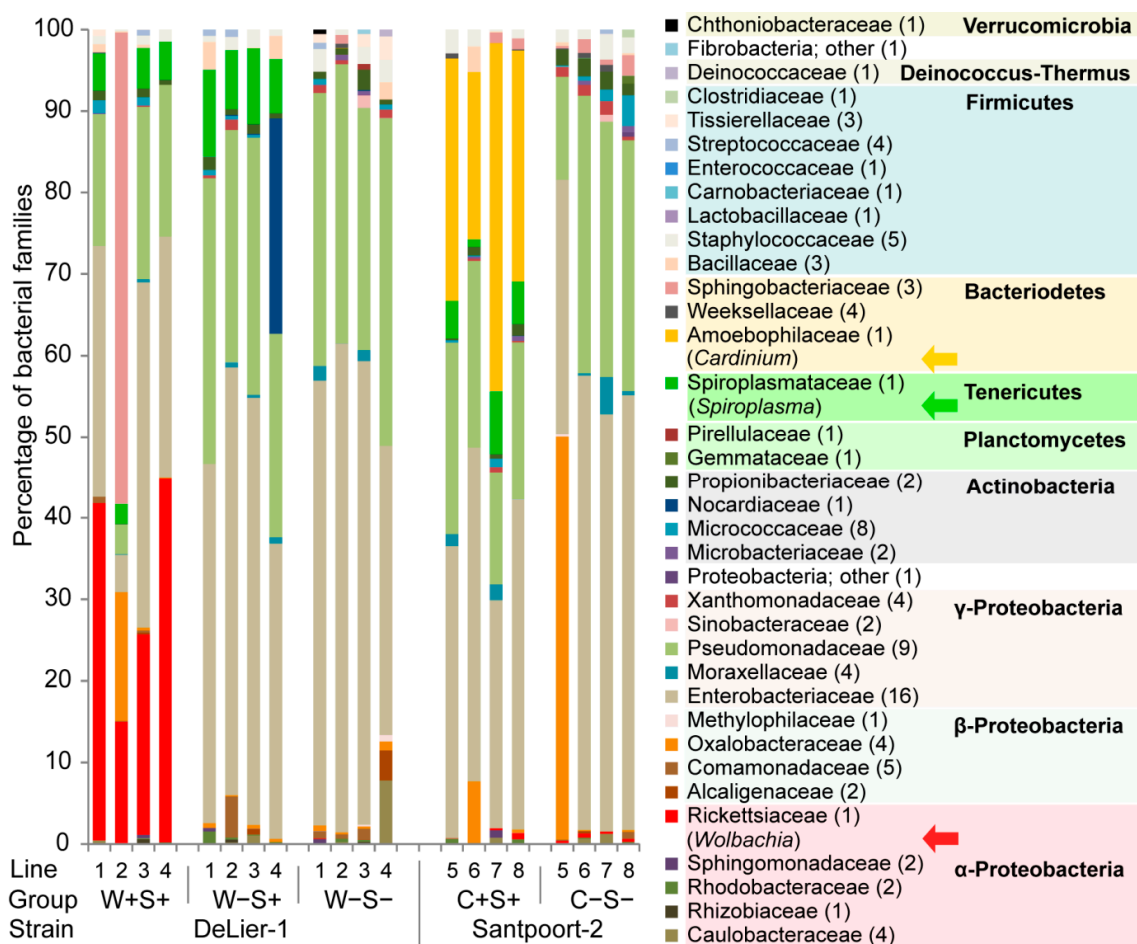
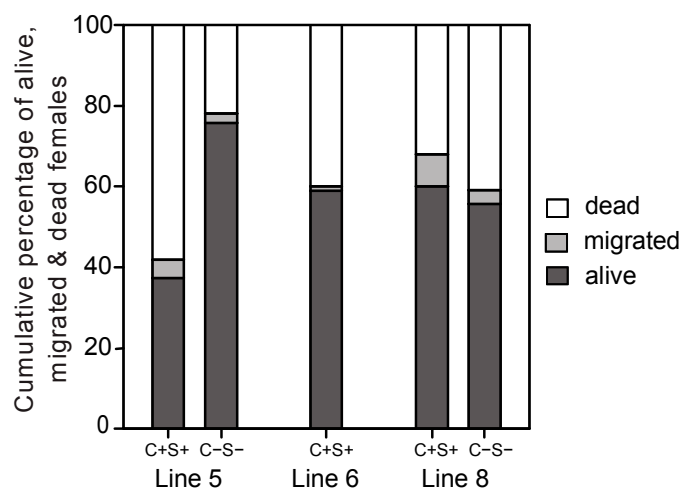


# Supplementary Materials: Independent Effects of a Herbivore's Bacterial Symbionts on Its Performance and Induced Plant Defences

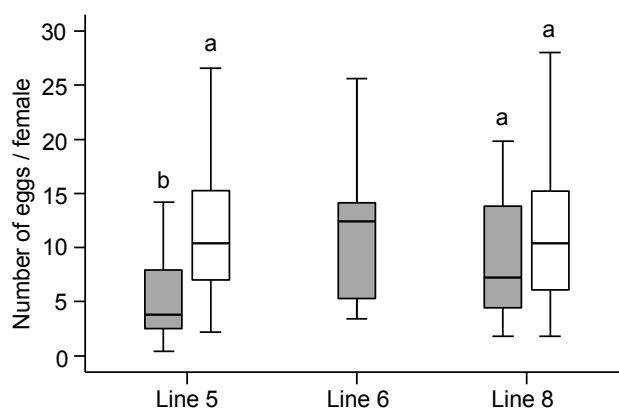
Heike Staudacher, Bernardus C. J. Schimmel, Mart M. Lamers, Nicky Wybouw, Astrid T. Groot and Merijn R. Kant



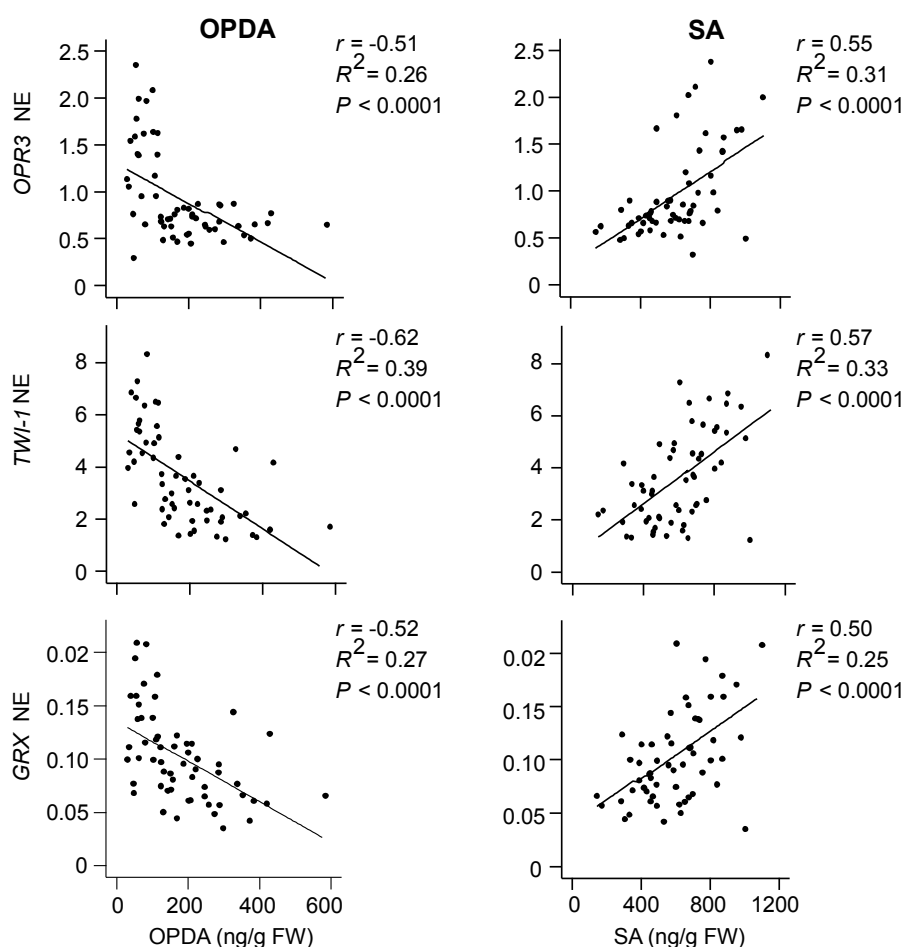
**Figure S1.** Bacterial community composition of two strains of the spider mite *Tetranychus urticae*, assessed with Illumina 16S rRNA amplicon-sequencing. The two strains had been treated with tetracycline and hence did (+) or did not (-) contain *Wolbachia* (W), *Spiroplasma* (S) and/or *Cardinium* (C); The DeLier-1 strain with three groups: W+S+, W-S+ and W-S-; The Santpoort-2 strain with two groups: C+S+ and C-S-; 4 lines per group. Bacterial OTUs were combined at the family level. Numbers after family names in parentheses indicate how many OTUs of one family were combined. Rare OTUs that were overall represented less than 0.5% are not shown.



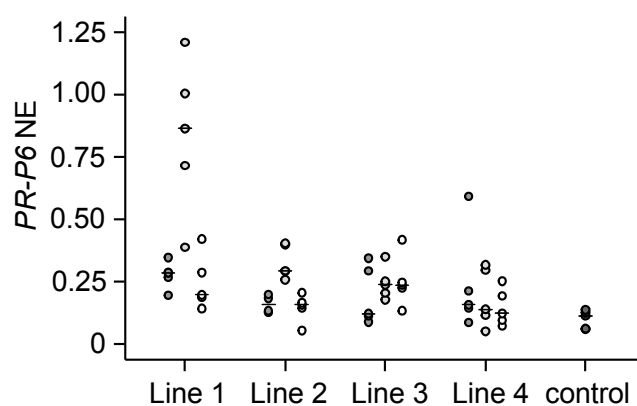
**Figure S2.** Survival, migration and mortality of the Santpoort-2 strain of the spider mite *T. urticae* which did (+) or did not (-) contain *Spiroplasma* and *Cardinium* after feeding on tomato (*Solanum lycopersicum*) for four days. Two lines (5 and 8) were tested with two mite groups: C+S+ and C-S-. Additionally, we tested C+S+ of line 6. C-S- of line 6 as well as both C-S- and C+S+ of line 7 went extinct before the experiment.



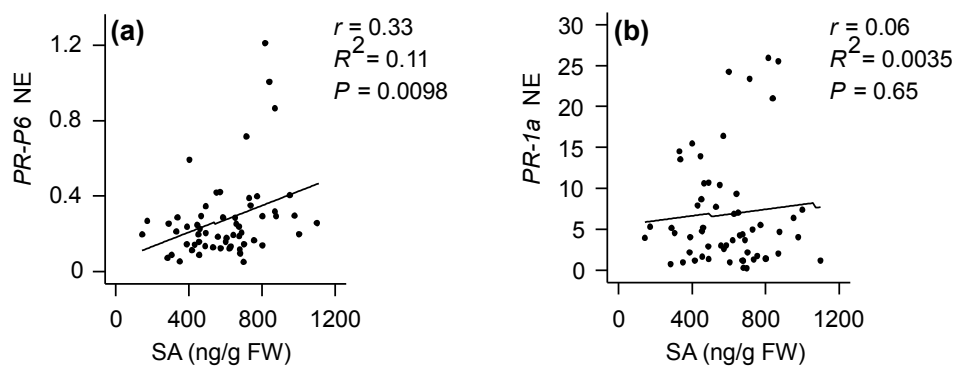
**Figure S3.** Reproductive performance (number of eggs produced per female in four days) of two lines (5 and 8) of the Santpoort-2 strain of the spider mite *T. urticae*. Each of the lines was subdivided into two groups which did (+) or did not (-) contain the bacteria *Cardinium* and *Spiroplasma*: C+S+ (grey) and C-S- (white). Additionally we tested C+S+ of line 6; C-S- of line 6 as well as both C-S- and C+S+ of line 7 went extinct before the experiment. Boxes span the 25–75 percentiles, horizontal lines in the boxes represent medians, whiskers span  $1.5 \times \text{IQR}$ , dots represent data points outside of this range. Different letters above the boxes indicate significant differences at a level of  $p \leq 0.05$  (tested per line) after applying a linear mixed model.



**Figure S4.** Correlations between phytohormone amounts of salicylic acid (SA) and 12-oxo-phytodienoic acid (OPDA) and the normalized expression levels of putative OPDA responsive genes (*OPR3*, *GRX* and *TWI-1*) measured in tomato leaflets after seven days of infestations with the Delier-1 strain of the spider mite *T. urticae*. *p*-Values were adjusted for multiple comparisons with the Holm method.



**Figure S5.** Normalized expression (NE) of salicylic acid defence marker gene *PR-P6* (qRT-PCR) in tomato (*S. lycopersicum*) leaflets after seven days of infestation with four lines (1, 2, 3 and 4) of the Delier-1 strain of the spider mite *T. urticae*. Each of the lines was subdivided into three groups which did (+) or did not (-) contain the bacteria *Wolbachia* and *Spiroplasma*: W+S+ (dark grey), W-S+ (light grey) and W-S- (white). Control plants were not infested (darkest grey); Circles represent individual data points, horizontal lines indicate the medians.



**Figure S6.** Correlations between phytohormone amounts of salicylic acid (SA) and the normalized expression levels of the two SA marker genes *PR-P6* and *PR-1a*, measured in tomato leaflets after seven days of infestations with the Delier-1 strain of the spider mite *T. urticae*. *p*-values were adjusted for multiple comparisons with the Holm method.

**Table S1.** Nucleotide sequence of primers used for PCR and qRT-PCR analysis.

Target Organism(s)	Target Gene	Name	Gene Identifier (BOGAS/ITAG2.3)	Forward Primer 5' → 3'	Reverse Primer 5' → 3'	References
<i>Wolbachia pipientis</i>	16S rRNA	16S ribosomal RNA	-	TTGTAGCCTGCTATGGTATAACT	GAATAGGTATGATTTTCATGT	[1]
<i>Cardinium</i> sp. (Cytophaga-Like Organism, CLO)	16S rRNA	16S ribosomal RNA	-	GCGGTGTA AAAATGAGCGTG	ACCTMTTCTTA ACTCAAGCCT	[2]
<i>Spiroplasma</i> sp.	<i>dnaA</i>	<i>DnaA</i>	-	ATTCTTCAGTAAAAATGCTTGGGA	ACACATTTACTTCATGCTATTGA	[3]
Bacteria (general)	16S rRNA	16S ribosomal RNA	-	TCCTACGGGNGGCWGCAG	TGACTACHVGGGTATCTAAKCC	LGC Genomics (Berlin, Germany); modified from [4]
<i>Tetranychus urticae</i>	<i>Actin</i>	<i>Actin</i>	Tetur03g09480	CAGCCATGTATGTTGCCATC	AAATCACGACCAGCCAAATC	[5]
<i>Solanum lycopersicum</i>	<i>OPR3</i>	<i>OPDA Reductase 3</i>	Solyc07g007870.2	GATCCAGTTGTGGGATACACAG	GCCCAACAAAATCAGGTTTC	[6]
<i>Solanum lycopersicum</i>	<i>TWI-1</i>	<i>Tomato Wound-Induced 1</i>	Solyc01g107820.2	CATCTTACAATGGATGGGCTAC	CGAGATGATTGATCTTGGATTC	[7]
<i>Solanum lycopersicum</i>	<i>GRX</i>	<i>Glutaredoxin</i>	Solyc07g053550.1	ATGATGCAACAAGCACTTCC	GATGATGTCCGATCAACTCTTGG	This study
<i>Solanum lycopersicum</i>	<i>JIP-21</i>	<i>Jasmonate-Inducible Protein 21</i>	Solyc03g098790.1	ACTCGTCTGTGCTTTGTCC	CCCAAGAGGATTTTCGTTGA	[8]
<i>Solanum lycopersicum</i>	<i>TD-2</i>	<i>Threonine Deaminase-2</i>	Solyc09g008670.2	TGCCGTAAAAAATGTCACCA	ACTGGCGATGCCAAAATATC	[9]
<i>Solanum lycopersicum</i>	<i>PR-1a</i>	<i>Pathogenesis-Related Protein 1a</i>	Solyc09g007010.1	TGGTGGTTCATTTCTGCAACTAC	ATCAATCCGATCCACTATCATTTTA	[10]
<i>Solanum lycopersicum</i>	<i>PR-P6</i>	<i>Pathogenesis-Related Protein 6</i>	Solyc00g174340.1	GTA CTGCATCTTCTTGTTTCCA	TAGATAAGTCTTGATGTGCC	[10]
<i>Solanum lycopersicum</i>	<i>PI-IIc</i>	<i>Proteinase Inhibitor IIc</i>	Solyc03g020050.2	CAGGATGTACGACGTGTGC	GAGTTTGCAACCCCTCTCCTG	[11]
<i>Solanum lycopersicum</i>	<i>Actin</i>	<i>Actin</i>	Solyc03g078400.2	TCAGCACATTCCAGCAGATGT	AACAGACAGGACACTCGCACT	[12]

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