Supporting Methods S1

High-throughput sequencing and de novo assembly

T. evansi whole transcriptome was sequenced from cDNA. Total RNA was isolated from mites of all stages using the Qiagen RNA extraction kit and 24 µg (S28/S18 = 1.71) was used for cDNA synthesis and library preparation. From the total RNA sample poly(A)+ RNA was isolated, which was used for cDNA synthesis. First strand cDNA synthesis was primed with a N6 randomized primer. Then 454 adapters A and B were ligated to the 5' and 3' ends of ds-cDNA. The cDNA was finally amplified with PCR using a proof reading enzyme. Normalization was carried out by one cycle of denaturation and reassociation of the cDNA, resulting in N1-cDNA. Reassociated ds-cDNA was separated from the remaining ss-cDNA (normalized cDNA) by passing the mixture over a hydroxylapatite column. After hydroxylapatite chromatography, the ss-cDNA was PCR amplified. For sequencing, the cDNA in the size range of 500–1100 bp was eluted from a preparative agarose gel and sequenced using 454 GS+ Titanium technology at Eurofins (MWG, Germany). The raw reads were submitted to the Sequence Read Archive (SRA) at NCBI under the accession number SRR2127882. After quality checks and filters a total of 1,558,090 high quality reads were subsequently used for transcriptome de novo assembly using MIRA (settings: denovo, est, accurate, 454, -SK:acrc=no, -CL:msvs) (Chevreux et al., 2004). The final assembly produced 31,263 isotigs, from which a subset of 17,663 isotigs assembled from 5 or more reads were used to predict their coding regions and protein sequences using ORF-predictor (Min et al., 2005).

Chevreux, B., Pfisterer, T., Drescher, B. et al. (2004) Using the miraEST assembler for reliable and automated mRNA transcript assembly and SNP detection in sequenced ESTs. *Genome Res*. **14**, 1147-1159.

Min, X.J., Butler, G., Storms, R. and Tsang, A. (2005) OrfPredictor: predicting protein-coding regions in EST-derived sequences. *Nucleic Acids Res*. **33**, W677-680.

Supporting Methods S2

RNA isolation and RT-qPCR

T. urticae and *T. evansi* main body parts (“idiosoma”) were collected after removal of the anterior body part (“gnathosoma”) using a scalpel on a glass Petri dish pre-cooled with liquid nitrogen. *N. benthamiana* agroinfiltrated or mock (i.e. the infiltration-buffer without bacteria) treated leaves were collected and immediately frozen in liquid nitrogen. After material grinding, total RNA was collected using the Qiagen RNA extraction kit and treated with Turbo-DNAse (Ambion) as described by the manufacturer. Subsequently, DNA-free samples were used for cDNA synthesis using M-MuLV RT (Fermentas), as described by the manufacturer. Reverse Transcriptase qPCR (RT-qPCR) was performed using EVA green (Biotium) by means of the ABI 7500 Real-Time PCR system (Applied Biosystems). Reactions were performed in a volume of 10-µl, containing 0.25 µM of each primer, 0.1 µl ROX reference dye and 1 µl of cDNA template (20 ng/µl). The cycling program was set to 5 min of pre-cycling stage (50°C), 5 min at 95°C, 45 cycles of 15 sec at 95°C and 1 min at

60°C. The program was followed by a melting curve analysis. For spider mite RT-qPCR assays *T. urticae* 18S rRNA, and *T. evansi* Ribosomal Protein 49 were used as housekeeping genes. For *N. benthamiana* RT-qPCR assays, actin was used as housekeeping gene. All primer pairs used are described in Supplemental Table S2. Statistical differences of transcript abundances shown in Figure 4, Supplemental Figures S6 and S7 were calculated by using a General Linear Model (GLM) analysis in SPSS 20 (IBM). Statistical differences shown in Supplemental Figure S1 were calculated using the Student's t-test in Excel (Microsoft).

Alba, J.M., Schimmel, B.C.J., Glas, J.J., Ataide, L.M.S., Pappas, M.L., Villarroel, C.A., Schuurink, R.C., Sabelis, M.W. and Kant, M.R. (2015) Spider mites suppress tomato defenses downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytol*. **205**, 828–840

Supporting Methods S3

Phytohormone extraction and LC-MS

Phytohormone analysis was performed as described in Alba et al. (2015). Briefly, between 80 to 150 mg of frozen leaf material was homogenized in 1ml of ethylacetate, which had been spiked with D6-SA and D5-JA (C/D/N Isotopes Inc., Canada) as internal standards to a final concentration of 100 ng/ml. After centrifugation at 13000 rpm for 10 min at 4°C, the supernatant was transferred to new tubes. The pellet was re-extracted with 0.5 ml of ethylacetate (without the two internal standards) and centrifuged for 10 min at 4°C at 13000 rpm. Both supernatants were combined and then evaporated to dryness on a vacuum concentrator. The residue was resuspended in 0.5 mL 70% methanol (v/v), centrifuged and the supernatants were transferred to glass tubes and then analyzed by LC-MS/MS. Measurements were performed on a liquid chromatography tandem mass spectrometry system (Varian 320 Triple Quad LC/MS/MS). SA and JA were quantified by comparing their peak area with the peak area of the respective internal standard. Statistical differences in the amounts of phytohormones among samples were calculated using log-transformed values by Fisher's LSD test after ANOVA (SPSS 20, IBM).

Alba, J.M., Schimmel, B.C.J., Glas, J.J., Ataide, L.M.S., Pappas, M.L., Villarroel, C.A., Schuurink, R.C., Sabelis, M.W. and Kant, M.R. (2015) Spider mites suppress tomato defenses downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytol*. **205**, 828–840

Supporting Methods S4

In situ hybridization

Primers for Te84 and Te28 probe-template amplification were designed using Primer3 (http://bioinfo.ut.ee/primer3/). The preferred amplicon length was about 300bp. The resulting

primers are listed in Supplemental Table II. RNA was extracted from T. evansi mites (Total RNA Isolation Mini Kit, Agilent), treated with TURBO DNA-freeTM Kit to remove contaminating genomic DNA and used for cDNA synthesis using Maxima First Strand cDNA Synthesis Kit. PCR products were cloned into pGEMT-T plasmids (Promega) and transformed into E. coli. Plasmids of liquid cultures were purified after which insert orientation and nucleotide sequence were checked by sequencing (LGC Genomics, Germany). A PCR was performed on the plasmids using pUC/M13 primers. PCR products, containing inserts flanked by T7 and SP6 promoter sites from the plasmid backbone, were checked by agarose gel electrophoresis and purified using Cycle Pure Kit (EZNA). Depending on the orientation, sense or anti-sense DIG-labeled probes were generated using T7 or SP6 RNA polymerase (Roche), using the pUC/M13 PCR product and DIG-UTPs (Roche) in the in vitro labeling reaction. Probes were then purified using SigmaSpinTM Sequencing Reaction Clean-Up Columns (Sigma).

T. evansi larvae and adults of both sexes were collected from tomato plants and fixed in a 1:1 mix of heptane and PTw (PBS with 0.1% Tween-20) containing 4% formaldehyde. The mites were then washed in methanol and PTw, followed by sonication in a sonic cleansing bath and treatment with 5 mg/ml Proteinase K during 10 minutes. The mites were then re-fixed with 4% formaldehyde in PTw. Mites were prehybridized in hybridization buffer (50% formamide (Sigma), 4x SSC (Sigma), 1x Denhardt's solution (Sigma), 250 μg/ml tRNA (wheat germ type V, Sigma), 250 μg/ml ssDNA (boiled salmon sperm DNA, Sigma), 50 μg/ml heparin (sodium salt, Sigma), 0.1% Tween-20 (Sigma), 5% dextran sulfate (sodium salt, Sigma) for 1h at 52°C. Hybridization buffer was refreshed and probe was added. The mites were then incubated overnight at 52°C. Washing occurred at 53°C (6 times 25 min) with wash buffer constituting of 50% formamide, 2x SSC and 0.1% tween-20. After washing at room temperature (RT) with PBTw (PTw with 0.1 % BSA (Sigma)), the mites were incubated at RT for 2h with 1:1000 dilution of anti-digoxigenin-AP (Fab fragments, Roche) in PBTw. The mites were then washed with PTw (5 times 20 minutes) and several times with AP buffer (100 mM Tris pH 9.5, 100mM NaCl, 1 M MgCl2, 0.1% Tween-20) until precipitation had disappeared. AP buffer containing the NBT/BCIP (nitro-blue tetrazolium/5-bromo-4-chloro-3'-indolyphosphate) (Roche) or FastRed substrate (SIGMAFASTTM Fast Red TR/Naphthol AS-MX tablets, Sigma) was added and mites were incubated several hours at 4°C in the dark, until blue or red staining was visible. Methanol was used to eliminate background staining and mites were cleared in 70% glycerol in PTw (pH 8.5) after washing with pure PTw. Finally, mites were mounted on microscopy glasses for further investigation.

FastRed-labeled spider mites were visualized using a laser-scanning microscope LSM510 (Zeiss), by exciting samples sequentially with the 458 nm (spider mite autofluorescence) and 543 nm (FastRed fluorescence) lasers. Mite autofluorescence and FastRed fluorescence emissions were detected sequentially by using the long pass filter LP 560. Z-stacks were performed by imaging up to 25 slices every 1-1.5 μm. Z-stacks were superposed by using ImageJ z-project (max intensity).