

# Supplementary materials

## A sex-specific trade-off between pesticide resistance and tolerance to heat-induced sterility in *Tetranychus urticae*

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**Table S1 Number of replicates analysed in each experiment performed.** a) the effect of high developmental temperatures on life-history traits; b) the effect of the interaction between pesticide resistance and high developmental temperature on life-history traits. “Resistance status”: the individuals tested could be from a population resistant or susceptible to the pesticide etoxazole; “Treatment”: developmental temperatures (25, 33, 35, 36 or 37°C in a), and 25 or 36°C in b)) of the offspring (in variables “Developmental time (♀) and (♂)” and “Number of surviving juveniles”) or of the female and male crossed (in variables “Daily fecundity”, “Offspring number” and “Offspring sex ratio”). In all response variables, replicates with damaged (*i.e.*, harmed during manipulation) mothers (in variables “Developmental time (♀) and (♂)” and “Number of surviving juveniles”) or damaged individuals forming the mating pairs (in variables “Daily fecundity”, “Offspring number” and “Offspring sex ratio”) were excluded from the analyses; in all response variables except “Daily fecundity”, females that did not lay eggs were excluded from the analyses. <sup>a</sup> Due to the low number of replicates (see Table S2), 37°C was not considered in the statistical analyses. <sup>b</sup> Due to logistic constraints, temperatures were not tested simultaneously; instead, three assays were performed, always including the control temperature, and testing 33 and 37 together, 35 alone or 36°C alone; as such, three values are provided, each corresponding to a different assay following the previously stated order. <sup>c</sup> The values of offspring number and sex ratio were taken from the same patches

a)

Response Variable	Resistance status	Treatment	No. of replicates
Developmental time (♀) <sup>a</sup>	Susceptible	25	31/32/31 <sup>b</sup>
		33	21
		35	23
		36	23
Developmental time (♂) <sup>a</sup>	Susceptible	25	29/31/30 <sup>b</sup>
		33	20
		35	23
		36	22
Number of surviving juveniles <sup>a</sup>	Susceptible	25	31/32/31 <sup>b</sup>
		33	22
		35	30
		36	30
Daily fecundity	Susceptible	♀25 x ♂25	37/36/36 <sup>b</sup>
		♀33 x ♂25	33
		♀35 x ♂25	34
		♀36 x ♂25	34
		♀37 x ♂25	39
Offspring number	Susceptible	♀25 x ♂25	37/36/33 <sup>b</sup>

	♀33 x ♂25	33
	♀35 x ♂25	28
	♀36 x ♂25	31
	♀37 x ♂25	25

b)

Response Variable	Resistance status	Treatment	No. of replicates
Developmental time (♀)	Susceptible	25	28
		36	20
	Resistant	25	28
		36	21
Developmental time (♂)	Susceptible	25	26
		36	20
	Resistant	25	27
		36	22
Number of surviving juveniles	Susceptible	25	28
		36	28
	Resistant	25	29
		36	29
Offspring number and Offspring sex ratio <sup>c</sup>	Susceptible	♀25 x ♂25	38
		♀25 x ♂36	40
		♀36 x ♂25	24
		♀36 x ♂36	21
	Resistant	♀25 x ♂25	39
		♀25 x ♂36	40
		♀36 x ♂25	13
		♀36 x ♂36	18

**Table S2 Survival and oviposition of adult females exposed to different temperatures.** Females developed at 25°C in cohorts were maintained at 25°C or were transferred to one of the four high temperatures (33, 35, 36 or 37°C) for 3 days, after which they were isolated to oviposit for 24 hours at the same temperature. A minimum of 60 and a maximum of 200 females were transferred to high temperature for 3 days. “No. alive ♀ at the time of isolation”: a maximum of 32 alive adult females (*i.e.*, maximum number of replicates created) were isolated on a patch after 3 days at high temperature. “No. ovipositing ♀ (% ovipositing ♀)”: number and percentage of females that laid at least one egg during the oviposition period. At 37°C, the survival and percentage of ovipositing of adult females was very low, which led to the exclusion of the data obtained at this temperature from the experiment. <sup>a</sup> Due to logistic constraints, temperatures were not tested simultaneously; instead, three assays were performed, always including the control temperature. 33 and 37°C were tested together, and 35 and 36°C were tested alone. As such, three values are provided, each corresponding to a different assay following the previously stated order.

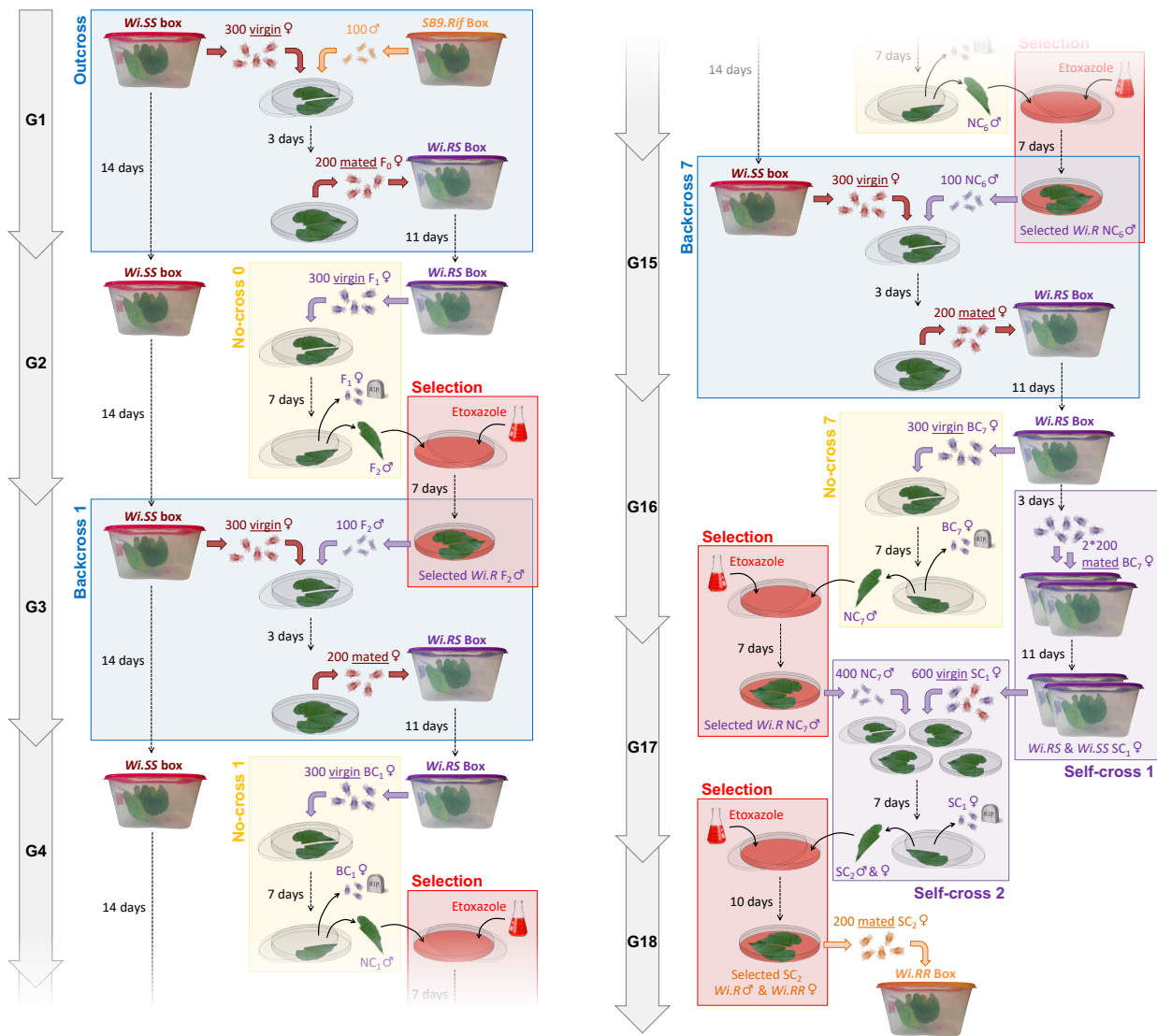
Temperature	No. alive ♀ at the time of isolation	No. ovipositing ♀ (% ovipositing ♀)
25°C	32/32/32 <sup>a</sup>	31/32/31 <sup>a</sup> (96.9/100/96.9%)
33°C	26	22 (84.6%)
35°C	32	30 (93.8%)
36°C	32	32 (100%)
37°C	6	3 (50%)

**Table S3 Description of the statistical models used to analyse the effect of high developmental temperatures on life-history traits.** “Sample size”: total number of replicates included in each analysis. “Maximal model”: complete set of explanatory variables included in the model. “Minimal model”: model containing only the variables that were statistically significant; round brackets indicate that the variable was included as a random factor. Within R subroutine, “lm”: linear model, “lmer”: linear mixed-effects model. Square brackets indicate the error structure (“g”: gaussian). “Developmental temperature”: temperature at which eggs were exposed until adulthood; “Fecundity”: the difference between the number of eggs laid at a high temperature and the mean number of eggs laid at control temperature (25°C); “Temperature of pair”: developmental temperature of both females and males paired to mate and oviposit (offspring developed at 25°C). “ $\bar{x}$ ”: mean trait value. <sup>a</sup> only includes replicates in which fecundity was above zero. <sup>b</sup> due to the low number of replicates (see Table S2), 37°C was not considered in the statistical analyses. Replicates with damaged (*i.e.*, harmed during manipulation) mothers or individuals forming the mating pairs were excluded in all analyses

Var. of interest	Response variable	Sample size	Maximal model	Minimal model	R subroutine [err struct.]
Developmental time (♀)	Day of 1 <sup>st</sup> adult ♀ at high developmental temperature - $\bar{x}$ (day of 1 <sup>st</sup> adult ♀ at 25°C)	161 <sup>a, b</sup>	Developmental temperature	Developmental temperature	lm [g]
Developmental time (♂)	Day of 1 <sup>st</sup> adult ♂ at high developmental temperature - $\bar{x}$ (day of 1 <sup>st</sup> adult ♂ at 25°C)	155 <sup>a, b</sup>	Developmental temperature	Developmental temperature	lm [g]
Number of surviving juveniles	Adult offspring at high developmental temperature - $\bar{x}$ (adult offspring at 25°C)	176 <sup>a, b</sup>	Developmental temperature + (Fecundity)	Developmental temperature + (Fecundity)	lmer [g]
Daily fecundity	Daily fecundity from ♀ developed at high temperature - $\bar{x}$ (daily fecundity from ♀ developed at 25°C)	249	Temperature of pair	Temperature of pair	lm [g]
Offspring number	Adult offspring from ♀ developed at high temperature - $\bar{x}$ (adult offspring from ♀ developed at 25°C)	223 <sup>a</sup>	Temperature of pair	Temperature of pair	lm [g]

**Table S4 Description of the statistical models used to analyse the interaction between pesticide resistance and the response to high developmental temperature.** “Sample size”: total number of replicates included in each analysis. “Maximal model”: complete set of explanatory variables included in the model. “Minimal model”: model containing only the variables that were statistically significant. Round brackets indicate that the variable was included as a random factor. Within R subroutine, “glm”: general linear model, “glmer” and “glmmTMB”: general mixed-effects models. Square brackets indicate the error structure used (“p”: Poisson; “qp”: quasi-Poisson; “bbI”: beta-binomial, accounting for zero inflation). “Developmental temperature”: temperature at which eggs were exposed until adulthood; “Resistance status”: resistance or susceptibility to etoxazole; “Fecundity”: number of eggs laid for four days; “♂”: number of sons; “♀”: number of daughters. “♀ temperature”: developmental temperature of the females paired with males to mate and oviposit (offspring developed at 25°C); “♂ temperature”: developmental temperature of the males paired with females to mate and oviposit (offspring developed at 25°C); “Age of male”: Age at which each male was paired; “Age of female”: Age at which each female was paired. All analyses include only replicates in which fecundity was above zero. Replicates with damaged (*i.e.*, harmed during manipulation) mothers or individuals forming the mating pairs were excluded all analyses

Var. of interest	Response variable	Sample size	Maximal model	Minimal model	R subroutine [err struct.]
Developmental time (♀)	Day of first adult female	97	Developmental temperature * Resistance status	Developmental temperature	glm [p]
Developmental time (♂)	Day of first adult male	95	Developmental temperature * Resistance status	Developmental temperature	glm [p]
Number of surviving juveniles	Number of adult offspring	114	Developmental temperature * Resistance status + (Fecundity)	Developmental temperature + (Fecundity)	glmer [p]
Offspring number	Number of adult offspring	233	♀ temperature * ♂ temperature * Resistance status + (Age of male) + (Age of female)	♀ temperature + ♂ temperature * Resistance status	glmmTMB [qp]
Offspring sex ratio	cbind (♀, ♂)	233	♀ temperature * ♂ temperature * Resistance status + (Age of male) + (Age of female)	♀ temperature + ♂ temperature * Resistance status	glmmTMB [bbI]



**Fig. S1 Detailed backcross procedure used to create the *Wi.RR* population replicates.** For each population replicate,  $F_0$  crosses (left panel) were performed by mixing 300 quiescent females (*i.e.*, female nymphs undergoing their last moulting stage) from each *Wi.SS* population box with 100 males from the SB9.rif donor strain, into a petri dish containing a bean leaf placed on water-saturated cotton. Females emerged as adult virgins and could mate for 3 days, after which 200 of them were transferred to an experimental box ( $14 \times 14 \times 20$  cm) containing two 17-day-old bean plants whose stem was imbibed in wet cotton. Eleven days later, 300 quiescent females were collected from each experimental box for a first ‘no-cross’ (NC) step. These females were placed without males (to remain virgin when emerging as adult) into a petri dish containing a bean leaf placed on water-saturated cotton and could lay eggs for 7 days (*T. urticae* is haplodiploid so virgin females produce male offspring). **Selection** was then applied to the offspring (all males) by transferring the bean leaf into a new petri dish containing cotton soaked with a 0.5g/L lethal concentration of etoxazole (trade name Borneo) (*cf.* van Leeuwen et al. 2012). Seven days later, 100 adult males (all resistant) were collected from this petri dish and **back-crossed** (BC) with 300 quiescent females collected from each *Wi.SS* population box (following the same procedure than for  $F_0$  crosses). The sequence ‘no-cross, selection, backcross’ was then repeated for six additional generations. At the seventh generation of backcross (right panel), all remaining  $BC_7$  females (not isolated as virgin to produce  $NC_7$  males) were kept in the experimental box for 3 more days to emerge as adult and mate with  $BC_7$  males, *i.e.*, ‘self-cross’ (SC). Then, 400 of these females were transferred into two new experimental boxes (200 females per box, each with two fresh bean plants). Eleven days later, 600 quiescent  $SC_1$  females were collected from these two boxes and equally distributed across 4 petri dishes with 400 selected  $NC_7$  males. These females emerged as adult, mated and laid  $SC_2$  eggs for 7 days. The bean leaves carrying this  $SC_2$  progeny were then transferred into new petri dishes with an etoxazole solution (as described above) to select resistant individuals (*Wi.RR* females and *Wi.R* males). Ten days later, 200 adult mated females were collected from these petri dishes and transferred into a new experimental box without pesticide to found a *Wi.RR* replicate. This entire procedure was repeated independently for the 5 *Wi.SS* population replicate to create the 5 *Wi.RR* population replicates.