Plants possess numerous traits that shield them from being consumed. Many of these traits are displayed independently from the circumstances. However, herbivory can induce reinforcement of these traits as well as initiate the formation of novel ones.1,2 Such traits pose selection pressure on herbivores to overcome them. Consequently, herbivores have evolved various ways to either avoid, resist or manipulate plant defenses, the latter for example via suppressing inducible defenses.3,4 Most— if not all – herbivores induce defense-associated changes when feeding from a plant, but whether or not these changes increase the plant’s level of resistance is determined by the herbivore’s degree of susceptibility.

Herbivory not only triggers the local activation of defenses; also in distal non-attacked (systemic) tissues defenses can be initiated. Furthermore, induced defenses often do not cease immediately after the attacker has left. Finally, plant defenses differ significantly in magnitude and/or in the timing of induction across plant age, developmental stage and tissue type, as well as across abiotic conditions.1,2,5 Heterogeneity in defensive responses is an important aspect of plant-herbivore interactions, as it may influence herbivore behavior and performance, yet these interactions are not well documented.6

The regulation of plant defenses mainly depends on two phytohormones: jasmonate (JA) and salicylate (SA). Previously, we have surveyed the magnitude of JA- and SA-dependent defenses over time in tomato leaflets infested with herbivorous spider mites. Whereas defenses were strongly induced during infestations with T. urticae,7,8 the T. evansi infestations were characterized by no or relatively low induction.9 Moreover, on leaflets simultaneously infested with mites from both species, defense levels were intermediate as a consequence of suppression by T. evansi. This defense suppression was demonstrated to occur downstream of hormone accumulation and independently of JA-SA crosstalk.8

Subsequently, we assessed the magnitude of spider mite-induced defenses directly at the feeding site and in adjacent non-attacked tissues of the same leaflet. We found that both defense induction by T. urticae (i.e., from a population not adapted to tomato), as well as suppression by T. evansi, were predominantly local events taking place at their respective feeding sites, despite near-saturating levels of visible feeding damage (i.e., 4 d after infestation with 25 mites).9

Here, following the same methodology, i.e., infestation of the leaflet middle section with either 25 T. urticae or 25 T. evansi (ref. 9), we present the within-leaflet defense responses as determined at an earlier time point of the infestations: 2 d after infestation. Doing so, we provide insight in the magnitude and timing of defense responses at and near the mite’s feeding sites.

Locally, both T. evansi and T. urticae induced a significant accumulation of the JA-precursor OPDA, JA itself, and SA, but not of JA-Ile (Fig. 1). Absolute amounts of OPDA and SA in

**ABSTRACT**

When feeding from tomato (Solanum lycopersicum), the generalist spider mite Tetranychus urticae induces jasmonate (JA)- and salicylate (SA)-regulated defense responses that hamper its performance. The related T. evansi, a Solanaceae-specialist, suppresses these defenses, thereby upholding a high performance. On a shared leaf, T. urticae can be facilitated by T. evansi, likely via suppression of defenses by the latter. Yet, when infesting the same plant, T. evansi outcompetes T. urticae. Recently, we found that T. evansi intensifies suppression of defenses locally, i.e., at its feeding site, after T. urticae mites were introduced onto adjacent leaf tissue. This hyper-suppression is paralleled by an increased oviposition rate of T. evansi, probably promoting its competitive population growth. Here we present additional data that not only provide insight into the spatiotemporal dynamics of defense induction and suppression by mites, but that also suggest T. evansi to manipulate more than JA and SA defenses alone.

**KEYWORDS**

Defense suppression; herbivore behavior; herbivore performance; induced plant defenses; local; spatiotemporal dynamics; systemic; Tetranychus evansi; Tetranychus urticae; tomato (Solanum lycopersicum)

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the T. urticae feeding sites were significantly higher than those in T. evansi's feeding sites. Adjacent to the feeding sites, the concentrations of the phytohormones were not significantly different from the controls, except for increased SA amounts in the basal sections of mite-infested leaflets.

Differences in tomato defenses in response to the two mite species were more apparent at the gene expression level; transcripts of the wounding/JA-responsive OPR3, PPO-D, JIP-21 and PI-IIc, as well as transcripts of the SA-responsive PR-1a and PR-P6, were most abundant in the middle section of T. urticae-infested leaflets (Fig. 2). Tetranychus evansi did not induce PI-IIc expression and only slightly, albeit significantly, upregulated expression of the other defense-associated genes. We did not observe upregulation of any of these genes in the leaflet parts adjacent to T. evansi feeding sites. By contrast, transcript levels of OPR3, PPO-D, JIP-21 and PR-1a were higher in at least one of the leaflet parts bordering T. urticae feeding sites than in their respective controls.

To assess the extent to which the magnitude of plant defenses correlates with mite performance, mites, eggs and web were removed from the middle section after 2 d of infestation, subsequently the 3 leaflet sections were cut out and each of the sections was infested for 2 d with either one T. urticae or one T. evansi female to assess their egg production and survival. The fecundity of T. evansi was higher on 2-d-old T. evansi feeding sites than on uninfested controls (Fig. 3A: leaflet middle section; the red bar versus the white bar), but was not significantly different from that on 2-d-old T. urticae feeding sites. No statistically significant differences were found between the fecundity of T. urticae on the middle section of leaflets from the 3 treatment groups (Fig. 3B). On systemic tissues the fecundity of T. urticae and T. evansi displayed similar trends: mite fecundity was lower, albeit not always statistically significant, on leaf parts adjacent to T. evansi feeding sites as compared with the uninfested controls (Fig. 3). Contrary to fecundity, the survival of the mites on each of the leaflet sections was not significantly affected by the pre-infestation treatment (linear mixed-effect models [statistical analysis as in ref. 9]; T. evansi on the basal leaflet section: $\chi^2_{[2,5]} = 5.00; P = 0.08$; T. urticae on the middle section: $\chi^2_{[2,5]} = 4.86; P = 0.09$; T. evansi on the tip section: $\chi^2_{[2,5]} = 0.99; P = 0.61$; T. urticae on the basal leaflet section: $\chi^2_{[2,5]} = 1.19; P = 0.55$; T. urticae on the middle section: $\chi^2_{[2,5]} = 1.78; P = 0.41$; T. urticae on the tip section: $\chi^2_{[2,5]} = 0.82; P = 0.67$; for all treatments $n = 30$).

The plant defense and mite performance data presented here show that both induction and suppression of defenses by T. urticae and T. evansi, respectively, are largely restricted to the mite's feeding sites. These results are consistent with those reported in our previous study,9 and when combined they suggest the JA- and SA-regulated induced defense responses, as well as the suppression thereof, to be mostly local events throughout the course of an infestation.

The combined data also reveal some spatiotemporal changes in the defense responses of mite-infested leaflets. For instance, whereas the concentrations of OPDA and SA in the T. evansi and T. urticae feeding sites were not significantly different from each other after 4 d of feeding,9 differences, i.e., higher concentrations in T. urticae feeding sites, were found after 2 d of infestation (Fig. 1). Similarly, PPO-D and JIP-21 were not differentially expressed in the 4-d-old feeding sites of both mite species,9 yet they were expressed approximately 15 times higher in T. urticae feeding sites 2 d after infestation (Fig. 2). Perhaps the most dynamic pattern was found for the transcript abundance of OPR3 in T. urticae-infested leaflets: OPR3 expression...
was upregulated in 2-d-old feeding sites and in adjacent tissues (Fig. 2A), but was downregulated in leaf tissues bordering 4-d-old feeding sites, while the same trend was visible locally. By contrast, the JA accumulation pattern as well as expression patterns of PI-IIc, PR-1a and PR-P6 were very similar at both time points of the infestations. Overall, the within-leaflet defense responses reported here align well with those previously found in whole leaflets, i.e., strong induction by *T. urticae* and relatively low or no induction by *T. evansi*. Hence, the absence vs. presence of significant differences in some defense responses between *T. evansi* - and *T. urticae*-infested leaf material likely depends on infestation regimes and sampling methods, as was suggested before.

In line with results from Sarmento et al. our mite performance data demonstrate that *T. evansi* has a higher fecundity on leaf tissue previously infested with conspecifics than on uninfested controls (Fig. 3A). However, contrary to Sarmento et al. we did not observe a negative effect of a local pre-infestation with *T. urticae* on the performance of *T. evansi*, neither did we find an effect of a local pre-infestation with either hetero- or conspecifics on the performance of *T. urticae*. Again, this might be due to differences in the infestation regimes, e.g. timing of the pre-infestation and/or number of mites used for the pre-infestation.

Surprisingly, the mite performance data presented here also suggest the nutritional quality of leaf tissue adjacent to *T. evansi* feeding sites to be reduced, as mites had, or tended to have, a reduced fecundity on these leaf tissues. Possibly, *T. evansi* induces undetected defenses systemically, e.g., defenses regulated by hormones other than JA and SA. Alternatively, like insect herbivores, it may manipulate the host's resource allocation, e.g., by recruiting nutrients from distal non-attacked tissues to the feeding site. If so, plant-mediated interactions between spider mites (both facilitation and interference) may be determined not only by the mite-directed spatiotemporal heterogeneity of defenses but also that of resources.

It is of note that although *T. evansi* had a statistically significant lower fecundity on the tip section of leaflets previously infested in the middle with *T. evansi* than on uninfested controls (Fig. 3A) and the fecundity of *T. urticae* on the leaflet tip showed a similar trend (Fig. 3B), we did not observe such a negative plant-mediated effect on mite performance in a similar set of experiments (Fig. S2 in ref. 9). We reason two differences in the experimental setup may explain the discrepancy in the results. (1) Here, mites, eggs and web were removed from the leaflet middle section after 2 d, i.e., before the secondary infestation, whereas these remained untouched in Schimmel et al. 9

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**Figure 2.** Relative transcript abundances of defense-associated marker genes in basal, middle and tip sections of spider mite-infested tomato (*Solanum lycopersicum*) leaflets. Using artificial barriers, leaflets of intact plants were divided into 3 sections: base, middle, tip. The middle section was infested with 25 specialist *Tetranychus evansi* mites (red letters), 25 generalist, non-adapted *T. urticae* mites (blue letters), or remained uninfested as a control. After 2 d leaflets were excised and leaflet sections were cut out for hormone extraction and RNA isolation. The figure shows the average (+ SEM) normalized transcript abundances of (A) 12-Oxo-phytodienoic acid reductase 3 (OPR3), (B) Polyphenol-oxidase-D (PPO-D), (C) Jasmonate-inducible protein 21 (JIP-21), (D) Proteinase inhibitor IIc (PI-IIc), (E) Pathogenesis-related protein 1a (PR-1a), and (F) Pathogenesis-related protein P6 (PR-P6) in each of the leaflet sections (n = 4–5, with 10 leaflet sections from 10 plants pooled to form one biological replicate). The leaflet section that was sampled is indicated in green in the diagram below the bar graphs. Transcript abundances were normalized to *Actin* and then scaled to the overall lowest average value per gene panel. Bars are colored according to the treatment of the middle section. Gene expression data was statistically evaluated per leaflet section. Different letters above the bars indicate significant differences at a level of P ≤ 0.05, after applying a linear mixed-effects model followed by Tukey multiple comparisons. Plant infestation, RNA isolation, DNAse treatment, cDNA synthesis, gene expression analysis by means of quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), and statistical analysis of the data were performed following the procedures described by Schimmel et al. 9

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*Note:* The image contains bar graphs showing the relative transcript abundances of defense-associated marker genes in basal, middle, and tip sections of spider mite-infested tomato (*Solanum lycopersicum*) leaflets. The graphs illustrate the expression levels of genes such as *OPR3*, *PPO-D*, *JIP-21*, *PI-IIc*, *PR-1a*, and *PR-P6* in different sections of the leaflets, with significant differences indicated by different letters above the bars. The leaflet section sampled is marked in green in the diagram below the bar graphs. The data were normalized to *Actin* and statistically evaluated per leaflet section, with significant differences indicated at P ≤ 0.05. Methods for plant infestation, RNA isolation, DNAse treatment, cDNA synthesis, gene expression analysis, and statistical analysis were described by Schimmel et al. 9.
As emphasized before, the timing of the infestations likely is an important determinant for the outcome of the plant-mediated interactions between \(T. \text{evansi}\) and \(T. \text{urticae}\). Here, mite performance was assessed on detached leaflet sections as opposed to leaflet sections of intact plants in Schimmel et al.\(^9\) Detached leaf tissues (e.g., leaf discs) are frequently used to assess the impact of plant defenses on mite performance,\(^9\) however the outcome may deviate from that of assays performed on intact plants, because detached leaf tissues suffer from wound-induced metabolic changes, accelerated desiccation, and the impaired transport of primary and secondary metabolites. In conclusion, our findings indicate the need to be cautious with detaching leaf tissues for investigating plant resistance, yet they also suggest the use of detached leaf tissues can uncover the potential involvement of biological processes (e.g., resource reallocation) in plant-mediated interactions between herbivores when comparing these directly to results obtained in parallel from intact plants. Such effects will be missed when working with detached leaves or with intact plants alone.

Similar to mite-infested tomato leaflets, defense patterns of predominantly local induction have been found in leaves of various plant species upon (simulated) herbivory by caterpillars.\(^{17-22}\) Generalist caterpillars are known to rapidly induce \(JA\)-mediated defenses at their feeding site and then move away to feed on distal, less-defended tissue.\(^{21,23-28}\) By contrast, specialist caterpillars, that have evolved physiological adaptations to the host’s defenses, usually do not show such defense-avoiding foraging behavior, and instead may even select and perform better on defense-rich tissues.\(^{21,25-27}\)

Such links between herbivore performance, herbivore behavior and the spatiotemporal patterns of plant defenses also exist between spider mites and tomato. On the one hand, the rapid, local induction of defenses by generalist \(T. \text{urticae}\) (i.e., individuals not adapted to tomato) hampers its performance,\(^8,8,11\) and this correlates well with it: (1) moving away from its feeding sites (the tendency of populations to disperse across their host plant),\(^8,28,29\) (2) having a low feeding intensity,\(^8\) and; (3) having a low population growth rate on tomato relative to \(T. \text{evansi}\).\(^8\) On the other hand, local host plant manipulation (i.e., defense suppression and possibly resource reallocation) by specialist \(T. \text{evansi}\) increases its performance (Fig. 3A),\(^9,11,30\) and this aligns well with it: (1) being gregarious;\(^9\) (2) having a high feeding intensity;\(^8\) (3) having a high population growth rate on tomato relative to \(T. \text{urticae}\),\(^10\) and; (4) preferring volatiles from tomato plants infested with conspecifics over those from uninfested plants.\(^10\) Nonetheless, when forced to feed on tomato, \(T. \text{urticae}\) can adapt to this unfavorable host within several generations, thereby markedly changing its behavior (less dispersal, more feeding) and increasing its fitness.\(^28,31\) Furthermore, \(T. \text{urticae}\) populations may harbor genotypes that are either resistant to induced defenses or that can suppress these, albeit less strong than \(T. \text{evansi}\) does.\(^8\)

Finally, how a possible negative effect of local host plant manipulation by \(T. \text{evansi}\) on mite performance on adjacent tissues (Fig. 3) fits into this framework is not so clear. It is conceivable that this negative effect can only be observed at very early time points of the infestation, thus not being representative for the whole interaction as such. That is because if not transient, such a systemic negative effect would likely impair \(T. \text{evansi}\)’s own population growth when mites run out of food at their local feeding site and are thus forced to move to non-attacked (adjacent) tissues. Accordingly, Sarmento et al.\(^10\) did not find a negative effect on the performance of \(T. \text{evansi}\) when feeding on non-attacked systemic leaflets of plants that had been pre-infested for 7 d with 200 conspecifics. However, more research is needed to determine under which circumstances mite performance decreases on leaf tissues adjacent to \(T. \text{evansi}\) feeding sites, what causes it to decrease, and whether this effect is herbivore- or plant-adaptive.

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The authors report no conflict of interest.

Author contributions
B.C.J.S. and L.M.S.A. conceived, designed and conducted the experiments; B.C.J.S. and L.M.S.A. analyzed the data; M.R.K. supervised the experiments. B.C.J.S. and M.R.K. wrote the manuscript with input from all co-authors. B.C.J.S. and L.M.S.A. contributed equally to this work.

Data availability
All raw data has been uploaded to FigShare (10.6084/m9.figshare.5319046) and is publicly available.

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