Consequences of russet mite-induced tomato defenses for community interactions

Glas, J.J.

Citation for published version (APA):
Glas, J. J. (2014). Consequences of russet mite-induced tomato defenses for community interactions

General rights
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 than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Russet mite-induced plant defenses increase tomato resistance to a phytopathogen but facilitate a competing herbivore


Plant parasites have to cope with their host’s defences and with each other, as competitors. Competition between individuals may be direct, but can also occur indirectly via induced changes in plant quality. Some parasites are able to manipulate their host plant to their own benefit but how this affects parasite natural communities remains a poorly understood question. Here, we show that plant parasites interact via the plant defenses they induce and that these indirect interactions can determine the performance of individual community members. As described in Chapter 2, we found that tomato russet mites (Aculops lycopersici) suppress jasmonic acid (JA)-related defenses, whereas they induce those that depend on salicylic acid (SA), and this can augment SA-defenses induced by competing herbivores, resulting in suppression of the JA-response via JA/SA-cross-talk. In this chapter, we show that absence of JA-defenses increases russet mite population growth, and thus, that by suppressing the JA pathway russet mites manipulate the plant defensive system. However, this metabolic hijack affects also the reproductive performance of other community members: while inhibiting the SA-sensitive bacterial leaf pathogen Pseudomonas syringae, it facilitates the spider mite Tetranychus urticae, a JA-sensitive food competitor of russet mites in the field. Furthermore, we found that while facilitating spider mites, russet mites experience reduced population growth. Thus, we conclude that whether or not host-defense manipulation improves a parasite’s fitness depends on interactions with other parasites via the sum of community-induced defenses, implicating bidirectional causation of community structure of parasites sharing a plant.

In nature as well as agriculture, plants suffer from a diverse community of parasites, involving herbivores as well as pathogens. Upon attack by parasites, plants undergo rapid physiological changes (Howe & Jander, 2008) which take place not only at the site of attack, but also in the undamaged parts of attacked leaves and in distal undamaged (systemic) leaves, thereby increasing resistance plant-wide (Wu & Baldwin, 2010). Two hormone signaling pathways play a major role in the regulation of these defense responses: the JA pathway, which is induced by herbivores and necrotrophic pathogens (Howe & Jander, 2008), and the SA pathway, which orchestrates defenses against biotrophic pathogens (Glazebrook, 2005) and some phloem-
feeding herbivores (Kaloshian & Walling, 2005). Herbivores and pathogen-induced plant responses can alter the plant in such a way that not only the initial attacker but also subsequent attackers are affected (e.g., De Vos et al. 2006; Stout et al., 2006; Long et al., 2007; Spoel et al., 2007; Thaler et al., 2010; Tack & Dicke, 2013). This implies that, apart from directly competing for the same resource, also referred to as ‘exploitation competition’, herbivores may also indirectly compete with each other via induced changes in the quality of the plant, i.e., via ‘plant-mediated indirect interactions’ (Ohgushi, 2005).

Here, we study how plant attackers influence each other’s performance by inducing and suppressing defense responses in a shared host plant. Our model system includes the tomato russet mite (Aculops lycopersici), a tiny eriophyoid mite species and specialist pest species on tomato (Solanum lycopersicum) (Gerson & Weintraub, 2012), and two of its natural competitors, i.e., the two-spotted spider mite (Tetranychus urticae) and the plant pathogenic bacterium Pseudomonas syringae pv. tomato (Pst DC3000). All three species are pests of tomato in countries where this crop is grown (Figure 3.1; Jones et al., 1991). We have observed that on field-grown tomato plants in Italy russet mite infestations were often followed by T.
urticae infestations on the same plants. This prompted us to hypothesize that russet mite infestations may alter the interactions between spider mites and their host, thereby influencing the reproductive performance of spider mites. Hence, we quantified defense responses in tomato plants infested with spider mites, russet mites, or the combination of spider mites and russet mites and found that russet mites suppressed spider mite-induced JA-dependent defense responses (Chapter 2). Here, we ask the question whether russet mite-induced responses in tomato change the ecological interactions occurring within their natural communities. First, we test the hypothesis that russet mites, by suppressing JA-defenses, benefit spider mites. Second, we assess the impact of russet mite-induced SA-defenses on Pst growth. Third, we investigate the role of JA-defenses in natural resistance of tomato against russet mites by measuring russet mite population growth on the def-1 mutant, which is defective in JA-dependent defense signaling (Howe et al., 1996). Finally, we investigate if, and if yes how, these host plant-mediated changes in the russet mite’s community feed back onto the russet mite itself.

MATERIAL AND METHODS

Plants, mites and bacteria

Tomato seeds (Solanum lycopersicum cv. Castlemart (CM), defenseless-1 (def-1), and a transgenic line 35S::prosystemin (prosys+), both in the genetic background of CM) and S. lycopersicum cv. Moneymaker (MM) as well as the transgenic line nahG, in the genetic background MM were germinated in soil and grown in a greenhouse compartment at a temperature of 25°C and a 15/9 h light/dark regime. One week prior to each experiment, plants were transferred to a climate room with day/night temperatures of 27°C/25°C, a 16/8 h light/dark regime and 60% RH. All experiments were performed on 21-day-old plants. The def-1 plants are deficient in wound- and systemin-induced JA accumulation and in the expression of downstream defense genes (Howe et al., 1996). Prosys+ plants overexpress the prosystemin gene, resulting in a constitutively activated JA-signaling pathway (Chen et al., 2006). The nahG plants are transformed with the bacterial gene nahG encoding salicylate hydroxylase which removes endogenous SA by converting it into catechol (Brading et al., 2000). Both the prosys+ and the nahG gene are under the control of the constitutively expressed CaMV 35S promoter. Experiments with wild-type (WT) and mutant/transgenic lines were always carried out in parallel.

Tomato russet mites (Aculops lycopersici, also referred to as russet mites or abbreviated as RM) were obtained from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands), who, in turn, had obtained them in the summer of 2008 from naturally infested plants in a greenhouse in the Westland area (The Netherlands), and was since reared in insect cages (BugDorm-44590DH, Bug Dorm...
Two-spotted spider mites (*T. urticae*, also called spider mites or abbreviated as SM) were originally obtained in 2001 from a single European spindle tree (*Euonymus europaeus*), in the dunes near Santpoort (The Netherlands) (GPS coordinates: 52 26.503 N, 4 36.315 E). The strain we used has been described as a JA-inducing mite genotype and as susceptible to these defenses (Kant *et al.*, 2008). Since its collection from the field, the strain has been propagated on detached bean (*Phaseolus vulgaris*) (cv. 746 Speedy) leaves that were placed with the abaxial surface on wet cotton wool and maintained in a climate room (temperature of 25°C, a 16/8 h light/dark regime and 60% RH).

*Pst* DC3000 bacteria were grown on King’s Broth (KB) medium (King *et al.*, 1954) agar plates, containing rifampicin (50 μg/ml), and grown at 28°C for 2-3 days. Subsequently, single colonies were picked and bacterial cultures were grown overnight at 28°C in liquid KB medium with rifampicin (50 μg/ml). Bacterial cells were collected by centrifugation (3000 rpm for 10 min), resuspended in 10 mM MgSO₄ and adjusted to the required optical density (OD) (see sections *Pst DC3000 growth assays* and *Russet mite population growth experiments*) before pressure infiltration into the leaflets.

**Samplings in tomato fields**

Tomatoes growing in the field were sampled to determine the co-occurrence of plant-eating mites. Samplings were performed in several areas of Italy that are well-known for their tomato production (Figure 3.2). On each location at least two samples were taken, the first one in July and the second one in September. Samplings were done at 93 different sites in total, in the summer of 1997 and 1998. For each sample, 80-100 leaves (i.e., compound leaves) were randomly collected. Subsequently, these leaves were examined under a stereomicroscope and on each leaflet the presence/absence of spider mites as well as that of russet mites was recorded.

**Spider mite reproductive performance assays**

To obtain RM-infested leaflets before the start of the SM-performance experiments, 21-day-old tomato plants were infested by transferring small (ca. 0.5 cm²) pieces of leaflets with RM to the leaflets of the clean plants. These pieces had been cut from leaflets picked from a well-infested tomato plant and each piece contained ca. 250 mobile stages of RM. In total, three leaflets per plant were infested, i.e., one leaflet of the second true compound leaf (counted from the bottom to the top of the plant), one from the third true compound leaf and the terminal leaflet of the fourth true compound leaf. To prevent mites from dispersing, a thin barrier of lanolin was applied on the petioles of leaflets that were chosen for infestation. Uninfested control plants received lanolin, but no mites.
Subsequently, four young adult female SM were placed on the adaxial surface of the RM-infested leaflets and on leaflets of the same age and position of non-infested control plants, using a soft bristle paintbrush. At this point in time, the plants had been infested with RM for 7 days. After 4 days (i.e., 11 days after the start of the experiment), infested leaflets were detached and SM adults and their eggs were...
counted using a stereomicroscope. The experiment was performed on WT, def-1 and prosys+ plants. The results presented in Figure 3.3 represent the mean number of eggs per mite per day of 24 leaflets that were obtained from 8 plants. The experiment was carried out twice, at different time-points, with similar results.

In order to obtain SM of the same age, egg waves were generated in a climate room (temperature of 25°C, a 16/8 h light/dark regime and 60% RH), as previously described (Kant et al., 2004). In short, 20-30 random adult female spider mites were selected from a rearing colony and allowed to produce eggs for a period of 48 hours on detached bean leaflets on wet cotton wool. After this period, the adults were removed but the eggs were maintained. After 14 days, the 2±2 days young adult females were collected and these were used for the oviposition assays.

**Pst DC3000 growth assays**

At the start of the assays, 21-day-old plants were infested with RM as described in the section Spider mite reproductive performance assays. Experiments were performed on WT (cv. MM) and SA-deficient nahG plants. After 7 days of infestation with RM, the left halves (bordering the midrib) of RM-infested leaflets and similar leaflets from non-infested control plants were pressure infiltrated with Pst DC3000 (OD600=0.001) in 10 mM MgSO4 using blunt 1-ml syringes (BD Plastipak, Franklin Lakes, USA). Three days after infection with Pst, leaflets were detached and two 1 cm2 circular leaf discs were punched out from the infiltrated leaflet halves and ground in 500 μl 10 mM MgSO4. Serial dilutions were prepared by taking 20 μl of the leaf disc solution and diluting it in 180 μl 10 mM MgSO4. Twenty μl of each serial dilution was plated on KB medium + Rifampicin (50 μg/ml) plates. The number of Colony Forming Units (CFU) was counted 2 days after incubation at room temperature.

Leaflets from the third and fourth compound leaf (counted from the bottom of the plant) were infected. Bacterial growth in both leaves was very similar. In Figure 3.5, data for leaf 3 are presented. In total, 7 plants were infected per genotype and per treatment and the experiment was repeated a second time with a similar result.

**Russet mite population growth experiments**

For the RM population growth experiments, 21-day-old tomato plants were infested by transferring 20 RM to each of three leaflets per plant. Thus, in total plants were infested with 60 RM each. To prevent mites from dispersing we applied a thin barrier of lanolin on the petioles of leaflets that were chosen for infestation. Uninfested control plants received lanolin, but no mites. We always picked the same leaflets for infestation, i.e., one leaflet of the second true compound leaf (counted from the bottom to the top of the plant), one from the third true compound leaf and the terminal leaflet of the fourth true compound leaf. Leaflets with the same position were used...
for the gene expression and phytohormone measurements. To assess RM performance, infested leaflets were detached and mites (all stages) were washed off by rinsing the leaflets one by one for 20 s in 25 ml 100% ethanol. Infested leaflets that came from the same plant were washed in the same solution. RM were counted by running 2 ml of the leaf washes through a particle counting system (PAMAS SVSS, PAMAS, Rutesheim, Germany). Leaf washes were counted 20 s after mixing them, to avoid that air bubbles enter the system. Adult RM are around 120-150 μm in size while their eggs are around 20 μm. Therefore, the number of particles measured in the range of 50-200 μm was used to quantify the number of adult mites. The number of mites per plant was calculated by multiplying the mean number of particles per ml with the total volume of 25 ml. This counting method was validated by means of a dose-response experiment. In this experiment, we infested leaflets on intact plants with 2, 4, 8, 16, 32 and 64 mites and after 14 days the mites were washed off, as described earlier. The numbers counted implied exponential population growth, corresponding to the starting conditions (data not shown). Only low numbers of particles (33±8) were counted in samples that came from clean uninfested leaflets.

For the RM-SM co-infestation experiment (FIGURE 3.6), plants were infested by transferring 20 RM to each of the three leaflets per plant. After 7 days of RM infestation, half of the plants were co-infested with five adult SM on the RM-infested leaflets. Thus, dual-infested plants were infested with in total 15 SM per plant. The population growth of RM was assessed after 14 days of infestation with RM (and hence after 7 days of infestation with SM) by counting the number of RM as described above. The values presented in FIGURE 3.6 represent the mean of 13-15 plants, obtained in two independent experiments.

For the RM- Pst co-infestation experiment (FIGURE 3.7), plants were infested by transferring 20 RM to each of three leaflets per plant. After 7 days of RM infestation, three leaflets of half of the RM-infested plants were infiltrated with Pst in 10 mM MgSO₄ and three leaflets of the other half of the RM-infested plants were infiltrated with mock 10 mM MgSO₄. The same three leaflets were chosen for infiltration from each plant. Since older leaflets are more susceptible to Pst compared to the younger leaflets (personal observation), the leaflets from the third and fourth compound leaf were infiltrated with a bacterial suspension which had an OD₆₀₀ of 0.0001, whereas the oldest leaflet (i.e., the leaflet on the second fully expanded leaf) was infiltrated with a lower OD₆₀₀ of 0.00005. Of each leaflet, approximately ¼ of the leaf area was infiltrated with bacteria. The population growth of RM was assessed after 14 days of infestation with RM (and hence after 7 days of infestation with Pst) by counting the number of RM as described above. The values presented in FIGURE 3.7 represent the mean of 15 plants, obtained from two independent experiments. Symptoms of Pst infection were visible at the time of sampling, with sometimes (minor) parts of the leaflet being senesced and/or necrotic.
For assessing RM performance on WT and def-1 plants (Figure 3.8), plants were infested by transferring 20 RM to each of the three leaflets per plant. RM population growth was assessed after 8, 12 and 16 days by counting the number of RM as described above. The values presented in Figure 3.8 represent the means of 5-10 plants per time-point, obtained from two independent experiments. In a third replicate of the experiment, using 10 plants per treatment, we sampled only at 16 days after infestation. This experiment gave a similar result (data not shown).

Statistical analysis
Spider mite oviposition data (Figures 3.3 and 3.4) were analyzed with ANOVA using ‘Treatment’ as fixed factor and including ‘Plant replicate’ as random factor. Means of each group were compared using Fisher’s LSD post hoc test and homogeneity of variances was assessed by means of the Levene’s test. Pst performance data were analyzed with the Student t-test (Figure 3.5). Russet mite population growth data were log-transformed before statistical analysis (Figures 3.6, 3.7 and 3.8). Values presented in the graphs represent untransformed data. Student t-tests were performed in Excel (Microsoft, Redmond, WA, USA) and ANOVA followed by Fisher’s LSD tests in SPSS 20.0 (SPSS, Chicago, IL, USA).

RESULTS
Russet mite infestations precede spider mite outbreaks in the field
Tomatoes growing in the field and in the greenhouse were sampled to determine the occurrence of plant-eating mites. Spider mites or russet mites were found at 77 sampling sites and in 33 cases the two species were found together on the same plant. Because we sampled repeatedly, we noticed that in 21 of those cases russet mite infestations had preceded spider mite infestations ($X^2=9.85$, d.f.=1, $P=0.0017$) (Table 3.1, Figure 3.2), whereas in only 5 of the cases spider mites were observed first. In seven cases the order of infestation was unknown (Table 3.1).

Russet mites increase reproductive performance of spider mites on WT tomato plants
To test the hypothesis that spider mites benefit from russet mite infestations, we co-infested plants with russet and spider mites and measured spider mite reproductive performance by counting the number of spider mite eggs produced on plants that had been pre-infested with russet mites and plants that had been infested with spider mites only. We found that spider mites produced significantly more eggs on def-1 than on WT plants ($P=0.002$; Figure 3.3), confirming that they are susceptible to JA-dependent defenses (Li et al., 2002; Kant et al., 2008). Strikingly, on WT plants that had been pre-infested with russet mites, spider mites produced significantly more
eggs as compared to plants infested with spider mites only ($P = 0.027$). In contrast, russet mites did not affect spider mite oviposition rate on def-1 plants (‘SM’ on def-1 vs. ‘Both mites’ on def-1; $P = 0.21$). Furthermore, the number of eggs produced by spider mites on co-infested WT plants was similar to the number of eggs produced

### Table 3.1

Russet mite infestations precede spider mite infestations significantly more often than the other way round.

<table>
<thead>
<tr>
<th>Location</th>
<th>Russet mites observed first</th>
<th>Spider mites observed first</th>
<th>Order of infestation unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 San Bonico, PC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Torre Mar., Montalto di Castro, VT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Valentini, S. Giorgio Plac., PC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Rurimoni, Montalto di Castro, VT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Saliceto di Cadeo, PC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Veneziani, S. Giorgio Plac., PC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Saliceto di Cadeo, PC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Monticello, Caorso, PC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Bondiocca di Caorso, PC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Casalloschino di Sissa, PR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 Trecasali, PR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Bianconeise di Fonteivivo, PR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 Prati B. Calabrina, Cesena, FC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 Capannaguzzo, Cesena, FC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 Milan, Montalto di Castro, VT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 Ania, Cesa, AR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 Meini, Poggetti, GR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 Ania, Rispesca, GR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 Dragoni, Poggio al Pino, GR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 Marioni, Poggio al Pino, GR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 Bondiocca di Caorso, PC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 Campo 4, Caorso, PC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23 Oliva, Aversana, Battaglia, SA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 Galassi R, Cannara, PG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 Dragoni, Sterpeto, GR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 Ania, Rispesca, GR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27 Meini, Vareop., GR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 Vallepega, Comacchio, FE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29 Dragoni, Favacchio, GR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 Gianandrea, Rosignano, Lj</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31 Di Francesco, La California, Bibbona, Lj</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32 Frolani, Bolgheri, Lj</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33 Di Nola, Lucera, FG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

List of samples documenting the (co-)occurrence of russet mites and spider mites on field-grown tomatoes in Italy. Spider mites or russet mites were found on tomato on 77 field sites. In the 33 cases shown in this table they co-occurred on the same host plant. In seven cases the order of infestation was unknown. In 21 out of the remaining 26 field observations, russet mite infestations preceded spider mite infestations ($X^2 = 9.85$, d.f. = 1, $P = 0.0017$).

Locations are indicated by the name of the farm (if applicable), followed by the name of the town and region (SA=Salerno, PG=Perugia, FG=Foggia, Lj=Ljorno, GR=Grosseto, AR=Arezzo, VT=Viterbo, FE=Ferrara, PC=Piacenza, PR=Parma, FC=Forlì-Cesena).
on def-1 plants infested with spider mites only ($P=0.28$). Finally, the number of eggs produced on co-infested def-1 plants was marginally significantly different from the number of eggs produced on WT plants infested with spider mites only (‘SM’ on WT vs. ‘Both mites’ on def-1; $P=0.053$) and not different from the number of eggs produced on WT plants infested with both mite species (‘Both mites’ on WT vs. ‘Both mites’ on def-1; $P=0.83$).

Spider mite performance is not affected by russet mite feeding on prosys+ plants

Subsequently, we performed the same experiment as described in b) but this time using prosys+ plants, which display constitutively activated JA-defenses (Chen et al., 2006; Kandoth et al., 2007). As in the previous experiment, spider mites produced significantly more eggs on WT plants that had been previously infested with russet mites than on clean plants ($P=0.011$; FIGURE 3.4). In contrast, the presence of russet mites did not affect the number of eggs produced by spider mites on prosys+ plants ($P=0.48$).

Spider mites produced significantly fewer eggs on prosys+ plants compared to WT plants (‘SM’ on WT vs. ‘SM’ on prosys+; $P=0.025$) confirming that they suffer from the strong JA-defense responses in these plants. Also, the number of eggs pro-
Reduced on co-infested prosys+ plants was significantly lower compared to the number of eggs produced on WT plants infested with spider mites only ($P = 0.006$) or with the two species simultaneously ($P < 0.001$).

Russet mite-induced SA inhibits growth of Pst DC3000 in tomato leaflets

Russet mites induce a significant accumulation of SA in tomato leaflets (FIGURE 2.5C). To investigate the consequence of russet mite-induced SA for growth of the bacterial pathogen Pst DC3000, we infiltrated uninfested control and russet mite-infested leaflets with Pst and determined growth of the bacterium 3 days after the start of the infection.

In leaflets that had been pre-infested with russet mites, bacterial growth was significantly reduced compared to uninfested control leaflets ($P < 0.001$). In contrast, on nahG plants the presence of russet mites did not have an effect on Pst bacterial growth ($P = 0.15$) (FIGURE 3.5). Furthermore, bacterial growth was significantly higher on nahG as compared to WT plants ($P < 0.001$; FIGURE 3.5), although in a second replicate of the experiment we did not observe a significant difference in bacterial growth between WT and nahG plants.
Spider mites inhibit population growth of russet mites

Spider mites display an increased reproductive performance on tomato WT leaflets previously infested with russet mites (Figure 3.3). To determine whether increased spider mite performance would have an effect on russet mite performance, we co-infested russet mite-infested plants with spider mites and measured russet mite population growth 14 days after infesting them with russet mites. Introducing spider mites on russet mite-infested WT leaflets had a significantly negative effect on russet mite population size (Student t-test; \( P = 0.033 \); Figure 3.6). The same effect was found on def-1 plants, where it turned out to be even more pronounced (Student t-test; \( P = 0.004 \); Figure 3.6). The same trend was found in a pilot experiment, in which we sampled leaflets 12 days after infesting them with russet mites (5 days after infesting with spider mites) (data not shown).

Pst infections do not interfere with russet mite population growth

We found that russet mite feeding strongly inhibited growth of Pst on WT tomatoes. The fact that this effect was absent on SA-deficient nahG plants (Figure 3.5) shows that the negative effect on Pst is due to SA-defenses induced by russet mites. To determine whether reduced bacterial performance would affect russet mite perform-
ance, we co-infiltrated russet mite-infested WT plants with Pst and measured russet mite population growth 14 days after infesting them with russet mites. Since on nahG plants russet mite infestations did not interfere with Pst growth (FIGURE 3.5), the same procedure was carried out on nahG plants as a control treatment. The results showed that russet mite performance was not significantly different between WT and nahG plants, suggesting that russet mites do not suffer from SA-mediated defenses.

Second, it appeared that Pst infections did not change russet mite population growth, neither on WT nor on nahG plants (ANOVA: \( P=0.83 \); FIGURE 3.7). The same result was found in a pilot experiment, in which leaflets were sampled 12 days after infesting them with russet mites (and 5 days after infiltrating them with Pst) (data not shown).

Russet mites display a higher growth rate on def-1 plants
To assess if defense suppression is costly for russet mites, we measured russet mite population growth rates on WT and mutant def-1 plants at different time-points after infestation. Def-1 plants are deficient in the induced expression of proteinase inhibitors (Ament et al., 2004) and therefore highly vulnerable to spider mite attack (Li et al., 2002). At day 8 and 12 after infestation, russet mite population sizes were not
significantly different between WT and mutant plants (Student t-test $P=0.27$ and $P=0.31$, respectively; FIGURE 3.8). However, at day 16 after infestation, the mean population size of russet mites was significantly higher on def-1 mutant plants as compared to WT plants ($P=0.036$).

**DISCUSSION**

By making use of tomato mutants in the JA- and SA-pathway, we have investigated the consequences of russet mite-induced defenses for the interactions with two naturally co-occurring species, i.e., the two-spotted spider mite and the bacterial phytopathogen *Pst* DC3000.

Russet mites often co-occur together with spider mites, and, strikingly, infestations of the latter often ‘follow’ the first (TABLE 3.1; cf. also Castagnoli *et al.*, 1998). To explain this observation, we hypothesized that russet mites change the interaction between spider mites and their tomato host, in such a way that spider mites benefit. We found that the oviposition rate of spider mites feeding from WT plants that had been pre-infested with russet mites was increased by ca. 30% as compared to the same rate on clean WT plants (FIGURES 3.3 and 3.4). Furthermore, we found that spider mites produced significantly more eggs on def-1 compared to WT plants, whereas oviposition...
rates on transgenic prosys+ plants were reduced. This confirms that the spider mite strain we used is sensitive to JA-defenses (Kant et al., 2008). Notably, the positive effect of the def-1 mutation on spider mite oviposition was similar to that of russet mite infestation (FIGURE 3.4), suggesting that russet mites, by suppressing JA-responses, ‘change’ WT plants in such a way that they are experienced by spider mites as if it were def-1 plants. On prosys+ plants, russet mite-infestation did not affect spider mite oviposition, which, in theory, could be due to (1) reduced performance of russet mites on prosys+, or (2) the inability of russet mites to suppress JA-defenses in prosys+ plants. The first explanation is not likely, since in preliminary experiments we did not find that russet mite performance was lower on prosys+ plants compared to WT plants. The second hypothesis could indeed explain why spider mites do not profit from russet mite infestations on prosys+ plants. Yet, given that russet mites seem to suppress JA-defenses downstream of JA-accumulation (CHAPTER 2), this explanation is perhaps not the most likely as they would then theoretically also be able to turn prosys+ plants into a better host for spider mites. To answer this question, a detailed investigation of the defensive status of prosys+ plants infested with spider mites, russet mites and the combination of russet mites and spider mites should be carried out, by measuring phytohormones and defense gene expression.

It should be noted that we are not the first to observe a positive effect of feeding by an eriophyoid mite on spider mite fecundity. Westphal et al. (1992) reported that
Aceria cladophthirus induced a hypersensitive reaction which lead to an increase in spider mite fecundity of about 40% when these fed on (detached) Solanum dulcamara leaves previously infested with the eriophyoid. Also in other systems evidence has been found that different species may positively affect each other via induced plant responses. For instance, Zhang et al. (2009) found that whiteflies suppress JA-defenses in Lima bean and this resulted in a better performance of spider mites on leaf discs that had been taken from whitefly-infested plants than on leaf discs from clean plants. Because the leaf disc assay is less laborious than whole plant assays, we also tried the leaf disc setup described by Zhang et al. (2009). However, on leaf discs we did not find that russet mites facilitate spider mites, in contrast to what we observed in the whole plant assays (data not shown). At present, we do not have a good explanation for why our results obtained in the whole plant assay differ from those obtained in the leaf disc assay. However, in general leaf discs are perhaps not the best to use for these induced defense experiments, since wounding, which occurs during preparation of the leaf discs, typically induces strong JA-dependent responses (Howe & Jander, 2008), which are likely to overrule herbivore-induced responses. Hence, in future work, it is recommended to assess spider mite fecundity using intact leaflets or on intact plants (as for instance in Li et al., 2004).

In Chapter 2, we showed that russet mites suppress spider mite-induced JA-responses on WT plants, but that this suppression is no longer significant on nahG plants (FIGURE 2.4). To study the potential consequences of this, we also performed an oviposition experiment on nahG plants with the expectation to find no, or even a negative, effect of russet mite pre-infestation on spider mite oviposition on these plants as compared to WT plants. Unexpectedly however, on the WT control plants (cv. MM, the genetic background of nahG) russet mite pre-infestations did not increase spider mite oviposition rates, in contrast to what we observed on the cv. CM. We observed that spider mites produced more eggs on MM than on CM plants (8.7 vs. 7.0 eggs/mite/day, and this difference was marginally significant: \( P=0.054 \)) (data not shown). Hence, it could be that MM plants are so much reduced in resistance to spider mites that the positive effect of russet mite infestation is no longer relevant. MM plants have a reduced number of type VI trichomes compared to CM plants (ca. 600 vs. ca. 1200 cm\(^{-2}\); Peiffer et al., 2009; Bleeker et al., 2012) and because trichomes are important in resistance of tomato to spider mites (Alba et al., 2009), it is likely that this plays a role in the reduced resistance of MM plants. In any case, since we did not find a difference in spider mite performance on the control WT plants, it is difficult to draw conclusions from this experiment.

Subsequently, we tested whether the presence of spider mites affected russet mite growth. We found that the russet mite population growth was significantly reduced on WT plants that had been co-infested with spider mites, and a similar
effect was found on def-1 plants (Figure 3.6), indicating that spider mite decrease russet mite growth. To which extent this effect is due to plant-mediated facilitation of spider mites by russet mites remains to be investigated. This could be done, for instance, by measuring russet mite performance on nahG plants with and without spider mites, as on these plant suppression of spider mite-induced JA-responses is disabled (Figure 2.4). In the case that competition of spider mites on russet mites is mediated via an effect on the JA-pathway, russet mite growth is unlikely to be affected by spider mite feeding on nahG, whereas russet mite growth is expected to be negatively affected in the case that the effect is due to direct competition.

To assess the consequence of russet mite-mediated SA-induction, additional experiments were performed with Pst DC3000, which is a virulent bacterial strain reported to elicit both JA-defenses as well as SA accumulation in tomato (Stout et al., 1999; Fidantsef et al., 1999; Thaler et al., 2010). Pst activates the JA-pathway via the phytotoxin coronatine (Zhao et al., 2003), which enhances bacterial virulence by attenuating the accumulation of SA (Uppalapati et al., 2007) and downstream SA-dependent defense responses (Zhao et al., 2003; Uppalapati et al., 2007). In line with this, a JA-insensitive mutant (jai1) of tomato is resistant to Pst DC3000 (Zhao et al., 2003). Both JA as well as SA-defense responses have been reported to negatively affect Pst growth (Thaler et al., 1999, 2004). Yet, the role of SA in the interaction between tomato and Pst has not been completely resolved, as Li et al. (2002) did not find convincing evidence that Pst growth was enhanced on tomato nahG plants, aligning with our observation that Pst growth was not always improved on nahG plants. Similarly, O’Donnell et al. (2001) did not find a difference in Xanthomonas campestris pv. vesicatoria growth rates between WT and nahG plants. Thus, tomato appears to differ in this respect from Arabidopsis and tobacco, where nahG consistently allows for increased bacterial growth as compared to WT plants (e.g., Delaney et al., 1994; Block et al., 2005).

We found that Pst growth rates were greatly reduced in russet mite-infested WT plants compared to uninfested WT plants. In contrast, this negative effect was not observed on nahG plants, which suggests that russet mite-induced SA can inhibit Pst growth rates. Since we did not see an effect of russet mite infestation on nahG plants, we can exclude the possibility that russet mites directly interfere with Pst. We also investigated the reciprocal interaction by investigating the effect of Pst on russet mite population growth and found that the latter was not negatively affected by Pst on WT plants, nor on nahG plants (Figure 3.8). This means we can exclude that the effect of russet mite infestation on Pst growth is caused by differences in mite density. Obviously, using a higher bacterial concentration or infecting a greater proportion of the leaflet may have resulted in a different outcome. Yet, since we were primarily interested in plant-mediated indirect effects we deliberately chose to work under these experimental conditions.
Finally, we tested the hypothesis that there are costs associated with the suppression of JA-defenses by russet mites. This was done by allowing them to build up a population on tomato *def-1* in comparison to WT plants. These experiments revealed significant costs since after 16 days of infestation, equivalent to two generation cycles, russet mite populations had grown larger on *def-1* than on WT tomatoes (Figure 3.8). An alternative explanation for this finding could be that defenses are effectively not suppressed fully down to levels in *def-1* by russet mites. Indeed, in WT plants, expression levels of the JA-marker genes were up-regulated slightly above control levels (Figure 2.2), suggesting that defense suppression by russet mites is not absolute. Russet mite population growth did not differ between WT and *nahG* plants indicating that they are not affected by SA-dependent defense responses (Figure 3.7). Since russet mites suppress JA-defenses independent from JA/SA-cross-talk (Chapter 2), this finding was in line with our expectation. Yet, it would be good to confirm this result in an independent tomato SA-mutant as catechol production in *nahG* plants may result in inadvertent effects (Van Wees & Glazebrook, 2003).

The importance of competition as a factor in shaping insect communities sharing a plant has been strongly debated (reviewed by Denno *et al.*, 1995; Kaplan & Denno, 2007). Initially, following the (at that time) prevailing view in ecology, competition was considered to be a central factor shaping the distribution and diversity of insect herbivore communities. At the most extreme side, Janzen (1973) argued that essentially all insects feeding from a common host plant are in competition with one another, since they will have to share resources. Later, this view was severely criticized, for instance by Lawton & Strong (1981) who argued that factors like climatic conditions, host plant phenology and the distribution of host plants should be considered much more important when one aims to explain the structure of insect communities. Consequently, in the early 1980s competition for resources among herbivores was considered to be the exception rather than the rule. Yet, from the 1980s onwards, many experimental studies have appeared suggesting that negative, but also positive, interactions may frequently occur between plant parasites, even when their densities are relatively low, when they feed from different plant tissues and at different times, or when belonging to different feeding guilds (e.g., Faeth, 1986; Karban, 1986, 1989; Harrison & Karban, 1986; Stout *et al.*, 2006; Tack & Dicke, 2013). Hence, the result of these studies was that competition regained its central status in insect community ecology. Yet, relatively few studies have investigated the specific roles of the different defense pathways (JA, SA, ET) in these ecological interactions. Studies in which plants were sprayed with JA and/or with the commercial SA analogue benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) have shown that artificial induction of defense responses can influence the structure of natural communities of herbivores and pathogens associated with plants in the field (Inbar *et al.*, 2004).
Also, it has been shown that responses induced by one particular species can result in resistance to another (e.g., Long et al., 2007; Poelman et al., 2008; Mouttet et al., 2013) and the order of herbivore arrival on the plant can influence the performance and number of herbivore species occurring later in the season (Van Zandt & Agrawal, 2004; Viswanathan et al., 2005; Erb et al., 2011).

In addition to these field studies, laboratory experiments have been performed with model organisms, usually with the aim to reveal the effectiveness of SA- and JA-regulated defenses against attackers with different feeding styles. De Vos et al. (2006), making use of mutants impaired in the JA, ET or SA-pathway, reported that feeding by the JA-inducing caterpillar *Pieris rapae* did not, as was expected, induce resistance against the necrotrophic fungus *Alternaria brassicicola*. In contrast, feeding by the caterpillar reduced disease symptoms caused by *Pst*, but this effect was independent from JA, ET and SA. Thaler et al. (2010) showed that *Pst* induces JA, SA and activity of proteinase inhibitors in tomato plants, which reduces the growth of *Spodoptera exigua* caterpillars. Tobacco mosaic virus infections induced SA, but not JA or proteinase inhibitor activity, and this caused induced susceptibility to *S. exigua* caterpillars but induced resistance to aphids (*Myzus persicae*). Summarizing, induction of the JA and/or SA pathway may decrease (De Vos et al., 2006; Thaler et al., 2010) or increase (Thaler et al., 2010; Sarmento et al., 2011) the performance of competing organisms on the same host.

Only a few studies, on spider mites, whiteflies and aphids, have examined the effects of defense suppression on other species. Kant et al. (2008) showed that JA-defense suppressing spider mites had a small, but significant, positive effect on the performance of ‘inducer’ mites (i.e., mite genotypes that normally induce the JA- and SA-pathway) when sharing the feeding site with ‘suppressor’ mites. Subsequently, Sarmento et al. (2011) showed that *T. urticae* had an higher oviposition rate on leaf discs on which *T. evansi* had fed previously, whereas induction of defenses by *T. urticae* resulted in decreased oviposition by *T. evansi*. Zhang et al. (2009) showed that JA-defense suppressing whiteflies improved the performance of spider mites on Lima bean and Soler et al. (2012) found that the cabbage aphid (*Brevicoryne brassicae*) inhibits the production of JA in cabbage (*Brassica oleracea*) plants, and this correlated with increased growth and development of caterpillars of the large cabbage white butterfly (*Pieris brassicae*). Notably, in the whitefly-spider mite, as well as in the aphid-caterpillar example, suppression of JA-defenses was shown to be independent from SA, although the latter was induced by whiteflies.

As shown here, russet mites, by inducing SA, may increase the plant’s resistance to bacterial phytopathogens. Yet, at the same time, russet mites increase the reproductive performance of spider mites when these invade their feeding sites, and this, in turn, slows down growth of the russet mites. Hence, the russet mite-induced
changes that feed back into its community are due to a shift in the relative resistance or susceptibility of the shared host plants and this means our data confirm previous predictions that interactions among different phytophagous organisms can indeed be indirectly mediated by plants (Ohgushi, 2005). The results of our study also show that, unlike in the two above examples, crosstalk between the SA and JA pathway is likely to play a role in the spider mite-russet mite interaction. Furthermore, it shows that the fitness benefits of defense suppression by russet mites depend on the community structure and that such suppression may backfire when competitors take advantage of it as well. Finally, these interactions can be bidirectional since one parasite population may respond to plant defenses induced or suppressed by another and, subsequently, also vice versa. Taken together, we show that parasite-plant-parasite interactions are subject to bidirectional causality in that plant parasites induce plant responses that may positively or negatively impact on them and their competitors implicating that ultimately a parasite’s fitness depends on how they themselves alter the community context. Hence, these findings have major implications for understanding under which conditions induction and suppression of host plant defenses can be adaptive.

Acknowledgements
We thank Harold Lemereis, Ludek Tikovsky and Thijs Hendrix for taking care of our tomato plants and Alessandra Scala (Plant Physiology, UvA) for providing P. syringae. Also, we would like to thank all members of the Population Biology group as well as the Plant Physiology group for many useful comments, suggestions and stimulating discussions.

REFERENCES


CHAPTER 3 | COMPLEX EFFECTS OF TOMATO DEFENSE MANIPULATION


