J. de Vries, R. A. Vos, G. Jacobs & J. A. J. Breeuwer



ABSTRACT – To understand the evolution of insect gut symbionts it is important to determine how they are passed on to the next generation. We studied this process in *Erwinia* species bacteria that inhabit the gut of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). This is a polyphagous herbivore and a world-wide pest in agricultural crops. With bacteria in the gut, the thrips larval development time can be shorter and its oviposition rate higher, compared to bacteria-free thrips. Bacteria are not directly transmitted from mother to offspring, but larvae acquire bacteria from the leaves right after they hatch. These gut bacteria are present on the leaves on feeding sites used by other thrips before the larvae arrive, probably because these other thrips have deposited bacteria via faeces or regurgitation. In this study we asked ourselves the question whether the transmission route of symbiotic bacteria influences the thrips feeding behaviour, and determined the feeding preference and oviposition preference of thrips, by giving them a choice between leaves with and leaves without prior grazing by other western flower thrips. This was studied for thrips with and thrips without gut bacteria. Young larvae prefer feeding from leaves that where grazed before by other thrips and females prefer to oviposit on these grazed leaves. The result is rather surprising because from earlier studies it is known that thrips larval fitness is lower on thrips-damaged plants than on clean plants. This behaviour does, however, promote the establishment of gut bacteria in the thrips. We speculate that the factors determining the preference for thrips-damaged leaves are due to the physical leaf damage or to odours that are produced by the plant, the bacteria or both.

KEY WORDS – Oviposition preference, feeding preference, host plant selection, symbiosis, mutualism, *Frankliniella occidentalis*, *Erwinia* species

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Gut bacteria in insects are usually commensals, but some of them are mutualists or parasites (Bignell, 1984; Campbell, 1990). Mutualistic gut bacteria may provide essential food components to the host, or enhance its internal defence against toxic compounds or parasites. Examples of the first are the gut symbionts of cockroaches and termites (Breznak, 1982; Cruden and Markovetz, 1984), and of the second are symbionts that eliminate pathogenic fungi from the gut of the desert locust (Dillon and Charnley, 2000). Earlier, we have described the bacterial species in the gut of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), which is capable of enhancing the population growth rate of its host (De Vries *et al.*, 2001a,b, 2004).

Hosts acquire symbiotic gut bacteria in various ways. In cockroaches, young larvae eat the eggshell or faeces that are infected with bacteria (Buchner, 1965; Koch, 1967). When the eggshell is infected with gut symbionts during oviposition, the bacteria are passed on directly from mother to offspring. In most cases, the bacteria are picked up when insects feed on plants or animals, contaminated with bacteria, such as the bacterial uptake by haematophagous insects (Demaio *et al.*, 1996; Hill *et al.*, 1976). Bacterial numbers in these bloodsucking insects increase right after a blood meal. For locusts and crickets, an equally strong coupling between feeding and increase in gut bacteria was observed (Dillon and Charnley, 1996; Kaufman *et al.*, 1989). For symbiotic bacteria, uptake and transmission of bacteria are linked because both use the food source as medium. After a visit of the insects, the plant leaf surface has bacterial species that are identical to the insect gut bacteria (Hunt and Charnley, 1981; McCollem *et al.*, 1992; Thibout *et al.*, 1995).

The question arises whether the uptake of bacteria from the surface of the food source is an accidental process, or whether the insect hosts are able to select the places to acquire the right bacteria. To determine if insects can actively select sites with potential symbiotic gut bacteria, we studied the preference of western flower thrips for plant leaves with and without gut bacteria. Western flower thrips is an important pest insect in agricultural crops all over the world. They feed on leaves, flowers, and pollen, of many plant species in different taxa (Bryan and Smith, 1956). Thrips harbour bacteria in the hindgut and Malpighian tubules (De Vries *et al.*, 1995; Ullman *et al.*, 1989), which belong to the Enterobacteriaceae on basis of 16S rDNA sequence data (De Vries *et al.*, 2001a). The insect gut is an open system suitable for different kinds of organisms, indeed in many cases a variety of bacterial species is found (Campbell, 1990). However, in the thrips gut one type of bacteria becomes dominant (De Vries *et al.*, 2001a). This type of gut bacterium is a near *Erwinia* species, and is further referred to as strain TAC (Thrips Amsterdam Chrysanthemum). Thrips with gut bacteria have a shorter larval development time and a higher egg production than thrips without gut bacteria (De Vries *et al.*, 2004). This benefit to the thrips of the presence of gut bacteria is diet-dependent. On plant leaves, thrips benefit from the presence of bacteria in their gut system, but they do not on leaves with pollen.

Freshly hatched thrips are free of gut bacteria. The thrips acquire these bacteria during feeding on leaves (De Vries *et al.*, 2001b). Thrips feeding causes small scars on leaves (Harrewijn *et al.*, 1996). Probably, thrips also release gut bacteria on leaves, *e.g.*, via the faeces. The small feeding scars will present a better habitat for heterotrophic bacteria than undamaged plants. These factors would explain the rapid uptake of similar right type of gut bacteria by newly hatched larvae that are grazing from sites where other thrips have been feeding before (De Vries *et al.*, 2001b). The prevalence of bacteria in thrips is the highest in second instar larvae (100%; de Vries *et al.*, 2001b). Possibly, larvae start eating at sites

where other thrips are present. But what would happen if the thrips were offered a choice between a fresh leaf and a 'used', but vacated, leaf? Would they select the leaf, earlier used by other thrips? In other words, do cues exist, other than the actual presence of conspecifics, which attract thrips to sites with potential gut bacteria? In this article we test the hypothesis that thrips prefer feeding from sites where other thrips have been eating, to fresh leaves. This hypothesis is tested by offering young larvae and young adult females the choice between a fresh leaf and a leaf that has been fed upon by other thrips before the test. We decided to use two different populations of thrips in this study, one from cucumber plants and one from chrysanthemum plants. We made sure that the two populations were tested on different plants throughout the experiment, in order to be able to draw some conclusions on host plant effects in preference as well.

MATERIALS AND METHODS

Thrips populations

The thrips used in this study were obtained from two different thrips populations. One population was started in 1994 with a batch of thrips kindly provided by the Centre for Plant Breeding and Reproduction Research (CPRO-DLO) in the Netherlands, and maintained on chrysanthemum plants. The second population was started on cucumber plants in 1995 with thrips collected from greenhouses with the same crop in the Dutch Westland area. Thrips were reared on small (max. 40 cm) intact plants in a climate box (25 °C, 60% r.h., and an L16:D8 photoperiod). These rearing conditions were used throughout this study for all experiments. The chrysanthemum plants (*Dendranthema grandiflora*) were obtained from local garden centres, whereas the cucumber plants (*Cucumis sativa*) were cultured in our own insect-free climate rooms. The populations were yearly checked for contamination with other thrips species (G. Vierbergen from the Plant Protection Service of The Netherlands, Wageningen). No contamination with other thrips species than *F. occidentalis* was detected.

Host plants

The experiments were done with cucumber leaves and bean leaves (*Phaseolus vulgaris*). These plants are known to support thrips growth very well, contrary to chrysanthemum leaves, on which thrips grow poorly (De Jager *et al*., 1995). The leaves were taken from plants that were grown in separate, insect-free compartments of the university greenhouse. In most experiments we used pollen as additional food. This pollen was obtained from Koppert Inc. (Berkel en Rodenrijs, The Netherlands), and was a mixture of pollen from different plant species, collected by bees.

Synchronised thrips rearing

The choice experiments were done with batches of thrips from roughly the same age. These batches were made by taking adult females from the thrips culture and allowing them to oviposit for 24 h on leaves. After this, these leaves were placed in large Petri dishes (200 mm

diameter) on an agar layer. The dishes were made insect-proof by sealing them with parafilm. The larvae emerged after ca. 3 days and developed on the same leaf. Pollen was added as additional food. The new thrips were transferred to fresh leaves at the end of the second larval stage, where they pupated. After moulting, adult female thrips were used in our experiments. The batches from the chrysanthemum and the cucumber thrips population were both reared on bean plants to create an equal starting positions.

Rearing thrips from isolated eggs

Rearing thrips from isolated eggs results in bacteria free thrips (De Vries *et al.*, 2004). Normally, eggs of thrips are inserted inside the leaf tissue and they are hard to detect. As soon as the larvae hatch from the eggs, they crawl on the leaf surface and start feeding. Isolated eggs can only be collected when adult thrips oviposit in Murai cages (Murai and Ishii, 1982), between the two sheets of parafilm, which contain a water layer. In these cages, the thrips feed on pollen. After removing the water, eggs were taken from the parafilm and incubated on wet filter paper for two more days. Thereafter the eggs were transferred to fresh leaves on an agar layer in a large Petri dish (200 mm diameter) and developed normally into larvae. These thrips are free of gut bacteria (aposymbionts).

Checking the presence of gut bacteria

The presence of gut bacteria was assessed in thrips used in our experiments, after the experiment had taken place. The presence of symbiotic bacteria was determined by incubating thrips homogenate on Luria Bertoni agar plates. This medium is reliable in supporting growth of *Erwinia* species TAC bacteria (De Vries *et al.*, 2001a). Thrips were individually homogenised after external sterilisation using a procedure described elsewhere (De Vries *et al.*, 2001a). We incubated bacteria for 24 h at 25 °C, after which number and type of colonies were scored. Plates were considered positive if bacteria present were morphologically similar to thrips gut bacteria, type strain *Erwinia* species TAC. Following De Vries *et al.* (2001a) these are small, white, opaque, and round colonies with small motile rods. Plates containing less than 30 colonies were considered negative. Such a low number may have been caused by accidental contamination during the isolation procedure. The number of bacteria per thrips typically ranges from 10^3 to 10^5 (De Vries *et al.*, 2001b).

Feeding preference of larvae

To determine whether thrips larvae prefer feeding sites where other thrips grazed before, a choice experiment was carried out. We used bacteria-free, isolated thrips eggs from the chrysanthemum thrips population. The eggs were placed individually on small stripes of wet filter paper (about 3 mm wide and 10 mm long), on wet cotton in a large Petri dish of 200 mm diameter. On both sides of this filter paper, one half of a 10-mm-diameter leaf disc was placed. The two halves were placed against the long sides of the filter paper. The thrips larvae could therefore move quite easily from one half to the other half of the leaf disc. The two halves of the same leaf disc had a different pre-treatment. One half had not been attacked by any phytophagous arthropod prior to the experiment. On the other half, second instar thrips larvae had been feeding for 24 h before the experiment, and were removed

thereafter. These thrips larvae were taken from the chrysanthemum thrips population and should all contain gut bacteria, because earlier studies have shown that the prevalence of thrips with bacteria is highest in the second larval phase (De Vries *et al.*, 2001b).

In one Petri dish, several (between 15 and 20) samples were put together. To avoid any bias to odours or light, the treated side of the leaf discs was alternately placed at the left or right-hand side (Figure 5.1). The experiment was incubated under standard conditions (25 °C, 60% r.h., L16:D8). After hatching of the thrips larvae, we scored the feeding preference of each larva by checking which half of the leaf disc it had selected to reside on. This was repeated every four h during a period of 12 h, and after one day (24 h). In each replicate experiment, at least 25 thrips larvae were tested.

Feeding preference of adult females

The feeding preference of adult females was determined in a choice test. Females of approximately the same age were used. We offered each individual female a choice between two 10-mm-diameter leaf discs in a 90-mm-diameter Petri dish, on agar. The leaf discs were placed close together but did not touch each other. Again, leaf discs were placed on alternating positions. The female was placed in between the two leaf discs and the dish was incubated at standard conditions. The presence of the females on any of the two leaf discs was monitored during one day for 12 h, at intervals of two h. Thrips were subjected to several choice tests: between cucumber and bean leaf discs, between leaf discs with and without pollen, and between leaf discs which were grazed by other thrips prior to the experiment, and non-grazed leaf discs. These tests were done to determine whether a preference for grazed leaf discs over fresh leaf discs was influenced by other preferences. Each experiment replicate contained at least 20 females and was repeated once. We tested females with and females without gut bacteria.

Oviposition preference

After testing the feeding preference of adult females, the same leaf discs were used to determine oviposition preference. The adult females were removed from the Petri dish and the leaf discs were transferred to individual wells of a macrowell plate, on agar. The wells were

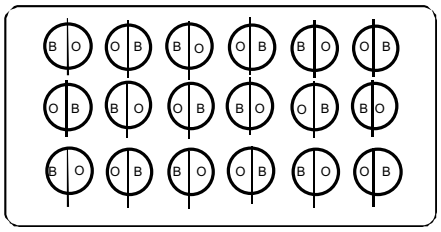

FIGURE 5.1 – Experimental set up of a choice test for western flower thrips larvae. Thrips were offered the choice between grazed leaves and clean leaves. Both leaf parts were semicircular and taken from the same ten mm diameter bean leaf disc. B = grazed by conspecific thrips larvae during 24 h before the experiment started; O = control group; clean leaf part without grazing. The line separating the two semicircular leaf discs was a 3 mm wide, 10 mm long piece of black filter paper, on which thrips eggs were put at the start of the experiment.

sealed with parafilm and the plates were incubated at standard conditions. After four days, all larvae present on each leaf disc were scored. All eggs should have hatched by that time (Van Rijn *et al.*, 1995). Oviposition preference was expressed as the number of larvae produced by all females on one type of leaf disc, vs. the number of larvae produced by the same set of females on the other type of leaf disc. We tested females with and females without bacteria.

Statistical analysis

Under the null hypothesis there is no preference, which implies a 50/50 ratio of feeding of larvae or female adults, or a 50/50 distribution of offspring, on the two types of leaf discs. A G-test was used to test for a significant deviation (Sokal and Rohlf, 1995). Within one replicate, all data for each treatment were pooled. The combined result of various replicates of an experiment was determined using a replicated goodness-of-fit test. This G-statistical test allows for separate testing of homogeneity of the replicates and goodness of fit to the 50/50 distribution of all replicates together (Sokal and Rohlf, 1995). We tested the results of the tests in our experiments; bean versus cucumber leaves, grazed versus non-grazed leaves, and leaves with pollen versus leaves without pollen. In several cases, it was found that the replicate results were heterogeneous, and overall significantly deviating from a 50/50 distribution. In these cases, separate results of G-tests on 50/50 distribution in the individual replicates were presented. It turned out that with this number of replicates in each test, the results could be heterogeneous even when all results showed the same trend, but not all of the individual results were significantly deviating from the 50/50 distribution.

RESULTS

Feeding preference of young thrips larvae

The experiment with clean bean leaves and grazed bean leaves showed a feeding preference of young thrips larvae for grazed leaf discs. In both replicates, significantly more thrips larvae were found on the leaf half that was grazed by other thrips before the experiment started (grazed leaf disc) (Table 5.1). These leaf discs contain *Erwinia* spec bacteria. Preference for non-grazed leaf discs was not observed at any of the eight measure points. The number of larvae taking part in the experiments increased over time because t = 0 was set at the time when the first larvae hatched from the egg, and there is quite a spread in hatching time.

Feeding preference of adult females and oviposition preference

The feeding preference of adult females was difficult to determine. Most of the time, females were not present on any of the two leaf discs but on the Petri dish or on the agar. The number of scores for any of the two leaf discs was not high enough to draw reliable conclusions about food preference. We therefore decided to concentrate on oviposition preference of the females. We determined various aspects of oviposition preference: host plant preference (bean or cucumber); influence of the presence of pollen as additional food source; and the preference for grazed or non-grazed leaf discs. Thrips of both populations

TABLE 5.1 – Preference of young western flower thrips larvae for grazed leaf discs over clean leaf discs. The grazed leaf discs had been used as food source by other thrips larvae during 24 h prior to the start of the experiment. Leaf discs were halves of the same original leaf disc (Figure 5.1). The percentage of thrips choosing for grazed leaves is presented. The other thrips were on clean leaf discs. The experiment was done with eggs that had roughly the same age. The time of experiment was set at zero at moment when the first ten eggs had hatched. Significant deviations from the expected 50/50 distribution are mentioned in the table (χ^2). n = number of larvae scored.

Replicate	Time of experiment (h)	% larvae on grazed leaf disc	χ^2	n
I	4	58	ns	25
	8	64	ns	33
	12	76	*	44
	24	82	**	38
II	4	91	**	11
	8	88	**	15
	12	83	**	18
	24	82	**	22

*, P<0.05; **, P<0.01; ns = non-significant

TABLE 5.2A – Preference of western flower thrips to oviposition on cucumber over bean leaf discs in a choice test. Two thrips populations (with either chrysanthemum or cucumber as original host plant) were tested. The oviposition preference is expressed as the sum of the number of eggs produced by 20 individually tested females. Thrips laid eggs for 24 h.

Thrips	Replicate	Leaf disc	
		Bean	Cucumber
Chrysanthemum population	I	11	36
	II	7	13
Cucumber population	I	8	41
	II	3	40

TABLE 5.2B – Statistical analysis of Table 5.2A with a relative goodness-of-fit test on all data, and on data per population separately. We tested the heterogeneity among replicates (G_H), the overall goodness of fit to a 50/50 distribution (G_P), and the total heterogeneity ($G_T = G_H + G_P$). d.f. = degrees of freedom.

Test	G value	d.f.	χ^2
Overall replicate heterogeneity (G_H)	7.82	3	ns
Overall goodness-of-fit to 50:50 (G_P)	69.36	1	***
Overall total heterogeneity (G_T)	78.04	4	**
Cucumber population G_H	2.0	1	ns
Cucumber population G_P	60.18	1	***
Cucumber population G_T	62.18	2	***
Chrysanthemum population G_H	2.12	1	ns
Chrysanthemum population G_P	14.91	1	***
Chrysanthemum population G_T	17.03	2	***

**, P<0.01; ***, P<0.001; ns = non-significant

TABLE 5.3A – Preference of western flower thrips females to oviposit on leaf discs with pollen rather than on leaf discs with no pollen. The pollen used in this experiment is bee-collected and derived from various host plants. The preference is expressed as the total number of eggs produced by 20 individually tested females. For each replicate, significant deviations from the expected 50/50 distribution are mentioned in the table (χ^2). The females were laying eggs for 24 h. Repl = replicate.

Thrips	Repl	Leaf disc					
		Bean + pollen	Cucumber – pollen	χ^2	Bean – pollen	Cucumber + pollen	χ^2
Chrysanthemum	I	37	12	**	9	43	***
population	II	32	21	ns	14	59	**
Cucumber	I	39	12	**	25	34	ns
population	II	64	23	**	19	61	**

**, P<0.01; ***, P<0.001; ns = non-significant

TABLE 5.3B – Statistical analysis of Table 5.3A with a relative goodness-of-fit test on all data, and on data per population separately. We tested the heterogeneity among replicates (G_H), the overall goodness of fit to a 50/50 distribution (G_P), and the total heterogeneity ($G_T = G_H + G_P$). Both the plant food source and the presence of pollen are tested. d.f. = degrees of freedom.

Test	Replicate heterogeneity			Goodness-of-fit			Total heterogeneity		
	G_H	d.f.	χ^2	G_P	d.f.	χ^2	G_T	d.f.	χ^2
POLLEN : NO POLLEN									
Overall	22.4	7	**	126.0	1	***	148.4	8	***
Cucumber population	19.8	3	***	52.82	1	***	72.62	4	***
Chrysanthemum pop.	8.6	3	*	61.1	1	***	69.7	4	***
CUCUMBER : BEAN									
Overall	51.5	7	***	0.5	1	ns	52.1	8	***
Cucumber population	71.6	3	***	1.1	1	ns	72.7	4	***
Chrysanthemum pop.	61.5	3	***	8.2	1	**	69.7	4	***

*, P<0.05; **, P<0.01; ***, P<0.001; ns = non-significant

have a clear oviposition preference for leaves fed upon by other thrips prior to the experiment, which were the leaves with bacteria (Table 5.4A). Apparently, this preference overrules other preferences such as the preference for cucumber leaves over bean leaves (Tables 5.2A and 5.4A).

First of all, thrips prefer to lay eggs in cucumber leaves rather than in bean leaves (Table 5.2A). This preference is independent of the original host plant of the thrips population under study, because in both populations, the deviation from the 1:1 distribution on cucumber and bean leaves was significant at 0.01 level (Table 5.2B). Secondly, it appeared that most thrips prefer leaf discs with pollen to leaf discs without pollen (Table 5.3A). For both thrips populations studied, this preference was significant at 0.01 level (Table 5.3B). Both thrips populations showed a significant heterogeneity among the replicates when the situation with pollen was compared to no pollen. However, the trend was clear and similar for each replicate. Only one replicate of each population did not show a significant deviation

TABLE 5.4A – Preference of western flower thrips females to oviposit on leaf discs with potential gut bacteria rather than on leaf discs without. Both thrips with (Sym) and without (Apo) gut bacteria were tested, from two populations. One population originated from cucumber, the other population from chrysanthemum. The cucumber and bean leaf discs in this experiment obtained bacteria by exposing them to grazing by a random sample of second instar thrips larvae in the 24 h prior to the test ('grazed'). Oviposition preference is expressed as the sum of the number of eggs produced by 20 individually tested females. Thrips were laying eggs for 24 h. For each replicate, significant deviations from the expected 50/50 distribution are mentioned in the table (χ^2). Bact = thrips with or without gut bacteria. Repl = replicate.

Thrips	Bact	Repl	Leaf disc					
			Bean grazed	Cuc. clean	χ^2	Bean clean	Cuc. grazed	χ^2
Chrysanthemum population	Apo	I	12	8	ns	9	30	**
		II	24	11	ns	25	48	**
		III	19	3	**	12	31	**
		IV	26	19	ns	9	41	**
	Sym	I	8	21	**	7	43	***
		II	10	34	**	12	31	**
		III	21	12	ns	3	23	***
		IV	34	19	*	6	37	**
Cucumber population	Apo	I	24	19	ns	14	38	**
		II	5	26	**	5	17	*
		III	35	24	ns	4	18	**
		IV	31	16	*	8	27	**
	Sym	I	25	12	*	10	37	**
		II	22	17	ns	7	29	**
		III	27	13	*	10	29	**
		IV	21	8	*	31	18	ns

*, P<0.05; **, P<0.01; ***, P<0.001; ns = non-significant

from the expected 1:1 distribution, but a trend towards pollen. This replicate probably caused the overall heterogeneity of the results.

This preference for pollen was overruling the aforementioned preference of cucumber over bean, as was confirmed by the overall relative goodness of fit to the 1:1 distribution in the thrips from both populations in this pollen experiment (Table 5.3B). In all these tests thrips contained gut symbionts, as was confirmed by isolating large amounts of bacteria from individual females after the test (data not shown).

Finally, it was measured whether adult female thrips prefer to oviposit in the leaf discs that have been used as food source by other thrips prior to the experiment ('grazed' leaves), instead of clean, insect-free leaf discs ('non-grazed' leaves). It appeared that in 21 out of 32 replicates there was a significant preference for the grazed leaf discs over the non-grazed leaves (Table 5.4A). The other replicates showed no significant deviation from the 1:1 distribution (but a trend towards the grazed leaf disc – except for one replicate), or a significant preference for the clean leaf disc (3 out of 32 replicates). Clearly, the data did not show

TABLE 5.4B – Statistical analysis of Table 5.4A with a relative goodness-of-fit test on all data, and on data per population separately. We tested the heterogeneity among replicates (G_H), the overall goodness of fit to a 50/50 distribution (G_P), and the total heterogeneity ($G_T = G_H + G_P$). Both the plant food source and the presence of pollen are tested. The aposymbiont thrips and symbiont thrips are treated separately, and combined. d.f. = degrees of freedom.

Test	Replicate heterogeneity			Goodness-of-fit			Total heterogeneity		
	G_H	d.f.	χ^2	G_P	d.f.	χ^2	G_T	d.f.	χ^2
Symbionts									
GRAZED : NON-GRAZED									
Overall	107.8	15	***	59.4	1	***	167.2	16	***
Cucumber population	28.8	7	***	32.2	1	***	61.0	8	***
Chrysanthemum pop.	80.9	7	***	27.3	1	***	108.2	8	***
CUCUMBER : BEAN									
Overall	140.9	15	***	26.3	1	***	167.2	16	***
Cucumber population	60.7	7	***	0.3	1	ns	61.0	8	***
Chrysanthemum pop.	100.9	7	***	7.2	1	**	108.1	8	***
Aposymbionts									
GRAZED : NON-GRAZED									
Overall	56.9	15	***	79.2	1	***	136.1	16	***
Cucumber population	41.8	7	***	20.3	1	***	62.1	8	***
Chrysanthemum pop.	103.3	7	***	57.4	1	***	160.7	8	***
CUCUMBER : BEAN									
Overall	4.2	15	ns	20.5	1	***	24.7	16	ns
Cucumber population	50.8	7	***	12.0	1	***	62.8	8	***
Chrysanthemum pop.	55.1	7	***	3.3	1	ns	58.4	8	***
All									
GRAZED : NON-GRAZED									
Overall	51.28	31	*	132.23	1	***	183.51	32	***
Cucumber population	71.28	15	***	51.28	1	***	122.56	16	***
Chrysanthemum pop.	7.42	15	ns	79.97	1	***	89.39	16	***
CUCUMBER : BEAN									
Overall	126.92	31	***	46.61	1	***	173.53	32	***
Cucumber population	121.6	15	***	0.2	1	ns	121.8	16	***
Chrysanthemum pop.	13.2	15	ns	47.78	1	***	60.98	16	***

*, P<0.05; **, P<0.01; ***, P<0.001; ns = non-significant

homogeneity when all replicates of a similar experiment were combined (Table 5.4B). However, we decided to still use the combined results because of the clear visible trend in the data.

The preference for grazed leaf discs over clean leaf discs was significant at 0.01 level in the test for the overall relative goodness of fit to a 1:1 distribution, and this effect was present in both populations of thrips studied (Table 5.4B). It was found that the preference for grazed leaf discs partly overruled the preference for cucumber leaf discs over bean leaf discs shown earlier (Table 5.2A). In the thrips of the cucumber population (all thrips and sym-

bionts only) and the thrips of the chrysanthemum population (aposymbionts) no significant deviation from the 1:1 distribution over cucumber and bean leaves was found (Table 5.4B). In 13 out of the 16 replicates where bean leaves were grazed and cucumber leaves were not grazed, the bean leaves were preferred. The overall heterogeneity between replicates was therefore higher when bean leaves were tested against cucumber leaves, than in the situation of grazed leaves vs. non-grazed leaves.

In this last set of experiments we used aposymbiotic and symbiotic females. The presence of bacteria was checked for each group, and indeed only the symbiotic females contained bacteria (data not shown). There was little difference in oviposition preference between bacteria-free and infected thrips (Table 5.4A). In two replicates, the symbiotic thrips of the chrysanthemum population preferred non-grazed cucumber leaves over grazed bean leaves, whereas in none of the replicates of aposymbiotic chrysanthemum thrips this was case. However, in the cucumber population this was not found at all. The comparison of data between aposymbiotic and symbiotic thrips showed that both had significant preference for the grazed leaves (significant at 0.01 level, Table 5.4B). The results did not differ from the overall results when aposymbiotic and symbiotic thrips were combined.

Variation between thrips populations

Overall, there was no difference in female oviposition choice between the thrips populations from cucumber or chrysanthemum. For both populations we found that cucumber leaves are preferred over bean leaves, leaves with pollen over leaves without, and leaves grazed by other thrips prior to the experiment over clean leaves (Tables 5.2-4).

DISCUSSION

Thrips acquire directly after hatching gut bacteria, which grow in number during the larval feeding stages (De Vries *et al.*, 2001b). With gut bacteria, thrips have a shorter larval period and a higher egg production on bean leaves than bacteria-free thrips (De Vries *et al.*, 2004). Adult thrips are not confined to leaves, but have the opportunity to move to flowers and can also feed on nectar or pollen. Larvae that hatched on leaves cannot move easily to other food sources and would probably benefit directly from the presence of the right type of gut bacteria normally present in thrips. So, it is more important for larvae to possess gut symbionts than for adults, which is consistent with the high number of bacteria per thrips in second instar larvae. Interestingly, as we described earlier (De Vries *et al.*, 2001b), transmission of bacteria to newly born thrips is horizontal. The route of transmission involves picking up bacteria from the plant surface, where they probably were deposited by other thrips during feeding.

Thus, it would be advantageous for newly hatched thrips larvae to find sites where potential gut symbionts are located if there is no pollen present. Indeed, our results indicate that young thrips larvae are able to select the particular feeding sites that other thrips had visited before (Table 5.1). This is not the first study to report insect feeding behaviour that enhances uptake of internal bacteria. A similar capacity to select sites with bacteria was described before in the fruit flies, which have other Enterobacteriaceae gut bacteria (MacCollem *et al.*, 1992; Martinez *et al.*, 1994). Several other mechanisms to enhance

uptake of gut bacteria have been described. In cockroaches the necessary gut methanogens are passed on via faeces. They produce a volatile compound that probably acts as an aggregation pheromone to attract the insect host (Cruden and Markovetz, 1984). Additionally, bacteria from bark beetles that are deposited in faeces are able to produce verbenol, the bark beetle aggregation pheromone (Brand *et al.*, 1975). In locusts, the beneficial gut bacteria also have to be obtained via the food source and these bacteria also produce aggregation pheromones in the faeces (Dilloen *et al.*, 2002). So both hosts and bacteria use different ways to promote symbiont uptake.

Aggregation behaviour has been described for thrips as well (Kirk, 1985; Terry and Dyreson, 1996). It is however primarily found with adult males and therefore it probably plays a role in mating and lekking behaviour rather than in feeding or oviposition (Terry, 1997, and references therein). We specifically decided not to study the preference of thrips for feeding sites with or without other thrips, but rather studied individual thrips, to exclude effects of aggregation behaviour. However, further studies may want to address this effect as well. Matteson and Terry (1992) describe how flower colour may affect aggregation in thrips. We have found no references regarding the role that odours and/or gut bacteria may play in thrips aggregation. The interesting question in this respect, whether thrips faeces (with or without interference of the gut bacteria) contains aggregation pheromones is not answered yet.

The thrips preference to feeding and ovipositing on sites where other thrips have been feeding, seems to be rather exceptional. Usually, it is found that phytophagous conspecifics avoid each other, and also avoid other competitors, see for example the spider mite *Tetranychus urticae* (Janssen *et al.*, 1998; Pallini *et al.*, 1997). Interestingly, Pallini (1999) found that thrips do not avoid nor prefer feeding sites invaded by spider mites. It is not known whether spider mites have gut bacteria and if so, whether these are the same to the bacteria of thrips. Preliminary investigations on the presence of this type of bacteria in spider mites showed no results (E. de Vries, data not shown).

In their study on thrips fitness on plants with and without infection of plant virus (TSWV), Belliure *et al.* (2005) found that thrips larvae have a better fitness on clean plants than on thrips damaged plants. They compared clean plants with plants of similar age that had been used by thrips in the two weeks prior to the experiment. In other words, it should not be in the interest of thrips to have the preference for damaged plants that we have found in this study. The damaged plants must offer other advantages to the thrips than direct fitness advantages. Other studies show that among the cues used by thrips to select feeding or oviposition sites are colour and odours. Thrips respond to several different odours that can be produced by plants (Terry, 1997; and references therein). The olfactory sensors were described in previous studies (Hunter and Ullman, 1992). Generally, it was found that thrips adults prefer flowers to leaves (De Jager *et al.*, 1993; Kirk, 1997, and references therein; Yudin *et al.*, 1988), which is confirmed by our data on female oviposition preference for leaves with pollen (Table 5.3). Plants infected with virus (TSWV) offer a more suitable environment for thrips larvae, even when these plants are damaged by thrips feeding (Belliure *et al.*, 2005; Maris *et al.*, 2004). Other studies have shown that these pathogens may be able to actively attract insect vectors to the host plants (Eigenbrode *et al.*, 2002).

The mechanism that causes the thrips preference for feeding sites occupied by other thrips is not clear. It could be the physical effect of grazing, or certain odours released as a result of grazing or deposition of bacteria (or both!). Thrips grazing causes crater-like scars

on the leaves, which may offer a better feeding site to any herbivore because it allows easier access to the plant tissue (Harrewijn *et al.*, 1996). Indeed, De Jager *et al.* (1995) found a positive correlation between the number of thrips eggs produced and the leaf feeding damage in chrysanthemum. If attractants or arrestment cues play a role in thrips feeding site selection, we cannot differentiate between compounds originating from bacterial activity, or compounds originating from thrips-damaged host plants. Odours emitted during or after feeding may be produced by the feeding thrips or by the plant itself, after induction by the thrips. In fact the question what came first, attraction to bacteria or attraction to feeding sites, is a chicken-and-egg problem that cannot be answered with this experiment. However, it is hard to circumvent this problem, and design experiments that do answer this question straightforwardly. Simply using aposymbiotic and symbiotic thrips is not enough because eliminating bacteria from the thrips does also influence the thrips itself, as was shown in our previous studies (De Vries *et al.*, 2004). Besides, aposymbiotic and symbiotic thrips do not seem to be behaving differently according to our experiments. Further studies could also include tests on whether thrips preference for grazed/damaged leaves is changed in the presence of pollen.

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