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Vector and virus induce plant responses that benefit a non-vector herbivore

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Abstract

The negative cross-talk between induced plant defences against pathogens and arthropod herbivores is exploited by vectors of plant pathogens: a plant challenged by pathogens reduces investment in defences that would otherwise be elicited by herbivores. This negative cross-talk may also be exploited by non-vector herbivores which elicit similar anti-herbivore defences in the plant. We studied how damage by the thrips *Frankliniella occidentalis* and/or infection with *Tomato spotted wilt virus* (TSWV) affect the performance of a non-vector arthropod: the two-spotted spider mite *Tetranychus urticae*, a parenchym feeder just like *F. occidentalis*. Juvenile survival of spider mites on plants inoculated with TSWV by thrips was higher than on control and on thrips-damaged plants. However, thrips damage did not reduce spider-mite survival as compared to the control, suggesting that the positive effect of TSWV on spider-mite survival is independent of anti-thrips defence. Developmental and oviposition rates were enhanced on plants inoculated with TSWV by thrips and on plants with thrips damage. Therefore, spider mites benefit from TSWV-infection of pepper plants, but also from the response of plants to thrips damage. We suggest that the positive effects of TSWV on this non-vector species cannot be explained exclusively by cross-talk between anti-herbivore and anti-pathogen plant defences.

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Zusammenfassung

Die negative gegenseitige Beeinflussung der induzierten Pflanzenabwehr gegen Pathogene und herbivore Arthropoden wird von Vektoren von Pflanzenpathogenen ausgenutzt: eine Pflanze, die von Pathogenen angegriffen wird, reduziert ihre Investition in Abwehrmechanismen, die ansonsten von den Herbivoren ausgelöst würden. Diese negative gegenseitige Beeinflussung kann ebenso von Herbivoren ausgenutzt werden, die keine Vektoren sind und die eine ähnliche Anti-Herbivoren-Reaktion der Pflanze auslösen. Wir untersuchten, wie eine Beschädigung durch den Trips *Frankliniella occidentalis* und/oder die Infektion mit dem Tomatenbronzefleckenvirus (TSWV) die Performanz eines Nicht-Vektor-Arthropoden beeinflusst, der Spinnenmilbe *Tetranychus urticae*, die genau wie *F. occidentalis* ein Parenchymfresser ist. Die Überlebensrate der juvenilen Spinnmilben war auf den Pflanzen höher, die durch den Trips mit TSWV inokuliert waren, im Unterschied zu den Kontroll- und den durch Tripse beschädigten Pflanzen. Die Beschädigung durch Tripse reduzierte die Überlebensrate der Spinnmilben jedoch im Vergleich zu den

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Kontrollen nicht und lässt vermuten, dass der positive Effekt des TSWV auf die Überlebensrate der Spinnmilben unabhängig von der Anti-Trips-Verteidigung ist. Die Entwicklungsraten und die Eiablageraten waren auf den Pflanzen verbessert, die durch Tripsen mit TSWV inokuliert oder von Tripsen beschädigt waren. Deshalb profitieren die Spinnmilben von einer TSWV-Infektion auf Paprikapflanzen aber auch von der Antwort der Pflanzen auf eine Beschädigung durch Tripsen. Wir vermuten daher, dass der positive Effekt von TSWV auf diese Nicht-Vektor-Art nicht ausschließlich durch die gegenseitige Beeinflussung der Pflanzenabwehr gegen Herbivore und Pathogene erklärt werden kann.

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Keywords: Induced plant defences; *Tetranychus urticae*; *Frankliniella occidentalis*; *Tomato spotted wilt virus*; Cross-talk

Introduction

Plants are targeted by various enemies, such as herbivorous arthropods and plant pathogens. Constitutive defences become too costly when attacks occur infrequently (Baldwin 1998). Therefore, many plants possess inducible defences, switched on upon attack (Karban & Baldwin 1987). Induced responses to herbivory are mediated via several signalling pathways, especially via the jasmonic acid (JA) pathway (Walling 2000). These responses involve changes in the quality of attacked plants, with negative effects on the fitness of the herbivore (direct defence) (Karban & Baldwin 1987), as well as the emission of plant volatiles, which attract natural enemies of the herbivore towards attacked plants (indirect defence) (Sabelis & Van der Baan 1983; Turlings, Tumlinson, & Lewis 1990; Turlings et al. 1995). As a result, herbivore-induced plants often become hosts of inferior quality (Agrawal 1998; Belliure, Janssen, Maris, Peters, & Sabelis 2005; Karban & Baldwin 1987), and are therefore less attractive for conspecific or heterospecific herbivores (Bernasconi, Turlings, Ambrosetti, Bassetti, & Dorn 1998; Pallini, Janssen, & Sabelis 1997). Pathogens also induce defences in plants, mainly through the salicylic acid (SA) pathway (Glazebrook 2005; Pieterse & van Loon 1999).

Negative cross-talk between anti-herbivore and anti-pathogen signalling pathways has been reported for several plant-pathogen systems: up-regulation of the SA pathway results in down-regulation of the JA pathway (Felton et al. 1999; Peña-Cortés, Albrecht, Prat, Weiler, & Willmitzer 1993; Pieterse & Van Loon 2004; Thaler, Fidantsef, Duffey, & Bostock 1999; Thaler, Karban, Ullman, Boege, & Bostock 2002). This negative cross-talk can be exploited by insect vectors of pathogens because induction of the anti-pathogen pathway reduces investment of the plant in anti-herbivore defence. Hence, herbivorous arthropods may profit from vectoring plant pathogens because pathogens decrease plant defence responses against the vector (Bautista, Mau, Cho, & Custer 1995; Belliure et al. 2005; Carter 1939; Maris, Joosten, Goldbach, & Peters 2004; Stumpf & Kennedy 2007). In turn, vector-borne pathogens can

benefit from this cross-talk because pathogen-infected plants with reduced anti-herbivore defences are good host plants for the vectors of the pathogen. This is the case for the thrips *Frankliniella occidentalis* (Pergande), the main vector of *Tomato spotted wilt virus* (TSWV). Thrips induce anti-herbivore defences in pepper plants (*Capsicum annuum* L.) that are detrimental to thrips (Belliure et al. 2005). However, thrips have a higher growth rate and juvenile survival on pepper plants infected with TSWV (Belliure et al. 2005). Hence, virus infection seems to reduce anti-herbivore defences, in agreement with the negative cross-talk between signalling pathways triggering anti-herbivore defences and those triggering anti-pathogen defences. Moreover, the increased juvenile growth rate results in a shorter period of vulnerability of juvenile thrips to predation (Belliure, Janssen, & Sabelis 2008). In line with this increased performance of juvenile thrips, adult thrips aggregate on virus-infected plants (Bautista et al. 1995; Carter 1939; Maris et al. 2004), and their offspring will vector the virus when adult (Ullman et al. 1992). Such mechanisms that reduce induced plant defences against vectors promote the spread of the virus by the vector and may therefore have evolved in vector-borne plant viruses (Belliure et al. 2005).

However, the lack of induced defence against herbivory could be exploited by other herbivorous arthropods that would otherwise induce the same plant defences as the vectoring herbivores, because they could also escape anti-herbivore plant defences by attacking virus-infected plants. Arthropods from different feeding guilds induce distinct changes in plant gene expression. Chewing and cell-content feeding herbivores like thrips activate wound-response pathways, whereas phloem-feeding whiteflies and aphids activate defence-response pathways induced by pathogen attack (Koornneef & Pieterse 2008; Walling 2000). Like *F. occidentalis*, the spider mite *Tetranychus urticae* Koch is a parenchym cell-content feeder. Both species pierce parenchym cells and consume their contents, causing substantial leaf damage. Spider mites induce a defence response detrimental to spider mites (Agrawal, Karban, & Colfer 2000; Gols, Roosjen, Dijkman, & Dicke 2003; Kant, Ament, Sabelis Haring, & Schuurink 2004; Kant,

Sabelis, Haring, & Schuurink 2008; Karban & Carey 1984), and thrips attacking TSWV-free plants induce a defensive plant response detrimental to thrips (Belliure et al. 2005). Both anti-thrips and anti-spider-mite defences involve the activation of the JA signalling pathway in several plant species (Abe et al., 2008; Ament, Kant, Sabelis, Haring, & Schuurink 2004; De Vos et al. 2005; Gols et al. 2003; Kant et al. 2004, 2008). Therefore, mechanisms that interfere with the induced response of plants against thrips could well benefit spider mites. Furthermore, thrips and spider mites often inhabit the same host plants in greenhouses and outdoors.

The aim of this work was to test whether spider mites perform better on host plants that are infected with TSWV. When TSWV triggers mechanisms that interfere with the induced defensive response against thrips (Belliure et al. 2005), this could likewise interfere with the induced defence against spider mites. We did not attempt to identify these underlying mechanisms of interference with plant defence, but concentrated on the effects of this interference on spider mites. To this end, we measured juvenile survival, development and oviposition of *T. urticae* on (a) plants with previous thrips damage, (b) plants inoculated with TSWV by thrips, (c) plants with mechanical damage, (d) plants that were mechanically inoculated with TSWV and (e) undamaged and uninfected control plants.

Materials and methods

Spider mites, thrips, plant material and virus isolate

A culture of spider mites was established on pepper plants, *C. annuum* L. (var. pikante Reuzen) in a climate room under controlled climatic conditions (25 °C, 50–70% RH, 16:8 h L:D). The culture was started with individuals reared on cucumber plants (*Cucumis sativa* L. (var. Ventura)), originally collected from a cucumber commercial greenhouse in Pijnacker, The Netherlands (1994), and refreshed with new individuals every 2 years. Thrips cultures were started from individuals collected at the same time and commercial greenhouse, and were reared on the same cultivar of pepper, in climate boxes at the conditions mentioned above since 2002.

All pepper plants used in the experiments were grown from seeds under similar conditions (25 °C, 50–70% RH, 16:8 h L:D). Clean pepper plants (*i.e.* free of virus and damage) were grown in plastic pots (11 cm high; 10 cm wide) in a climate room. To obtain plants with thrips damage, yet without virus (subsequently referred to as thrips-damaged plants), we incubated clean plants (2 weeks old) in a climate box with uninfected plants and TSWV-free thrips. The response of plants to thrips

damage is induced already 12 h after exposure (De Vos et al. 2005), but in the experiments we used plants exposed to thrips for at least 2 weeks, in order to obtain a higher level of thrips damage.

The *Tomato spotted wilt virus* isolate used was ‘Brazilian BR01’ (DeAvila et al. 1990). To obtain virus-infected plants inoculated by thrips and therefore with thrips damage (henceforth referred to as thrips-inoculated plants), clean plants (2 weeks old) were placed for 2 weeks in a climate box (conditions as above) containing a population of virus-infected thrips from cohorts living on virus-inoculated plants. To obtain virus-infected plants without thrips damage, we inoculated plants mechanically. Mechanically damaged and inoculated treatments were used as control for the effect of damage and of TSWV in the absence of thrips. An inoculum was prepared by triturating 1 g of leaf material from systemically infected leaves from thrips-inoculated pepper plants in 10 ml of inoculation buffer (0.01 M NaPO₄, pH 7.0) (Maris et al. 2004). The two first real leaves of clean plants were mechanically damaged by dusting them with carborundum powder (500 mesh) and subsequently rubbing them with a sponge previously embedded in the inoculum. These plants are referred to as mechanically inoculated. To establish a control treatment for the effect of mechanical damage, plants were treated as above, but with a virus-free buffer (mechanically damaged plants). TSWV can be detected in the top leaves of pepper plants 4 days after inoculation (Soler, Díez, & Nuez 1998). Hence, infection is systemic after 4 days. Infection of plants by the virus was checked by the development of symptoms typical for TSWV: chlorotic rings, chlorotic mosaic patterns, mottling on the leaves, growth reduction and deformation. To our best knowledge, there is no literature about the influence of virus titer in plants on the effect of TSWV on *F. occidentalis* or other arthropods such as *T. urticae*. We did not find either an effect of virus titer in the plant on transmission efficiency of several TSWV isolates by *F. occidentalis* (Belliure et al., unpublished data). Two weeks after inoculation, we made leaf discs of the top leaves, which showed symptoms of infection.

Survival and development of juvenile spider mites

Survival from larva to adult and developmental time of spider mites was measured on leaf discs from pepper plants that were treated in different ways: clean plants, thrips-damaged plants, thrips-inoculated plants, mechanically damaged plants and mechanically inoculated plants. The response of plants to inoculation or damage changes with time in several systems, and this affects the community of herbivores living on them. It is likely that performance of spider mites would change with time after treatment of the plants. To avoid variability due to

different responses in time, we used the plants at the same time after treatment in the different experiments.

Leaf discs ($\varnothing=1.5$ cm) were obtained from undamaged leaves of these plants and were put floating on tap water, in separate plastic cups (volume=20 ml), filled with a piece of wet cotton wool to anchor the leaf disc, thus preventing it from touching the cup wall. Five to ten plants were used to obtain discs for each treatment. To avoid the effect of induced responses due to the cutting of the leaf-disc, all leaf discs from a plant were cut at the same time and the plant was not used a second time. Effects of cutting of the leaf disc were unavoidable, but consistent in all treatments, including clean plants.

Female spider mites were allowed to oviposit on the leaf discs. Females and eggs were removed 24 h later, except for one egg that was left on each leaf disc. Survival and developmental stage of each larva were checked daily under the stereomicroscope until they reached adulthood. Every 5 days, juvenile spider mites were transferred to new leaf discs from plants that had received the same treatment as the plants from which their former disc was cut. The discs were incubated at 25 °C, 50–70% RH and 16:8 h L:D. Each treatment consisted of 38–48 larvae placed individually on a leaf disc.

Juvenile survival (proportion of larvae reaching adulthood) and developmental time (from newly hatched larvae to adulthood) were subjected to Kaplan–Meier survival analysis to identify an overall effect. Functions describing the cumulative proportion of larvae surviving to reach adulthood or the number of days required to develop from larva to adulthood were estimated using the Kaplan–Meier method and subject to survival analysis (Hosmer & Lemeshow 1999). Log-rank tests were used for pairwise planned comparisons (Hosmer & Lemeshow 1999). Sequential Bonferroni corrections were applied when the same treatment was compared more than once (Sokal & Rohlf 1995).

Oviposition

Young adult female spider mites were obtained from cohorts on clean pepper leaves as follows: teleiochrysalids (the last resting stage before reaching adulthood) were assigned to leaf discs from pepper plants that had received one of the treatments as described above. Subsequently, pre-oviposition period and oviposition rate of the females obtained were recorded daily during the first 7 days after moulting to adulthood. Per treatment, 17–20 female spider mites were used. Because not all females laid eggs, comparisons of pre-oviposition period and oviposition rate were performed only with those female spider mites that actually reproduced (see sample size in Results). A Kruskal–Wallis analysis was

used to identify an overall effect in pre-oviposition period, and planned comparisons were performed with the Kruskal–Wallis ANOVA (Siegel & Castellan 1988). Oviposition rates through time were $\ln(x+1)$ transformed to stabilize variance, and a linear mixed effect model with time as a random factor nested in individual mites was used to correct for repeated measures (R Development Core Team 2006; Crawley 2007). Treatments were compared using a posteriori model simplification (Crawley 2007). Models were checked for goodness-of-fit and non-normality of error distributions.

Results

Survival and development of juvenile spider mites

Survival of spider mites from larva to adulthood differed among treatments ($\chi^2=29.2$; d.f.=4; $p<0.00001$; Fig. 1). Survival on thrips-damaged plants was higher, although not significantly different from survival on clean plants (Table 1). This indicates that previous thrips damage on plants was not detrimental for spider mite survival. On thrips-inoculated plants, survival of spider mites was significantly higher than on clean plants and also higher than on thrips-damaged plants (Table 1). Hence, infection of the plant with TSWV had a positive effect on juvenile survival of spider mites.

Mechanical damage did not affect survival of spider mites (Table 1). Although survival on mechanically

Fig. 1. Cumulative proportion of spider mite larvae surviving to adulthood (Kaplan–Meier) on pepper leaf discs under different treatments: clean ($n=43$), thrips-damaged ($n=41$), inoculated with TSWV by thrips ($n=42$), mechanically damaged ($n=39$) and mechanically inoculated with TSWV ($n=37$). Curves accompanied by the same letter were not significantly different (planned comparisons, log-rank test).

Table 1. Effects of plant damage, virus infection with *Tomato spotted wilt virus* (TSWV) and method of inoculation on juvenile survival and developmental time of two-spotted spider mites on pepper.

Effect of	Comparison ^a	Juvenile survival		Developmental time	
		Test statistic ^b	Pvalue ^c	Test statistic ^b	Pvalue ^c
Thrips damage	Clean vs. thrips-damaged	1.94	0.05224	−2.62	0.02700
Thrips-inoculated TSWV	Clean vs. thrips-inoculated	4.34	0.00001	−3.88	0.00040
TSWV infection	Thrips-damaged vs. thrips inoculated	2.68	0.02200	−1.76	0.07796
Mechanical damage	Clean vs. mechanically damaged	−1.15	0.50242	0.74	0.91240
Mechanically inoculated TSWV	Clean vs. mechanically inoculated	0.47	0.64163	1.84	0.12994
TSWV infection	Mechanically damaged vs. mechanically inoculated	1.78	0.15066	0.64	0.52235
Type of damage	Thrips-damaged vs. mechanically damaged	−3.04	0.00700	2.47	0.04000
Type of inoculation	Thrips-inoculated vs. mechanically inoculated	−4.28	0.00060	5.49	0.00001

^aClean = pepper plants with neither thrips damage nor virus infection; thrips damaged = pepper plants with thrips damage, without virus infection; thrips inoculated = pepper plants with both thrips damage and virus infection; mechanically damaged = pepper plants treated as mechanically inoculated but without virus inoculum; mechanically inoculated = pepper plants with mechanical damage and virus infection.

^bShown are the results of planned comparisons using a log-rank test.

^cP-values were corrected for multiple comparisons using sequential Bonferroni corrections. P-values indicating significant differences are given in bold.

inoculated plants was not significantly higher than survival on mechanically damaged plants, it showed the same trend as thrips-inoculated plants vs. thrips-damaged plants (Table 1), indicating a positive effect of TSWV on spider-mite survival. Comparisons of spider-mite survival between thrips-damaged and mechanically damaged plants, and between thrips-inoculated and mechanically inoculated plants showed that previous damage by thrips increased survival of spider mites as compared to previous mechanical damage (Table 1).

The developmental period was longer for female than for male spider mites for all treatments together (females: 12.57 ± 0.34 days; males = 11.86 ± 0.23 days; log-rank test; test statistic = -3.9 ; $p = 0.00009$). Because the sex ratio was similar (fraction of females = 0.53) for all treatments, we decided to pool data of males and females in order to be able to use data from individuals that did not reach adulthood (hence, whose gender was unknown) in the survival analysis to compare developmental time. There was an overall difference in developmental time between treatments ($\chi^2 = 43.1$; d.f. = 4, $p < 0.00001$; Fig. 2). Development of spider mites on clean plants took longer than on thrips-damaged plants and on thrips-inoculated plants, whereas there was no significant difference between thrips-damaged and thrips-inoculated plants (Table 1). This suggests that previous thrips damage had a positive effect on spider mite development, whereas the virus did not affect the developmental rate of spider mites. This is further confirmed by the similar developmental period of spider mites found on mechanically damaged plants and on mechanically inoculated plants (Table 1). The developmental rate of both these treatments did not differ significantly from that on clean plants (Table 1).

Fig. 2. Average developmental time in days (plus standard error of the mean, s.e.m) from larva to adulthood on pepper leaf discs under different treatments: thrips-damaged (number of larvae reaching adulthood, $n = 26$), inoculated with TSWV by thrips ($n = 36$), clean ($n = 18$), mechanically damaged ($n = 8$) and mechanically inoculated with TSWV ($n = 11$). Bars accompanied by the same letter were not significantly different (planned comparisons, log-rank test).

Spider mites had a shorter developmental period on plants that were induced by thrips than on plants that were mechanically damaged (Fig. 2, Table 1), again suggesting a positive effect of previous thrips damage on development.

Oviposition

The pre-oviposition period (number of days from moulting to adulthood until first oviposition) differed significantly among females developing on plants that received different treatments (Kruskal–Wallis ANOVA; d.f. = 4; $\chi^2 = 32.13$; $p = 0.000002$; Fig. 3). The pre-

oviposition period on clean plants was longer than on thrips-damaged plants and on thrips-inoculated plants, whereas it did not differ on thrips-damaged plants vs. thrips-inoculated plants (Table 2). Pre-oviposition period was also not significantly different between mechanically damaged, mechanically inoculated and clean plants. Previous thrips damage reduced the pre-oviposition period of spider mites as compared to previous mechanical damage, whereas inoculation of TSWV by thrips did not reduce it significantly as compared to mechanical inoculation (Table 2).

There was a significant effect of plant treatment on oviposition rate of the spider mites through time (linear mixed effects model: $F_{4,65}=45.0$; $p<0.0001$; Fig. 4). Oviposition rate on thrips-damaged plants was highest, followed by thrips-inoculated plants ($p=0.0238$; Fig. 4).

Fig. 3. Average pre-oviposition period (in days, plus s.e.m.) of spider mite females on pepper leaf discs under different treatments: thrips-damaged ($n=19$), inoculated with TSWV by thrips ($n=17$), clean ($n=7$), mechanically damaged ($n=9$) and mechanically inoculated with TSWV ($n=17$). Bars accompanied by the same letter were not significantly different in planned comparisons (Kruskal–Wallis ANOVA).

Oviposition on mechanically inoculated plants was significantly higher than on clean and mechanically damaged plants together. The latter two treatments did not result in differences in oviposition ($p=0.5617$; Fig. 4). This indicates that the response of plants to thrips damage and to TSWV infection enhanced oviposition rate of spider mites.

Discussion

We found evidence for a positive effect of virus infection of a host plant on a non-vector herbivore; survival of juvenile spider mites was significantly higher on pepper plants that were inoculated with TSWV by thrips than on clean plants or on plants damaged by

Fig. 4. Average oviposition rate (eggs per female spider mite per day $\pm$ s.e.m.) during the first 7 days of oviposition on leaf discs from the differently treated pepper plants. Numbers per replicate are the same as for Fig. 3. Curves accompanied by the same letter were not significantly different in planned comparisons (linear mixed effects model).

Table 2. Effects of plant damage, virus infection with *Tomato spotted wilt virus* (TSWV) and method of inoculation on the pre-oviposition period of two-spotted spider mites.

Effect of	Comparison ^a	Pre-oviposition period	
		Test statistic ^b	Pvalue ^c
Thrips damage	Clean vs. thrips-damaged	27.4812	<0.01
Thrips-inoculated TSWV	Clean vs. thrips-inoculated	25.2815	<0.05
TSWV infection	Thrips-damaged vs. thrips inoculated	2.1997	>0.05
Mechanical damage	Clean vs. mechanically damaged	11.5714	>0.05
Mechanically inoculated TSWV	Clean vs. mechanically inoculated	8.9874	>0.05
TSWV infection	Mechanically damaged vs. mechanically inoculated	20.5588	>0.05
Type of damage	Thrips-damaged vs. mechanically damaged	39.0526	<0.00001
Type of inoculation	Thrips-inoculated vs. mechanically inoculated	16.2941	>0.05

^aSee legend to Table 1 for explanation of treatments.

^bShown are the results of planned comparisons using a Kruskal–Wallis ANOVA.

^cP-values were corrected for multiple comparisons using sequential Bonferroni corrections. P-values indicating significant differences are given in bold.

thrips. Oviposition was also enhanced by TSWV-infection, both on mechanically inoculated and on thrips-inoculated plants. Other life-history parameters, however, were not positively affected by virus infection as compared to previous thrips damage. Because previous thrips damage did not significantly affect spider-mite survival, the positive effect of TSWV on survival is not related to an alleviation of anti-thrips defence in TSWV-infected plants, which is in disagreement with our hypothesis.

Contrary to what we expected, there was a positive effect of previous damage by thrips on developmental rate, pre-oviposition period and oviposition rate of spider mites. This suggests that the primary and secondary metabolic pathways induced by thrips alter the quality of the plant (e.g. in terms of nutritional and/or defensive status), thereby benefiting the spider mites. This positive effect of previous thrips damage on the life history of spider mites is surprising, because it was expected that such damage would induce plant defences that are detrimental not only to thrips, but also to spider mites. It was found earlier that previous thrips damage did have a negative effect on survival and developmental rate of juvenile thrips (Belliure et al. 2005). Moreover, the defensive response of plants induced by thrips involve the induction of the JA signalling pathway (De Vos et al. 2005; Abe et al. 2008), which is also activated by spider-mite feeding (Ament et al. 2004, Kant et al. 2004, 2008), and causes detrimental effects on spider mites (Agrawal et al. 2000; Gols et al. 2003; Kant et al. 2004, 2008; Karban & Carey 1984).

Oviposition of spider mites was highest on thrips-damaged plants, followed by oviposition on thrips-inoculated plants. This contrasts with the described reduction in oviposition of spider mites on cotton plants induced by application of JA (Omer, Granett, Karban, & Villa 2001). Further research is needed to unravel the mechanisms explaining the different effects of previous thrips damage and thrips inoculation on spider mites performance.

The females used in the oviposition experiments came from whole clean pepper plants. However, there were large differences in the pre-oviposition period on the leaf discs from the different experimental plant treatments, suggesting that the pre-oviposition period is mainly affected by the diet during, not before, this period.

The use of leaf discs in our experiments has unavoidable effects on plant responses as compared to whole plants. We tried to minimise these side effects by applying the treatments and cutting the leaf discs in all treatments at the same time. However, it would be interesting to corroborate our results in experiments using whole plants.

Research on plant-pathogenic viruses has mainly focused on interactions between virus and vector and between the virus and the plant. We previously showed that such a virus can affect the interaction between the vector and its natural enemies (Belliure et al. 2008). Here, we show that a plant-pathogenic virus can also affect non-vector herbivores

through changes in plant quality. This stresses the importance of studying the interactions of plants, plant pathogens and vectors within the context of the web of interactions among arthropods and the plants they inhabit.

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