# Abstract:

Many parasites can interfere with their host’s defences to maximize their fitness. Here we investigated if there is heritable variation in the spider mite, *Tetranychus evansi* for traits associated with how they interact with their host plant. We also determined if this variation correlates with mite fecundity. This mite has the ability to interfere with jasmonate (JA) defences which is the main determinant of anti-herbivore immunity in plants. We investigated (i). variation in fecundity in the presence and absence of JA defences, making use of a wild-type tomato cultivar and a JA-deficient mutant (*defenseless-1*), and (ii) variation in the induction of JA defences, in 4 *T. evansi* field populations and in 59 inbred lines created from an outbred population conceived from controlled crosses of the four field populations. We observed a strong positive genetic correlation between fecundity in the presence (on WT) and in the absence of JA-defenses (on *def-1*). However, fecundity did not correlate with the magnitude of induced JA-defenses in WT plants. Our results suggest that JA-defences have a minimal impact on the performance of the specialist *T. evansi*, either because all lines can adequately reduce levels of defences, or because they are resistant to such defences.

# Keywords:

*Tetranychus evansi*, inbred lines, defence suppression, plant-herbivore interactions

# Introduction

Antagonistic interactions between organisms, such as between parasites and their hosts, are abundant in nature. Hosts have evolved numerous traits relating to resistance or avoidance to minimise the negative effects on fitness caused by parasites (Duncan & Little, 2007; Klein, 2003; Lopes et al., 2016; Råberg et al., 2009; Vale et al., 2014; Martins et al., 2014; Faria et al, 2015). This, in turn, selects for parasites that can overcome, resist or interfere with host resistance (Agrawal et al., 2002; Ezenwa et al., 2010; Schmid-Hempel, 2009), which can lead to the maintenance of genetic variation in host and parasite populations (Haldane 1949).

Interference of host defences is a strategy that has evolved in diverse plant parasites, including viruses (e.g. Kalyandurg et al., 2019), nematodes (e.g., Gheysen & Mitchum, 2011), lepidopteran larvae (e.g., Musser et al., 2022; Eichenseer et al., 2010), mites (e.g., Sarmento et al., 2011a; Alba et al., 2015; Villarroel et al., 2016) and aphids (e.g., Will et al., 2013); and animals parasites, such as parasitoid wasps (e.g., Colinet et al., 2013), *Plasmodium* (e.g., Boëte et al., 2004) and HIV (e.g., Arany et al., 1998). Immune interference is often associated with the production of molecules by the parasite, that alter host-cell structure and function (in plants: Hogenhout et al 2009, in animals: Ke et al, 2015) referred to as “effectors”. Many effectors have been identified and some of these have been characterised in detail (review in Jones et. al 2022), but for many the mode of action, genetic variation and their costs are unclear. Theory predicts that their production may incur direct physiological costs for the parasite (Poulin, 1994). There may also be ecological costs due to parasite-mediated changes in host immune traits, as a disarmed host may be more suitable for competing parasites (e.g., helminths: Cornet, 2011; reviewed for spider mites: Blaazer et al., 2018; reviewed in: Zélé et al., 2018; theoretically shown: Kamiya et al., 2018). These physiological and ecological costs may be responsible for the maintenance of genetic diversity for immune interference of host defences (Dupas & Boscaro 1999), although evidence for this is still scarce (Senshu et al., 2009; Colinet et al., 2010; Kalyandurg et al., 2019).

Plant immunity largely depends on the upstream action of plant hormones, with salicylic acid (SA) and jasmonic acid (JA) being the central players in mediating defences such as the production of toxic secondary metabolites and proteins that interfere with herbivore digestion and development (Kant et al., 2015; Petersen et al., 2011; Walling, 2000). The SA pathway is mainly involved in the response against biotrophic pathogens and phloem-feeding herbivores, while the JA pathway is involved in defence against necrotrophic pathogens and chewing or cell-content feeding herbivores (Walling, 2000). Several herbivores have been shown to interfere with these defence pathways and this has been linked to increases in arthropod fitness (e.g., weight, fecundity or survival; lepidopteran larvae*:* Musser *et al.*, 2002; white flies: Zarate *et al.*, 2007; aphids: Bos *et al.*, 2010; Escudero-Martinez *et al.*, 2020; spider mites: Kant *et al.*, 2008; Sarmento *et al.*, 2011a; Alba *et al.*, 2015). In some cases, interference occurred after the initial onset of induced defences (Alba *et al.*, 2015, de Oliveira et al., 2016).

There is evidence that plant defence interference (e.g., spider mites: Schimmel *et al.*, 2017a; aphids:Mathers et al., 2017) and effector production by herbivores (spider mites: Jonckheere *et al.*, 2018, Liu et al., 2020a,b; lepidoptera: Paudel et al., 2020) are plastic traits that can be influenced by environmental cues such as competition (Schimmel et al., 2017a), light-dark cycles (Liu et al; 2020a) or temperature (Paudel et al., 2020). This plasticity may serve to limit physiological or ecological costs associated with immune interference. For instance, suppressed host defences can promote competition with other parasites on the same host plant (Sarmento et al., 2011b; Alba et al., 2015), which in turn may drive selection against immune interference traits. However, modulation of immune interference during competition has been demonstrated in spider mites, such as stronger suppression of defences locally in plant leaves after the arrival of competitors (Schimmel et al., 2017a). In addition, mites were also shown to shield their feeding site from competitors via physical barriers (through webbing, Sarmento *et al.*, 2011b, or by using leaf hairs as refuge, Glas *et al.,* 2014), and to mitigate competitive population growth via reproductive interference (Sato et al., 2014, 2016). This may act to buffer competitor-driven selection against immune interference (Blaazer et al., 2018).

Several studies have reported immune interference (often referred to as defence suppression) in tetranychid spider mites (Sarmento et al., 2011a,b; Alba et al. 2015; Godinho et al., 2016; Paulo et al., 2018). Knegt et al., (2020) observed levels of immune interference to differ among *T. evansi* populations collected on different continents. In this study, we measure intraspecific variation in immune interference at the population and genotype level, and how it relates to fitness differences. We used 4 field populations collected in Portugal and 59 inbred lines generated from an outbred population, created from controlled crosses of all four field populations (Godinho et al., 2020), thus capturing the genetic variation present in them. First, we compared fecundity of the 4 field populations and inbred lines on a common tomato variety (referred to as wild-type, *WT*) and on a mutant tomato unable to activate the JA pathway (*defenseless-1*, *def-1,* (Bergey et al., 1996; Howe et al., 1996; Li, 2002) to assess how they cope with JA-defences. We then assessed the magnitude of induced defences in the WT for 19 lines using marker genes involved in the mite-induced JA and SA pathways. We observed that fecundity across the 4 field populations differed marginally, with no difference in the expression of the tomato defence-genes. Yet we did find variation in fecundity and in their effect on expression of defence genes across for the lines, with some lines clearly inducing immune responses. This variation in fecundity has a genetic basis but did not correlate with their effect on induced tomato defences.

# Material and Methods

## The study system

*Tetranychus evansi* is an arrhenotokous mite species. *T. evansi* feeds mostly on *Solanaceae* plants (Migeon et al., 2010). In the laboratory, this species has a life cycle of approximately 13 days (egg to adult) at 25ºC (de Moraes & McMurtry, 1987), with all life-stages residing on the host plant. We performed the experiments on tomato plants (*Solanum lycopersicum* L.) cv. Castlemart (wildtype; WT) and the mutant *defenseless-1* in the Castlemart genetic background (*def-1*; Howe et al., 1996). *def-1* is unable to mount JA-related defenses. All plants were grown in a climatic chamber (photoperiod of 16:8h, 25:18°C, day:night, 50-60% relative humidity). Infestation with *T. evansi* occurred when plants were 28 days old, with four fully expanded leaves.

### T. evansi field and Outbred populations:

The collection of the 4 field populations and the generation of the outbred mite population has been described in detail previously in Godinho et al. (2020). In summary, at four locations in Portugal in 2017 *T. evansi* mites were collected from the field and greenhouse grown tomato. We refer to these four mite populations as VIT, 6M1, QG and PBS. All four populations had the same ITS haplotype (Godinho et al., 2020). To remove *Wolbachia*, these populations were heat shocked at 33°C for 42 daysand then used to create an outbred laboratory population with a maximal level of genetic diversity by performing controlled crosses while avoiding over-representation of genotypes from a given population (Godinho *et al.* 2020). Field and outbred populations were maintained on detached tomato leaves (28 days old, cv Moneymaker) with the petioles submerged in water. All the females used in the experiment were obtained from cohorts of 100 mated females maintained on tomato cv. Moneymaker leaves.

### T. evansi inbred lines

The generation of the 59 inbred mite lines has been described in detail previously in Godinho et al. (2020). In summary, *Tetranychus evansi* inbred lines were created from 400 mated females randomly collected from the outbred population. Brother-sister mating was imposed for each line for 15 generation resulting in 59 lines giving an expected level of 93.6% homozygosity (Godinho et al., 2020). All lines were maintained on detached tomato leaves (28 days old, cv Moneymaker) placed on water-saturated cotton wool in Ø10cm Petri dishes. All the females used in the experiment were obtained from cohorts of 60 mated females maintained on tomato cv. Moneymaker leaves.

### Benchmark mite strains

For all our experiments, *T. urticae* Santpoort-2 (“KMB” in Kant *et al.*, 2008, "Santpoort-2" in Alba *et al.,* 2015) and *T. evansi* Baker & Pritchard Viçosa-1 ("Viçosa-1" in Sarmento et al., 2011a,b; Alba *et al.* 2015) were used as benchmark controls for the induction and suppression of plant defences, respectively. These lines are referred to as the ‘inducer’ and ‘suppressor’ benchmarks as they have been shown in previous experiments to induce and suppress JA defences in tomato, respectively (e.g., Sarmento et al., 2011a, b, Alba *et al.* 2015). The *T. urticae* Santpoort-2 strain was maintained on detached bean leaves (10 days old, cv Speedy), and the *T. evansi* Viçosa-1 population on detached tomato leaves (28 days old, cv Castlemart). All strains, prior to and for the experiments, were maintained in a temperature-controlled room (photoperiod of 16:8h, 23.5±2°C, 70% RH).

## The populations

### Fecundity of T. evansi field and outbred populations on WT and def-1 tomato plants:

First, we assessed fecundity in the 4 field populations and the outbred population in the presence (WT) or absence of functional JA mediated defences (*def-1*). To this end, 25 mated females (15 ± 1 day old) from each of the *T. evansi* populations were placed on one non-terminal leaflet of one fully expanded leaf of *WT* or *def-1* tomato plants (day 0). Mite dispersal was prevented by isolating the adaxial surface of this leaflet with a 1:1 mix of entomological glue (Tanglefoot, The Scotts Company LLC, OH, USA), and lanolin (Sigma-Aldrich, St Louis, MO, USA), which was distributed around the adaxial edge of the leaflet. For each population and replicate, individual plants were used. The number of surviving females and their eggs were assessed four days after infestation. With these two measures, we calculated fecundity per female assuming linear mortality (Li & Zhang, 2022) by using: [total eggs] / [(alive females + total females)/2] and using these numbers as the average per female. This equation accounts for differential female mortality, which is only measured at the end of the assay and thus enables a more accurate representation of per capita fecundity. For each mite population we included eight to 11 replicates (i.e., one leaflet of different plants). Assays regarding *WT* and *def-1* plants were not performed simultaneously due to logistical constraints and were each done in three or four temporal experimental blocks.

### Expression of salivary effectors and tomato defences after infestation with the field and outbred populations

We measured the expression of salivary effector *84* in the different spider mite populations and of genes implicated in JA-defences (*Proteinase Inhibitor IIc*, *WIPI-*IIc, and *Proteinase Inhibitor IIf*, *WIPI-IIf*, Alba et al., 2015) in WT plants infested with mites. The mite *Ribosomal protein 49* (*RP49*) and the tomato *Actin* were used as housekeeping reference genes for spider mites and tomato plants, respectively (see Table S1 for primer sequences). For this we used the leaflets from WT plants from the performance assay described above. In parallel, we had also infested plants with the inducer benchmark strain Santpoort-2 and the immune suppressor benchmark strain Viçosa-1 in the same way as described above to have a quantitative reference for induction and suppression (Alba et al., 2015). The part of the leaflet with glue and lanolin was discarded and the rest of the leaflet (plant and mite material) was collected, flash-frozen in liquid nitrogen and stored at -80°C for RNA extraction. For each mite population (field and outbred) we included five to 11 replicates. Clean plants (i.e., without mite infestation) were used as a control.

Total RNA from the sampled WT leaves was isolated using a protocol adapted from Verwoerd et al. 1989. Our protocol differs from it in that: (i) we used phenol at room temperature instead of hot phenol (heated to 80°C) and (ii) the five-minute sample incubation step was completed at room temperature, instead of at 80°C. Next, 2µg of RNA was DNAse-treated with Ambion Turbo DNA-free kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and cDNA was synthesised with RevertAid H Minus Reverse Transcriptase (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Next, 1µL of ten-times-diluted cDNA was used as a template for a 20µL quantitative reserve-transcriptase polymerase chain reaction (RT-qPCR) using the SsoFast™ EvaGreen®Supermix (Bio-Rad, Hercules, California, USA) and the CFX96 Real-Time system (Bio-Rad, Hercules, California, USA). Gene expression was normalized using the ΔCt method (Alba et al. 2015) and, for graphical representation, scaled to the value with the lowest normalized average expression per gene.

### Impact of infestation with the field and outbred populations on T. urticae Santpoort-2 and on host immune responses

Immune interference can be investigated by comparing the fecundity of a mite strain sensitive to JA-defences (Santpoort-2) on plants previously exposed to spider mites that suppress defences, with their fecundity on clean plants (Kant et al., 2008). We used this method to assess the immune interference of the 4 populations, and the outbred population.

We first infested WT plants with females from the field or outbred population for four days of infestation and then removed adults and eggs. Next, cleaned leaflets were re-infested with three *T. urticae* inducer matedfemales (15±1 days old). Female survival and the number of eggs were recorded 48h later (so six days after the primary infestation). Plants pre-infested with the *T. urticae* inducer Santpoort-2 and *T. evansi* suppressor Viçosa-1 strains were used as benchmarks for induction and suppression. Uninfested plants were used as a control. We also assessed whether immune interference of the field and outbred populations can persist following secondary infestation by measuring gene expression on day 6 using the same assay as described above. There were six replicates (n = six) for each pre-infestation treatment divided across seven experimental blocks. Note, although this was set up in parallel to the experiment measuring gene expression 4 dpi described above, two more complete replicates were performed.

## The inbred lines

### Genetic variation in T. evansi inbred lines for fecundity on WT and def-1 tomato plants:

As for the field and outbred populations, we assessed variation in fecundity between lines on the different plant types: in the presence (WT) and absence (*def-1*) of functioning JA immune defences following the same experimental setup described for outbred populations with some modifications. These were (i) 12 mated females (13±1 days old) were placed on each leaflet, (ii) leaves were detached from plants with the petiole in water, and (iii) infestations were only for two days. Mean fecundity per surviving female (referred to as fecundity) was calculated as described above.

Due to the large number of inbred lines, we randomly tested subsets of inbred lines (incomplete block design). In total, we assessed mite performance across thirty-five temporal blocks over the period of a year. Each block included twelve inbred lines placed on both *WT* and immunocompromised plants, (*WT:* n= four - seven; *def-1*: n= four - seven). In each block, there were also two to three replicates of each benchmark control population, i.e., *T. urticae* inducer (*WT:* n= 75; *def-1*: n=72) and *T. evansi* suppressor (*WT:* n= 76; *def-1*: n=67) populations also on both plant types, in addition to clean plants (*WT:* n= 27; *def-1*: n=25).

### Expression of tomato defences in response to infestation by T. evansi inbred lines

The expression of marker genes implicated in JA defences (*WIPI-IIc, WIPI-IIf*) was measured to assess variation in the defensive response of WT plants. To this aim, we chose 19 of the inbred lines (n = five - seven) for which we tested the fecundity, such as to ensure variation in this trait across the *WT* and *def-1* plants and to reduce the number of lines on which to test gene expression, due to logistic constrains. Induction of defences was also measured for a subset of the benchmark control populations (i.e., inducer and suppressor populations, n = four) and clean plants (n= four).

## Statistical Analysis

All statistical analyses were performed with the software R (version 4.2.2, R Development Core Team 2022, Chichester, UK). All models for gene expression were repeated including or excluding the benchmark controls for immune induction (*T. urticae* inducer), immune suppression (*T. evansi* suppressor) and uninfested plants. This was mostly to ensure that induction of immune defences occurred as in previous experiments (Alba *et al.*, 2015; Knegt *et al.*, 2020). Details and results for both models of each variable are shown in the Supplementary Materials. This also enabled us to test whether there were differences among *T. evansi* populations or inbred lines, i.e., in models excluding the benchmark controls.

We fitted two independent generalized mixed linear mixed models (GLMM) (i.e., one for each host plant type) with a normal error structure (lmer, lme4 package, Bates et al. 2015) to investigate whether fecundity (4 dpi) varied among the field and outbred populations on *WT* and *def-1*. The models included *population* (*T. evansi* Outbred, VIT, 6M1, QG and PBS) as a fixed explanatory variable, and *block* as a random variable.

To analyse transcript accumulation of salivary effector 84, we fitted a GLMM with a gamma distribution and a log link function (glmmTMB package, Brooks et al. 2017). The model included *population* (*T. urticae* inducer, *T. evansi* suppressor, Outbred, VIT, 6M1, QG and PBS) as a fixed explanatory variable, and *block* as a random variable. To investigate whether transcript accumulation changed in tomato plants infested with field or outbred populations (4dpi) and if the levels changed when subsequently infested with the *T. urticae* inducer population (6dpi), GLMMs with a gamma distribution and a log link function (glmmTMB package, Brooks et al. 2017) were fitted for each gene separately (*WIPI-IIc, WIPI-IIf*). These models included *population, time of gene expression analysis* and their interactionin the model as a fixed explanatory variable and *block* as a random variable. glmmTMB models were used instead of glmer to improve model convergence.

To evaluate if the fecundity of *T. urticae* inducer Santpoort-2 was affected by a previous infestation with the *T. evansi* field and outbred populations, we fitted a GLMM assuming a Gamma distribution and a log-link function (lme4 package, Bates et al. 2015) since normality was not met (Shapiro-Wilk test: p = 0.008), and variances were not homogeneous. In these models the pre-infestation *population* (*T. evansi* Outbred, VIT, 6M1, QG, PBS, and the inducer and manipulator benchmark populations) was included as a fixed explanatory variable and *block* as a random variable.

A GLMM assuming a Gamma distribution and a log-link function (lme4 package, Bates et al. 2015) was used to investigate whether there was variation in fecundity of the inbred lines on the different host plant types since normality was not met, but here variances were homogeneous. *Inbred line*, *host plant* (i.e., WT or *def-1*) and their interaction (*inbred line:host plant*) were included in the model as fixed explanatory variables and *block* as a random variable. A similar model was repeated for the subset of 19 inbred lines selected to study genetic variation on induction of plant defences.

We calculated broad-sense heritability (*H2*) (Falconer 1961) for fecundity of the inbred lines on WT and *def-1* by performing separate generalized mixed linear models for each host type with a Gamma distribution and a log link function (lme4 package, Bates et al. 2015) with *inbred line* and *block* included in the models as random explanatory variables. From the summary of the models, we extracted the variance for *inbred line, block* and the *residual variance* of the modeland calculated *H2* as follows: (. To determine the significance of *H2* we compare models, using anova, including and excluding the *inbred line* random factor. The benchmark control populations were excluded from these analyses.

To analyse if there is variation in the induction of plant defences after infestation with the inbred lines, GLMMs with a gamma distribution and a log link function (glmmTMB package, Brooks et al. 2017) were fitted for each gene separately (*WIPI-IIc or WIPI-IIf*). *Inbred line* was included in the model as a fixed explanatory variable and *block* as a random variable. glmmTMB models were used instead of glmer to improve convergence of the models.

To test how JA defences influence *T. evansi* fecundity, a genetic correlation between fecundity on WT and *def-1* plants was performed using a generalized mixed linear model assuming a Gamma distribution and a log link function (lme4 package, Bates et al. 2015) with *host plant* as a fixed variable and *inbred line* nested within plant(0+*host plant* | *inbred line*) and *block* as a random explanatory variable. From the summary of the model, we extracted the correlation obtained in the random effects section. To determine the significance of the genetic correlation, we compare models with and without the correlation using an anova.

We investigated whether fecundity of the inbred lines on WT plants correlated with normalized gene expression for the defence genes *WIPI-IIc* and *WIPI-IIf* in the JA pathway using Pearson correlations (M. J. Crawley, 2013). Normalized gene expression was log-transformed to improve the normality of data.

For all the analyses, when significant differences were found, multiple comparisons were performed using estimated marginal means (emmeans, emmeans package) (Lenth et al. 2019) and the p-values corrected using the false discovery rate (FDR) method (α= 0.05) (Benjamini & Hochberg, 1995).

# Results

## Fecundity, expression of salivary *effector 84* and of plant defences after infestation with *T. evansi* field and outbred populations

There was variation in levels of fecundity among field and outbred populations on WT (*population*:  = 12.204, p = 0.016, Figure 1A) and on *def-1* (*population*:  = 15.928, p = 0.003, Figure 1B)plants. On WT, 6M1 had the highest fecundity, while PBS had the lowest. On *def-1* VIT had the highest fecundity and differed significantly from PBS and QG. The outbred population did not differ from any of the field populations on either plant type.

There was a significant effect of population on the expression of salivary effector 84 ( = 116.290, p<0.001, Figure 1C). This was mainly explained by low levels of expression of this effector for the *T. urticae* inducer benchmark control, with all *T. evansi* populations having similar levels of expression. There was no difference in transcript accumulation levels among *T. evansi* populations.

We observed differences between populations for *WIPI-IIc* ( = 64.097, p < 0.001, Figure 1D) and *WIPI-IIf* ( = 53.344, p < 0.001, Figure 1E), in analyses including the benchmarks; both JA marker genes were induced by *T. urticae* inducer. Expression levels of JA marker genes in plants infested with field or outbred populations were similar to the defence levels found in plants infested with the *T. evansi* suppressor control, and sometimes to uninfested clean plants (i.e., all populations for *WIPI-IIc* and VIT and 6M1 for *WIPI-IIf*).

*Chart, box and whisker chart

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Figure 1. **Phenotypic characterization of the field populations and the outbred population.** Mean fecundity (± SE) of each population on (A) WT and (B) *def-1* plants at 4 dpi, and the mean normalized gene expression (± SE) of the (C) salivary *effector 84,* and JA related genes (D) *WIPI-IIc* and (E) *WIPI-IIf* at 4 dpi. The black circle denotes the mean and each grey circles a replicate. Different lowercase letters indicate statistical differences among populations according to multicomparison analysis performed using estimated marginal means. The benchmark control treatments are denoted as “I” for the *T. urticae* inducer control, “S” for the *T. evansi* suppressor control, and “C” for uninfested plants.

## Impact of infestation with the field and outbred populations on a JA-susceptible *T. urticae* population

Fecundity (6dpi) of the JA-susceptible *T. urticae* inducer population was not affected by pre-infestation with any of the field, outbred populations, or benchmark controls (*population*: = 7.7907, p = 0.3514, Figure 2A). However, the expression of JA marker-genes changed following this second infestation depending on the pre-infestation treatment (pre-infestation population treatment\*time of gene expression analysis; *WIPI-IIc*: = 24.516, p = 0.001; *WIPI-IIf:* = 56.020, p < 0.001; Figure 2B and C). The expression of these genes only increased in previously uninfested plants and in plants pre-infested with the inducer benchmark control population. In contrast, plants pre-infested with each of the *T. evansi* populations had no increase in expression, with levels remaining the same or being lower than at 4dpi.

Chart, scatter chart

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Figure 2. **Effect of the prior infestation with *T. evansi* populations on a JA-susceptible *T. urticae* population.** Mean (A) fecundity (± SE) at 6 dpi and mean normalized gene expression (± SE) of JA-related genes (B) *WIPI-IIc* and (C) *WIPI-IIf* at 6 dpi. Each grey circle represents a replicate and the black circle the mean. Different lowercase letters indicate statistical differences between populations according to multicomparison analysis performed using estimated marginal means. The benchmark control treatments are denoted as “I” for the *T. urticae* inducer mite population, and “S” for the *T. evansi* suppressor population control, and “C” for uninfested plants.

## Genetic variation in *T. evansi* inbred lines for fecundity and expression of plant defences:

There were significant differences among inbred lines for fecundity (*inbred line*: = 156.611, p<0.001, Figure 3A) with overall a higher fecundity on the immunocompromised plants than on WT (*host plants*: = 5.371, p=0.021, Figure 3A). The interaction between line and host plant was not significant (*inbred line\*host plants*: = 42.549, p=0.936). The genetic component for this observed phenotypic variation in fecundity was calculated on both WT (H2= 0.094, = 75.435, p < 0.001) and *def-1* (H2= 0.1217, = 136.490, p < 0.001) plants.

On the subset of 19 inbred lines selected to study the expression of JA plant defences (highlighted in black in Figure 3A), fecundity varied among inbred lines but did not differ on WT or *def-1*plants(*inbred line*: = 45.952, p<0.001; *host plants*: = 3.232, p=0.072; *inbred line:host plants*: = 18.235, p=0.440).

There were differences in levels of expression for *WIPI-IIc* (= 78.470, p < 0.001, Figure 3B) and *WIPI-IIf* (*WIPI-IIf*:  = 98.584, p < 0.001, Figure 3C), with the *T. urticae* inducer inducing the highest and the *T. evansi* suppressor showing lower levels of induced defences. Post-hoc comparisons revealed that most of the *T. evansi* inbred lines (17/19) had similar levels of *WIPI-IIc* expression to the suppressor control, with 7/19 lines also not differing from uninfested plants. For *WIPI-IIf,* levels of induction for most inbred lines did not differ from either of the benchmark controls (15/19), with 18/19 *T. evansi* inbred lines not differing from the *T.* *urticae* inducer benchmark. However, only three inbred lines (lines 1, 8 and 42) had higher levels of expression that the *T. evansi* suppressor benchmark. In analyses excluding the benchmark controls, there were no differences among lines for the expression of each gene.

Chart, histogram

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Figure 3. **Phenotypic variation of the 59 inbred lines.** (A)Mean fecundity (± SE) in the WT (circles) and *def-1* (triangles), and for the mean normalized gene expression (± SE) of JA-related genes(B) *WIPI-IIc* and (C) *WIPI-IIf* on WT plants. The 19 inbred lines selected to measure the response of the JA marker genes after infestation are highlighted in black in panel (A). Panel C has two graphs, to account for the high variation in normalized gene expression of *WIPI-IIf* among lines. Each grey circle represents one replicate. The benchmark control treatments are denoted as “I” for the benchmark inducer population, “S” for the benchmark suppressor population, “O” for the outbred population, and “C” for uninfested plants.

## Influence of JA defences on *T. evansi* fecundity

Chart, diagram, scatter chart

Description automatically generatedThere was a strong positive genetic correlation (rg = 0.72, p < 0.001, Figure 4A) between fecundity in the presence (WT) and the absence of JA defences (*def-1*) across inbred lines. There was no phenotypic correlation between the fecundity of the 19 inbred lines on *WT* plantsand the normalized gene expression for either of the genes implicated in JA defences after mite infestation (*WIPI-IIc*: r= -0.079, t(107)= - 0.821, p = 0.414; *WIPI-IIf*: r= 0.077, t(107)= 0.811, p = 0.419, Figure 4B and C).

Figure 4. **Influence of JA defence levels on *T. evansi* fecundity.** (A)Genetic correlation between fecundity on WT and *def-1.* Black points correspond to the 19 inbred lines selected to study induction of defences. Pearson’s correlation between fecundity on WT and (B) normalized expression of *WIPI-IIc* or (C) normalized expression of *WIPI-IIf*. In panels (B) and (C) points represent all biological replicates for the 19 inbred lines selected to study induction of defences.The benchmark control treatments are denoted as “I” for the benchmark inducer population, “S” for the benchmark suppressor population, and “O” for the outbred population, and highlighted in grey in all panels. In all panels the black line represents the correlation between traits, excluding benchmark controls, and the grey shadow the 95% confidence intervals.

# Discussion

We investigated intraspecific variation, in different field populations and individual lines derived from these populations, for the ability to interfere with host immune responses and how this relates to fecundity. We found no differences among 4 field populations in the gene expression of salivary effector 84 and the immune interference of JA defences, despite differences in fecundity among populations on WT and *def-1* plants. We did though find genetic variation among the inbred lines for oviposition on WTplants and variation in induction of gene expression for both marker genes implicated in the JA pathway (*WIPI-IIc* and *WIPI-IIf*). We also found that overall, the different lines had higher fecundity on immunocompromised host plants, indicating that the active JA pathway in WT plants negatively affects *T. evansi* mites. However, higher levels of fecundity in the inbred lines did not correlate with lower levels of immune defences (i.e., higher levels of immune interference).

## Fecundity of *T. evansi* does not correlate with levels of immune interference

JA defences reduced the fecundity of the inbred lines of *T. evansi* as, overall, fecundity was higher on *def-1* than on WT plants. Indeed, plants with a functioning immune response are a more hostile environment for several herbivores, as has been shown for mites and caterpillars (Howe et al., 1996; Li, 2002; Alagarmalai et al., 2007; Tian et al., 2014; Alba et al., 2015). Similarly, plants pre-infested by *T. evansi* have generally been found to increase the fecundity of both *T. evansi* and *T. urticae* (Sarmento *et al.*, 2011a; Alba *et al.*, 2015; de Oliveira *et al.*, 2016; Godinho *et al.*, 2016) although this effect can depend on the timing of the co-infestation (Schimmel et al 2017b). Taken together with our results, it seems that immunocompromised plants, either artificially or via herbivore immune interference mechanisms, confer obvious benefits to the herbivores feeding on them, including *T. evansi*.

However, despite genetic variation in the propensity of the *T. evansi* inbred lines to induce immune responses, we did not find a correlation between the degree of immune interference and fecundity. Most inbred lines induced defences to levels that were intermediate to that of the suppressor and inducer benchmarks, but some suppressed defences down to levels of clean plants and others induced them to higher levels than the inducer benchmark.

Fecundity is often used as a proxy for performance, is heritable, and can be influenced by the environment (Tien et al., 2009, Magalhães et al, 2007, Li & Margolies, 1993). In a sister species, *T. urticae,* fecundity has been observed to have variable levels of narrow-sense heritability (*h*2=0.72, Tien et al., 2009; *h*2=0.11, Magalhães et al, 2007; *h*2=0.05, Li & Margolies, 1993). Although these studies revealed genetic and environmental correlations among fecundity and other life-history traits (e.g., development time and juvenile mortality), fecundity was found to be independent of longevity (Magalhães et al, 2007) and web production (Tien et al., 2009). Similarly, our results suggest that fecundity is independent of the overall magnitude of JA-defences, as heritability was similar on WT and immunocompromised plants and fecundity on WT and *def-1* across lines was positively correlated. Possibly using absolute expression of two marker genes at an arbitrary time point does not adequately reflect the complexity and dynamics of the relevant downstream induced-defence response of tomato plants. In addition, defence induction may affect other traits. A recent study found that *T. urticae* mites that suppress defensive bubble formation in honeysuckle did not have higher fecundity than inducer mites, but that there were differences in developmental survival in response to plant defences (Villacís-Perez et al., 2022). It may also be the case that variation in the ability of *T. evansi* to interfere with immune defences is correlated with other life-history traits (longevity, lifetime reproductive performance, juvenile survival, etc). As such, how other life-history traits (e.g., developmental rate, Tien et al., 2009 and survival, Villacís-Perez et al., 2022) co-vary with immune induction, in longer-term experiments, should be considered in future.

It is generally found that *T. evansi* can suppress tomato immune responses, with corresponding beneficial effects on fecundity, juvenile survival and development rate compared to *T. urticae* (Sarmento et al., 2011a; Alba et al., 2015; de Oliveira et al., 2016; Godinho et al., 2016; de Oliveira et al., 2017). Previous studies that identified a link between defence induction and herbivore performance included comparisons either among *T. urticae* lines (Kant *et al.,* 2008) or between *Tetranychus* species (Alba *et al.,* 2015).

It is possible that some of the inbred lines with higher fecundity may be more resistant to tomato defences than other lines. Plant defence resistance has been demonstrated in *T. urticae* whereby some lines induce host immune genes but maintain high levels of fecundity (Kant et al., 2008; Dermauw et al., 2013; Snoeck et al., 2018). If this is the case, resistance to plant defences may be via detoxification mechanisms (e.g., metabolite modification, degradations and/or secretion) (Després et al., 2007; Dermauw et al., 2013; Heckel, 2014; Njiru et al., 2022). One study found *T. urticae* to have increased expression of genes involved in detoxification following only 5 generations on tomato (a novel stressful environment after bean), with correlated beneficial effects in other toxic environments such as the presence of pesticides (Dermauw et al., 2013).

## Genetic variation for immune interference among lines, but not among populations

There were differences in immune interference among the different inbred lines, but the four field populations did not differ in the extent to which they upregulated tomato defences. It is most probable that this is because the different populations contain similar levels of genetic variation that was captured across the different lines. This is supported by the observation that the variation in defence gene induction by the outbred population overlaps with variation across inbred lines. Thus, in our lines, we probably fixed the genetic variation present within the field populations.

## Immune interference in *T. evansi* populations and consequences for heterospecifics

Not only the magnitude of defence activation but also the expression levels of effector 84 was similar across the field populations of *T. evansi*. This was despite variation in levels of fecundity among the field populations on both WT and *def-1* plants. *Effector 84* is a salivary effector protein that is involved in the suppression of the JA and SA pathways in plants (Villarroel et al., 2016; Schimmel et al., 2017a). To date, no study has addressed variability in the expression of this effector within the *Tetranychus* genus, as most studies addressing effector genes mainly focus on the mechanism and mode of action of such molecules (reviewed in Jones et al., 2022). The absence of significant variation in the expression of this effector may be explained by the fact that all these populations belong to the same haplotype (ITS lineage I, Godinho et al., 2020). Another haplotype (ITS lineage II) might suppress plant defences more strongly (Knegt et al., 2020), although it is challenging to compare across different studies. However, Knegt et al. (2020) also observed little variability across populations. It would be interesting to assay these populations for variation in expression of the effector and see how it relates to the expression of plant defence genes and *T. evansi* life-history traits. More studies investigating the dynamics in effector gene expression in herbivores and linking this to the expression of immune genes in host plants could improve our understanding of the mechanisms of co-evolution between species.

We did find that infestation with the *T. evansi* field and outbred populations prevented plants from mounting an effective immune response against the JA-defence susceptible *T. urticae* Santpoort-2 (Alba et al., 2015). In plants pre-infested with mites from these *T. evansi* populations, levels of induction for all defence genes were maintained at the same or lower levels after subsequent infestation with the *T. urticae* inducer population. In comparison, infestation of plants with *T. urticae* that were clean, or had been pre-infested with *T. urticae,* induced significantly higher levels of defence-gene transcript accumulation. Such a lasting effect of defence interference could result from a latency period required for the host plant to re-establish its normal defensive status in response to a secondary infection. Moreover, in several co-infection studies when infections are sequential, host-mediated facilitation by a suppressor parasite has been shown to increase fitness related traits in a second parasite (Enzenwa et al., 2010; Sarmento et al., 2011a; Alba et al., 2015; de Oliveira et al., 2016; Godinho et al., 2016). This facilitation can in turn promote competition with herbivores co-habiting the same host (e.g., spider mites: Sarmento et al., 2011a, b, Glas et al., 2014; beetles: Erwin et al., 2014). Investigating how the lasting effect of defence interference persists through time and its benefits for con- and heterospecific individuals increase our understanding of how species evolve in communities. In the experiments presented here, however, despite defences being maintained at low levels, we did not observe facilitation of the JA-susceptible *T. urticae* population (Fig 2A). Previous studies showed that the oviposition of this heterospecific competitor of *T. evansi* is higher on suppressed and clean plants, compared to plants with induced defences (Kant et al., 2008; Sarmento et al., 2011a; Alba et al., 2015; Godinho et al 2016). As previously reported, the outcome of facilitation experiments may be variable and strongly depend on the timing of the infestation and the numbers of mites used (de Oliveira et al., 2016; Schimmel et al., 2017a, b;Blaazer et al., 2018; Fragata et al., 2022).

## Plasticity of immune interference

We found no evidence for metabolic costs of immune interference; lines showing higher levels of immune interference did not have lower levels of oviposition on *def-1* plants. This type of trade-off has been investigated in other parasites, with varying results. Immune interference by a parasitoid was lost following selection on a diversity of hosts, possibly due to being ineffective, and/or costly to maintain on some host species (Dupas & Boscaro, 1999). Another study found metabolic detoxification to trade-off with increased population growth rate in the *Sitobion avanae* aphid (Castañeda et al., 2010). In contrast, no trade-off was found between the growth rate of *Depressaria pastinacella* or *Heliothis zea* caterpillars and the production of detoxification enzymes (Neal & Berenbaum, 1987; Berenbaum & Zangerl, 1994). Also, there was no apparent cost for *Schistocephalus solidus* manipulating the behaviour of their intermediate host (to increase predation by its definitive host, Hafer-Hahman, 2019). Another study, with *T. evansi,* showed the maintenance of suppression after evolution on immunosuppressed (*def-1)* plants for 60 generations suggesting marginal costs associated with this trait in this species (Knegt, 2019). Low costs for the maintenance of immune interference may be explained through trait plasticity. Indeed, responses to induced plant defences are phenotypically plastic for some herbivores, only being expressed on plants when needed (Després et al. 2007, Broadway 1997, Li et al. 2002). It would be interesting to see if this is the same for *T. evansi*. In line with this the expression of effector 84 was found to be highly plastic in a related species, *T. urticae* (Schimmel et al 2017a; Liu et al 2020a, b).

Spider mites also show plasticity in other traits related to defence suppression (Schimmel et al., 2017a). An ecological cost for parasites may be the presence of competitors in the within-host environment that benefit from, but do not contribute to, immunosuppression (Mideo, 2009; Kamiya et al., 2018, Blaazer et al., 2018). In the presence of competitors, *T. evansi* mites can increase web density, local levels of defence suppression and their own oviposition, showing plasticity in traits facilitating their monopolization of the immunosuppressed environment (Sarmento et al., 2011b; Schimmel et al., 2017). This indicates that trait plasticity may help mitigate ecological costs associated with immune interference.

## Immune interference as a public good

Immune interference could be seen as a public good, as it benefits other herbivores sharing the same host (Enzenwa et al., 2010; Sarmento et al., 2011a; Alba et al., 2015; de Oliveira et al., 2016; Godinho et al., 2016). This could lead to the emergence of cheaters/exploiters of the same or different species, which do not suppress defences but have higher fitness when it occurs, since they pay no energy costs associated with defence suppression. A theoretical study revealed that it is possible for two strains with extreme defence suppression strategies (i.e., zero and maximum) to coexist in a population (Kamiya et al., 2018). This could produce antagonistic coevolutionary dynamics between defence suppressor parasite strains that strive to monopolise the suppressed environment and cheaters that aim to reap the benefits (Diard et al., 2013). Cheaters that benefit from, but do not contribute to defence suppression have been identified in *Pseudomonas aeruginosa* and *Yersinia pestis* (Rundell et al., 2016). The emergence of cheaters exploiting public goods has also been identified in vitro in the bacteria *Pseudomonas flourescens* (Griffin et al., 2004; Kümmerli et al., 2015). Selection for suppression and cheating would be possible in *T. evansi* considering the existing standing genetic variation for this trait. However, support for this hypothesis in this system would require identifying the (benefits) and costs of immune interference either energetically (i.e., via the production of interfering molecules, such as salivary effectors), or the presence of competitors exploiting and benefitting from the resource more than suppressor lines. It would be interesting to establish the relationship or co-occurrence between the fecundity of putative cheaters and their proximity to suppressors in natural populations.

The fact that suppression enables overcoming host immune responses, and can be beneficial for competitor parasites of the same or different species, means this trait may coevolve in response to both the host and other parasites (Schmid-Hempel, 2009; Zélé et al., 2018). These may also be important factors simultaneously responsible for the maintenance of genetic variation in this trait. For instance, selection for immune interference may depend on the host environment, and the frequency at which parasites encounter hosts upon which immune interference is effective, or in coinfections with other parasites that exploit the manipulated host environment.

## Conclusion & Perspectives

Our results show genetic variation for fecundity within a *T. evansi* population, but that this does not correlate with variation in levels of induction of immune defences. This might be because intraspecific variation for immune interference might be linked to other *T. evansi* life-history traits that, for example, are more targeted by host immune defences. We advocate that more studies should be conducted to investigate the presence and causes of intraspecific variation for immune interference and consequences for parasite life-history traits, both in the absence and presence of competitors. This should contribute to a better understanding of how variation in traits related to immune interference are maintained in parasite populations, its role as a driver for coevolution with hosts and competitors and how it relates to outbreaks of parasites or pest species.

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