Supplementary Material

1 Supplementary methods

1.1 Time-lapse video recording of mites on honeysuckle

To produce a time-lapse video that showing the sequence of steps leading to the production of sticky droplets on honeysuckle, the bottom (abaxial) surface of a leaf in one experimental arena was infested with five adult females from a specialist line (C1N1a), and a separate arena was infested with five adult females from a generalist line (C2N3a). A Nikon D750 camera with a macro 60mm lens and a ring LED lamp was mounted on a vertical stand and positioned above the experimental arena. Recording started one minute after mites were added to the arenas. Pictures were taken every 30sec for 24 consecutive hours with an automated shutter. We assembled the pictures into time-lapse videos with a speed of 10 frames per second (fps). A similar methodology was used to produce a time-lapse video of a honeysuckle arena with sticky droplets that had formed following herbivory by generalist *T. urticae* females, and into which two adult *A. andersoni* females were introduced, except that the pictures were taken every 20 seconds in order to record predation events.

1.2 Quantification of sticky droplet production upon herbivory by different mite populations and treatments

We infested experimental arena with five adult females from the specialist or the generalist lines to quantify the number of sticky droplets produced. The experiment was separated into two blocks; leaves for each arena were collected from three to four plants. We included three controls to dissect the main effect of mite genotype on the amount of sticky droplets produced: 1) a non-infested control to assess the effect of the overall set-up, 2) a control where we punctured the leaves with a patterned wheel to control for the effect of physical damage to the leaves, and 3) a treatment with western flower thrips (*Frankiniella occidentalis*) larvae to assess whether thrips feeding also induced droplet production (n=4 replicates for all treatments and controls). The number of droplets was counted every day for three consecutive days. Since the droplets of each day remained on the leaf surface throughout the duration of the experiment, we compared the total number of droplets between treatments by the 3rd day of the experiment using a linear mixed-effects model (package *lme4* in R v3.6.1), (log+1)-

transformed to improve model fit, with mite population with three levels (one specialist and two generalists) as fixed factor, and the experimental block (2 levels) and iso-female line as random factors. A p-value was obtained using a Satterthwaite's approximation. Significant differences were further analysed between treatments using generalized linear hypothesis with a Tukey correction for multiple testing.

1.3 Sticky droplet production relative to mite density

We investigated whether the number of droplets produced on honeysuckle leaves upon mite herbivory depended on the density of mites, and whether any differences found would depend on the mite genotype. Experimental honeysuckle arenas with the abaxial surface up were infested for 24 hours with either adult specialist females (line C1N1a) or with adult generalist females (line C2N3a) to create three density treatments. To account for differences in the number of droplets produced by specialist or generalist mites we adjusted the number of mites used per line: for the generalist line, either one, six or twelve mites were placed on each arena (n = 10 replicates per treatment). For the specialist line, either five, fifteen or thirty mites were placed on each arena (n = 6 replicates per treatment). We performed a linear regression on the number of droplets against the number of females alive after 24 hours and compared the slope of the regression line between mite lines using a linear model with an interaction term between number of mites and mite line as fixed factor.

1.4 Effect of phytohormones on sticky droplet production

To determine whether the production of sticky droplets is regulated by phytohormones, and whether this effect depends on mite genetic background, we collected multiple honeysuckle twigs (stem with one leave) from five different plants, and embedded their basal end into a 15ml tube containing sterilised tap water. The lid of each tube was punctured and the stem of each twig was passed through the puncture to hold it upright; a lanoline barrier was added to the junction of lid and stem. All replicates (n=120 twigs in a tube) were kept in sterilized tap water for three days. Then, water was discarded and replaced with one of three solutions: a jasmonic acid [0.05mM] + isoleucine [1mM] solution, a salicylic acid solution [0.05mM], or a mock treatment as a control solution of sterilized water with 1% methanol, which was used because the JA and SA stock solutions were diluted in methanol. Concentrations were

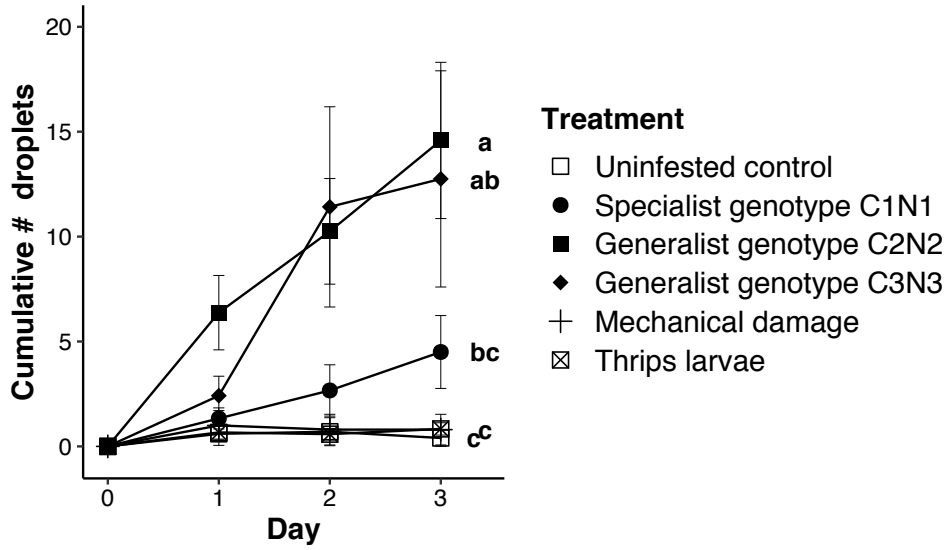
based on the experimental design described by Ataide *et al.* (2016). We infested 30 replicates, 10 replicates per treatment solution, with five adult specialist females (line C1N1a), and 30 replicates with five adult generalist females (line C2N3a). Another set of 30 replicates were not infested with mites, but were subjected to mechanical damage daily by using a fine leaf puncher to assess the effect of physical damage, and a fourth set of 30 replicates were left undamaged and without mites to assess the impact of the overall set-up. All treatments started at the same time, but half of the replicates across treatments were sampled two days post infestation (dpi) and the other half were sampled 4 dpi. Females that died or escaped from the leaves were replaced with females from the same line daily. The total number of sticky droplets was counted at each time point (2 or 4 dpi). The number of sticky droplets was compared between the three treatment solutions within each day post infestation, within each treatment, using a linear model (package *aov* in R v3.6.1) with 'treatment solution' as the fixed factor. Significant differences were further analysed using a generalized linear hypothesis test with a Tukey correction for multiple testing.

1.5 Sugar and amino acid quantification of sticky droplets

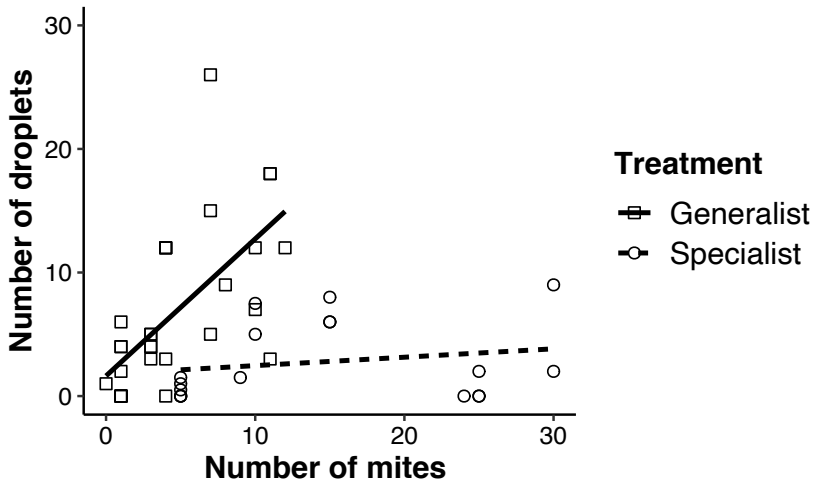
The amounts of sucrose, fructose, glucose and amino acids in the droplets were analysed using High-Performance Liquid Chromatography (HPLC). For the quantification of the three sugars, honeysuckle leaves collected from three plants were infested for 24hr with 5-10 adult females from the specialist (C1N1a) or generalist (C2N3a) line. We collected three samples from leaves infested with each mite line using capillary tubes, each sample containing 0.5-1µl of droplets, placed immediately after collection in 150µl double-distilled water in a HPLC vial for 24hr to allow the sugars to dissolve. Samples were analysed with HPLC-UV/VIS_RID (LC-20AT, Prominence, Shimadzu) on an ion-exclusion Rezex ROA-Organic Acid H+(8%) column (300x 7.8mm; Phenomenex) with guard column (Phenomenex) and 5mN H_2SO_4 in double-distilled water (18.2MΩ) as mobile phase. The isocratic flow rate was set at 0.2ml/min for 40min to allow full separation of the sucrose, glucose and fructose peaks. The injection volume was 15µl (Autosampler: SIL-20AC, Prominence, Shimadzu) and the column oven temperature was set at 55ºC (CTO-20A, Prominence, Shimadzu). Detections were performed with RID (Refractive Index Detector: RID 20A, Shimadzu). For amino acid quantification, we used the protocol described in Gao *et al.* (2016), except we did not clean up samples before analyses. Honeysuckle leaves were infested for 24hr with 5-10 adult specialist females (line C1N1a) or adult generalist females (line C2N3a) females. Approximately 5µl of droplets were collected per sample in

capillary tubes, placed inside an HPLC vial with 50µl double-distilled water immediately after collection (n = 3 samples per mite line). This solution was further diluted with 450µl acetonitrile in H_2O (90%) with formic acid 0.1% [v/v]. Internal standards for the three sugars and amino acids were run in parallel with the samples. Amounts of each compound obtained after peak integration relative to internal standards were subsequently corrected for the volume of droplet collected per sample. We were unable to collect enough droplets for the amino acid quantification procedure in replicates upon which specialist mites fed.

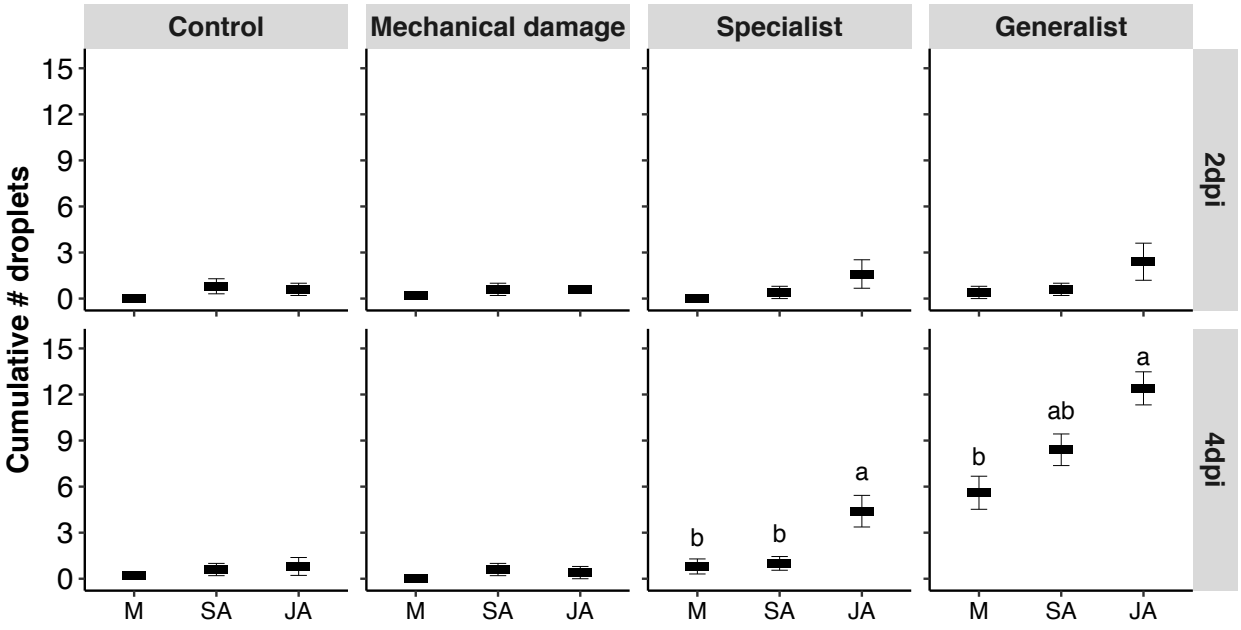
2 Supplementary Figures



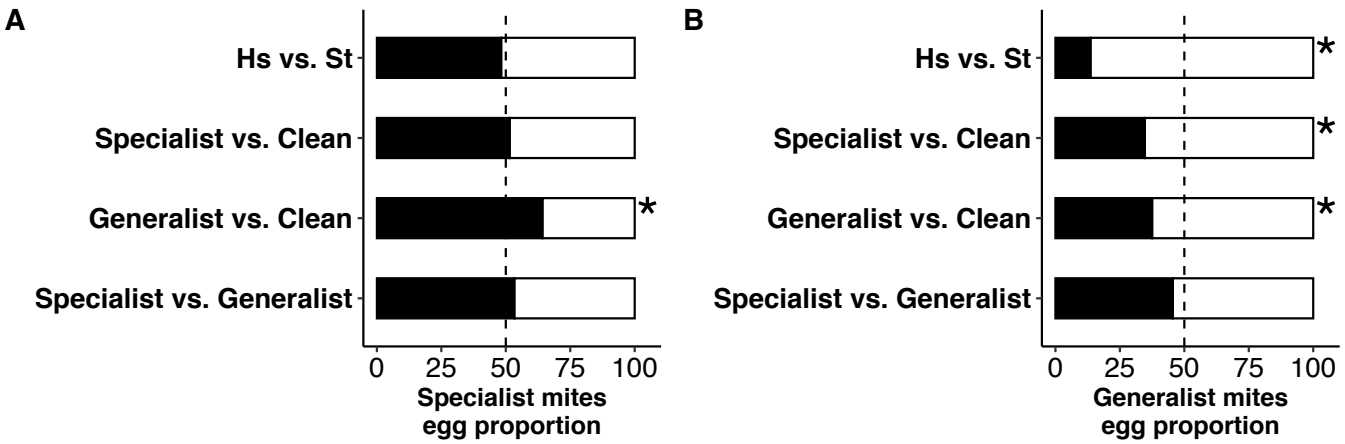
Supplementary Figure 1. Cumulative number of droplets (mean ± one standard error of the mean [SEM]) exuded per day on honeysuckle leaves (x-axis). *Tetranychus urticae* populations of the honeysuckle race (specialist genotype C1N1), and two sympatric generalist populations (generalist genotypes C2N2 and C3N3) were represented by 3 field-derived lines with 4 replicates each, averaged for plotting. Controls include arenas without mites; arenas with mechanical damage inflicted manually; and arenas with thrips (*Frankliniella occidentalis*) larvae. Letters represent significant differences between treatments.



Supplementary Figure 2. Sticky droplet production relative to mite density**.** Each data point represents the number of mites present on a honeysuckle leaf for 24 hours (x-axis) and the number of droplets produced (y-axis). Mites belonged either to a generalist or a specialist line (legend).



Supplementary Figure 3. Cumulative number of droplets (mean ± SEM) produced on honeysuckle leaves in four different treatments at two time points. Treatments: Control, leaves without mite herbivory; Mechanical damage, inflicted mechanically with a puncturing tool; Specialist, herbivory by a honeysuckle specialist line; Generalist, herbivory by a generalist line. Number of droplets were counted at two time points, 2 days post infestation with mites (dpi) and at 4dpi. Per treatment, twigs were embedded in either a Mock solution (M), a JA+ile dilution (JA) or a SA dilution. Letters represent significant differences between solutions, within a panel.



Supplementary Figure 4. Preference for oviposition by *Tetranychus urticae*. In a two-choice experiment, either choice 1 (black bars, left) or choice 2 (white bars, right) in each row were given to adult females; 'choice1 vs. choice2' in the labels of the y-axis. **A**) Specialist mite preference. **B**) Generalist mite preference. Hs = honeysuckle; St = spindle tree; Specialist = leaves with previous herbivory by specialist mites; Generalist = leaves with previous herbivory by generalist mites; Clean = leaves without previous herbivory. Stars represent significant differences from a 50:50 probability.

3 Supplementary Tables

Supplementary Table 1. Sugar composition of the sticky droplets produced by honeysuckle leaves upon mite herbivory. Concentrations of three major sugars (average ± SEM) were quantified from droplets produced upon feeding by a specialist and a generalist line of *Tetranychus urticae* (n=3).

Mite genotype	[ηg/µL]	Glucose	Fructose	Sucrose
Specialist	Average	30.47	42.46	8077.91
	SEM	3.44	10.15	896.27
	%	<0.001	<0.001	99
Generalist	Average	27.01	53.43	14487.27
	SEM	2.32	24.82	2636.24
	%	<0.001	<0.001	99

Supplementary Table 2. Amino acid composition of the sticky droplets produced by honeysuckle leaves upon mite herbivory.

	Average [µg/ml]	SEM	Percentage
asp	12.19	2.02	25.68
glu	9.26	0.56	19.51
gln	6.87	1.11	14.47
lys	5.16	0.45	10.88
ala	2.42	0.43	5.10
thr	2.39	0.40	5.03
hit	2.31	0.63	4.88
ser	1.29	0.33	2.73
val	1.13	0.37	2.38
asn	0.96	0.28	2.03
phe	0.79	0.22	1.67
tyr	0.63	0.17	1.32
leu	0.57	0.21	1.19
ile	0.49	0.08	1.03
gly	0.46	0.05	0.98
arg	0.29	0.05	0.62
pro	0.24	0.06	0.51
met	NA	NA	NA
sar	NA	NA	NA
trp	NA	NA	NA

4 Supplementary Videos

Supplementary video 1. Generalist spider mites on honeysuckle leaves. Time-lapse video showing the sequence of steps leading to the production of sticky droplets on honeysuckle leaves after herbivory by generalist *Tetranychus urticae* mites. https://doi.org/10.21942/uva.c.5617300

Supplementary video 2. Specialist spider mites on honeysuckle leaves. Time-lapse video showing the lack of production of sticky droplets on honeysuckle leaves after herbivory by honeysuckle-specialist *Tetranychus urticae* mites. https://doi.org/10.21942/uva.c.5617300

Supplementary video 3. Predatory mites on honeysuckle leaves. Time-lapse video showing the interaction between honeysuckle leaves with sticky droplets, the herbivore mites (*Tetranychus urticae*) feeding on the leaves, and omnivore mites (*Amblyseius andersoni*). https://doi.org/10.21942/uva.c.5617300