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Interaction with plant defences in spider mites with different degrees of specialization

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Abstract

Plants have evolved several defensive strategies (e.g. secondary metabolites, wound-inducible proteins) to limit the damage caused by herbivory. However, some herbivores have evolved means to circumvent such plant defences. Indeed, while most herbivores trigger plant defences, some can suppress or even down-regulate them, and examples of this strategy have been mainly reported in herbivores interacting with plants from the Solanales order. Unlike *Tetranychus urticae*, a polyphagous spider-mite species that induces wound-responses on tomato plants, *T. evansi* and *T. ludeni*, specialists of the Solanaceae family and the Solanales order, respectively, can down-regulate such defences. However, little is still known about the defence targets of this manipulative ability. By using tomato plants with impaired defences, results shown that *T. evansi* and *T. ludeni* may manipulate similar defence targets. Additionally, to further understand how broad this down-regulation ability is and how different degrees of specialization (host range) can influence its presence and intensity down-regulation, four plants from the Solanales order, namely tomato, datura, tobacco and purple (three Solanaceae and one Convolvulaceae), and bean plants (Fabales: the outgroup) were selected. It was observed that *T. evansi* and *T. ludeni* were locally adapted to tomato and purple plants, respectively, and that *T. urticae* performed worse in Solanales order plants than in the outgroup. Moreover, no evidences of down-regulation were found contrarily to what was expected. Indeed, only suppression of plant defences was observed, being present for the three mite species on at least one of the five plants tested. Finally, to understand what may be the ecological consequences of down-regulation, choices between plants either clean or pre-infested with *T. evansi* and *T. urticae* were performed. This experiment revealed that, despite the benefits of down-regulation, spider mites preferred plants pre-infested with *T. urticae.* With this project, it was shown that the manipulation of plant defences is highly dependent on both host and herbivore, but not on the degree of specialization of the latter. As such, a co-evolutionary approach of this complex phenomenon can be the key to understand the evolution of defence manipulation.

Keywords: Plant-herbivore interactions; down-regulation; *Tetranychus*; Solanales

Resumo

As plantas evoluíram várias estratégias defensivas (ex. metabolitos secundários e inibidores de proteases) para diminuir os efeitos negativos causados pelos herbívoros. Em resposta, os herbívoros evoluíram estratégias para resistir às defesas das plantas. Enquanto que alguns evitam tecidos ricos em compostos tóxicos ou os digerem, outros conseguem manipular a indução da resposta defensiva das plantas, suprimindo ou mesmo reduzindo as defesas. Em alguns ácaros-aranha do género *Tetranychus* a indução e a redução das defesas do tomate foram descritas. Enquanto que *T. urticae* é conhecido por induzir as defesas relacionadas com o ácido jasmónico, *T. evansi* e *T. ludeni* conseguem reduzir essas defesas, aumentando a sua própria capacidade reprodutiva e a de competidores. Contudo, o método como estas espécies manipulam as defesas das plantas é ainda pouco conhecido. Em *T. evansi*, estudos anteriores observaram que esta espécie atua em defesas dependentes tanto do ácido jasmónico como do ácido salicílico. Contudo, estudos referentes a *T. ludeni* ainda não foram realizados. Para perceber se os alvos de manipulação são semelhantes para *T. evansi* e *T. ludeni* tomateiros com defesas modificadas foram utilizados: *def-1*, tomateiros em que a expressão génica associada à indução de defesas está mutada; e *35S::prosys*, tomateiros em que a expressão génica associada à indução de defesas está constitutivamente expressa. Os resultados obtidos sugerem que *T. evansi* e *T. ludeni* parecem ter o mesmo alvo defensivo e que, provavelmente, *T. ludeni* poderá ainda modificar outros alvos defensivos independentes daquele já caracterizado.

Apesar de, na maioria dos casos, *T. urticae* ser caracterizado como um indutor de defesas, em algumas populações desta espécie a supressão de defesas foi descrita. Esta variação intraespecífica e a evidência de redução de defesas nas outras duas espécies de ácaros-aranha, sugere que a capacidade de reduzir as defesas pode estar relacionada com a sua distribuição em diferentes hospedeiros. Sendo *T. urticae* uma espécie polifágica é provável que a sua estratégia manipulativa seja diferente consoante a família da planta em que se encontra. Por sua vez, em *T. evansi* e *T. ludeni*, o grau de redução de defesas poderá estar associado ao seu grau de especialização, sendo que a espécie especialista da família das Solanaceae (*T. evansi*) apresenta uma maior redução das defesas de tomate do que a espécies especialista da ordem das Solanales (*T. ludeni*). Estas evidências levantam as seguintes questões: (i) como é que diferentes graus de especialização pode influenciar a presença de redução de defesas; e (ii) como é que a variabilidade das defesas do hospedeiro podem influenciar e modular esta estratégia. Para perceber isto, estudámos a performance e a capacidade de redução de defesas em quatro plantas da família das Solanales, nomeadamente, tomate, datura, tabaco e *purple* (três Solanaceae e uma Convolvulaceae) e uma Fabaceae (feijão:*outgroup*). Primeiro, a performance das três espécies de ácaros-aranha nas diferentes plantas hospedeiras foi medida. Os resultados da performance demonstraram que *T. evansi* e *T. ludeni* estavam localmente adaptados a tomate e *purple* e que *T. urticae* não teve uma boa fecundidade em nenhuma das Solanales testadas, apresentado uma fecundidade mais elevada para feijão. Em datura, apesar dos seus elevados níveis de tropanos, a fecundidade das três espécies parece não ter sido afetada, sendo semelhante para *T. ludeni* e *T. urticae* e mais elevada em *T. evansi*. Isto sugere que tomate e datura têm um perfil químico semelhante, contudo menos tóxico que o de tomate para *T. ludeni* e *T. urticae*. Em tabaco, a fecundidade para as três espécies de ácaros-aranha foi, no geral, reduzida, sugerindo que esta planta pode ser altamente tóxica para estas espécies. Finalmente, em feijão, a fecundidade das três espécies foi semelhante. Contudo, o *sex-ratio* (proporção de fêmeas) de *T. evansi* nesta planta foi menor comparativamente às restantes espécies. Isto, juntamente com a baixa proporção de eclosão de ovos de *T. evansi*, comparativamente com *T. urticae*, poderá explicar o porquê desta espécie não ser encontrada em feijão na natureza e não ser possível mantê-la em feijão em laboratório.

Para testar a presença ou ausência de redução das defesas, as várias plantas foram pré-infestadas com as três espécies de ácaros. Para tomate, em plantas pré-infestadas tanto por *T. evansi*, *T.ludeni* ou *T. urticae* não houve um aumento da performance dos con- ou heterospecíficos como sugerido anteriormente. Resultados referentes à actividade de inibidores de proteases demonstraram que uma redução das defesas não estava presente, mas sim uma supressão em todas as plantas pré-infestadas. Contudo, é

importante referir que, devido à elevada mortalidade de *T. ludeni* em tomate, nenhumas conclusões concretas conseguiram ser delineadas. Por sua vez, o resultado obtido para *T. urticae* poderá ser explicado pela manutenção da população utilizada, por mais de 20 gerações, em tomate. Como sugerido em estudos anteriores, uma adaptação a este hospedeiro poderá levar ao aparecimento de supressão das suas defesas. Para datura e *purple* não se verificaram diferenças tanto ao nível da performance de con- ou heterospecíficos como na actividade dos inibidores de proteases para todas as plantas pré-infestadas. Isto sugere, novamente, uma supressão das defesas. Contudo, devido ao número reduzido de réplicas de datura e á grande mortalidade de *T. evansi* em *purple*, conclusões sobre todos os tratamentos de datura e sobre *purple* pré-infestada com *T. evansi* não poderam ser delineadas Em tabaco, apesar de não ser visível uma redução da performance dos conspecíficos, *T. evansi* induziu a actividade dos inibidores de proteases. Isto sugere que a manipulação das defesas é altamente dependente do hospedeiro em que o herbívoro se encontra. Para tabacos pré-infestados por *T. ludeni* ou *T. urticae*, houve um aumento da performance de heterospecíficios, contudo, a actividade dos inibidores de proteases apareceu tendencialmente induzida. Assim sendo, os resultados de performance poderão ter sido afetados pela grande variabilidade no crescimento destas plantas. Os resultados face ao tabaco sugerem que estas plantas possuem uma estrutura defensiva muito particular, impedindo a supressão das suas defesas por parte dos ácaros-aranha. Para complementar estes resultados, medições da refletância de folhas limpas e pré-infestadas foram realizadas. Os resultados mostraram uma redução da refletância na zona dos UV-B em todas as plantas pré-infestadas. Isto sugere que as plantas, independentemente do efeito supressor observado nos ácaros-aranha, conseguem induzir a produção de compostos secundários, como compostos fenólicos, respondendo à herbivoria. Assim, a capacidade manipulativa dos ácaros-aranha, parece estar restrita a um nível de defesas, não suprimindo outras respostas defensivas por parte das plantas. Adicionalmente, numa tentativa de perceber se a herbivoria por parte dos ácaros-aranha levava a uma modificação no metabolismo primário das plantas, vários índices de refletância baseados na concentração relativa de clorofilas e na eficiência do uso de radiação pelo fotossistema II foram calculados. Os resultados sugerem que, no geral, o metabolismo primário das plantas não foi modificado. Contudo, um dos índicies utilizados (Cig) revelou uma redução da concentração relativa da clorofila em plantas pré-infestadas com *T. evansi.* Isto pode sugerir um maior dano foliar por parte desta espécie.

Finalmente, para perceber quais as consequências ecológicas da redução de defesas, testes de escolha entre plantas limpas ou infestadas com uma espécie indutora (*T. urticae*) ou redutora (*T. evansi*) de defesas foram realizados. Os resultados revelaram que, apesar dos benefícios previamente descritos da redução das defesas, os competidores preferem plantas previamente ocupadas por *T. urticae*. Isto poderá ser explicado pela: (i) ausência de evidências, ao longo deste projeto, da redução ou indução de defesas por *T. evansi* e *T. urticae;* (ii) grande produção de teias, por *T. evansi*, que afetam a capacidade reprodutiva dos competidores. Adicionalmente, também conspecíficos de *T. evansi* parecem preferir plantas pré-infestadas por *T. urticae.* Contudo, foi previamente observado que competidores interespecíficos tendem a preferir plantas pré-infestadas com *T. urticae.*

No geral, não foram encontradas evidências de redução de defesas. Contudo, parece que todas as espécies de ácaros-aranha testadas possuem a capacidade de fazer supressão de defesas em algumas das cinco plantas hospedeiras testadas. Contudo, este resultado parece não ser dependente do grau de especialização dos ácaros testados. Adicionalmente, dados de refletância sugeriram que apesar da supressão da actividade dos inibidores de proteases, as plantas continuam a responder à herbivoria através de outras estratégias defensivas, como compostos secundários. Os resultados deste projeto sugerem que ambos herbívoro e planta possuem um papel activo na evolução da supressão das defesas. Assim sendo, uma abordagem co-evolutiva deste fenómeno complexo poderá ser a chave para perceber a evolução da supressão.

Palavras chave: Interações planta-herbívoro; redução de defesas; *Tetranychus*; Solanales

Index

List of tables and figures

Introduction

The plant kingdom is one of the richest in terms of chemical compounds, such as specialized and secondary metabolites (Strauss et al. 1999). Some of these metabolites, together with other induced defences, play a role as defensive plant traits, as they limit the damage caused by herbivory (Fraenkel 1959). Herbivores, in turn, have evolved diverse strategies to circumvent plant defences, allowing them to counter the negative effect caused by such defences and maximize the conversion of plant material into offspring (Karban et al. 1989). Such history of evolution of defence and counter-defence may lead to a plant-herbivore coevolutionary arms race (Ehrlich & Raven 1964).

How herbivores cope with defensive plant traits is highly variable. While some species have evolved means to avoid tissues with high levels of chemical compounds (Paschold et al. 2007) or to digest these toxic compounds (Despres et al., 2007), others can manipulate the induction of plant defences by fully or partially supressing them (Musser et al. 2002) or even down-regulate them (Sarmento et al. 2011a). Suppression was found in several plant pathogens, insects and arthropods (Kant et al. 2015) and it is characterized by the ability to lower the rate of production of defensive compounds, operating up-or downstream of a defensive pathway (Alba et al. 2015). However, its target defence and mode of action seems to be conserved across herbivore species. As such, studies have revealed that most of the herbivores can suppress jasmonic acid (JA) related defences (Kant et al. 2015).

Suppression can be paralleled with an increase in the reproductive performance of the suppressor (Kant et al. 2015, Sarmento et al. 2011a). However, since suppression of defences, as with induction, is expressed in distal tissues (Kant et al. 2008; Alba et al. 2015), con- and heterospecifics can benefit from such manipulative strategy, increasing their performance (Sarmento et al. 2011a, Alba et al. 2015). This can lead to an increase in competition by con- and heterospecifics (Sarmento et al. 2011a), which can affect the suppressor negatively (Glas et al. 2014), and, consequently, modulate ecological interactions and community composition (Kant et al. 2015).

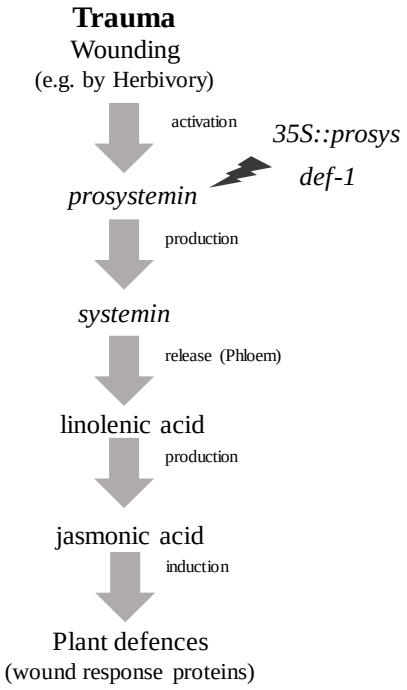

Figure 1.1. **Trauma or wounding response in tomato plants.** Wound-signaling pathway leads to the synthesis of systemic wound response proteins as proteinase inhibitors. (based on Ryan and Pearce 1998).

Some spider-mite species of the genus *Tetranychus* have been shown to induce or down-regulate tomato plant defences. These herbivores are important crop pests that feed by piercing mesophyll cells and sucking the intracellular content (Mothes and Seitz 1981; Albert and Crooker 1985). While, *T. urticae* is known to induce plant defences in JA pathway, *T. evansi* and *T. ludeni* are the spider-mite species known to down-regulate plant defences, increasing the reproductive performance of both con- and heterospecifics. The mode of action of these species is still poorly understood, but it is known that the *T. evansi* species acts, simultaneously, downstream of both jasmonic acid (JA) and salicylic acid (SA) pathways in tomato plants (*Solanum lycopersicum*) (Sarmento et al. 2011a; Alba et al. 2015). However, for *T. ludeni*, the target defence is still unknown. Tomato plants, with impaired plant defences, can be a powerful tool to better understand this issue. On tomato plants, a compound named systemin (18-amino-acid peptide) was found to induce the wound-induced expression of wound response proteins (e.g. proteinase inhibitors or PIs), defence proteins that can inhibit the digestive enzymes of herbivores (Ryan 1990; Pearce et al. 1991) (*Figure 1.1*). It has been proposed that systemin, as well as wounding, regulate JA synthesis which, in turn, regulates the JA-induced pathway (Farmer et al. 1992; Lee et al. 1997; Walling 2000) (*Figure 1.1*). To allow for a better

understanding of the role of the systemin signalling pathway, two tomato strains with different levels of defences – *35S::prosys* and *def-1* - were established by changing the expression of prosystemin (200-amino-acid), the precursor of systemin (McGurl et al. 1992; Howe et al. 1996) (*Figure 1.1*). While the former, through the constitutive expression of the *35S::prosystemin* transgene, has higher levels of wound response proteins, even in absence of wounding, the latter has wound-inducible gene expression inhibited due to the mutation *defenseless-1* (Howe et al. 1996; Bergey et al. 1996). These mutant plants were already used to describe the effect of *T. urticae* on tomato defences. It was observed that in *def-1* plants, since no wound-response is present, *T. urticae* causes more damage and has higher performance than in *wild-type* plants (Li et al. 2002). However, when exogenous JA was applied, the wound response was activated down-stream of the *defenceless-1* mutation, and the damage and performance were reduced (Ament et al. 2004). The same was obtained when using *35S::prossys* plants (Ament et al. 2004). This type of studies is lacking for *T. evansi* and *T. ludeni*, and can be important to gain insight on the potential similarity of the mechanisms underlying suppression in both species.

Despite the previous observations of induction in *T. urticae*, it has been found that some populations of this species can suppress the JA pathway in tomato (Kant et al. 2008; Alba et al. 2015). This suggests that an intraspecific variation may be presence in the induction of this species (Kant et al. 2008). This intraspecific variation in *T. urticae* and the clear evidences of down-regulation in *T. evansi* and *T. ludeni* might be related with their different host ranges. Indeed, *T. urticae* is a polyphagous species, feeding on a broad range of host plants (Raworth et al. 2002), which can lead to different strategies depending on the host-family-specific defensive pathways (Schulz 1988). Although in *T. ludeni* and *T. evansi*, the degree of specialization seems to affect the intensity of down-regulation of plant defences. It was observed that *T. ludeni*, a specialist of the Solanales order (Migeon et al 2011), has a less pronounced level of down-regulation in tomato plants than *T. evansi*, a specialist of the Solanaceae family (Godinho et al. 2016). From this, the following questions can be asked: (i) how can different degrees of specialization (host range) influence the presence of down-regulation, and (ii) how can host variability on the defensive plant traits modulate this down-regulation mechanism differently.

The variability of inducible defensive traits (e.g. secondary metabolites, *Table S1.1*) in the plant taxa in which *T. ludeni* and *T.* evansi are specialized, could contribute to understanding these questions. Plant species belonging to the Solanaceae and the Convolvulaceae families (Solanales order), manly endemic from South America (Wink 2003), share secondary metabolites that are rarely observed in the plant kingdom (Eich 2008), but can be highly specific for others. Due to their large economical relevance in agriculture, plant-herbivore interactions on plants from those families are well studied (Eich 2008). Additionally, since they are highly used in pharmaceutics, the blend of secondary compounds on several plant species, that sometimes can act as herbivore defences, are well characterized. Datura plants (*Datura stramonium*), from the same subfamily (Solanoideae) as tomato plants, have high levels and a variety of tropane alkaloids (Tepfer et al 1988). These alkaloids can be highly toxic for many herbivores, functioning as strong defences against them (Eich 2008). On tobacco plants (*Nicotiana tabacum*) the production of nicotine, a highly specific secondary metabolite, occurs downstream in the JA pathway and can be induced after herbivore attack (Stepphuhn et al. 2004; Baldwin et al. 2001). Regarding the Convolvulaceae family, purple plants (*Ipomoea purple*), on which *T. ludeni* is generally found, in the field (Santos et al. in prep), have, in their epigeal vegetative parts, compounds from the hydroxycinnamic acid amides family (Trumm et al. 1991). As previously suggested, compounds from this family can have an important role in chemical and physical defences (Pearce et al. 1998). With this variability in defensive traits and several mite/host quantification techniques the questions raised above can be addressed. Con- and heterospecifics performance upon pre-infestation was identified as a good proxy for down-regulation/induction of defensive plant responses (Kant et al. 2004, Sarmento et al. 2011a). However, direct measurements of plant defences are required to confirm defences manipulation. As

previously referred, wound inducible proteins (PI) are one of the inducible defences most affected by down-regulation. As such, measurements of PI activity are of great importance to this type of studies (Sarmento et al. 2011a, Godinho et al. 2016). However, investigating down-regulation from the plant's perspective can provide important insights to this mechanism. Since it was previously shown that reflectance spectroscopy can successfully characterize plant performance (Couture et al. 2016), this may be a powerful tool to understand how the primary and secondary metabolism is affected by the several spider mite species.

As referred above, suppression can benefit both the suppressor and other species in the community. Indeed, both down-regulator mites (*T. evansi*) and inducer mites (*T. urticae*) perform better in leaves pre-infested by *T. evansi* (Sarmento et al. 2011a, b). This ability of *T. evansi* may explain the rapid invasion of this species in the Mediterranean region, affecting resident spider mites such as *T. urticae* (Ferragut et al. 2013). To address how down-regulation can be one of the drivers of *T. evansi* invasion, it is important to understand how the spider mite communities can be affected, in their host choice and dispersion, by these defence manipulative strategies.

Overall, this study aims to address three levels of knowledge: mechanistic, by understanding how similar is the suppression target defence of both *T. evansi* and *T. ludeni*; physiological, by characterizing the presence and intensity of down-regulation in several spider-mite species, with different degrees of specialization (host range), on several Solanales hosts; and ecological, by understanding how the presence of down-regulation can affect host choice and possibly modulate spider-mites' communities.

Materials and Methods

Plants

All plants used in this thesis were sown in an Aralab climatic chamber, where they grew under controlled conditions (25ºC; 70% RH; photoperiod of 16L:8D). The taxonomic description, the age at which each plant was used and which leaf was selected for the experiment are summarized in *Table 2.1*.

Table 2.1. **Taxonomic description, age and leaf number of plants used.**

Order	Family	Subfamily	Genus	Species	Variety (strain)	Producer	Age (weeks)	Leaf number (from below)
Solanales	Solanaceae	Solanoideae	*Solanum*	*lycopersicum*	Moneymaker (*wild-type)*	Johnsons	5	3 or 4
					Castlemart (*35S::prossys*)	MSU[1]		
					Castlemart (*def-1*)	UVA[2]		
			Datura	*stramonium*		UTAD[3]	5	4
		Nicotianoideae	*Nicotiana*	*tabacum*	Virginia	FCUL[4]	5	6
	Convolvulaceae	Ipomoeeae	*Ipomoea*	*spp*	Vigorous	Vilmorin	5	3
Fabales	Fabaceae	Faboideae	*Phaseolus*	*vulgaris*	Contender	Germisem	2	1

Seeds kindly provided by:
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[4]Dr. Fernando Dias from Faculty of Sciences of University of Lisbon.

Spider-Mite Cultures

T. evansi and *T. ludeni* were collected from datura plants (*D.* stramonium), in 2013, in Portugal. These species were maintained on four-week-old tomato plants (*S. lycopersicum*, var. Moneymaker) and on two-week-old bean plants (*P. vulgaris*), respectively, in the University of Lisbon during approximately 80 generations. *T. urticae* was collected in Portugal from tomato plants (*S. lycopersicum*), in 2010, and, in the University of Lisbon, was firstly reared on two-week-old bean plants (*P. vulgaris*), during approximately 160 generations, and then reared on four-week-old tomato plants (*S. lycopersicum*, var. Moneymaker) during approximately 20 generations.

From these populations, several subsets were created, to be used for each experiment. All stocks and subset populations were reared in large numbers (>2000) at controlled conditions (25ºC; photoperiod of 16L:8D).

In all experiments, the age of the females was controlled. To this aim, 300 mated females from the population of interest were placed in leaf patches and allowed to oviposit for 2 days. From these cohorts, 15±1 days-old mated females were obtained.

Manipulation of plant defences on tomato strains

Effect of pre-infestation on mite performance

This experiment was performed using *T. evansi* and *T. ludeni* population subsets created with 200 mated females from the stock populations and maintained in detached four-week-old tomato leaves until the time of this experiment.

To shed some light on the potential similarity of the down-regulation mechanism between *T. evansi* and *T. ludeni*, *wild-type* tomato plants (*S. lycopersicum*, var. Moneymaker) and two mutant strains: *def-1* (gene expression, induced by wounding, blocked) and *35S::prosys* (overexpression of prosystemin, constitutive wound-induced gene expression), were used. Additionally, to measure exogenous induction of the JA pathway (down-stream of *defenseless-1* mutation) and how this can affect down-regulation, on half of the *def-1* plants used, 10 µl of a 0.25 mM jasmonate solution (Ament et al. 2004), diluted with 100% ethanol, were applied to the node of the selected leaf *(def-1 + Jasmonic acid)*. For the remaining plants, an equal volume of 100% ethanol was applied instead, as a control for this procedure. These treatments were used to understand if down-regulation can also act upon a defence target independent of the JA pathway (*def-1*) and if this ability is still present when high levels of wound defences (PIs) are constitutively present on the plant (*35S::prosys*) or are exogenously induced (*def-1 + Jasmonic acid*).

Mite infestations were performed in one fully-expanded leaf of each tomato variety (*Table 2.1*). This leaf was isolated by applying vaseline to the petiole, and infested with 60 mated females from each mite species during 48h (*Figure 2.1*). Then, the females, the eggs and the web were removed. As a control, clean plants, also treated with vaseline, were tested at the same controlled conditions (25ºC; photoperiod of 16L:8D). After removing the females, the eggs and the web, 7 leaf discs (11 mm^2 Ø) were made from each leaf and placed in water-saturated cotton. The remaining plant material was stored at -80ºC, preserving the tissue biochemical properties, for further physiological analyses. Since performance upon pre-infestation is a good proxy for suppression/induction of defensive plant responses (Kant et al. 2004, Sarmento et al. 2011a), the performance of con- and heterospecific *T. evansi* females was measured. As such, 15±1 days-old mated females were added to each leaf disc and allowed to oviposit for 4 days.

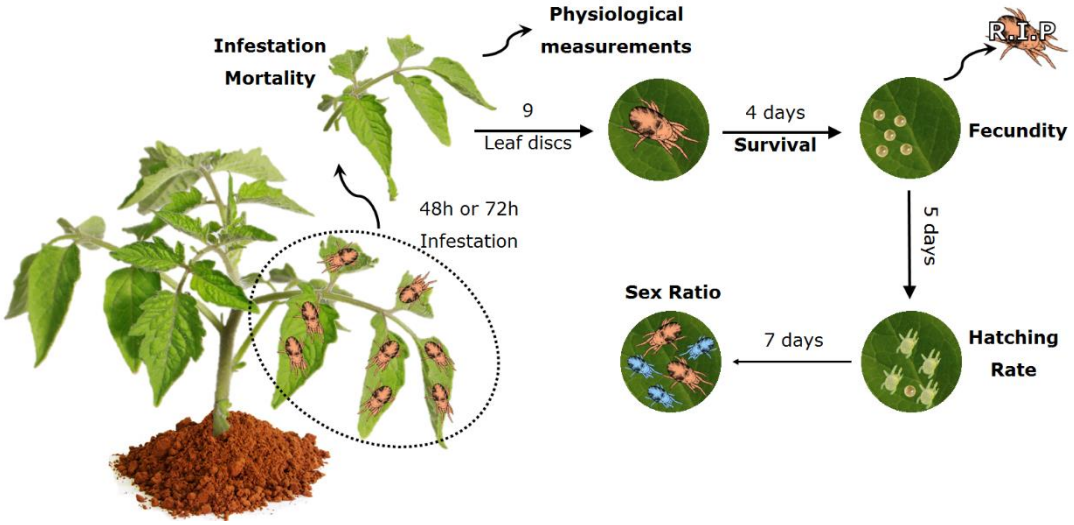

Figure 2.1. **Schematic representation of the protocol used to measure the effect of pre-infestation on mite performance.** The orange and blue mites represent *T. evansi* females and males, respectively.

Every day, it was recorded whether the female was alive, dead (the female died naturally on the leaf disc) or drowned (the female drowned in the water). This data was used to calculate female survival and to calibrate daily fecundity. On the fourth day, the females that were still alive were disposed of and the

number of eggs oviposited on each leaf disc (fecundity) was assessed. After 5 days, the number of juveniles that hatched from the eggs was measured (hatching rate). 7 days later, the number of males, females and dead juveniles on each leaf disc was measured (Sex-ratio) (*Figure 2.1*). Due to problems of moisture in the incubator in which the performance of this experiment was followed, the leaf discs rotted often, which prevented the proper following of the development of the eggs until adulthood (hatching rate and sex ratio measurements were discarded). All these measurements were obtained under controlled conditions (25ºC; photoperiod of 16L:8D).

This experiment was performed in 9 blocks in a total of 5 to 9 plant replicates and 31 to 44 mite replicates per plant treatment.

Proteinase Inhibitors

To characterized directly the manipulation of defences by spider mites, PIs activity was measured. As previously reported, PI activity is a good indicator of the level of expression of plant defences and of their suppression by spider-mites (Kant et al. 2004; Sarmento et al. 2011a; Godinho et al. 2016). The activity of PIs can be estimated by their ability to inhibit the hydrolytic activity of trypsin upon a synthetic substrate as the N-Benzoyl-D,L-arginin-4nitroamilide hydrochloride (BapNA) that originates a colored compound that can be easily detected by spectrophotometry (Erlanger et al. 1961). To quantify PI activity, at the beginning of this thesis, the protocol used was based on the one reported in Sarmento et al (2001a). However, due to constant problems in replicating this protocol, the protocol suggested by Kassel (1970) was used instead. As such, using a Quiagen TissueRuptor, ~300 mg of the vegetal material, previously stored at -80ºC, was grounded and homogenized with 600 µL of extraction buffer (0.1M Tris-HCl, pH 8.2; 20 mM $CaCl_2$; 1:3). After centrifuging each sample at 4ºC, 16.0xG for 25 minutes, the supernatant was separated from the pellet and maintained at 4ºC during the entire procedure (to preserve the sample from autohydrolysis).

To quantify PI activity through trypsin inhibition, several measures were defined: i) Positive control: controls for the full hydrolytic activity of trypsin upon BApNA in the absence of an inhibitor; ii) Trypsin negative: controls for the autohydrolysis of trypsin; iii) BApNA negative: controls for the autohydrolysis of BApNA; iv) Sample negative: controls for the hydrolytic activity intrinsic to the sample and for the supernatant color; and v) Sample positive: the quantification assay per se, in which the hydrolytic activity of trypsin is partially inhibited by the PIs present in the plant extracts.

Immediately before use, a trypsin solution containing 2000-1300 units/mL of trypsin and a 0.1% (w/v) BApNA solution was prepared. In a 96 well plate, each of the measures described above were performed in triplicate. To do so, the trypsin solution, extraction buffer and sample were mixed as presented in *Table 2.2*. The plate was incubated 10 minutes at room temperature and then BApNA was added. Immediately after, the plate was read at 405 nm (t=0 min) and left to incubate at room temperature for another 5 minutes. After that, the plate was re-read (t=5). The proportion of trypsin inhibition was calculated with the difference between the two readings (Δ) (8).

***Table 2.2*. Reagents and sample proportions used in the quantification of PIs.** The presented volumes are a down rescale of Kassel (1970) protocol in other to be applied at 96 well plate level.

	Measurements	Trypsin (µL)	Extraction Buffer (µL)	Sample (µL)	BApNA (µL)
Controls	Positive	15	135	---	75
	Trypsin negative	15	210	---	---
	BApNA negative	---	150	---	75
	Sample negative	15	135	15	75
	Sample positive	15	120	15	75

(8) $$Inibibition = 1 - \left[\frac{\Delta Sample\ positive - (\Delta Sample\ negative + \Delta Trypsin\ negative + \Delta BApNA\ negative)}{\Delta Control\ positive - (\Delta Trypsin\ negative + \Delta BApNA\ negative)}\right]$$

To determine the PI concentration that leads to inhibition, the relative quantification of total protein present in each sample is required. To do so, the Bradford method (Bradford 1976) was applied. Two major changes were applied to the classic Bradford method. First, due to technical constraints, the spectrophotometry readings were performed at 630nm and not at 595nm. However, the accuracy and resolution of the method at this wavelength was tested and verified (*Figure S1.2*). Second, due to a reduced volume of supernatant (the same used in the previous protocol) and of total protein content (reveled in pilot assays), the proportion of 1:50 of sample to Bradford reagent was changed to 1:10. As such, 100µL of Bradford reagent were added to a 96 well plate and, subsequently, 2µL of sample were added until a change of coloration was observed. Note that, the volume of sample did not exceed the 10µL and the dilution factor was registered. Despite the assay only being performed one time for each sample, this protocol allowed to circumvent the reduced volume of sample and time constrains by applying a direct dilution in the reading plate. To obtain a standard curve (*Figure 2.2*), solutions of bovine serum albumin (BSA) were used in the following concentrations (µg/mL): 0, 3, 10, 30, 50, 75, 100, 150, 250, 350, 450, 500. These solutions were measured in triplicate.

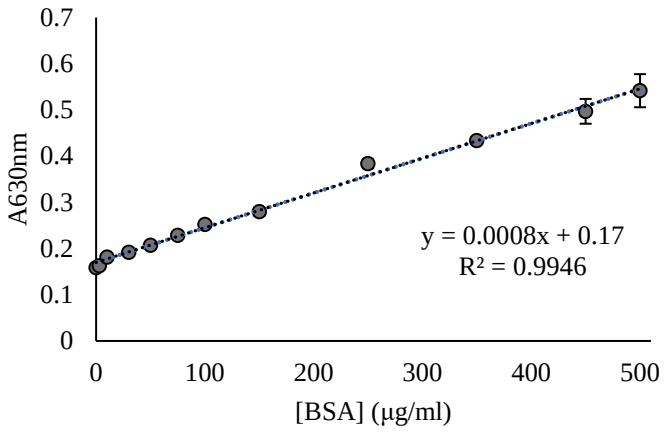

Figure 2.2. **Standard curve for Bradford assay.** Markers represent the average (± s.e.) of the three replicated measurements of the known protein content present in the several BSA solutions.

From the absorbances obtained for each sample, the relative total protein content was calculated (9).

(9) $$[Total\ protein] = \left(\frac{A_{630nm} - 0.17}{0.0008}\right) \times dilution\ factor\ (\mu g/ml)$$

Knowing that the trypsin solution (2000-1300 units/mL) has ~100µg/mL of protein (diluted from a stock powder with 20 000-13 000 units per mg/mL) the relative content of PIs in each sample could be assessed and normalized to the total protein content (10).

(10) $$[PIs] = \frac{Inibition \times 10E^5}{[Total\ protein]}\ (\mu g/ml)\ per\ (mg/mL)\ of\ total\ protein$$

Down-regulation ability of *Tetranychus* species in other Solanales plants

To understand how different degrees of specialization of three *Tetranychus* species (*T. evansi, T. ludeni* and *T. urticae*) can influence their ability to down-regulate plant defences, how broad this ability is and how different hosts can modulate the down-regulation mechanism differently, four plant species from the Solanales order were selected. This selection aimed at obtaining a good representation of these two phylogenetically-close families, Solanaceae and Convolvulaceae (*Figure S1.1*), and the presence of different toxic secondary metabolites, while keeping workload and plant rearing feasible. The selected plants were: tomato (*S. lycopersicum*, var. Moneymaker), datura (*D. stramonium*), tobacco (*N. tabacum*, var. Virginia) and purple (*Ipomoea spp*). Bean plants (*P. vulgaris*, var. Contender) from the Fabales order were selected as an outgroup of the Solanales order (*Table 2.1*).

To perform this experiment, the creation of population subsets of all mite species on the same host plant was attempted. However, a first population of *T. ludeni* placed on tomato plants was lost due to a contamination, and, after consecutive attempts, the new population was unable to colonize this host plant. Since *T. ludeni* was frequently collected from purple plants (*Ipomoea sps*) (Santos et al. in prep.), a new subset was created on this host plant, with success. However, all attempts to rear *T. evansi* on this host plant failed. Therefore, at the time of the experiment, the species used were not reared on a common host plant: (1) a subset of *T. evansi* population, which was maintained on detached four-week-old tomato leaves (~30 generations); and (2) two subsets of *T. ludeni* and *T. urticae* populations, which were founded with, approximately, 200 mated females from the stock populations and maintained in purple plants (~10 generations). After detecting a *Wolbachia* contamination in the subset of *T. urticae* population, a rifampicin treatment was applied. To do so, 300 females from the purple subset were installed in bean leaves placed in petri-dishes with a solution of rifampicin (0.05% w/v). The treatment occurred during one generation and, subsequently, 300 mated females were transferred to purple plants to create a new population. After three generations, this population was tested to confirm that the rifampicin treatment was effective.

As such, across this experiment, the following populations were used: (1) *T. evansi*, rearing in detached tomato leaves, (2) *T. ludeni*, maintained in purple plants and (3) *T. urticae*, maintained in purple plants and treated with rifampicin. In the latter population, induction of plant defences was previously observed (Godinho personal observations). As such, this population was used as a negative control for down-reguation.

All experiments were performed under controlled conditions (25ºC; 70% RH; photoperiod of 16L:8D).

Host Range of Tetranychus species

It is of great importance to first characterize how the populations of the three spider mite species can perform and compete in the host range selected. To do so, performance measurements were done as previously stated (*Figure 2.1*), allowing the following of the development of a generation in the several plants.

This experiment was performed in 2 blocks, in a total of 4 to 8 plant replicates and 35 to 59 mite replicates per plant treatment.

Effect of pre-infestation on mite performance

To test whether down-regulation by *T. evansi* and *T. ludeni* and induction by *T. urticae*, increases or reduces the reproductive performance of con- and heterospecifics, the performance of mites on either infested and uninfested (clean) plants, on the selected plants was measured.

Mite infestations were performed on one fully expanded leaf of each plant (*Table 2.1*). The infestation protocol was similar to the one described above (*Figure 2.1*). However, for this experiment, the protocol was optimized by increasing the number of females placed on the plant (100 mated females) and the time of infestation (72h). After removing the females, the eggs and the web, 9 leaf discs (12 mm^2 Ø) were made from each leaf and placed in water-saturated cotton. 15±1 days-old *T. evansi* mated females were added to each leaf disc and their performance was followed as described in the previous experiments.

As before, the remaining plant material was stored at -80ºC, preserving the tissue's biochemical properties, for further physiological analyses.

This experiment was performed in two sub-experiments: (a) *Effect of pre-infestation by* T. evansi *on mite performance*, and (b) *Effect of pre-infestation by* T. ludeni *and* T. urticae *on mite performance*. Both sub-experiments were performed in 8 blocks in a total of 4 to 9 plant replicates and 36 to 81 con- and heterospecific mite replicates per treatment. The data concerning the performance of *T. evansi* species in the *Host Range in Tetranychus species* experiment, was obtained from the clean treatment of the latter sub-experiment.

Proteinase inhibitors

To directly measure the presence of down-regulation or induction in the pre-infested plants, the concentration of PIs and total protein content in each sample were measured. The protocols described above were applied.

Spectral analysis

Reflectance spectroscopy was shown to successfully characterize plant performance (Couture et al. 2016). As such, it may allow to understand how the primary and secondary metabolism of pre-infested plants can be affected by herbivory in a powerful and non-invasive way (Herrmann et al. 2017). Using a UniSpec spectroradiometer (PP-Systems, Haver Hills, MA, USA), the reflectance spectra of the selected leaves was assessed in the range of UV-B light (300.4 nm) to near-infrared (1148.1 nm) wavelengths, with an optimized integration time of 30ms. Five measurements were performed per plant replicate.

Spectral reflectance factors (ρ) were obtained by normalizing the reflected radiation from the leaves by a reflectance white standard. With ρ integrated over the spectra, several measurements were calculated. With values of ρ obtained in the UV-B spectra, changes in the relative levels of secondary metabolites due to mite herbivory could be assessed (Lattanzio et al. 2006). Additionally, since UV-B radiation was shown to induce the production of JA-associated defences (e.g. proteinase inhibitors and phenolic compounds), modulating plant-herbivore interactions (Demkura et al. 2009 and Izaguire et al. 2007), down-regulation and induction of such defences, may affect the reflectance in this spectra region, being easily detected.

Since spider mites feeding can affect photosynthetic pigments and leaf structures, leaf damage and modification in the primary metabolism can be present. As such, several reflectance index were calculated using ρ. **Stress index** (1) was used as a measurement of the possible stress response to herbivory. This index is based in the decrease of relative chlorophyll content due to stress conditions (e.g. pathogens), which would increase leaf reflectance in wavelengths with lower absorption of chlorophylls (695nm). To minimize confounding effects, the wavelength in which the chlorophylls absorption is higher (420nm) was used. (Carter 1994). Water index or **WI** (2) was used as a measurement of water stress in the plants to check this possible abiotic stress during the experiments. This index is

based on the ratio between the reflectance at 970 nm, one of the water absorption bands, and a reference wavelength (900nm) (Peñuelas et al 1993). The higher the WI, the lower is the water content in the plant and higher the water stress. Green chlorophyll index or **CIg** (3) was used to determine the relative chlorophyll content present in the assessed leaves, and how it can be affect by the different species. This index is based on a conceptual model at leaf level that, by applying ρ at wavelengths (NIR=700; Green=550) in which the absorption of chlorophylls is smaller but deeper in the leaf, it is more sensitive to variations on the leaf relative chlorophyll content than the broadly used NDVI index (Gitelson et al. 2002). Physiological reflectance index or **PIR** (4) was used as an indirect indicator of relative photosynthetic function and photosystem II (PSII) radiation use efficiency (Gamon et al. 1997). This index incorporates the reflectance at 531 nm, which can detect the interconversion of xanthophyll cycle pigments that are closely linked to PSII (Gamon et al. 1992), normalized by a reference wavelength (570 nm), for other factors besides the xanthophyll cycle (e.g. pigment content and chloroplast movement) (Gamon et al. 1993).

(1) $Stress = \frac{\rho_{695}}{\rho_{420}}$

(2) $WI = \frac{\rho_{970}}{\rho_{900}}$

(3) $CIg = \frac{\rho_{NIR}}{\rho_{Green}} - 1$

(4) $PIR = \frac{\rho_{531} - \rho_{570}}{\rho_{531} + \rho_{570}}$

(5) $[Chl\ a] = (-\log(\rho_{663.6}) \times 13.71) - (-\log(\rho_{646.6}) \times 2.85)$

(6) $[Chl\ b] = (-\log(\rho_{646.6}) \times 22.39) - (-\log(\rho_{663.6}) \times 5.42)$

(7) $[Chl\ a+b] = (-\log(\rho_{663.6}) \times 8.29) - (-\log(\rho_{646.6}) \times 19.54)$

To complement the two earlier indexes, the estimation of the concentration of chlorophyll *a* (4), *b* (5) and total chlorophyll (*a*+*b*) (6) present in each treatment, was calculated. To do so, equations based on the extinction coefficients of chlorophylls *a* and *b* were used (Porra et al. 1989). Note that these equations use absorbance values that were calculated through -log(ρ) (transmittance is negligible). Together Cig, PIR and estimated chlorophylls content were used as a proxy for leaf damage.

Choice between infested and un-infested plants

To understand how the presence of down-regulation can affect host choice, *T. evansi* and *T. urticae* populations subsets were used. Both population subsets were created with 200 mated females from the stock populations and reared in detached four-week-old tomato leaves, until the time of this experiment. With the different defence manipulative ability of these two populations, it is possible to verify how these two competitive mite species can distinguish between environments in which the quality was reduced by *T. urticae* (induction) or improved by *T. evansi* (down-regulation), from environments that did not have previous mite infestations, choice experiments were performed (*Figure 2.3*). To do so, tomato (*S. lycopersicum*, var. Moneymaker) and bean plants (*Phaseolus vulgaris*, var. Contender) were used. These plants were selected due to the presence or absence, respectively, of down-regulation by *T. evansi* as previous observed (Godinho et al. 2016; Godinho personal observations).

Tomato and bean plants were pre-infested as described in the *Suppression ability of* Tetranychus *species in other Solanales plants* experiment. After the disinfestation of these plants, 100 mated females (15±1 days-old) of *T. evansi* or *T. urticae* species were placed in a small petri dish, between a clean and a pre-infested plant, either tomato or bean plants. As such, the mites were presented with the choice between clean plants and plants pre-infested with either con- or heterospecifics (*Figure 2.3*). After 3 days, the number of females present in each leaf was registered. This experiment was performed in 14 blocks (6 to 8 choice replicates) at controlled conditions (25ºC; photoperiod of 16L:8D).

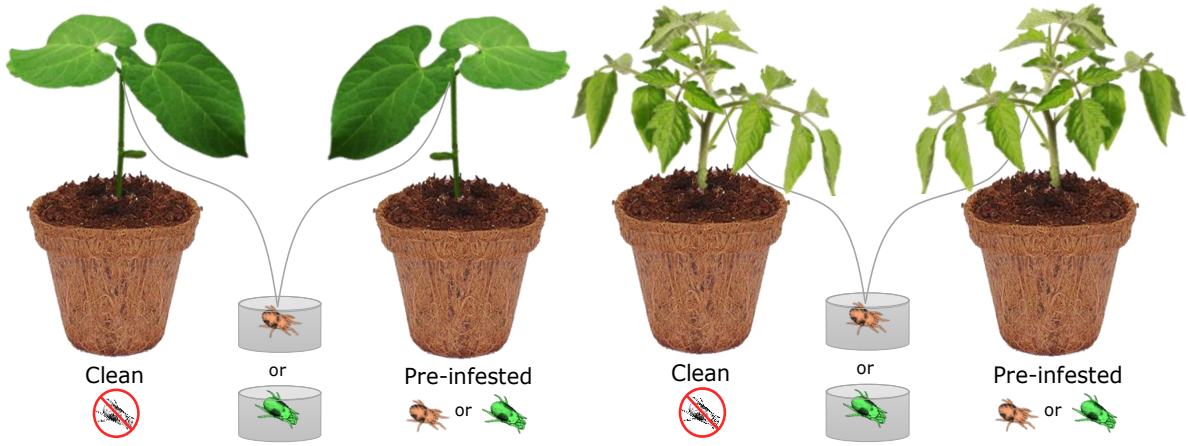

***Figure 2.3*. Schematic representation of the choice protocol performed with tomato and bean plants (right and left, respectively).** The orange and green mites represent *T. evansi* and *T. urticae*, respectively.

Statistical analyses

All statistical analyses were performed with the software R (version 3.2.5, R Development Core Team 2016). The description of the statistical models is available in *Table S1.2.*

Manipulation of plant defences on tomato strains

Effect of pre-infestation on mite performance

For the analysis of daily fecundity, Box-cox transformation (Crawley 2012) was performed to improve normality and linear mixed-effect models (lm, lme4 package) were used (Bates et al. 2015). For this analysis, the offspring of females that died accidentally during the 4 days were excluded. Tomato plant variety and infestation status were used as fixed explanatory variables and the block and the ID number of the plants (num) were used as random explanatory variables.

Proteinase inhibitors

For the analysis of the concentration of PIs and total protein content in each sample, Box-cox transformations (Crawley 2012) were performed to improve normality and linear mixed-effect models (lm, lme4 package) were used (Bates et al. 2015). For this analysis, samples in which the vegetal material was less than 100mg were excluded. Tomato plant variety and infestation status were used as fixed explanatory variables.

Suppression ability of* Tetranychus *species in other Solanales plants

Host Range in *Tetranychus* species

To analyse female survival over the 4-day oviposition period, a cox proportional hazard mixed-effect model (coxme, coxme package) (Therneau 2012), with accidental deaths as censored, was used. Additionally, the proportion of females that drowned in the water-saturated cotton when trying to escape from the unfavorable patch was analyzed. To do so, the proportion of drowned and live females was computed by the cbind function and analyzed using a generalized linear model with a binomial error distribution (glm, lme4 package). Subsequent analyses on the offspring of these females (see below) excluded the offspring of females that died accidentally during the 4 days.

To analyse the number of eggs oviposited per day (daily fecundity), a Box-cox transformation (Crawley 2012) was performed to improve normality and linear mixed-effect models (lmr, lme4 package) were used (Bates et al. 2015). To test for differences in the hatching rate, the number of unhatched eggs, adults and dead juveniles was computed using the cbind function. To account for overdispersion, a generalized linear mixed model with a beta-binomial error distribution (zeroInflation=TRUE added when the model needs to consider a big number of zeros present in the dataset) was used (glmmadmb, glmmADMB package) (Fournier et al. 2012; Skaug et al. 2013).

Finally, to analyse sex-ratio, the number of males and females was computed using the cbind function and analysed using a generalized linear model with a binomial error distribution (glm, lme4 package) (Bates et al. 2015). For both hatching and sex ratio analyses, all the leaf discs that rotted were excluded.

In all analyses, plant and mite species were defined as fixed explanatory variables and block was defined as a random explanatory variable.

Effect of pre-infestation on mite performance

Data from this experiment was analysed as above. However, as the experiment with *T. evansi* was done independently from that with *T. ludeni* and *T. urticae* (due to differences in mite rearing, as mentioned

above), two sub-experiments were analyzed separately. Plant species and infestation status were used as fixed explanatory variables and the block and the ID number of the plant (num) were used as random explanatory variables.

Proteinase inhibitors

The statistical analysis of the concentration of PIs and total protein content in each sample was performed as described above.

Spectral analysis

To analyze spectral measurements, Box-cox transformations (Crawley 2012) were performed to improve normality and linear mixed-effect models (lm, lme4 package) were used (Bates et al. 2015). Plant species and infestation status were used as fixed explanatory variables.

Choice between infested and un-infested plants

To test the significance of the choice between infested and uninfested plants, choice treatment (clean vs pre-infested with *T. evansi* or *T. urticae*), plant species (bean or tomato) and the mite species that choose (*T. evansi* or *T. urticae*) were used as fixed explanatory variables and the block as a random explanatory variable.

To analyze if the proportion of mites that disperse to the plants differed between treatments, the number of mites that choose and that did not choose were computed using the cbind function. To account for overdispersion of the data, generalized linear mixed models with a beta-binomial error distribution were used (glmmadmb, glmmADMB package) (Fournier et al. 2012; Skaug et al. 2013). From the number of mites that dispersed to the choice plants, the proportion of those that choose clean or infested plants was computed using the cbind function and analyzed using generalized linear mixed models with a beta-binomial error distribution (zeroInflation=TRUE) (glmmadmb, glmmADMB package) (Fournier et al. 2012; Skaug et al. 2013).

For all the analyses described before, when a significant interaction between the two fixed variables was observed, *a posteriori* contrasts, with Bonferroni corrections, were performed (testInteractions, phia package) (De Rosario-Martinez 2013). When these contrasts were not robust enough to verify a significant interaction, comparison analyses were performed (likelihood ratio test with X^2 -like distribution). If a significant interaction was not found or the analysis was established with a minimal model with a single fixed explanatory variable, *a posterior* contrasts, with Bonferroni corrections, were tested using a General Linear Hypothesis Test (glht, multcomp package) (Hothorn et al. 2008).

Results

Manipulation of plant defences on different tomato strains

Effect of pre-infestation on mite performance

Overall, there was no significant effect of the tomato strains on the daily fecundity of *T. evansi* females ($F_{3,0.58}$= 0.651, P= 0.7396; *Figure 3.1*). However, a significant effect of the infestation status ($F_{2,441.18}$= 6.436., P= 0.002) and of the interaction of both response variables ($F_{6,441.32}$= 3.252., P= 0.004) was observed. Contrast analyses (*Table S2.2)* revealed that, on *def-1* plants, females had higher daily fecundity on *T. ludeni* pre-infested plants than on either clean or *T. evansi* pre-infested plants. In *wild-type* plants the same was observed between clean and *T. ludeni* pre-infested plants.

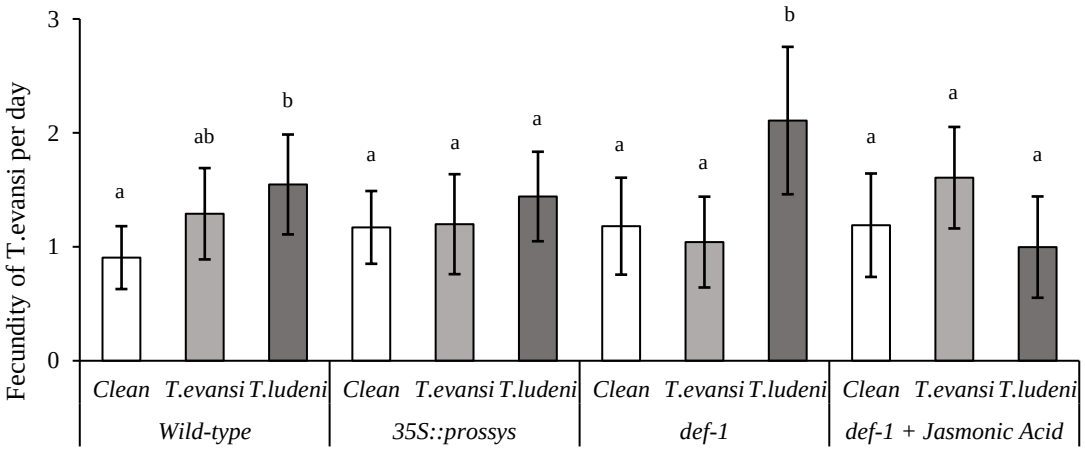

Figure 3.1. **Effect of tomato strain and infestation status on daily fecundity of *T. evansi* females.** Bars represent the average (± s.e.; *Figure S2.1*) daily fecundity per female (the number of eggs oviposited per *T. evansi* female/number of days that the female was alive) on leaf discs of *wild*-type, *35S::prosys*, *def-1* or *def-1* + Jasmonic acid tomato plants either clean, infested by *T. evansi* or infested by *T. ludeni*. Different small letters indicate significant differences among mite species within plant species.

The measurements of female survival in the different treatments are not present since they did not reveal significant differences and, overall, did not increase the clarification in terms of plant defence manipulation (data not shown).

Proteinase inhibitors

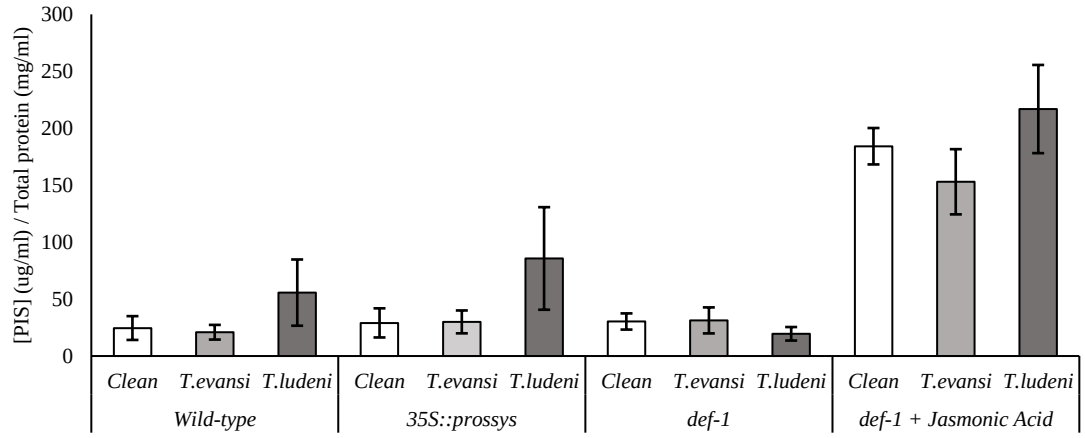

Figure 3.2. **Effect of infestation status on the concentration of PIs (µg/ml) per total protein content (mg/ml) of the different tomato strains.** Bars represent the average (± s.e.) of the concentration of proteinase inhibitors (µg/ml) per total protein content (mg/ml) on each tomato strains (*wild*-type, *35S::prosys*, *def-1* or *def-1* + Jasmonic acid) either clean, infested by *T. evansi* or infested by *T. ludeni*.

Regarding the concentration of PIs (µg/ml) present in each treatment, only tomato strains had a significant effect ($F_{3,106.788}$= 17.162, P< 0.001). Contrast analysis revealed that, overall, *def-1* plants in which exogenous jasmonic acid was added had higher PI level than the remaining tomato strains (*def-1 + Jasmonic acid – wild-type:* t= 6.261; P<0.001; *def-1 + Jasmonic acid – 35S::prossys:* t= 5490; P<0.001; *def-1 + Jasmonic acid – def-1:* t= 6.010; P<0.001).

Suppression ability of *Tetranychus* species in other Solanales plants

Host Range of Tetranychus species

Female survival during the 4 days of the experiment was similar across spider-mite species (X_2^2= 3.280, P= 0.194). However, host plant species affected this trait (X_4^2= 11.149, P= 0.025; *Figure S2.1*) and this effect differed across spider mite species (interaction: X_8^2= 31.336, P<0.001). Analyses within each plant species (*Table S2.4A*) showed that *T. ludeni* females had lower survival than *T. urticae* on tobacco plants, but higher survival than this species on datura plants and *T. evansi* on purple plants. Furthermore, comparison analyses within mite species (*Table S2.4B)* revealed that: (1) *T. evansi* died less on tobacco plants and more on purple plants, compared to other plants; (2) *T. ludeni* females had higher mortality on tomato and tobacco plants, compared to the remaining plants; and (3) *T. urticae* had similar survival on all host plants.

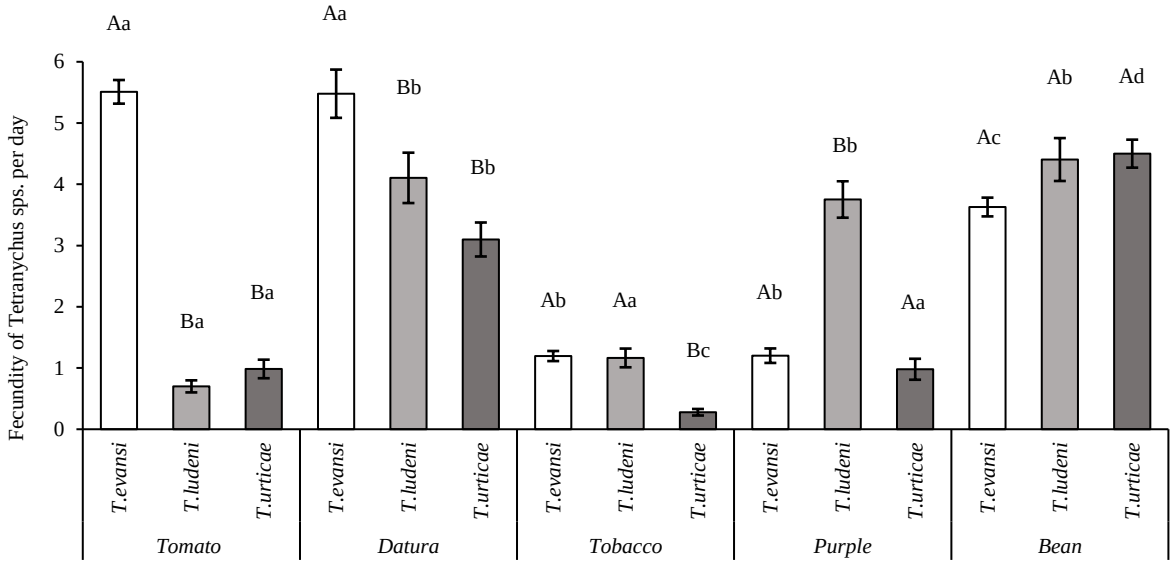

Figure 3.3. **Effect of plant species on daily fecundity of *T. evansi*, *T. ludeni* and *T. urticae* females.** The bars represent the average (± s.e.) daily fecundity per female (the number of eggs oviposited per female, per number of days that the female was alive) in leaf discs of Tomato, Datura, Tobacco, Purple or Bean plants. Different capital letters indicate significant differences among mite species within plant species. Different small letters indicate significant differences among plant species within mite species.

Additionally, the proportion of females that drowned (i.e., escaped from the leaf disc) over the 4 days of survival (*Figure S2.2)* was significantly affected by plant (X_4^2= 13.033, P= 0.011), mite species (X_2^2= 7.017, P= 0.030) and their interaction (X_8^2= 65.544, P< 0.001). Contrast analysis within plant species (*Table S2.5A*) revealed that *T. ludeni* escaped more from tomato plants than the two other mite species. Additionally, contrast analysis within mite species (*Table S2.5B*) showed that *T. ludeni* females escaped more from tomato and tobacco plants than from bean and purple plants.

Tetranychus spp. females had, overall, similar daily fecundity ($F_{2,544.38}$= 1.625, P= 0.198; *Figure 3.3*). However, oviposition was affected by host plant species ($F_{4,512.89}$= 95.985, P<0.001) and this effect was different across mite species (interaction: $F_{8,654.29}$= 62.178, P<0.001).

Contrast analyses within plant species (*Table S2.6A*) showed that: (1) *T. evansi* females had higher daily fecundity on tomato and datura plants than the other mite species; (2) the daily fecundity of *T. urticae* on tobacco plants was lower than that of other mite species; and (3) the daily fecundity of *T. ludeni* females on purple plants was higher than that of other mite species. No differences were found on bean. Additionally, contrast analysis within mite species (*Table S2.6B*) revealed that the daily fecundity of *T. evansi* females was higher on tomato and datura plants than on the remaining plants, with the lowest values on tobacco and purple plants and intermediate on bean. Daily fecundity of *T. ludeni* females was similar on datura, purple and bean plants and greater on tomato and tobacco plants. Regarding *T. urticae* females, daily fecundity was lowest on tobacco, then on tomato and purple, intermediate on datura and the highest on bean.

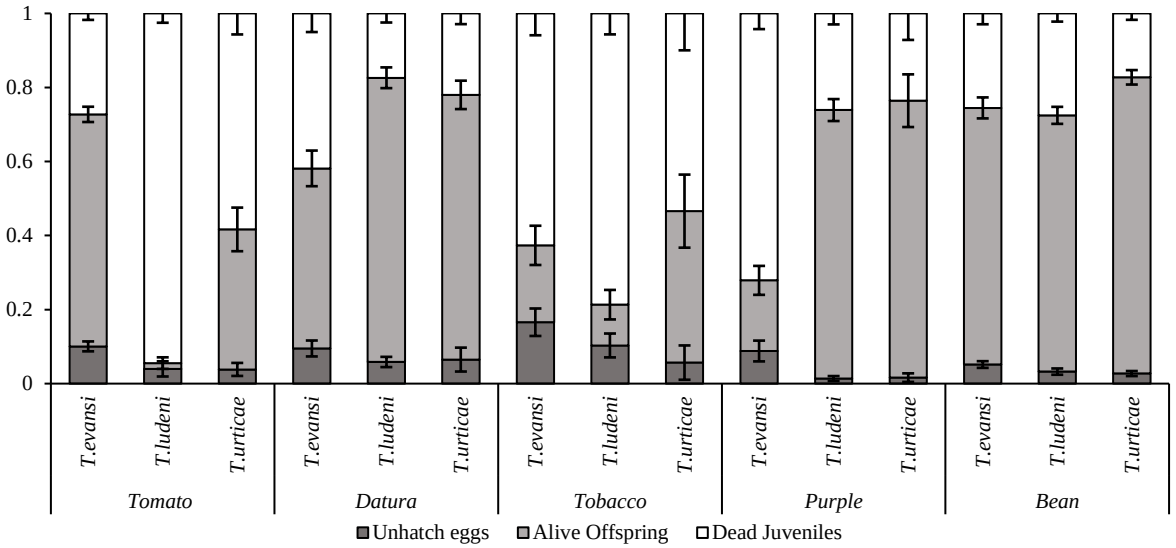

Figure 3.4. **Hatching rate of *Tetranychus spp* on different host plants.** Bars represent the average (± s.e.) proportion of unhatched eggs, alive offspring and dead juveniles on leaf discs of Tomato, Datura, Tobacco, Purple or Bean plants.

There was a significant effect of plant species (X_4^2=34.693, P<0.001) and mite species (X_2^2=12.766 P= 0.002), but not of their interaction (X_8^2= 7.493, P= 0.486), in the proportion of unhatched eggs (*Figure 3.4*). Contrast analyses (*Table S2.7*) showed that the proportion of unhatched eggs was higher on tobacco than on purple and bean plants. Moreover, it was higher for *T. evansi* than for *T. urticae.*

Plant species and mite species and their interaction significantly affected the proportion of dead juveniles (X_4^2=175.193, P<0.001; X_2^2= 6.693, P= 0.035; X_8^2= 246.761, P<0.001, respectively). Further contrast analyses within plant species showed that juvenile mortality was lower for *T. evansi* and higher for *T. ludeni* on tomato plants and the highest for *T. evansi* on purple plants (*Table S2.5A*). Additionally, contrast analyses within mite species revealed that juvenile mortality was higher in tobacco and purple plants for *T. evansi* compared to other plants, higher on tobacco and tomato plants for *T. ludeni* and higher on tomato than on bean plants for *T. urticae* (*Table S2.5B*).

Overall, the proportion of females and males in the offspring (sex-ratio) was similar in all plant species tested (X_4^2=9.075, P= 0.059; *Figure S2.3*). However, this proportion was significantly affected by the mite species (X_2^2= 21.156, P<0.001) and the interaction between mite and plant species (X_8^2= 22.683, P= 0.038). Contrast analyses within plant species (*Table S2.8A*) revealed that *T. ludeni* produced more

females than the other mite species on purple plants. On bean plants, this proportion was lower for *T. evansi*. Contrast analysis within mite species (*Table S.2.8B*) revealed that *T. evansi*, produced a lower proportion of females on purple compared to tomato plants, and that *T. urticae* produced fewer proportion of females on tobacco and purple plants than on tomato plants.

Effect of pre-infestation on mite performance

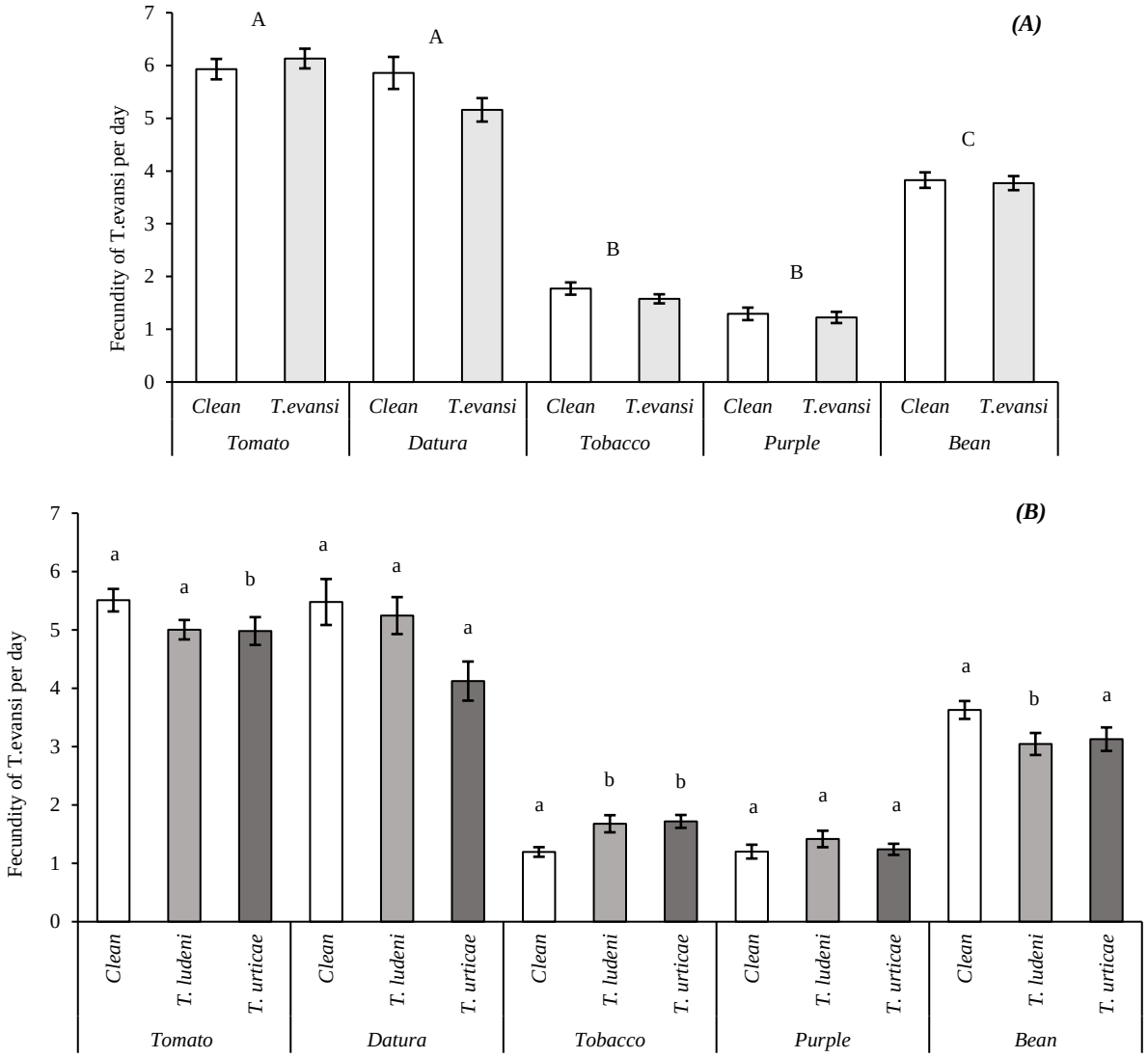

Figure 3.5. **Effect of plant species and infestation status on daily fecundity of *T. evansi* females.** Bars represent the average (± s.e.) daily fecundity per female (the number of eggs oviposited per *T. evansi* female/number of days that the female was alive) on leaf discs of Tomato, Datura, Tobacco, Purple or Bean plants that were either (A) clean or infested by *T. evansi* or (B) clean or infested by *T. ludeni* or *T. urticae.* Different capital letters indicate significant differences among plant species. Different small letters indicate significant differences among mite species within plant species.

Plant species significantly affected daily fecundity of *T. evansi* females on either clean and conspecifics pre-infested plants ($F_{4,67.241}$= 181.850, P<0.001; *Figure 3.5A*). However, infestation status ($F_{1,62.269}$= 0.481, P= 0.491) and its interaction with host plant were not significant ($F_{4,58.384}$= 0.377, P=0.824).

The plant species, infestation status and their interaction significantly affected the daily fecundity of *T. evansi* females on clean plants and on plants previously infest with heterospecifics (*T. ludeni* and *T. urticae)* ($F_{4,277.890}$ = 139.453, P<0.001; $F_{2,141.840}$= 3.654, P= 0.028; $F_{8,335.73}$= 3.122, P= 0.002; *Figure 3.5B*).

Further contrast analyses (*Table S2.11*) showed that the fecundity of *T. evansi* females in sub-experiment (a) follows same pattern shown in the *Host range of* Tetranychus *species* experiment. However, regarding the infestation status on sub-experiment (b), the daily fecundity was lower on tomato and bean plants pre-infested with *T. urticae* and higher in both pre-infested status on tobacco plants.

The remaining measurements, concerning the female survival and the development of the offspring of each female in the different treatments, are not presented since they follow a similar pattern to those in the previous experiment and, overall, did not increase the information in the terms of plant defence manipulations (data not shown).

Proteinase inhibitors

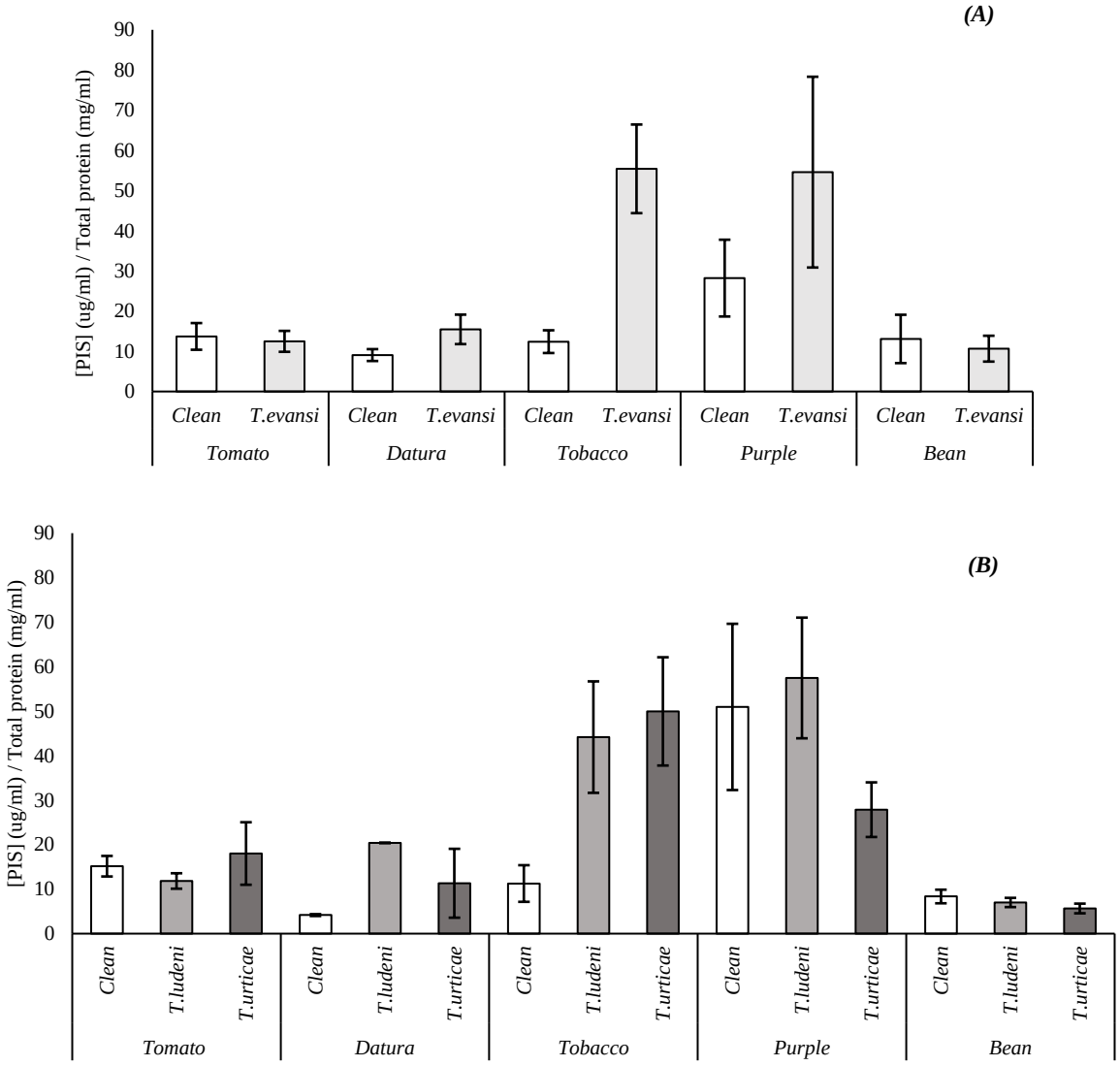

Figure 3.6. **Effect of infestation status on the relative concentration of PIs (µg/ml) per total protein content (mg/ml) of the different plant species.** Bars represent the average (± s.e.; *Table S2.9, S2.10*) of the concentration of proteinase inhibitors (µg/ml) per total protein content (mg/ml) on each plant species (Tomato, Datura, Tobacco, Purple or Bean plants) either (A) clean or infested by *T. evansi* or (B) clean or infested by *T. ludeni* or *T. urticae.*

Regarding sub-experiment (a), there was only a significant effect of the interaction between plant species and infestation status ($F_{4,5471.4}$= 3.010, P= 0.026) on the relative concentration of PIs (µg/ml) present in each treatment (*Figure 3.6)*. Contrast analysis among plants revealed that, in most of plants, the levels of PIs were similar in clean and pre-infested plants. On tobacco plants, an increase in PIs levels was

observed in pre-infested plants (*Table S2.12A*). Regarding sub-experiment (b), there was only a significant effect of plant species ($F_{4,13.519}$= 11.431, P<0.001). Considering sub-experiment (b), contrast analysis showed that tobacco and purple plants had higher levels of PIs compared to the remaining plant species (*Table S2.12B*)

Spectral analysis

No significant effects for stress index, WI and PRI of either plant species, infestation status and their interaction (P>0.05) were found. For CIg, there was a significant effect of the infestation status in sub-experiment (a) ($F_{1,0.334}$ = 5.531, P= 0.024). This suggests that plants pre-infested with *T. evansi* have a lower CIg than clean plants.

Concerning the chlorophyll concentrations, for either [Chl *a*], [Chl *b*] and [Chl *a+b*], there was a significant effect of plant species in both sub experiments (sub-experiment (a)*:* [Chl *a*]: $F_{4,16.430}$ = 3.052, P= 0.028; [Chl b]: $F_{4,37.210}$ = 3.764, P= 0.011; [Clh *a+b*]: $F_{4,101.740}$ = 3.433, P= 0.017; sub-experiment (b)*:* [Chl *a*]: $F_{4,4.297}$ = 16.720, P<0.001; [Chl *b*]: $F_{4,97.880}$ = 10.463, P<0.001; [Clh *a+b*]: $F_{4,87.84}$ = 12.715, P<0.001).

Concerning the effect of the plant species and infestation status on the leaf reflectance factor (*ρ)* on several wavelengths of the UV-B spectra (*Figure 3.7*), plant species had a significant effect in all the wavelengths for sub-experiment (b) (P<0.005) and infestation status had a significant effect in all wavelengths tested for both sub-experiments (P<0.005). There was not a significant interaction of the fixed explanatory variable in all wavelengths tested for both sub-experiments (P>0.005).

The results concerning the infestation status in sub-experiment (a) suggest that plants pre-infested by *T. evansi* had significantly lower *ρ* in all UV-B wavelengths than clean plants (*300.4*: $F_{1,<0.001}$ = 21.727, P<0.001; *303.7*: $F_{1,<0.001}$ = 40.745, P<0.001; *310.5*: $F_{1,1.820}$ = 24.180, P<0.001; *313.9*: $F_{1,0.002}$ = 39.871, P<0.001). Regarding sub-experiment (b), further contrast analysis between clean plants and plants either pre-infested by *T. ludeni* or *T. urticae* revealed the same pattern observed in sub-experiment (a). However, no significant differences between plants pre-infested by *T. ludeni* and plants pre-infested by *T. urticae* were revealed (*Table S2.13*).

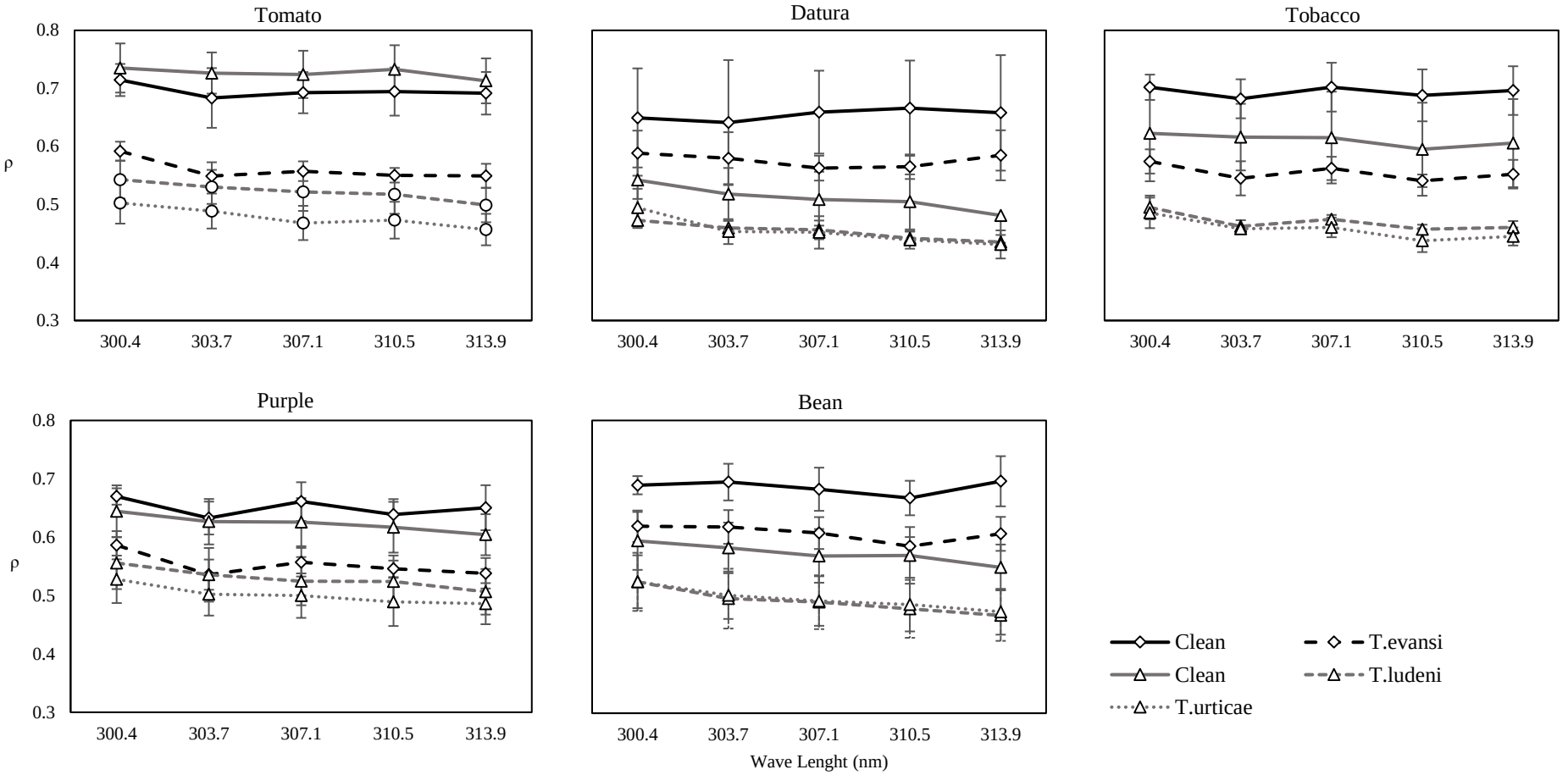

Figure 3.7. **Effect of plant species and infestation status on the leaf reflectance factor (ρ) on wave lengths of the UV-B spectra.** The markers represent the average (± s.e.) of ρ for each plant species either clean or infested by *T. evansi* (black line,◊) or clean and infested by *T. ludeni* or *T. urticae* (grey line,Δ).

Choice between infested and uninfested plants

Significantly more *T. urticae* females were found on the plants, compared to *T. evansi* (X_1^2=4.492, P= 0.034; *Figure S2.4*).

Regarding the choice between clean or infested plants (*Figure 3.8*), only the choice pairs (Clean vs *T. urticae* or Clean vs *T. evansi*) had a significant effect (X_1^2=4.311, P= 0.038). Further contrast analysis suggested that mites that dispersed prefer plants pre-infested with *T. urticae*, rather than plants clean plants (z= 2.076, P= 0.038).

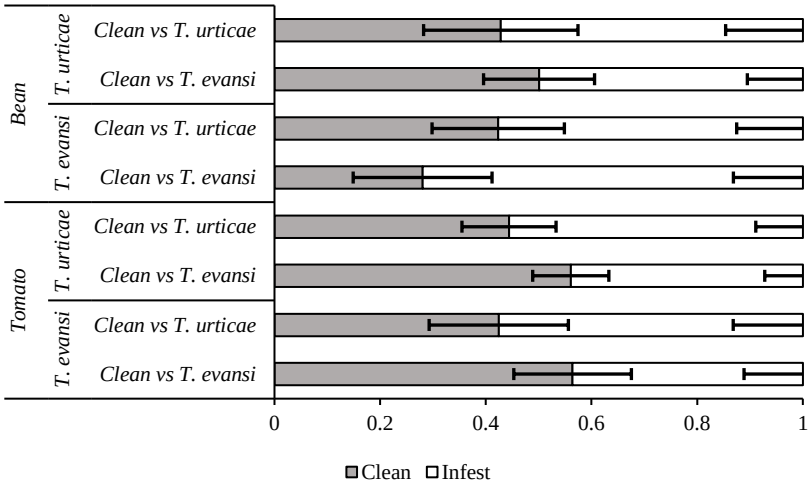

Figure 3.8. **Proportion of mites on each plant.** Bars represent the average (± s.e.) proportion of *T. evansi* or *T. urticae* between clean or pre-infested (with *T. evansi* or *T.urticae*) plants. This proportion only considers the mites that have dispersed to the presented plants. This choice was performed using bean and tomato plants.

Discussion

This project aimed at testing whether the ability of some *Tetranychus* spider mites to down-regulate plant defences would correlate to their host range. To address this, it was first tested how the down-regulating species, *T. evansi* and *T. ludeni*, interacted with tomato mutant plants with different degrees of defences. Results suggested that these two species probably manipulate the same defence target. However, the first evidence in which *T. ludeni* may also manipulate another defence target should not be excluded. Moreover, by testing the host range of *T. evansi, T. ludeni* and *T. urticae* on several host plants from the Solanales order, results suggested that: (i) *T. evansi* and *T. ludeni* were locally adapted to tomato and purple plants, respectively, and (ii) *T. urticae* performed worse on Solanales plants than on the outgroup. However, differences did not translate into a pattern of down-regulation across plants. Indeed, no down-regulation was found even on tomato plants, where it was originally described. Additionally, by spectral analysis, the results suggested that, despite suppression, plants respond to herbivory through changes in the reflectance of leaves in the area of secondary metabolites. Finally, unlike what was expected, both *T. evansi* and *T. urticae* preferred to colonize plants pre-infested with *T. urticae*.

Manipulation of plant defences on different tomato strains

No evidence of down-regulation by *T. evansi* was found on *wild-type* tomato plants. Direct physiological measurements of the PI activity revealed similar levels for both clean and *T. evansi* pre-infested *wild-type* tomato plants, suggesting suppression. However, performance on *wild-type* plants pre-infested with *T. ludeni* was higher than on clean plants (Godinho et al. 2016). However, the non-significance of the infestation status in PI activity suggests that *T. ludeni*, as *T. evansi*, only suppressed the induction of PIs, not confirming the presence of down-regulation as previously shown (Sarmento et al. 2011a, Godinho et al. 2016).

Regarding *def-1* plants it would be expected that, due to the lack of response of the JA pathway (suppression always present), the fecundity on *def-1* plants either clean or pre-infested would be the same. This was verified for *T. evansi* pre-infested *def-1* plants. However, on *def-1* plants, *T. evansi* performance was similar when plants were pre-infested with *T. evansi* or clean plants, but higher for plants pre-infested with *T. ludeni*. This can be explained by a possible suppression or down-regulation in another pathway, independent of the JA pathway, in which the *defenseless-1* mutation is present (*Figure 1.1*). However, no differences were found concerning PI activity. Therefore, this hypothesis needs further investigation.

Regarding *35S::prosys* plants, PI activity measurements revealed no differences between *wild-type* and this mutant plant. *35S::prosys* plants are supposed to constitutively express PIs (McGurl et al. 1994), which would result in a lower fecundity and higher PI levels of these plants relative to *wild-type* plants. As this was not the case, perhaps the seeds used were *wild-type* or not fully transgenic. Therefore, no conclusion can be taken from this treatment.

Finally, regarding the *def-1* plants in which exogenous JA was applied, the significant increase of PI activity confirmed that the 0.25mM jasmonate solution applied was effective (Ament et al. 2004). It was expected that daily fecundity would be reduced due to the exogenous induction of the JA pathway (down-stream of *defenseless-1* mutation). However, non-significant differences of the infestation status in both daily fecundity and PI activity were found, which suggests: (i) both species can suppress the induction of PIs even when at higher levels; or (ii) PI activity is not affected by either species since the exogenous induction is stronger than the effect of the defences manipulation.

These results suggest that *T. ludeni*, as *T. evansi*, can only suppress plant defences in the JA pathway and not down-regulate them. This contradicts earlier findings. A possible explanation for this discrepancy may be that the protocol for PI activity quantification used was different from the ones previously used with this system (Sarmento et al. 2011a, Godinho et al. 2016). It may be that earlier

studies had some protocol problems. For example, PI activity in the controls (clean plants) in Sarmento et al. 2011a were highly discrepant. Additionally, a more recent study, using molecular tools of gene expression, revealed that *T. evansi* only suppresses the gene expression of *PI-II* (serine PIs) (Alba et al. 2015), which is in accordance to our results based in the activity of these PIs. Additionally, since moisture issues greatly reduced the overall fecundity of the *T. evansi* females tested, the beneficial effect of suppression (Kant et al. 2015) could not be fully observed. In sum, this experiment needs to be repeated in more controlled conditions and with complementary molecular measurements of plant defences to test whether down-regulation is truly present in *T. evansi* and *T. ludeni*. However, this does not invalidate the first line of evidence of an alternative mechanism in *T. ludeni* that can be complemented with the use of proper *35S::prosys* seeds.

Down-regulation ability of Tetranychus species in other Solanales plants

Host range of Tetranychus species

The performance of the three-mite species on five host plants revealed local adaptation of *T. evansi* and *T. ludeni* populations to tomato (Solanaceae) and purple (Convolvulaceae) plants, respectively. This local adaptation can be one of the reasons why *T. evansi* and *T. ludeni* are not found in the field on purple and tomato plants, respectively (Santos et al. in prep) and why it was not possible to maintain both species in a common host in the laboratory under standard conditions (Magalhães group personal observations). On datura plants, despite their high levels of tropane alkaloids (Tepfer et al 1988), the oviposition of *T. evansi* was the highest. However, the fecundity of *T. ludeni* and *T. urticae* was still relatively high when compared with tomato. Although tomato and datura plants share several secondary metabolites (*Figure S1.1*) (Eich 2008) these plants seem to affect the performance of the three spider mite species differently. To better understand these results, the chemical and nutritive profile of these plants should be analysed more deeply. Regarding tobacco plants, all spider-mite species showed low daily fecundity and a high proportion of unhatched eggs. This may be due to high levels of nicotine present in this host plant, which may be as toxic for spider mites as for other herbivores (Baldwin et al 2001). Finally, on bean plants (outgroup), all mite species showed similar fecundity and *T. urticae* had significantly higher performance on this plant as compared to all other Solanales and Solanaceous plants. However, the sex ratio of *T. evansi* on bean plants was lower than that of other species (lower production of females). This, together with the higher proportion of unhatched eggs of *T. evansi*, compared with *T. urticae,* on all plants tested, may explain why this species is not present on bean plants in nature (Santos et al. in prep) and cannot be reared on this host under standard laboratorial conditions (Magalhães group personal observations).

In sum, testing the host range of the three-spider mite species led to the following conclusions: (i) *T. evansi* and *T. ludeni* are locally adapted; (ii) some plants, such as tobacco, are bad host plants for all mites species; and (iii) some plants, such as bean, are overall permissive. However, these results may be partly due to the host plant at which each spider mite species has been reared on and/or to maternal effects (Magalhães et al. 2011). Repeating this experiment with a common rearing host plant, will confirm the presented results.

Effect of pre-infestation on mite performance and plant defences

Reproductive performance of *T. evansi* was not modified after pre-infestations with one of the three-mite species, as previously suggested (Sarmento et al. 2011a, Godinho et al. 2016). Direct measurements of PI activity revealed that suppression is indeed present in tomato plants pre-infested with either *T. evansi*, *T. ludeni* or *T. urticae.* These results contradict previous findings (Li et al. 2002, Ament et al. 2004, Kant et al. 2004, Sarmento et al. 2011a, Godinho et al. 2016). However, results concerning *T. ludeni* need to be carefully analysed due to the high mortality of this species on tomato plants. If *T. ludeni* died due to the constitute chemical profile of tomato plants, the mites will probably die before

feeding, leading to no defensive manipulations (induction or suppression) of PI activity. To confirm this hypothesis, measurements of leaf damage, as a proxy for feeding and wounding, would be of extreme importance. Regarding the results of *T. urticae* pre-infestations, some suppressor *T. urticae* populations have been already found (Kant et al. 2008, Alba et al. 2015). Since the *T. urticae* population used in this project has been reared on tomato plants for more than 20 generations, this population could have evolved the ability to suppress tomato defences, as previously observed (Wybouw et al. 2015). Performance on datura plants pre-infested by *T. evansi, T. ludeni* or *T. urticae* was not different from that of on clean plants. Regarding the measurements of PI activity, due to the highly reduced number of replicates quantified, no conclusions could be drawn. Concerning purple plants pre-infested with either *T. evansi, T. ludeni* or *T. urticae*, no differences in performance and PI activity were observed, suggesting suppression. However, results concerning plants pre-infested with *T. evansi* need to be carefully analysed due to the high mortality of *T. evansi* females on purple plants. If this mortality was due to the chemical profile of purple plants, the mites will probably die before feeding, leading to no induction of PI activity. Again, measuring leaf damage would be crucial to interpret these results. On tobacco plants, although a reduction in conspecific performance was not observed, possibly due to the already reduced fecundity of *T. evansi* in this plant, there was a clear induction of PI activity by *T. evansi*. On *T. ludeni* and *T. urticae* pre-infested tobacco plants, an increase of heterospecifics performance was observed. However, both species showed a clear tendency to induce PI activity, suggesting that the results obtained in fecundity can be a byproduct of the big variability in plant growth. These results suggest that tobacco plants may have a particular defensive structure that cannot be as easily circumvented. Finally, on bean plants pre-infested by either *T. evansi* or *T. urticae*, no differences in con- and heterospecific performance were observed. However, on bean plants pre-infested with *T. ludeni*, a decrease on the heterospecific performance was revealed. In PI activity measurements, a general suppression was observed for all mite species. As such, these results may suggest that: (i) *T. evansi* and *T. urticae* can suppress PI activity; (ii) *T. ludeni* can suppress PI activity but may induce other defensive traits, affecting heterospecifics performance; or (iii) the wound-response on bean plants is so subtle that cannot be detected by the PI activity protocol used.

The non-significant differences in the stress index, PRI and chlorophyll concentrations between clean and pre-infested plants revealed that: (i) the stress caused by spider mites was not as easily detected as the one caused by pathogens (Carter 1994); and (ii) the primary metabolism (relative content of chlorophyll and PSII radiation use efficiency) was not significantly affected by spider-mites, revealing a lower leaf damage. In contrast, Cig revealed a lower relative content of chlorophyll in plants pre-infested with *T. evansi*, which may suggest a higher leaf damage by this species. More measurements of primary metabolism (Carbon: Nitrogen rations, direct chlorophyll quantifications) need to be performed to corroborate these results. Additionally, no significant differences were shown in WI, suggesting that the plants were not under water stress during the infestation procedure.

Regarding UV-B measurements, we found that the controls (clean plants) between sub-experiments were not replicable. Indeed, only reflectance regarding tomato plants seemed to be similar across sub-experiments. This could be due to growth conditions, since in the second sub-experiment, the space available to sow plants was limited, which probably resulted in abiotic stresses (e.g. competition for light). If a plant is under an additional stress, its response to herbivory may be reduced because it cannot afford to allocate as many resources to defense against herbivores (Feeney 1976, Stamp 2003). This variability in plant quality could influence the expression of plant defences and, hence, how mites responded to them. Additionally, since most plants used are non-model laboratory plants, the knowledge and requirements needed to grow these plants was still not optimized. However, working with non-model plants can be a great step forward in the understanding of plant-herbivore interactions in nature. Despite all this, the three spider mite species seemed to induce the same response in all host plants by

reducing the leaf reflectance. Since plants respond similarly to UV-B and herbivory by inducing the production of flavonoids and phenolic acids (Izaguire et al. 2007), this reduction in reflectance can be translated into the accumulation of such secondary metabolites, increasing their absorbance. Thus, plants may have other layers of response to herbivory, besides those culminating in PI production, to respond to spider-mite attacks.

Put together, these results point to a complex interaction between mites and plants, which is only starting to be understood. Indeed, the absence of down-regulation shown in this project may be due to either variation across populations or to the use of a more optimized quantification of PI activity. Also, the clear relation between daily fecundity and PI activity previously shown (Sarmento et al. 2011a, Godinho et al. 2016) was not recovered in our study, probably because it is highly dependent on the condition in which fecundity is assessed. Fecundity of the same *T. evansi* population is highly dependent on the environmental conditions (*Figure 4.1A*). As previously shown, a high relative humidity interferes with water loss and concentration of nutrients, decreasing spider-mite feeding and, consequently, oviposition rate (Boudreaux 1957). As such, it is possible that, when the daily fecundity was measured in a drier environment (~70% RH), the *T. evansi* females had their fecundity in the maximum plateau (*Figure 4.1B*). Thus, only measuring daily fecundity in conditions at which females did not reach the fertility plateau (higher humidity) would allow to see the increase in fecundity due to suppression.

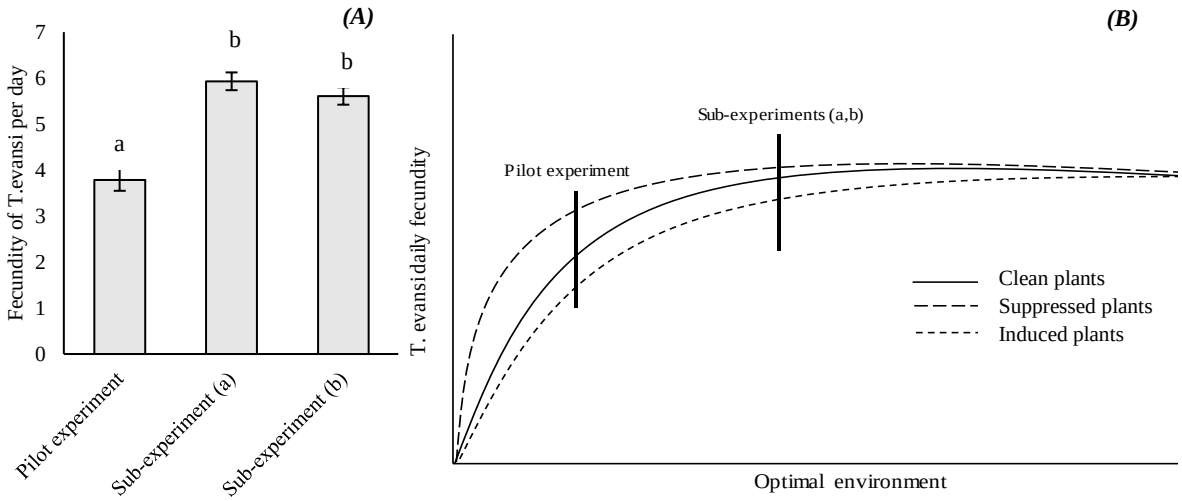

Figure 4.1. **Comparison of fecundity measured at 90% (pilot experiment) and 70% (sub-experiments) and possible daily fecundity curve in clean, suppressed or induced plants.** (A) Bars represent the average (± s.e.) daily fecundity per female (the number of eggs oviposited per *T. evansi* female/number of days that the female was alive) on leaf discs of clean tomato plants at different humidity conditions; Different small letters indicate significant differences among experiments (sub-experiment(a):pilot experiment: t= 7.234, P< 0.001; sub-experiment (b):pilot experiment: t= 6.156, P< 0.001; sub-experiment(a):sub-experiment(b): t=1.150, P= 0.754). (B) Theoretical representation of daily fecundity curves on clean (solid line), down-regulated (dashed line) and up-regulated (pointed line) plants.

Choice between infested and uninfested plants

Both *T. urticae* and *T. evansi* preferred plants pre-infested with *T. urticae* over clean plants, regardless of the host plant. It would be expected that both con- and heterospecifics would prefer: (i) clean plants rather plants pre-infested with *T. urticae,* as this species induces tomato plant defences; and (ii) plants pre-infested with *T. evansi* rather than clean plants, as this species is known to down-regulate tomato plant defences (Sarmento et al 2011a, Godinho et al. 2016). However, during this project, no differences between PI activity on bean and tomato plants, either clean or pre-infested by *T. urticae* or *T. evansi*, were shown. As such, down-regulation and induction of defences may not be present in this experiment. Additionally, *T. evansi* can produce massive amounts of dense webbing, impenetrable to competing

species, that interferes with the reproduction of competitors (Sarmento et al. 2011b). Hence, it may be that the lack of preference for plants with T. evansi is related to the presence of this web. The results corroborate those found within plants, in which *T. urticae* avoids leaves with *T. evansi* (Godinho et al. in prep). The preference of *T. evansi* for plants with *T. urticae* was unexpected. However, it was previously shown that interspecific competitors of *T. urticae* are attracted to plants pre-infested with this mite species (Pallini et al 1997).

Conclusions and perspectives

With this project, it was possible to understand that *T. evansi* and *T. ludeni* may have similar defence targets on tomato defences (*Figure 4.2*). However, it is possible that *T. ludeni* could also act in another defence target, independent of the JA pathway. Furthermore, results highlight the presence of local adaptation of *T. evansi* and *T. ludeni* to tomato and purple plants, respectively, and of a low performance of *T. urticae* on Solanales plants. However, the interaction with plant defences seems not to be related with the different degrees of specialization (host range). Additionally, no relation between the effect of mites on plant defences to their host choices was found. Overall, we found several possible sources of variation in both plant and mite responses that call for more investigation on this system.

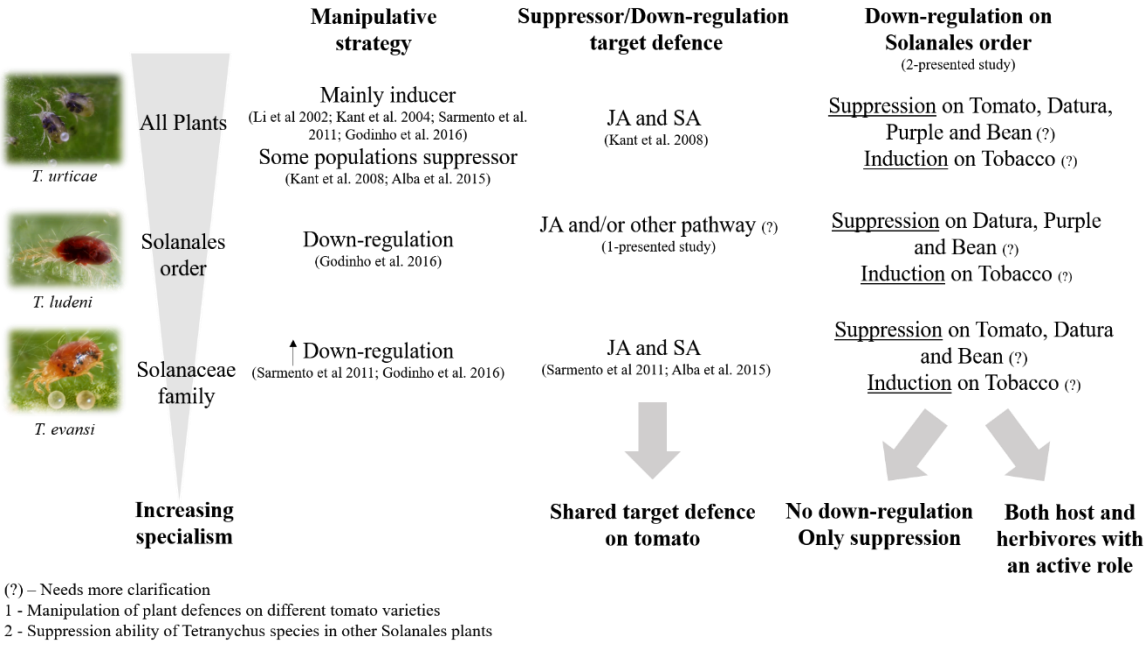

Figure 4.2. **Summary of principal conclusions regarding defences manipulations.**

In future experiments, to eliminate the effect of the rearing host plant, it is important to compare the several mite species adapted to the same host plant. Additionally, an optimal characterization of plant defences should be achieved (e.g. molecular tools) to further understand whether down-regulation is indeed present in spider-mites and at what defensive level. For the selection and pre-characterization of plant species used (e.g. chemical profile, nutritive value) it is also fundamental to understand, not only the herbivore perspective, but also the plant perspective, in defence manipulation.

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Appendix I

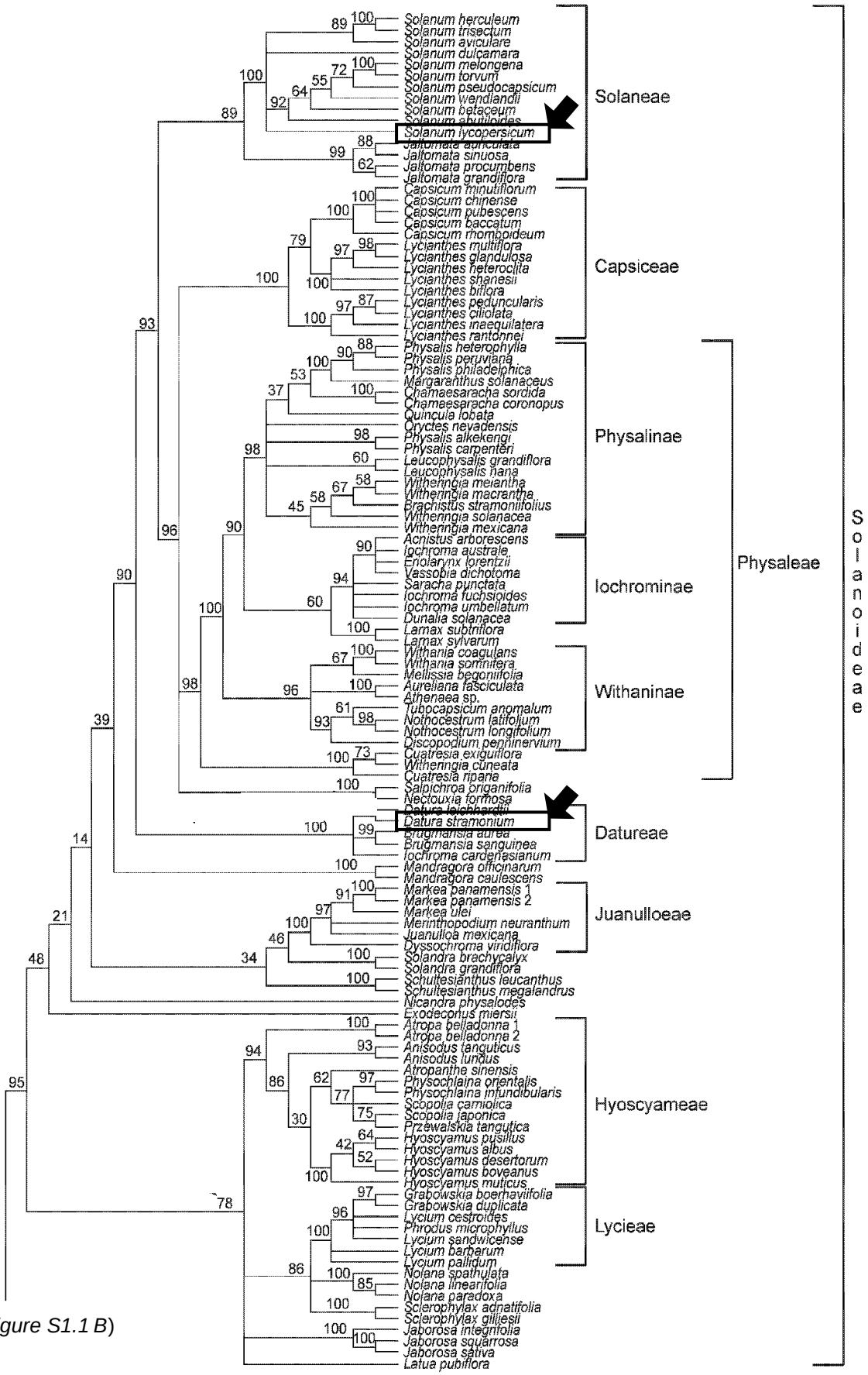
Supplementary Material

Introduction

Materials and Methods

Table 1.1 **Summary of secondary metabolites present four plants of Solales order (based on Eich 2008).** N1: Pyridylpyrrolidines (nicotine, nornicotine); N2: Pyridylpiperidines (e.g., anabasine); T1: Aliphatic esters of 3α-hydroxytropane; T1-A: Tropan-3-one (tropinone), 3α-hydroxytropane (tropine), 3β-hydroxytropane (pseudotropine), and/or their nor derivatives; T2/T3: Esters of 3α,6ß-dihydroxytropane (T2) / 3α,7ß-dihydroxytropane (T3); T4: Esters of 3α,6ß,7ß-trihydroxytropane; T5: Esters of 3α-hydroxytropane/-nortropane with Solanaceae-specific phenylpropanoid acids, e.g., hyoscyamine/atropine, anisodamine (6β-hydroxyhyoscyamine), littorine; T6: Esters of 6β,7β-epoxy-3α-hydroxytropane (scopine)/-norscopine with Solanaceae-specific phenylpropanoid acids (e.g., scopolamine (hyoscine)); T7: Acylated 3α,6β- / 3α,7β-dihydroxytropanes 3b-Acyloxytropanes; T7-B: N-Oxides of T5- and T6-type alkaloids; A3, A5: trihydroxynortopane,; B1-4: tetrahydroxy congeners; C1: pentahydroxy derivate; +: compound detected; −: compound not detected; (−): compound assumed to be absent since no further differentiation is given in the literature; NA: non-tested

Secondary Metabolites			**Solenaceae**			**Convolvulaceae**
			Solanoideae		***Nicotianoideae***	***Ipomoeeae***
			Solanum lycopersicum	***Datura stramonium***	***Nicotiana tabacum***	***Ipomoea purpurea***
Ornithine-Derived Alkaloids	Nicotinoids	N1	+	+	+	-
		N2	NA	NA	+	
	Tropane alkaloids	T1	NA	+	-	A
		T2/T3	NA	+	-	
		T4	NA	+	-	
		T5	NA	+	-	-
		T6	NA	+	-	
		T7	NA	B	-	
	Calystegines	A3	+	+	(-)	-
		A5	NA	(-)	(-)	-
		B1	NA	+	(-)	-
		B2	+	+	(-)	-
		B3	NA	(-)	(-)	-
Phenylalanine-derived Metabolites/ Phenyllpropanoids	N-acylphenylethylamines and derivatives	Cyanogenic Glycosides	Not present			-
	Cinnamate, Hydroxycinnamates and derivatives	Benzoates	+	NA	+	+
	Hydroxycoumarins	Scopoletin	NA	NA	+	NA
	Caffeic Acid Derivates/ Hydroxycinnamate conjugates	O-glucosides	NA	NA	+	NA
		Chlorogenic acid	+*	+*	+	-
		Hydroxycinnamic acid amides	+	NA	+	+
		Polyamine-HCA conjugates	+	NA	+	NA
Terpenoids	Monoterpenoids		+	+	NA	NA
	Sesquiterpenoids		+	+	+	NA
	Diterpenoids		NA	NA	+	NA
	Glycoalkaloids		+	NA	NA	NA
Fatty Acids and Carbohydrate	Fatty acids Derived		+	NA	+	+ (seeds)
	Secondary Carbohydrates	Sucrose esters	NA	NA	+	NA
	Resin Glycosides		-	-	-	+



(*Figure S1.1 B*)

Figure S1.1. **Solanaceae phylogeny depicted as strict consensus tree based on combined *ndhF* and *trnLF* sequences (adapted from Olmstead et al. 2008)**. (A) closer look to Solanoideae subfamily phylogeny; (B) Solanaceae and Convolvulaceae families' phylogeny. Numbers above branches represent bootstrap values. Suprageneric groups recognized here are labeled to the right.

Figure S1.1A

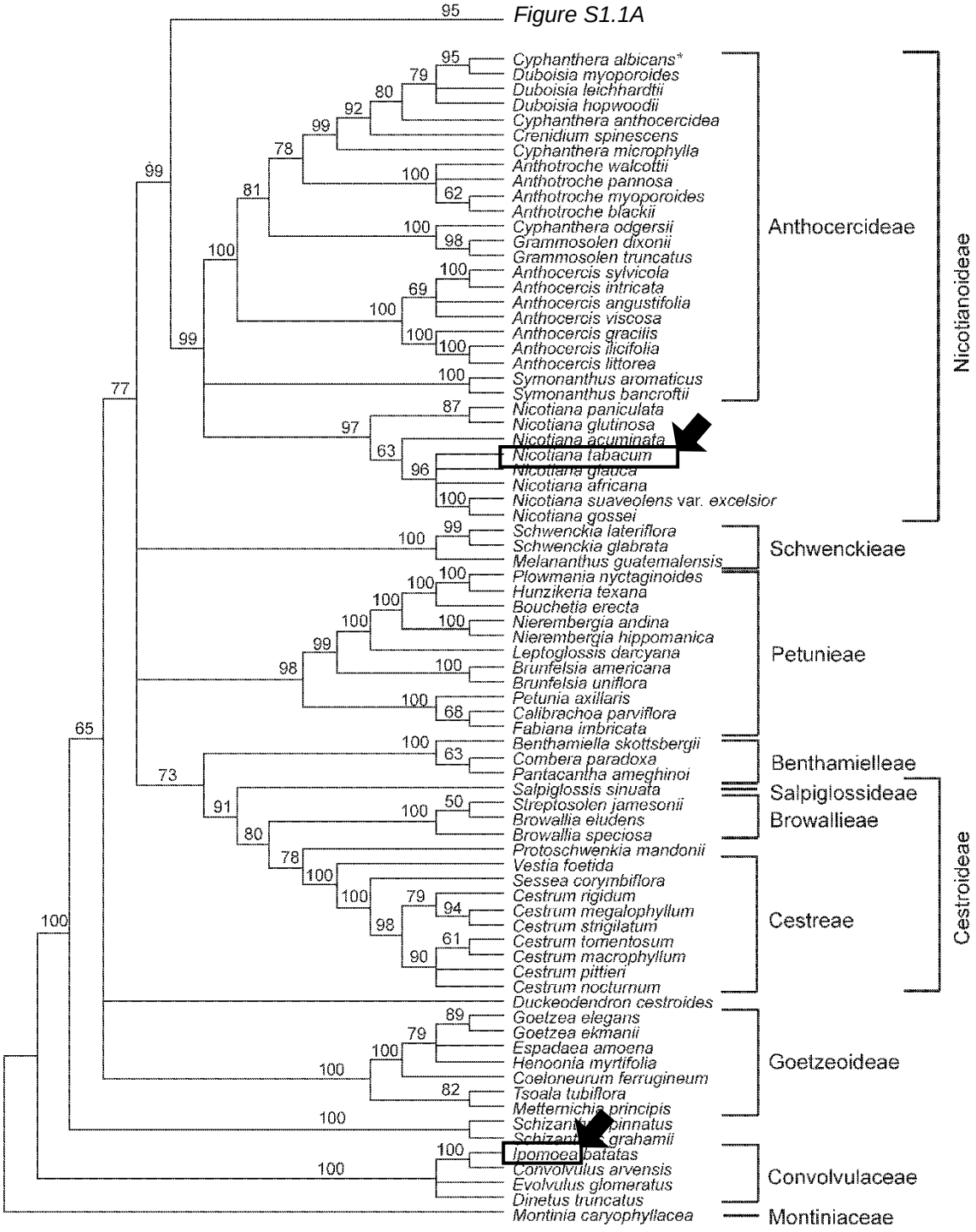

Cyphanthera albicans*
Duboisia myoporoides
Duboisia leichhardtii
Duboisia hopwoodii
Cyphanthera anthocercidea
Crenidium spinescens
Cyphanthera microphylla
Anthotroche walcottii
Anthotroche pannosa
Anthotroche myoporoides
Anthotroche blackii
Cyphanthera odgersii
Grammosolen dixonii
Grammosolen truncatus
Anthocercis sylvicola
Anthocercis intricata
Anthocercis angustifolia
Anthocercis viscosa
Anthocercis gracilis
Anthocercis ilicifolia
Anthocercis littorea
Symonanthus aromaticus
Symonanthus bancroftii
Nicotiana paniculata
Nicotiana glutinosa
Nicotiana acuminata
Nicotiana tabacum
Nicotiana glauca
Nicotiana africana
Nicotiana suaveolens var. excelsior
Nicotiana gossei
Schwenckia lateriflora
Schwenckia glabrata
Melananthus guatemalensis
Plowmania nyctaginoides
Hunzikeria texana
Bouchetia erecta
Nierembergia andina
Nierembergia hippomanica
Leptoglossis darcyana
Brunfelsia americana
Brunfelsia uniflora
Petunia axillaris
Calibrachoa parviflora
Fabiana imbricata
Benthamiella skottsbergii
Combera paradoxa
Pantacantha ameghinoi
Salpiglossis sinuata
Streptosolen jamesonii
Browallia eludens
Browallia speciosa
Protoschwenkia mandonii
Vestia foetida
Sessea corymbiflora
Cestrum rigidum
Cestrum megalophyllum
Cestrum strigilatum
Cestrum tomentosum
Cestrum macrophyllum
Cestrum pittieri
Cestrum nocturnum
Duckeodendron cestroides
Goetzea elegans
Goetzea ekmanii
Espadaea amoena
Henoonia myrtifolia
Coeloneurum ferrugineum
Tsoala tubiflora
Metternichia principis
Schizanthus pinnatus
Schizanthus grahamii
Ipomoea batatas
Convolvulus arvensis
Evolvulus glomeratus
Dinetus truncatus
Montinia caryophyllacea
Anthocercideae
Schwenckieae
Petunieae
Benthamielleae
Salpiglossideae
Browallieae
Cestreae
Goetzeoideae
Convolvulaceae
Montiniaceae
Nicotianoideae
Cestroideae

Table S1.2. **Description of the statistical models used in the analysis of the experiments presented in this thesis.** In Cox proportional hazard models (coxme) the response variables include censored individuals (whose death was accidental or that were lost before the end of the survival study). Models with binomial error structure require a concatenated response variable binding together the number of successes and failures for a given outcome. Sampling size gives the number of plants or female mites included in each analysis. "Maximal model" gives the complete set of explanatory variables (and their interactions) included in the model. "Minimal model" gives the model containing only the significant variables and their interactions. Round brackets indicate that the variable was fitted as a random factor. Square brackets indicate the error structure used (n: normal errors, b: binomial errors, coxme does not do predictions); zeroInflation indicates that the model accounts for a high proportion of zeros in the dataset; λ indicates the lambda value used in Box-Cox tranformations. Survival - survival: day at which females die, drowned: females that drowned in the water, natural: females that die naturally; Daily Fecundity - fecundity: number of eggs laid by each females; Hatching Rate - unhatch: number of unhatched eggs (=fecundity-hatch), juvdead: number of dead juveniles; Sex Ratio - male: number of males, female: number of females; Stress: stress index given by formula (1); WI: water index given by formula (2); Cig: Green Chlorophyll index given by formula (3); PRI: Physiological reflectance index given by formula (4); [Chl a], [Chl b], [Chl a+b]: estimate chorophyll concentration given by formula (5) to (6); Proteinase inhibitors: estimate concentration of PIs given by formula (10); Total protein: estimate concentration of total protein given by formula (9); Choice – choice: number of mites that choose one of the two plants, unchoice: number of mites that do not choose any plant, clean: number of mites that choose clean plants, infest: number of mites that choose infested plants. plant: plants species, strain: tomato strain; infest: infestation status, treat: choice present to the mites (clean vs infested), sps: spider mite species tested, block: block, num: number the plant used. I: Manipulation of plant defences on tomato mutant strains, IIa: Host Range in *Tetranychus* species, IIb: Effect of pre-infestation by *T. evansi* on mite performance, IIc: Effect of pre-infestation by *T. ludeni* and *T. urticae* on mite performance, III: Choice between infested and uninfested plants.

Variable of Interest		Response variable	Experiment	Data subset	Sampling size	Maximal Model	Minimal Model	R subroutine
Female survival		(survival,censor)	I	Complete	460[2]	plant*infest+(1|block)+(1|num)	1+(1|block)+(1|num)	coxme
			IIa	Complete	694[2]	plant*sps+(1|block)	plant*sps+(1|block)	
				by plant		sps+(1|block)	sps+(1|block)	
				by sps		plant +(1|block)	plant +(1|block)	
			IIb	Complete	663[2]	plant*infest+(1|block)+(1|num)	plant +(1|block)+(1|num)	
			IIc	Complete	818[2]	plant*infest+(1|block)+(1|num)	plant+(1|block)+(1|num)	
	Drowned	cbind(drowned,accidental+natural)	I	Complete	460[2]	strain*infest	infest	glm [b]
			IIa	Complete	694[2]	plant*sps	plant*infest	
			IIb	Complete	663[2]	plant*infest	plant	
			IIc	Complete	818[2]	plant*infest	1	
Daily Fecundity		fecundity/survival	I	Complete	460[2]	strain*infest+(1|block)+(1|num)	strain*infest+(1|block)+(1|num)	lmer[n] (λ=0.040)
			IIa	Complete	694[2]	plant*sps +(1|block)	plant*sps+(1|block)	(λ=0.153)
			IIb	Complete	663[2]	plant*infest +(1|block)+(1|num)	plant +(1|block)+(1|num)	(λ=0.263)
			IIc	Complete	818[2]	plant*infest +(1|block)+(1|num)	plant*infest+(1|block)+(1|num)	(λ=0.250)
				by plant		infest+(1|block)+(1|num)	infest+(1|block)+(1|num)	
Hatching Rate	Unhatch	cbind(unhatch,juvdead+adult)	IIa	Complete	620[2]	plant*sps+(1|block)	plant+sps+(1|block)	glmmadmb[betab]
			IIb	Complete	654[2]	plant*infest+(1|block)+(1|num)	plant +(1|block)+(1|num)	glmmadmb[betab]+zeroInflation
			IIc	Complete	760[2]	plant*infest+(1|block)+(1|num)	plant+(1|block)+(1|num)	glmmadmb[betab]
	Juvenile Mortality	cbind(juvdead,unhacth+adult)	IIa	Complete	620[2]	plant*sps+(1|block)+(1|num)	plant*sps+(1|block)+(1|num)	glmmadmb[betab]+zeroInflation
			IIb	Complete	654[2]	plant*infest+(1|block)+(1|num)	plant*infest+(1|block)+(1|num)	glmmadmb[betab]
			IIc	Complete	760[2]	plant*infest+(1|block)+(1|num)	plant*infest+(1|block)+(1|num)	
				by plant		infest+(1|block)+(1|num)	infest+(1|block)+(1|num)	
Sex Ratio	Female	cbind(female,male)	IIa	Complete	460[2]	plant*sps+(1|block)+(1|num)	plant*sps+(1|block)+(1|num)	glmer[b]
			IIb	Complete	508[2]	plant*infest+(1|block)+(1|num)	plant*infest+(1|block)+(1|num)	
			IIc	Complete	558[2]	plant*infest+(1|block)+(1|num)	plant+(1|block)+(1|num)	
	Male	cbind(male,female)	IIa	Complete	460[2]	plant*sps+(1|block)+(1|num)	plant*sps+(1|block)+(1|num)	glmer[b]
			IIb	Complete	508[2]	plant*infest+(1|block)+(1|num)	plant*infest+(1|block)+(1|num)	
			IIc	Complete	558[2]	plant*infest+(1|block)+(1|num)	plant+(1|block)+(1|num)	
Spectral Analysis	Stress	(1)	IIb	Complete	45[1]	plant*infest	1	lm[n]
			IIc	Complete	71[1]	plant*infest	1	(λ=0.200)
	WI	(2)	IIb	Complete	45[1]	plant*infest	1	lm[n]
			IIc	Complete	71[1]	plant*infest	1	(λ=-2)
	CIg	(3)	IIb	Complete	45[1]	plant*infest	1	lm[n]
			IIc	Complete	71[1]	plant*infest	1	(λ=-0.350)
	PIR	(4)	IIb	Complete	45[1]	plant*infest	1	lm[n]
			IIc	Complete	71[1]	plant*infest	1	(λ=-22.2)
	[Chl *a*]	(5)	IIb	Complete	45[1]	plant*infest	plant	lm[n]
			IIc	Complete	71[1]	plant*infest	plant	(λ=0.350)
	[Chl *b*]	(6)	IIb	Complete	45[1]	plant*infest	plant	lm[n]
			IIc	Complete	71[1]	plant*infest	plant	(λ=0.950)
	[Chl *a*+*b*]	(7)	IIb	Complete	45[1]	plant*infest	plant	lm[n]
			IIc	Complete	71[1]	plant*infest	plant	(λ=0.750)
	UV-B (300.4)	ρ300.4	IIb	Complete	45[1]	plant*infest	infest	lm[n] (λ=-2.850)
			IIc	Complete	71[1]	plant*infest	plant+infest	
	UV-B (303.7)	ρ303.7	IIb	Complete	45[1]	plant*infest	infest	lm[n] (λ=-0.700)
			IIc	Complete	71[1]	plant*infest	plant+infest	
	UV-B (310.5)	ρ310.5	IIb	Complete	45[1]	plant*infest	infest	lm[n]
			IIc	Complete	71[1]	plant*infest	infest	
	UV-B (313.9)	ρ313.9	IIb	Complete	45[1]	plant*infest	infest	lm[n] (λ=-0.700)
			IIc	Complete	71[1]	plant*infest		
Proteinase Inhibitors		(10)	I	Complete	49[1]	strain*infest	strain	lm[n] (λ=0.140)
			IIb	Complete	63[1]	plant*infest	plant	
			IIc	Complete	71[1]	plant*infest	plant	(λ=-0.100)
Total Protein		(9)	I	Complete	49[1]	strain*infest	strain	lm[n] (λ=0.010)
			IIb	Complete	63[1]	plant*infest	plant	(λ=-0.380)
			IIc	Complete	71[1]	plant*infest	plant	(λ=-0.300)
Dispersion	Choice	cbind(choice,choice+unchoice)	III	Complete	56[1]	plant*treat*sps+(1|block)	sps +(1|block)	glmmadmb[betab]
	Unchoice	cbind(unchoice,choice+unchoice)		Complete	56[1]	plant*treat*sps+(1|block)	1+(1|block)	
Choice	Clean	cbind(clean,infest+clean)	III	Complete	56[1]	plant*treat*sps+(1|block)	1+(1|block)	glmmadmb[betab]+zeroInflation
	Infest	cbind(infest,infest+clean)		Complete	56[1]	plant*treat*sps+(1|block)	treat +(1|block)	

[1]Corresponds to all plants used

[2]Corresponds to all females used (*i.e.*, the number of leaf discs)

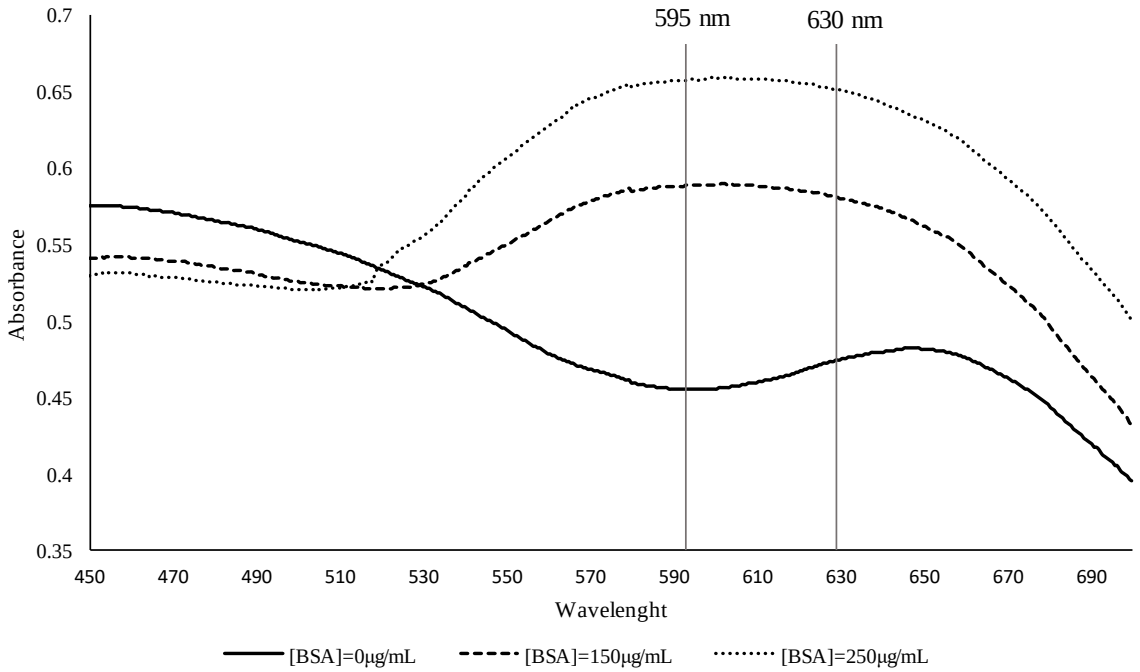


Figure S1.2. **Absorbance of three Bradford assays with different concentrations of BSA (0, 150 and 250 µg/mL) in a spectral range of 450 to 700nm.** Vertical lines point out the wavelength at which Bradford assay is usually performed (595nm) and the wavelength at which the assay was performed in this project. As represented in figure, the differences in absorbance in the quantification of 150 and 250 µg/mL of protein are minimal.

Appendix II

Supplementary Material

Results

Manipulation of plant defences on tomato mutant strains

Table S2.1. **Effect of con and heterospecifics on the performance *T. evansi* females in mutant strains of tomato plants.** Average (± s.e.) of daily fecundity per female over 4 days for wild-type and mutant tomato plants with different infestation status.

Plant	**wild-type**			**35S::prossys**			**def-1**			**def-1+Jasmonic Acid**		
Infest	**Clean**	***T. evansi***	***T. ludeni***	**Clean**	***T. evansi***	***T. ludeni***	**Clean**	***T. evansi***	***T. ludeni***	**Clean**	***T. evansi***	***T. ludeni***
N	44	31	37	44	39	40	41	36	37	40	39	32
Daily Fecundity	0.91 ± 0.14	1.29 ± 0.20	1.55 ± 0.22	1.17 ± 0.16	1.20 ± 0.22	1.44 ± 0.19	1.18 ± 0.21	1.04 ± 0.20	2.11 ± 0.32	1.19 ± 0.22	1.61 ± 0.22	1.00 ± 0.22
N	4	4	5	3	5	5	4	4	4	3	3	5
Total Protein	698.44 ± 289.31	659.38 ± 68.60	415.00 ± 73.54	672.92 ± 79.80	455.00 ± 78.94	420.88 ± 91.52	463.28 ± 142.16	589.06 ± 62.01	400.26 ± 64.10	385.42 ± 22.41	308.33 ± 27.14	232.08 ± 11.46
Proteinase Inhibitors	24.56 ± 10.45	20.90 ± 6.44	55.68 ± 29.08	29.08 ± 12.79	29.97 ± 10.05	85.68 ± 45.03	30.32 ± 7.11	31.28 ± 11.43	19.52 ± 5.93	184.22 ± 15.98	153.01 ± 28.63	216.86 ± 38.73

Effect of pre-infestation on mite performance

Table S2.2. **Multiple comparisons of daily fecundity between tomato strains using (phia package).** *** P<0.001, * P<0.05, **.** P marginally significant.

Contrast		**Deviance**	**Df**	**Chisq**	**Pr (>Chisq)**
def-1	Clean-*T.evansi*	0.020	1.000	0.035	1.000
	Clean-*ludeni*	-0.330	1.000	9.370	0.026*
	T.evansi-T. ludeni	-0.350	1.000	10.020	0.019*
def-1 + JA	Clean-*T.evansi*	-0.229	1.000	4.624	0.378
	Clean-*ludeni*	0.069	1.000	0.379	1.000
	T.evansi-T. ludeni	0.298	1.000	7.033	0.096.
35S:prossys	Clean-*T.evansi*	-0.044	1.000	0.177	1.000
	Clean-*ludeni*	-0.103	1.000	0.989	1.000
	T.evansi-T. ludeni	-0.059	1.000	0.302	1.000
wild-type	Clean-*T.evansi*	-0.206	1.000	3.444	0.762
	Clean-*ludeni*	-0.304	1.000	8.307	0.047*
	T.evansi-T. ludeni	-0.098	1.000	0.721	1.000

Down-regulation ability of *Tetranychus* species in other Solanales plants

Host Range of Tetranychus species

Table S2.3. **Performance of three *Tetranychus spp.* on a range of host plants**. Average (± s.e.) of females that were alive and drowned after 4 days, of daily fecundity per female over 4 days, of eggs that did not hatch (unhatch), of dead juveniles, of adults, of females, males on Tomato, Datura, Tobacco, Purple or Bean leaf discs.

	Plant	Tomato			Datura			Tobacco			Purple			Bean		
	Infest	*T. evansi*	*T. ludeni*	*T. urticae*	*T. evansi*	*T. ludeni*	*T. urticae*	*T. evansi*	*T. ludeni*	*T. urticae*	*T. evansi*	*T. ludeni*	*T. urticae*	*T. evansi*	*T. ludeni*	*T. urticae*
	N	71	55	54	35	35	33	34	37	39	70	39	37	59	46	50
Survival	Alive	0.94 ± 0.23	0.43 ± 0.50	0.86 ± 0.35	0.96 ± 0.20	1.00	0.92 ± 0.28	1.00	0.44 ± 0.51	0.75 ± 0.44	0.80 ± 0.40	0.97 ± 0.16	0.70 ± 0.47	0.93 ± 0.25	0.88 ± 0.33	1.00
Survival	Drowned	0.06 ± 0.23	0.58 ± 0.50	0.14 ± 0.35	0.04 ± 0.20	0.00	0.08 ± 0.28	0.00	0.56 ± 0.51	0.25 ± 0.44	0.20 ± 0.40	0.03 ± 0.16	0.30 ± 0.47	0.07 ± 0.25	0.12 ± 0.33	0.00
	Daily Fecundity	5.51 ± 0.19	0.70 ± 0.10	0.98 ± 0.15	5.48 ± 0.39	4.10 ± 0.41	3.10 ± 0.28	1.20 ± 0.08	1.16 ± 0.15	0.28 ± 0.05	1.20 ± 0.12	3.75 ± 0.30	0.98 ± 0.17	3.63 ± 0.15	4.40 ± 0.35	4.50 ± 0.23
	N	71	44	36	35	33	32	32	34	22	64	38	28	59	44	48
Hatching Rate	Unhatch	0.10 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.09 ± 0.02	0.06 ± 0.01	0.06 ± 0.03	0.17 ± 0.04	0.10 ± 0.03	0.06 ± 0.05	0.09 ± 0.03	0.01 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Hatching Rate	Dead Juveniles	0.27 ± 0.02	0.94 ± 0.03	0.58 ± 0.06	0.42 ± 0.05	0.17 ± 0.02	0.22 ± 0.03	0.63 ± 0.06	0.79 ± 0.06	0.53 ± 0.10	0.72 ± 0.04	0.26 ± 0.03	0.24 ± 0.07	0.26 ± 0.03	0.28 ± 0.02	0.17 ± 0.02
Hatching Rate	Adult	0.63 ± 0.02	0.02 ± 0.02	0.38 ± 0.06	0.49 ± 0.05	0.77 ± 0.03	0.71 ± 0.04	0.21 ± 0.05	0.11 ± 0.04	0.41 ± 0.10	0.19 ± 0.04	0.73 ± 0.03	0.75 ± 0.07	0.69 ± 0.03	0.69 ± 0.02	0.80 ± 0.02
Sex Ratio	Female	0.27 ± 0.02	0.18 ± NA	0.29 ± 0.07	0.24 ± 0.04	0.23 ± 0.03	0.28 ± 0.04	0.19 ± 0.08	0.17 ± 0.13	0.47 ± 0.14	0.56 ± 0.09	0.15 ± 0.02	0.29 ± 0.07	0.29 ± 0.02	0.18 ± 0.02	0.20 ± 0.02
Sex Ratio	Male	0.73 ± 0.02	0.82 ± NA	0.71 ± 0.07	0.76 ± 0.04	0.77 ± 0.03	0.72 ± 0.04	0.81 ± 0.08	0.83 ± 0.13	0.53 ± 0.14	0.44 ± 0.09	0.85 ± 0.02	0.71 ± 0.07	0.71 ± 0.02	0.82 ± 0.02	0.80 ± 0.02

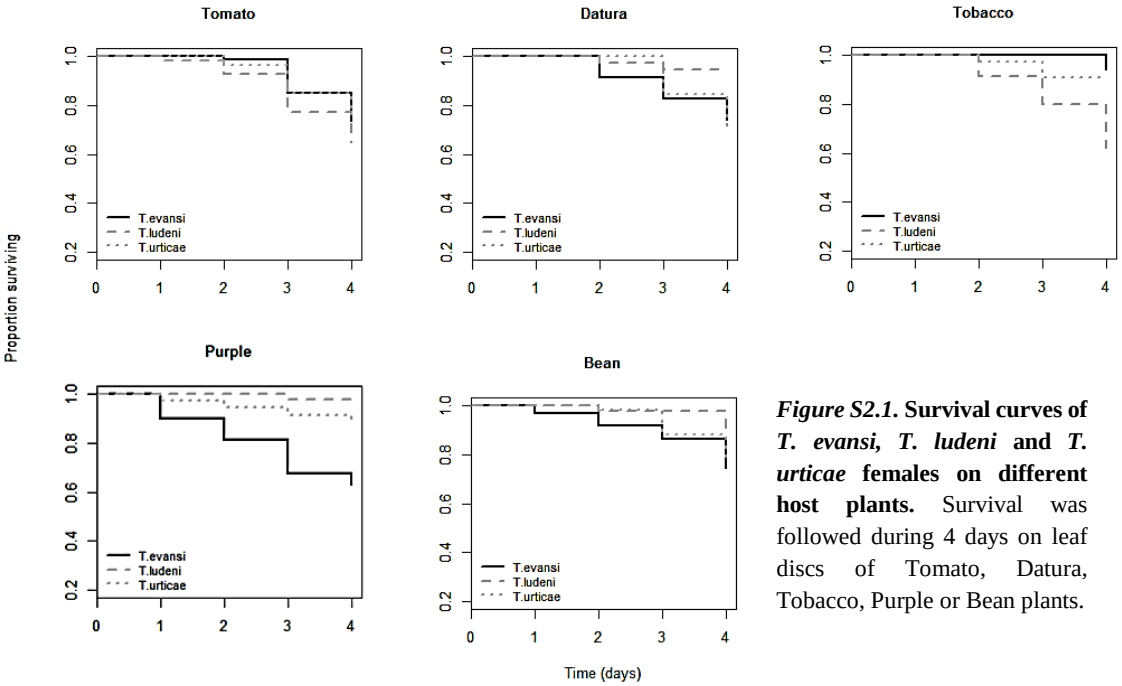


Figure S2.1. **Survival curves of *T. evansi, T. ludeni* and *T. urticae* females on different host plants.** Survival was followed during 4 days on leaf discs of Tomato, Datura, Tobacco, Purple or Bean plants.

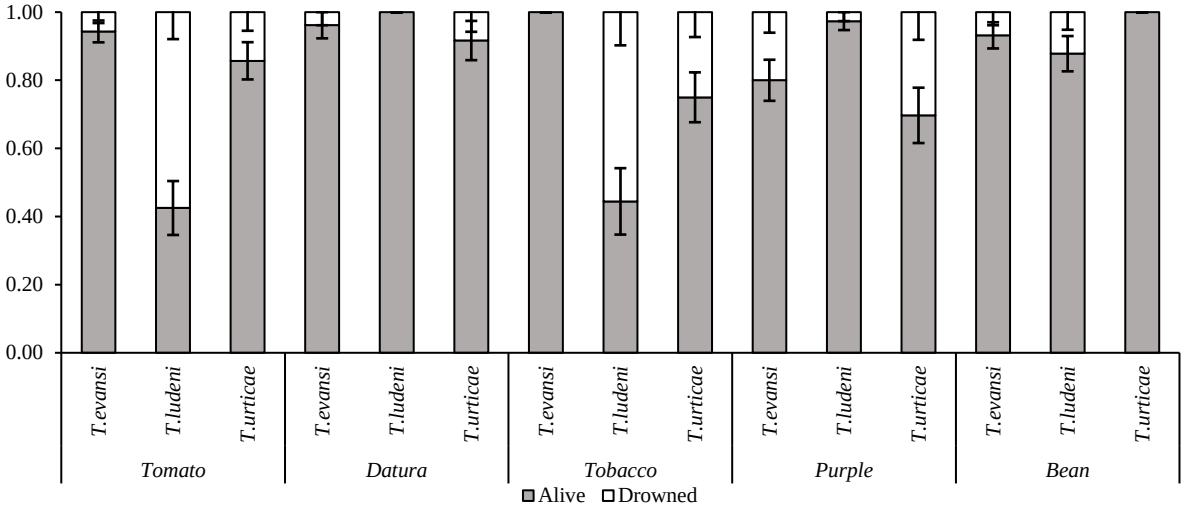


Figure S2.2. **Proportion of drowned females on the selected plant range.** the proportion of females that died drowned in the water saturated cotton when trying to escape the unfavorable patch. Alive gives que proportion of females that were alive in the end of the survival followed during 4 days. Bars represent the average (± s.e.) of each death type of *T.evansi*, *T. ludeni* and *T. urticae* females in leaf discs of Tomato, Datura, Tobacco, Purple or Bean plants.

Table S2.4. **Analysis of female survival.** (A) within plant species and between mite species; (B) within mite species and between plant species. *** P<0.001, * P<0.05, . P marginally significant.

(A)	Contrast	Df	Chisq	Pr (>Chisq)	(B)	Contrast	Df	Chisq	Pr (>Chisq)
Tomato	*T. ludeni-T. evansi*	1.000	1.231	0.267	*T. evansi*	Tomato-Datura	1.000	0.006	0.940
	T. ludeni-T. urticae	1.000	1.913	0.167		Tomato-Tobacco	1.000	8.263	0.004*
Datura	*T. ludeni-T. evansi*	1.000	3.401	0.065.		Tomato-Purple	1.000	4.242	0.039*
	T. ludeni-T. urticae	1.000	6.001	0.014*		Tomato-Bean	1.000	0.003	0.956
Tobacco	*T. ludeni-T. evansi*	1.000	3.133	0.077.	*T. ludeni*	Tomato-Datura	1.000	11.083	<0.001***
	T. ludeni-T. urticae	1.000	6.578	0.010*		Tomato-Tobacco	1.000	0.008	0.928
Purple	*T. ludeni-T. evansi*	1.000	4.507	0.034*		Tomato-Purple	1.000	19.441	<0.001***
	T. ludeni-T. urticae	1.000	2.570	0.109		Tomato-Bean	1.000	9.867	0.002*
Bean	*T. ludeni-T. evansi*	1.000	2.737	0.098.	*T. urticae*	Tomato-Datura	1.000	0.203	0.652
	T. ludeni-T. urticae	1.000	2.067	0.151		Tomato-Tobacco	1.000	3.621	0.057.
						Tomato-Purple	1.000	0.970	0.325
						Tomato-Bean	1.000	0.026	0.872

Table S2.5. **Test interactions, for the proportion of drowned females and juvenile mortality, with Bonferroni corrections (phia package).** (A) within plant species, between mite species; (B) within mite species, between plant species. *** P<0.001, * P<0.05, **.** P marginally significant.

Response variable		Survival Drowned				Unhatch Rate Juvenile Mortality			
(A) Contrast		**Value**	**Df**	**Chisq**	**Pr (>Chisq)**	**Value**	**Df**	**Chisq**	**Pr (>Chisq)**
Bean	*T. ludeni-T. evansi*	0.305	1.000	1.176	1.000	-0.121	1.000	0.334	1.000
	T. evansi- T. urticae	1.000	1.000	0.000	1.000	0.386	1.000	3.225	1.000
	T. ludeni-T. urticae	1.000	1.000	0.000	1.000	0.507	1.000	6.293	0.182
Datura	*T. ludeni-T. evansi*	1.000	1.000	0.000	1.000	0.523	1.000	1.709	1.000
	T. evansi- T. urticae	0.313	1.000	0.395	1.000	0.164	1.000	0.115	1.000
	T. ludeni-T. urticae	0.000	1.000	0.000	1.000	-0.359	1.000	0.519	1.000
Purple	*T. ludeni-T. evansi*	0.849	1.000	2.576	1.000	2.428	1.000	67.894	<0.001***
	T. evansi- T. urticae	0.285	1.000	3.202	1.000	2.592	1.000	34.312	<0.001***
	T. ludeni-T. urticae	0.066	1.000	6.011	0.213	0.164	1.000	0.100	1.000
Tobacco	*T. ludeni-T. evansi*	0.000	1.000	0.000	1.000	-0.321	1.000	0.660	1.000
	T. evansi- T. urticae	0.000	1.000	0.000	1.000	0.865	1.000	2.547	1.000
	T. ludeni-T. urticae	0.694	1.000	2.627	1.000	1.186	1.000	2.647	1.000
Tomato	*T. ludeni-T. evansi*	0.058	1.000	18.421	<0.001***	-3.412	1.000	57.679	<0.001***
	T. evansi- T. urticae	0.261	1.000	2.025	1.000	-1.348	1.000	15.062	0.002*
	T. ludeni-T. urticae	0.852	1.000	11.668	0.010*	2.064	1.000	12.625	0.006*
(B) Contrast		**Value**	**Df**	**Chisq**	**Pr (>Chisq)**	**Value**	**Df**	**Chisq**	**Pr (>Chisq)**
T. evansi	Bean-Datura	0.646	1.000	0.260	1.000	-0.245	1.000	1.179	1.000
	Bean-Purple	0.266	1.000	2.144	1.000	-2.421	1.000	128.494	<0.001***
	Bean-Tobacco	1.000	1.000	0.000	1.000	-2.153	1.000	46.997	<0.001***
	Bean-Tomato	0.548	1.000	0.054	1.000	-0.161	1.000	0.986	1.000
	Datura-Purple	0.166	1.000	2.248	1.000	-2.176	1.000	43.982	<0.001***
	Datura-Tobacco	1.000	1.000	0.000	1.000	-1.908	1.000	23.377	<0.001***
	Datura-Tomato	0.400	1.000	0.119	1.000	0.084	1.000	0.095	1.000
	Purple-Tobacco	1.000	1.000	0.000	1.000	0.268	1.000	0.533	1.000
	Purple-Tomato	0.770	1.000	3.065	1.000	2.260	1.000	69.085	<0.001***
	Tobacco-Tomato	0.000	1.000	0.000	1.000	1.992	1.000	29.692	<0.001***
T. ludeni	Bean-Datura	1.000	1.000	0.000	1.000	0.399	1.000	0.878	1.000
	Bean-Purple	0.823	1.000	1.880	1.000	0.129	1.000	0.117	1.000
	Bean-Tobacco	0.152	1.000	8.803	0.090.	-2.353	1.000	19.671	<0.001***
	Bean-Tomato	0.145	1.000	10.520	0.035*	-3.451	1.000	43.816	<0.001***
	Datura-Purple	0.000	1.000	0.000	1.000	-0.270	1.000	0.222	1.000
	Datura-Tobacco	0.000	1.000	0.000	1.000	-2.752	1.000	14.906	0.003*
	Datura-Tomato	0.000	1.000	0.000	1.000	-3.850	1.000	30.937	<0.001***
	Purple-Tobacco	0.037	1.000	9.304	0.069.	-2.482	1.000	23.477	<0.001***
	Purple-Tomato	0.035	1.000	9.935	0.049*	-3.580	1.000	35.597	<0.001***
	Tobacco-Tomato	0.487	1.000	0.015	1.000	-1.098	1.000	2.820	1.000
T. urticae	Bean-Datura	0.000	1.000	0.000	1.000	-0.467	1.000	1.293	1.000
	Bean-Purple	0.000	1.000	0.000	1.000	-0.214	1.000	0.254	1.000
	Bean-Tobacco	0.000	1.000	0.000	1.000	-1.675	1.000	7.432	0.192
	Bean-Tomato	0.000	1.000	0.000	1.000	-1.895	1.000	28.290	<0.001***
	Datura-Purple	0.148	1.000	4.563	0.980	0.253	1.000	0.168	1.000
	Datura-Tobacco	0.177	1.000	3.490	1.000	-1.207	1.000	2.364	1.000
	Datura-Tomato	0.340	1.000	0.608	1.000	-1.427	1.000	6.459	0.331
	Purple-Tobacco	0.552	1.000	0.158	1.000	-1.460	1.000	3.777	1.000
	Purple-Tomato	0.748	1.000	3.635	1.000	-1.680	1.000	7.769	0.159
	Tobacco-Tomato	0.706	1.000	2.309	1.000	-0.220	1.000	0.082	1.000

Table S2.6. **Contrast analysis of daily fecundity of *Tetranchus spp* (phia package).** (A) within plant species and between mite species; (B) within mite species and between plant species. *** P<0.001, * P<0.05, **.** P marginally significant.

(A)	Contrast	Value	Df	Chisq	Pr (>Chisq)
Tomato	*T. ludeni-T. evansi*	1.604	1.000	288.125	<0.001***
	T. evansi- T. urticae	1.504	1.000	251.070	<0.001***
	T. ludeni-T. urticae	-0.100	1.000	1.217	1.000
Datura	*T. ludeni-T. evansi*	0.516	1.000	13.565	0.003*
	T. evansi- T. urticae	0.734	1.000	26.904	<0.001***
	T. ludeni-T. urticae	0.218	1.000	3.583	0.876
Tobacco	*T. ludeni-T. evansi*	0.038	1.000	0.084	1.000
	T. evansi- T. urticae	0.563	1.000	18.514	<0.001***
	T. ludeni-T. urticae	0.525	1.000	23.198	<0.001***
Purple	*T. ludeni-T. evansi*	-0.943	1.000	83.149	<0.001***
	T. evansi- T. urticae	0.114	1.000	1.185	1.000
	T. ludeni-T. urticae	1.057	1.000	94.018	<0.001***
Bean	*T. ludeni-T. evansi*	-0.111	1.000	1.043	1.000
	T. evansi- T. urticae	-0.195	1.000	3.374	0.994
	T. ludeni-T. urticae	-0.085	1.000	0.765	1.000
(B)	**Contrast**	**Value**	**Df**	**Chisq**	**Pr (>Chisq)**
T. evansi	Bean-Datura	-0.501	1.000	19.718	<0.001***
	Bean-Purple	0.957	1.000	121.392	<0.001***
	Bean-Tobacco	0.892	1.000	58.526	<0.001***
	Bean-Tomato	-0.409	1.000	22.749	<0.001***
	Datura-Purple	1.459	1.000	160.811	<0.001***
	Datura-Tobacco	1.393	1.000	113.477	<0.001***
	Datura-Tomato	0.092	1.000	0.631	1.000
	Purple-Tobacco	-0.065	1.000	0.357	1.000
	Purple-Tomato	-1.366	1.000	283.677	<0.001***
	Tobacco-Tomato	-1.301	1.000	131.041	<0.001***
T. ludeni	Bean-Datura	0.126	1.000	1.330	1.000
	Bean-Purple	0.125	1.000	1.468	1.000
	Bean-Tobacco	1.041	1.000	98.149	<0.001***
	Bean-Tomato	1.306	1.000	189.423	<0.001***
	Datura-Purple	-0.001	1.000	0.000	1.000
	Datura-Tobacco	0.915	1.000	61.994	<0.001***
	Datura-Tomato	1.180	1.000	125.914	<0.001***
	Purple-Tobacco	0.916	1.000	70.614	<0.001***
	Purple-Tomato	1.180	1.000	140.352	<0.001***
	Tobacco-Tomato	0.265	1.000	6.823	0.270
T. urticae	Bean-Datura	0.429	1.000	15.485	0.002*
	Bean-Purple	1.267	1.000	151.145	<0.001***
	Bean-Tobacco	1.651	1.000	263.336	<0.001***
	Bean-Tomato	1.290	1.000	191.730	<0.001***
	Datura-Purple	0.838	1.000	51.349	<0.001***
	Datura-Tobacco	1.222	1.000	110.090	<0.001***
	Datura-Tomato	0.861	1.000	64.302	<0.001***
	Purple-Tobacco	0.384	1.000	12.396	0.013*
	Purple-Tomato	0.023	1.000	0.053	1.000
	Tobacco-Tomato	-0.360	1.000	12.978	0.009*

Table S2.7. **Multiple comparisons of the proportion of unhatch eggs using Tukey contrasts with Bonferroni corrections (multcomp package)***** P<0.001, * P<0.05, . P marginally significant.

Response variable	Fixed explanatory variable	Contrast	Estimate	Std. Error	z value	Pr(>|z|)
Hatching Rate	plant	datura - bean	0.496	0.206	2.404	0.162
		purple - bean	-0.360	0.263	-1.369	1.000
		tobacco - bean	1.126	0.239	4.703	<0.001***
		tomato - bean	0.453	0.184	2.454	0.141
		purple - datura	-0.856	0.361	-2.371	0.177
		tobacco - datura	0.630	0.318	1.980	0.477
		tomato - datura	-0.043	0.280	-0.153	1.000
		tobacco - purple	1.486	0.364	4.087	<0.001***
		tomato - purple	0.813	0.328	2.476	0.133
		tomato - tobacco	-0.673	0.301	-2.239	0.251
	infest	*T. ludeni-T. evansi*	-0.471	0.223	-2.107	0.105
		T. urticae-T evansi	-0.730	0.245	-2.985	0.009*
		T. urticae-T. ludeni	-0.260	0.323	-0.803	1.000

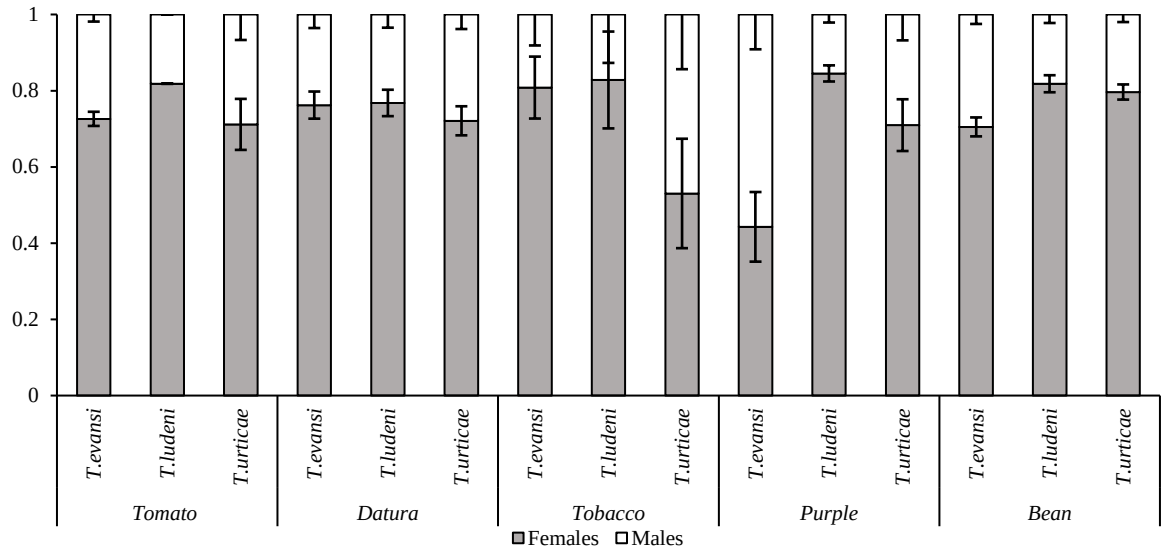


Figure S2.3. **Sex Ratio** Bars represent the average (± s.e.) proportion of females and males on leaf discs of Tomato, Datura, Tobacco, Purple or Bean plants.

Table S2.8. **Comparison analysis.** (A) within plant species, between mite species; (B) within mite species, between plant species. *** P<0.001, * P<0.05, . P marginally significant.

Response variable / **(A) Contrast**		**Sex-Ratio Females and Males**		
		Df	**Chisq**	**Pr (>Chisq)**
Tomato	*T. ludeni-T. evansi*	1.000	0.489	0.484
	T. ludeni-T. urticae	1.000	0.282	0.595
Datura	*T. ludeni-T. evansi*	1.000	1.593	0.207
	T. ludeni-T. urticae	1.000	3.001	0.083.
Tobacco	*T. ludeni-T. evansi*	1.000	0.024	0.876
	T. ludeni-T. urticae	1.000	2.366	0.124
Purple	*T. ludeni-T. evansi*	1.000	16.269	<0.001***
	T. ludeni-T. urticae	1.000	16.297	<0.001***
Bean	*T. ludeni-T. evansi*	1.000	12.959	<0.001***
	T. ludeni-T. urticae	1.000	2.475	0.116
(B) Contrast		**Df**	**Chisq**	**Pr (>Chisq)**
T. evansi	Tomato-Datura	1.000	0.754	0.385
	Tomato-Tobacco	1.000	0.636	0.425
	Tomato-Purple	1.000	7.088	0.008*
	Tomato-Bean	1.000	0.471	0.497
T. ludeni	Tomato-Datura	1.000	2.173	0.141
	Tomato-Tobacco	1.000	0.281	0.596
	Tomato-Purple	1.000	1.478	0.224
	Tomato-Bean	1.000	0.007	0.936
T. urticae	Tomato-Datura	1.000	3.248	0.072.
	Tomato-Tobacco	1.000	4.632	0.031*
	Tomato-Purple	1.000	6.664	0.010*
	Tomato-Bean	1.000	0.793	0.373

Effect of pre-infestation on mite performance

***Table S2.9*. Effect of infestation by *T.evansi* on mite performance**. Average (± s.e.) of daily fecundity per female over 4 days, of total protein (mg/mL), of proteinase inhibitors (µg/mL) per total protein, of reflectance index (Stress, WI, CIg, PRI), of concentrations of chlorophyll a and b ([Chl a], [Chl b] and [Chl a+b]) and of ρ in several wave lengths in the UV-B spectrum for different plant species with different infestation status.

Plant		**Tomato**		**Datura**		**Tobacco**		**Purple**		**Bean**	
Infest		**Clean**	***T. evansi***	**Clean**	***T. evansi***	**Clean**	***T. evansi***	**Clean**	***T. evansi***	**Clean**	***T. evansi***
N		79	80	62	62	71	72	62	54	62	59
Daily Fecundity		5.93 ± 0.19	6.13 ± 0.19	5.86 ± 0.30	5.16 ± 0.22	1.77 ± 0.12	1.58 ± 0.09	1.29 ± 0.12	1.22 ± 0.11	3.83 ± 0.15	3.77 ± 0.13
N		8	7	5	3	7	9	4	5	5	6
Total Protein		466.08 ± 47.48	556.85 ± 33.75	788.13 ± 30.63	1207.29 ± 227.32	297.07 ± 34.64	220.73 ± 19.93	251.88 ± 42.91	229.56 ± 26.66	787.08 ± 253.48	778.04 ± 230.14
Proteinase Inhibitors		13.71 ± 3.32	12.48 ± 2.60	9.98 ± 1.43	17.91 ± 5.97	12.41 ± 2.83	55.41 ± 11.03	28.22 ± 9.55	54.59 ± 23.73	15.69 ± 6.64	10.66 ± 3.22
N		6	6	3	3	5	5	5	4	4	4
Reflectance Index	**Stress**	1.13 ± 0.05	1.18 ± 0.06	1.11 ± 0.07	1.08 ± 0.02	1.06 ± 0.12	1.14 ± 0.03	1.09 ± 0.09	1.13 ± 0.06	0.95 ± 0.06	1.11 ±0.03
	WI	1.05 ± 0.12	1.02 ± 0.34	1.27 ± 0.12	1.18 ± 0.05	1.09 ± 0.06	0.90 ± 0.10	1.05 ± 0.20	1.06 ± 0.05	1.03 ± 0.12	1.14 ± 0.08
	CIg	0.91 ± 0.13	0.78 ± 0.08	0.83 ± 0.13	0.67 ± 0.08	0.82 ± 0.15	0.57 ± 0.04	0.96 ± 0.14	0.85 ± 0.18	1.01 ± 0.07	0.79 ± 0.09
	PRI	<0.01 ± <0.01	<0.01 ± <0.01	<0.01± <0.01	0.01± <0.01	0.01± <0.01	0.01± <0.01	<0.01± <0.01	<0.01± <0.01	0.01± <0.01	0.01± <0.01
	[Chl a]	8.98 ± 0.40	8.90 ± 0.41	8.78 ± 0.92	7.37 ± 1.10	7.54 ± 0.49	8.17 ± 0.66	7.15 ± 0.49	7.39 ± 0.33	7.70 ± 0.670.67	7.75 ± 0.45
	[Chl b]	12.63 ± 0.91	12.60 ± 0.62	12.25 ± 1.00	10.58 ± 1.33	10.12 ± 0.71	11.05 ± 1.02	10.07 ± 0.73	10.57 ± 0.43	10.63 ± 0.75	10.86 ± 0.42
	[Chl a+b]	21.61 ± 1.01	21.50 ± 1.02	21.03 ± 1.92	17.96 ± 2.42	17.66 ± 1.20	19.22 ± 1.68	17.21 ± 1.22	17.96 ± 0.75	18.33 ± 1.42	18.61 ± 0.86
UV-B Reflectance	**300.4**	0.71 ± 0.03	0.59 ± 0.02	0.65 ± 0.09	0.59 ± 0.04	0.70 ± 0.02	0.57 ± 0.02	0.67 ± 0.01	0.59 ± 0.02	0.65 ± 0.04	0.59 ± 0.03
	303.7	0.68 ± 0.05	0.55 ± 0.02	0.64 ± 0.11	0.58 ± 0.04	0.68 ± 0.03	0.54 ± 0.03	0.63 ± 0.03	0.54 ± 0.03	0.66 ± 0.05	0.59 ± 0.03
	307.1	0.69 ± 0.04	0.56 ± 0.02	0.66 ± 0.07	0.56 ± 0.02	0.70 ± 0.04	0.56 ± 0.02	0.66 ± 0.03	0.56 ± 0.02	0.64 ± 0.03	0.58 ± 0.02
	310.5	0.69 ± 0.04	0.55 ± 0.01	0.67 ± 0.08	0.56 ± 0.02	0.69 ± 0.04	0.54 ± 0.01	0.64 ± 0.03	0.55 ± 0.01	0.63 ± 0.02	0.56 ± 0.02
	313.9	0.69 ± 0.04	0.55 ± 0.02	0.66 ± 0.10	0.58 ± 0.04	0.70 ± 0.04	0.55 ± 0.01	0.65 ± 0.04	0.54 ± 0.03	0.66 ± 0.03	0.58 ± 0.03

***Table S2.10*. Effect of infestations by *T. ludeni* and *T. urticae* on mite performance**. Average (± s.e.) of daily fecundity per female over 4, of total protein (mg/mL), of proteinase inhibitors (µg/mL) per total protein, of reflectance index (Stress, WI, CIg, PRI), of concentrations of chlorophyll a and b ([Chl a], [Chl b] and [Chl a+b]) and of ρ in several wave lengths in the UV-B spectrum for different plant species with different infestation status.

Plant		Tomato			Datura			Tobacco			Purple			Bean		
Infest		Clean	*T. ludeni*	*T. urticae*	Clean	*T. ludeni*	*T. urticae*	Clean	*T. ludeni*	*T. urticae*	Clean	*T. ludeni*	*T. urticae*	Clean	*T. ludeni*	*T. urticae*
N		71	72	62	35	35	33	34	42	44	70	70	71	59	58	62
Daily Fecundity		5.51 ± 0.19	5.00 ± 0.17	4.98 ± 0.24	5.48 ± 0.39	5.25 ± 0.32	4.12 ± 0.33	1.20 ± 0.08	1.68 ± 0.15	1.72 ± 0.11	1.20 ± 0.12	1.42 ± 0.14	1.24 ± 0.10	3.63 ± 0.15	3.05 ± 0.19	3.13 ± 0.20
N		7	8	6	2	1	2	3	5	5	5	7	8	3	4	5
Total Protein		285.49 ± 16.54	334.11 ± 40.39	348.54 ± 58.92	1325.00 ± 537.50	903.13	809.38 ± 53.13	205.00 ± 31.10	247.90 ± 48.08	246.50 ± 20.32	323.58 ± 43.08	194.99 ± 58.90	197.98 ± 42.40	382.64 ± 96.62	572.40 ± 139.02	541.15 ± 54.36
Proteinase Inhibitors		15.16 ± 2.31	11.84 ± 174	18.01 ± 7.02	4.17 ± 0.21	20.39	11.32 ± 7.74	11.26 ± 4.12	44.17 ± 12.53	49.95 ± 12.16	50.95 ± 18.68	57.46 ± 13.56	27.86 ± 6.14	8.35 ± 1.53	7.00 ± 1.03	5.66 ± 1.09
N		7	7	6	3	3	3	2	3	3	6	5	5	6	6	6
Reflectance Index	Stress	1.57 ± 0.16	1.72 ± 0.16	1.86 ± 0.18	1.66 ± 0.24	1.68 ± 0.34	1.65 ± 0.34	1.97 ± 0.48	1.89 ± 0.34	1.91 ± 0.30	1.60 ± 0.15	1.62 ± 0.17	1.69 ± 0.17	1.76 ± 0.18	1.85 ± 0.20	1.82 ± 0.17
	WI	0.99 ± 0.10	1.11 ± 0.10	0.88 ± 0.15	1.06 ± 0.11	0.98 ± 0.10	0.83 ± 0.05	0.97 ± 0.02	0.85 ± 0.04	0.70 ± 0.12	1.24 ± 0.16	1.04 ± 0.18	1.20 ± 0.13	0.98 ± 0.11	1.16 ± 0.11	1.12 ± 0.13
	CIg	1.42 ± 0.28	1.64 ± 0.27	1.61 ± 0.26	1.40 ± 0.36	1.48 ± 0.31	1.79 ± 0.27	1.15 ± 0.37	1.00 ± 0.29	0.95 ± 0.28	1.37 ± 0.30	1.28 ± 0.33	1.33 ± 0.32	1.67 ± 0.24	1.62 ± 0.25	1.71 ± 0.24
	PRI	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	0.01 ± <0.01	0.01 ± <0.01	0.01 ± <0.01	<0.01 ± 0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01
	[Chl a]	8.79 ± 0.22	9.01 ± 0.28	9.20 ± 0.33	10.18 ± 0.51	10.09 ± 0.54	11.56 ± 2.05	8.33 ± 0.38	9.57 ± 0.44	9.01 ± 0.21	6.74 ± 0.44	7.03 ± 0.48	7.45 ± 0.41	9.17 ± 0.51	8.91 ± 0.52	9.17 ± 0.54
	[Chl b]	12.18 ± 0.50	12.65 ± 0.48	12.90 ± 0.56	13.88 ± 0.51	13.93 ± 0.61	15.77 ± 2.71	11.09 ± 0.07	12.78 ± 0.49	11.96 ± 0.37	9.72 ± 0.83	10.05 ± 0.89	10.60 ± 0.75	12.79 ± 0.80	12.43 ± 0.81	12.85 ± 0.78
	[Chl a+b]	20.97 ± 0.72	21.66 ± 0.75	22.10 ± 0.88	24.06 ± 1.01	24.02 ± 1.15	27.33 ±4.76	19.42 ± 0.45	22.35 ± 0.91	20.97 ± 0.53	16.46 ±1.27	17.08 ±1.37	18.05 ± 1.14	21.96 ± 1.30	21.34 ± 1.33	22.02 ± 1.31
UV-B Reflectance	300.4	0.74 ± 0.04	0.54 ± 0.03	0.50 ± 0.04	0.54 ± 0.03	0.47 ± 0.01	0.49 ± 0.03	0.62 ± 0.08	0.49 ± 0.02	0.49 ± 0.03	0.64 ± 0.04	0.56 ± 0.04	0.53 ± 0.04	0.56 ± 0.03	0.50 ± 0.04	0.50 ± 0.03
	303.7	0.73 ± 0.04	0.53 ± 0.03	0.49 ± 0.03	0.52 ± 0.05	0.46 ± 0.01	0.45 ± 0.02	0.62 ± 0.06	0.46 ± 0.01	0.46 ± <0.01	0.63 ± 0.04	0.54 ± 0.05	0.50 ± 0.04	0.55 ± 0.03	0.48 ± 0.04	0.48 ± 0.03
	307.1	0.72 ± 0.04	0.52 ± 0.03	0.47 ± 0.03	0.51 ± 0.05	0.46 ± 0.02	0.45 ± 0.03	0.62 ± 0.08	0.47 ± 0.01	0.46 ± 0.02	0.63 ± 0.04	0.52 ± 0.04	0.50 ± 0.04	0.54 ± 0.04	0.47 ± 0.03	0.47 ± 0.03
	310.5	0.73 ± 0.04	0.52 ± 0.03	0.47 ± 0.03	0.50 ± 0.05	0.44 ± 0.01	0.44 ± 0.02	0.60 ± 0.08	0.46 ± 0.01	0.44 ± 0.02	0.62 ± 0.04	0.52 ± 0.04	0.49 ± 0.04	0.54 ± 0.03	0.46 ± 0.04	0.47 ± 0.03
	313.9	0.71 ± 0.04	0.50 ± 0.03	0.46 ± 0.03	0.48 ± 0.05	0.44 ± 0.01	0.43 ± 0.02	0.61 ± 0.08	0.46 ± 0.01	0.44 ± 0.02	0.60 ± 0.04	0.51 ± 0.04	0.49 ± 0.04	0.52 ± 0.03	0.45 ± 0.03	0.46 ± 0.03

***Table S2.11*. Multiple comparisons of daily fecundity across treatments using Tukey contrasts (multcomp package).** Sub-experiment (a): *Effect of pre-infestation by T. evansi on mite performance*, sub-experiment (b): *Effect of pre-infestation by T. ludeni and T. urticae on mite performance.* *** P<0.001, * P<0.05, **.** P marginally significant.

Contrast		Contrast analysis			
Sub-experiment (a)		**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
datura - bean		0.462	0.081	5.683	<0.001**
purple - bean		-1.059	0.084	-12.653	<0.001**
tobacco - bean		-0.842	0.077	-10.880	<0.001**
tomato - bean		0.601	0.077	7.788	<0.001**
purple - datura		-1.520	0.087	-17.501	<0.001**
tobacco - datura		-1.304	0.081	-16.082	<0.001**
tomato - datura		0.140	0.080	1.740	0.818
tobacco - purple		0.216	0.079	2.746	0.060.
tomato - purple		1.660	0.076	21.701	<0.001**
tomato - tobacco		1.444	0.072	19.915	<0.001**
Sub-experiment (b)		**Deviance**	**Df**	**Chisq**	**Pr (>Chisq)**
Tomato	Clean-*T. ludeni*	249.280	1.000	1.732	0.188
	Clean-*T. urticae*	249.280	1.000	4.204	0.040*
Datura	Clean-*T. ludeni*	137.850	1.000	0.061	0.805
	Clean-*T. urticae*	137.850	1.000	2.661	0.103
Tobacco	Clean-*T. ludeni*	90.936	1.000	4.213	0.040*
	Clean-*T. urticae*	90.936	1.000	5.447	0.020*
Purple	Clean-*T. ludeni*	248.240	1.000	3.344	0.067.
	Clean-*T. urticae*	248.240	1.000	1.291	0.256
Bean	Clean-*T. ludeni*	205.540	1.000	4.891	0.027*
	Clean-*T. urticae*	205.540	1.000	3.184	0.074.

Proteinase inhibitors

Table S2.12. **Contrast analysis of plant PIs and total protein concentration across different infestation status (A-phia or B-multcomp packages)** Sub-experiment (a): *Effect of pre-infestation by T. evansi on mite performance,* sub-experiment (b): *Effect of pre-infestation by T. ludeni and T. urticae on mite performance* *** P<0.001, * P<0.05, **.** P marginally significant.

(A) Contrast [PIs]		**Contrast analysis**				
Sub-experiment (a)		**Value**	**Df**	**Sum of Sq**	**F**	**Pr (>F)**
Bean	*T. evansi- Clean*	2.426	1.000	17.700	0.039	1.000
Datura	*T. evansi- Clean*	-6.393	1.000	111.500	0.245	1.000
Purple	*T. evansi- Clean*	-26.368	1.000	1545.000	3.400	0.354
Tobacco	*T. evansi- Clean*	-43.004	1.000	7281.800	16.024	0.001*
Tomato	*T. evansi- Clean*	1.229	1.000	5.600	0.012	1.000

(B) Contrast [PIs]		**Contrast analysis**			
Sub-experiment (b)		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
plant	datura - bean	0.135	0.289	0.467	1.000
	purple - bean	1.119	0.199	5.634	<0.001***
	tobacco - bean	1.055	0.218	4.846	<0.001***
	tomato - bean	0.480	0.197	2.439	0.174
	purple - datura	0.983	0.272	3.617	0.006*
	tobacco - datura	0.920	0.286	3.214	0.020*
	tomato - datura	0.345	0.271	1.274	1.000
	tobacco - purple	-0.064	0.194	-0.330	1.000
	tomato - purple	-0.639	0.170	-3.760	0.004*
	tomato - tobacco	-0.575	0.192	-2.996	0.039*

Spectral analysis

Table S2.13. **Multiple comparisons of plant species (fixed explanatory variable), concerning ρ in all UV-B wave lengths, using Tukey contrasts with Bonferroni corrections (multcomp package).** sub-experiment (b): *Effect of pre-infestation by T. ludeni and T. urticae on mite performance.* *** P<0.001, * P<0.05, . P marginally significant.

Response variable	**Contrast**	**Contrast analysis**			
		Sub-experiment (b)			
		Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
300.4	*T.ludeni* – Clean	-0.396	0.078	-5.101	<0.001***
	T. urticae -Clean	-0.443	0.079	-5.645	<0.001***
	T. urticae – T. ludeni	-0.047	0.078	-0.601	1.000
303.7	*T.ludeni* – Clean	-0.410	0.084	-4.875	<0.001***
	T. urticae -Clean	-0.480	0.085	-5.649	<0.001***
	T. urticae – T. ludeni	-0.070	0.085	-0.830	1.000
310.5	*T.ludeni* – Clean	-0.435	0.088	-4.938	<0.001***
	T. urticae -Clean	-0.485	0.089	-5.442	<0.001***
	T. urticae – T. ludeni	-0.050	0.089	-0.557	1.000
313.9	*T.ludeni* – Clean	-0.430	0.077	-5.557	<0.001***
	T. urticae -Clean	-0.526	0.078	-6.723	<0.001***
	T. urticae – T. ludeni	-0.096	0.078	-1.230	0.670

Choice between infested and uninfested plants

***Table S2.14*. Effect of the infestation status on mite host choice and dispersion.** Average (± s.e.) of females that die by accidental, drown of natural causes and of daily fecundity per female over 4 days for wild-type and mutant tomato plants with different infestation status.

Plant		**Bean**				**Tomato**			
Mite Species		***T. evansi***		***T. urticae***		***T. evansi***		***T. urticae***	
Infest		**Clean vs *T. evansi***	**Clean vs *T. urticae***	**Clean vs *T. evansi***	**Clean vs *T. urticae***	**Clean vs *T. evansi***	**Clean vs *T. urticae***	**Clean vs *T. evansi***	**Clean vs *T. urticae***
	N (mites/choice)	8 / 790	7 / 700	7 / 700	7 / 700	7 / 700	7 / 700	7 / 700	6 / 600
Dispersion	**Choice**	0.28 ± 0.11	0.37 ± 0.08	0.40 ± 0.08	0.50 ± 0.08	0.18 ± 0.09	0.14 ± 0.05	0.48 ± 0.07	0.53 ± 0.11
	Unchoice	0.72 ± 0.11	0.63 ± 0.08	0.60 ± 0.08	0.50 ± 0.08	0.82 ± 0.09	0.86 ± 0.05	0.52 ± 0.07	0.47 ± 0.11
	N (mites/choice)	8 / 119	7 / 101	7 / 336	7 / 317	7 / 227	7 / 257	7 / 281	6 / 355
Choice	**Clean**	0.56 ± 0.11	0.42 ± 0.13	0.56 ± 0.07	0.44 ± 0.09	0.28 ± 0.13	0.42 ± 0.13	0.50 ± 0.11	0.43 ± 0.15
	Infest	0.44 ± 0.11	0.58 ± 0.13	0.44 ± 0.07	0.56 ± 0.09	0.72 ± 0.13	0.58 ± 0.13	0.50 ± 0.11	0.57 ± 0.15

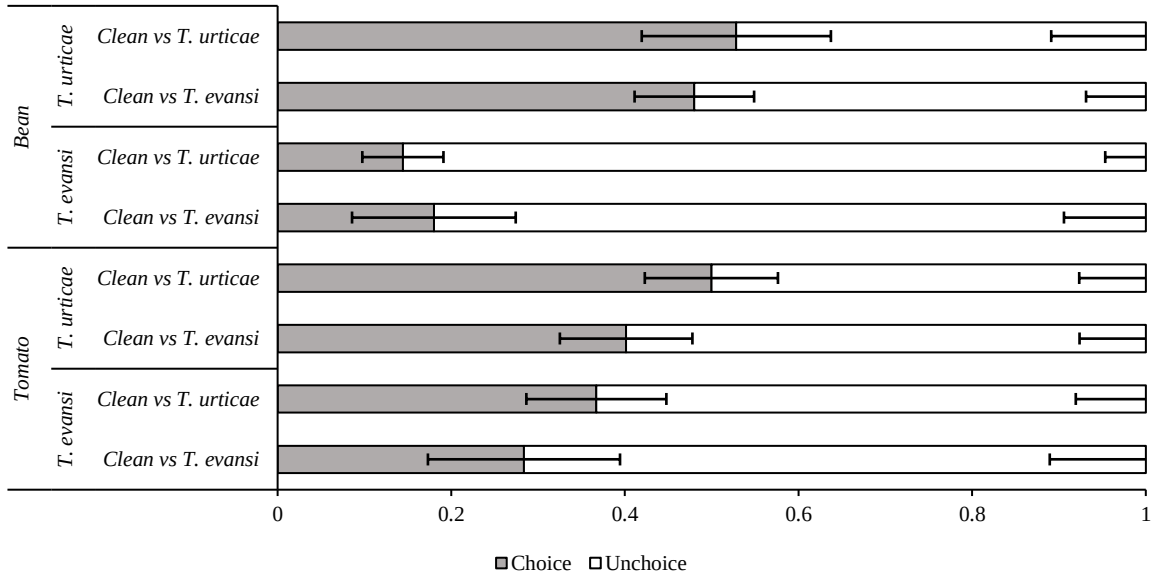


***Figure S2.4.* Proportion of mite dispersion.** Bars represent the average (± s.e.) of the proportion of choice or unchoice (dispersion or not) of *T. evansi* or *T.urticae* to both presented choice plants (clean or pre-infested with *T. evansi* or *T.urticae*). This choice was performed in bean and tomato plants.