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Tetranychus urticae adaptation to tomato

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Abstract

The arms race between plants and herbivores has resulted in a great diversity of plant compounds to act as defences against attackers. It has concurrently resulted in herbivorous pest adaptations to host defences, including plant-host defence suppression through the action of secreted effectors, and detoxification of phytochemicals ingested during feeding. While these two mechanisms of herbivore adaptation are relatively well studied, they have not been tested for use at the same time. This study uses the model plant species *Solanum lycopersicum* (tomato), and the model arthropod species *Tetranychus urticae* (two-spotted spider mite), to characterize the utilization of the above-mentioned mechanisms in an experimental adaptation set-up. Two spider mite strains, non-adapted (ancestral) and tomato-adapted, were used to infest tomato under different experimental conditions to interrogate the adaptation process. Tomato adaptation was validated through plant damage and mite performance assays. Transcriptional analysis of differentially expressed genes demonstrated an attenuation of the response to non-adapted mites by adapted ones, indicating the defence response to be deficient in induced defence programs, such as jasmonic acid biosynthesis and protease inhibitor biosynthesis. This was supported with marker gene and hormone quantification. However, inhibition activity was found to be differentially induced in different tomato cultivars, being highly induced in Moneymaker and attenuated in Heinz samples fed on by adapted mites, suggesting mites still encounter protease inhibitors as a plant defence in certain tomato cultivars despite being adapted to tomato in general. A mite co-infestation experiment was used to demonstrate that any benefit to host-plant modulation occurs only at the feeding site. Characterization of mite protease activity and fecundity post-inhibition by a synthetic inhibitor, E-64, suggest that mites increase their protease activity to overcome tomato protease inhibitors. Detoxification was also found to be involved in tomato adaptation, whereby inhibiting different classes of enzymes (cytochrome P450s, esterases, or glutathione-S-transferases) resulted in decreased fecundity on tomato.

Keywords: Plant-herbivore interaction, *Tetranychus urticae*, *Solanum lycopersicum*, plant-host adaptation, detoxification, defence suppression

Summary for lay audience

Insight into the molecular mechanisms of plant host adaptation by herbivores can inform future agricultural practices and technologies to ensure continued food production in a sustainable, ecologically friendly way. This research investigates two such adaptation mechanisms. First, suppression of plant defences. Using this mechanism, a herbivore can utilize a host plant by suppressing the plant response to herbivory, decreasing the amount of defences a plant produces in response to attack, and making the plant a more hospitable host. Detoxification of plant compounds is the second mechanism of adaptation studied here. Detoxification of toxic plant compounds can also make a host plant suitable for development and reproduction. Detoxification does not decrease the amount of plant defences produced, but it renders toxic metabolites that are ingested during feeding to be non- or less-functional against the herbivore. I use the two-spotted spider mite as a model herbivore that has been documented to use these two mechanisms of suppression to feed on tomato plants, and investigate whether these two mechanisms can be used simultaneously. Previous research has only studied these two mechanisms independently, but I hypothesize they can be used concurrently. I used a variety of techniques to characterize the adaptation status of a tomato adapted mite population by comparing it to a non-adapted mite population sharing genetic ancestry. Quantification of gene expression and plant hormone accumulation indicated that the adapted mite population can attenuate the tomato response to mite feeding, compared to the non-adapted strain. A co-infestation experiment revealed that any physiological benefit to adapted mites must occur at the feeding site and is not transmitted systemically throughout the plant. I also characterized tomato protease inhibitor activity and mite protease activity to ascertain how mites were overcoming tomato protease inhibitors (an anti-digestive plant defence). Results suggests that mites have high protease activity to overcome tomato protease inhibitors and may not be relying on suppression of this plant defence class. Finally, I characterized the involvement of three prominent detoxification enzyme classes, namely carboxyl/choline esterases, glutathione-S-transferase, and cytochrome P450, using synthetic inhibitors of these classes. Results from detoxification inhibitor experiments support adapted mites also using detoxification as a mechanism to overcome tomato toxin metabolites. Overall, this research supports the conclusion that spider mites, and probably herbivores generally, can use multiple mechanisms of adaptation concurrently.

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Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

η^2p	partial eta squared: effect size
µmol m^{-2} s^{-1}	µmol of photons per second and square meter
ABA	abscisic acid
CCE	carboxyl/choline esterases
cDNA	complementary deoxyribonucleic acid
Ct	cycle threshold
CYP	cytochrome P450
DAMPs	damage associated molecular patterns
DEF	S,S,S tributyl-phosphorotrithioate
DEG	differentially expressed gene
DEM	diethyl maleate
dpi	dots per inch
GO	gene ontology
GST	glutathione-S-transferases
HAMPs	herbivory associated molecular patterns
HTI	herbivore triggered immunity
JA	jasmonic acid
M	million/molar
MAPK	mitogen-activated protein kinase
n	sample size
NRQ	normalized relative quantity
OS	oral secretions
PAMPs	pathogen associated molecular patterns
PBO	piperonyl butoxide
PI	protease inhibitor
RNA	ribonucleic acid
RNAi	RNA interference
RT-qPCR	Quantitative reverse transcription PCR
SA	salicylic acid
SE	standard error

Preface

The investigation into plant-herbivore interaction is valuable across disciplines. It is valuable in the study of ecological systems as both plants and arthropod herbivores play an enormous role in almost every ecosystem found on this planet. The interaction between plants and their pests has shaped both of them extensively over hundreds of millions of years of evolution, and the enormous variation, from the species, to the molecular and genetic levels can provide us with valuable tools as we face problems in agriculture regarding the loss of crops to herbivores. Ultimately, knowledge gained from basic research in this field can be applied in industry and biotechnology developed using this knowledge could improve agricultural systems and have enormous economic benefits. This study is one contribution I make in the hopes of improving our knowledge of natural systems and human endeavours to live within and alongside them.

1 An introduction to plant-herbivore interaction

1.1 The agricultural perspective

The dawn of civilization has largely been attributed to approximately 20 agricultural revolutions that occurred around the world between 10 000 – 5 000 years ago, leading to the domestication of plant species as widely used and distributed crops (Smith, 2002; Fuller et al., 2014). It was the ability to grow more than enough food for a population and store it for future use that allowed humans to grow to far greater numbers than ever before. Unfortunately, a lack of collective forethought has since led humanity to a point where we must face the negative consequences our development has had on the natural world we continuously use to sustain our numbers (Larsen, 2006; Campbell et al., 2017). Indeed, resource shortages, including declining crop yields, have been characterized as an underlying problem during the fall of previous civilizations (Taylor, 2008).

Nevertheless, since the dawn of agriculture, a good life has included food security. Whether at the level of the individual, family, community or empire, the knowledge that food would always be on the table allowed humanity to dwell on other things besides hunting, gathering or scavenging enough food to make it through the day. It was then that a standard of living was born that even today is strived for across cultures. As we attempt to provide that standard of living to a human global population expected to reach almost 11 billion by the end of this century (UN, 2019), we need to consider the agricultural requirements of sustaining such a population. The problem is exacerbated by climate change (Arbuckle et al., 2015; Burton and Lim, 2005). Parry and Hawkesford (2010) predict that a doubling in food production is required by 2050 to attain global food security. Sustainable food production, food distribution and economics are all major challenges to achieving this goal (Parry and Hawkesford, 2010). One inescapable conclusion from an objective view of the current and not too distant future is that we will need to increase food production and do so in a sustainable way that does not compromise the ability of future generations to combat current and future problems.

Obstacles that food producers face are varied as different crop plants experience a variety of biotic and abiotic stresses, including pests and drought, respectively. Biotic stressors

such as invasive weeds and herbivorous pests in addition to pathogens represent challenges that affect crop yield and are costly to producers. Yield loss due to arthropod infestation of annual crops is estimated to be 18-26% globally (Culliney, 2014). However, plants have evolved an extensive array of defensive strategies to combat the myriad of predators and parasitoids that inevitably come to the plant for the purpose of taking nutrients that plants have absorbed and/or synthesized. While previous endeavours to domesticate crops through breeding have focused on the development of traits such as high yield and higher nutritional content, this has been at the cost (or lack of interest) of defensive traits we now recognize as important (Moreira et al., 2018).

Pest control in the past several decades has largely relied on the use of synthetic pesticides. However, this is accompanied by a variety of undesired outcomes that include negative effects on non-target and beneficial arthropods, the development of resistance in target pests, and secondary pest emergence. This is in addition to the cost associated with purchasing pesticides, equipment and fuel (Ekström & Ekbom, 2011). Additionally, an increasing number of products have become unusable and unavailable due to target resistance and/or regulatory changes (Van Leeuwen et al., 2010a; Marcic, 2012). Given this outlook, it seems obvious that more environmentally sound pest control methods are required for the sustainable production of food. New methods should both prolong the effectiveness of current and new pesticides as well as ameliorate any associated negative impacts. Providing novel and varied modes of action against pests can slow the development of resistance to any individual method.

1.2 The biological perspective

The conflicting interests of plants and the herbivores that feed on them has resulted in an immensely complex relationship between them accompanied by genotypic and phenotypic variation that is remarkably impressive. The plant has the goal of acquiring enough resources to defend and/or tolerate attack and stress to ensure reproductive success. The details of plant life-history strategies beyond that of defence against herbivores is not addressed here. Likewise, the strategies herbivores utilize outside of overcoming plant defences are not covered in this work; however, a brief summary of the interaction is included to give context. The interaction between arthropod herbivores and plants begins

when the herbivore locates a potential host plant through visual or olfactory cues, for example by the flower colour or volatile compounds released, respectively. Using contact chemoreceptors on mouthparts, antennae, and tarsi, the suitability of a host plant for feeding and oviposition can then be physiologically and chemically assessed by the arthropod (Howe and Jander, 2007). Through various modes of probing or initial feeding, the herbivore either accepts or rejects the plant as a suitable host (Stout, 2013). The final phase can be considered host utilization, characterized by the ability of the herbivore to survive, develop and reproduce on the host plant. Success of the herbivore is greatly influenced by the nutritional quality and types of plant defences in the host plant (Duffey and Stout, 1996). Regarding these defenses, plant allelochemicals can act to either excite or inhibit herbivore feeding, which in turn affects the rate and duration of feeding, and eventually influences the fecundity of the herbivore (Miller & Strickler, 1984). Allelochemicals are defined as chemical emitted by an organism of one species that influences the physiology or behavior of an organism of a different species.

To add even more complexity to the scenario, herbivores must also interact with other organisms (directly or indirectly), in addition to its host plant. These other organisms can be of the same trophic level, and represent competing herbivores, or they can be those of different trophic levels and represent predators or parasitoids of the herbivore. There are also pathogens the herbivore may have to overcome, although some microbes are beneficial and act as symbionts of the herbivore. These herbivore-associated organisms can affect how the plant responds to herbivory (Chung et al., 2013; Zhu et al., 2014). Additionally, it must be acknowledged, that these interactions are dynamic in time, where the defensive phenotype of the host plant will change depending on previous, current and future biotic and abiotic stressors.

1.3 The plant-herbivore “arms race”

Arthropod herbivory came in two phases. The first phase occurring 417 to 403 million years ago, shortly after the colonization of land by plants and is supported by evidence for consumption of sporangia and stems. The second occurred 327 to 309 million years ago following the origination and expansion of roots, leaves, wood and seeds tissues of plants (Labandeira, 2007). This long-term competition has been coined an “arms race” between

plants and herbivores where the former produce defences to guard against the latter which in turn develops mechanisms to adapt to the defenses (Ehrlich and Raven, 1964). In this way, plants have become an incredible source of diverse defence compounds, ranging in effectiveness across pest species and mode of action against the target attacker (Futuyma and Agrawal, 2009). In fact, plants are one of the best, largely un-tapped, resources for development of bio-pesticides and this has become an area of high interest and development (Walia et al., 2017). Additionally, the prospects for generating herbivore resistant crops using the genetic diversity of traits found in wild varieties is very promising (Mitchell et al., 2016). For herbivores to successfully utilize host plant species, they must evolve methods of overcoming those defences. This successful utilization of a host plant is considered to be a compatible interaction (Rioja et al., 2017), a term adopted from plant-pathogen studies, occurring when the herbivore is either not detected by the plant, or is detected but has evolved the ability to avoid or overcome the defences. Incompatible interactions, conversely, occur when the plant is resistant and can successfully defend itself against the herbivore upon recognizing it and leads to that plant being defined as a non-host. The classifications of herbivores based on the range of host plants are: generalists (feeding on many hosts from different families, also termed polyphagous); oligophagous herbivores (feeding on several plant species, usually from the same family); and specialists that feed on one or a few species within the same genus (Barrett and Heil, 2012; Bernays and Graham, 1988; Futuyma and Gould, 1979).

One evolutionary consequence of the arms race is hypothesized to be the observed specialization of insect species on certain plant hosts, where they adapt to the defence compounds specific to a plant species/family. In fact, the majority of herbivorous arthropods are restricted to feeding on a single or very few plant species and are considered specialists for that reason (Wheat *et al.*, 2007). It is in this way that the evolution of new defensive traits by plants and the counteradaptations to them by herbivores accounts for the patterns of variation in plant defence and has played a role in the specialization and diversification of both plants and herbivores concurrently (Stout, 2013).

Both plant defense and herbivore adaptation involve metabolic costs, so most plant-insect interactions reach a stand-off, where both host and herbivore survive although their

development is suboptimal (Fürstenberg-Hägg et al., 2013). This is consistent with the balance of biological forces throughout the biosphere.

1.4 Plant defence response

Due to the motile nature of herbivores, plant defence strategies generally come down to a go-away-or-die strategy or a slow-them-down strategy. Toxic and repellent plant compounds can be employed in the go-away-or-die strategy, whereas anti-digestive compounds or plant re-allocation of resources can lead to delayed development of the herbivore (Kant et al., 2015). Millions of years of the arms race has led to a wide range of plant defences, and these can be characterized generally as either direct or indirect. Direct defences are those that act directly against the attacker, and include physical barriers, such as trichomes or cuticle wax, and compounds that have a direct toxic effect, or antifeedant property that deters herbivory (Howe and Jander, 2008; Santamaria et al., 2013). Alternatively, indirect defences are those which protect the plant through the attraction of predators/parasitoids of the herbivore feeding on the plant in the form of released volatile compounds (Mithöfer and Boland, 2012). The cost of producing secondary metabolites serving as direct or indirect defences applies a selection pressure on the plant, as they cost energy to produce and deplete stores of valuable amino acids, like nitrogen. To decrease the metabolic cost to the plant and allow it to focus on growth or reproduction when no threat is present, many of these defense responses are induced only upon perception of herbivore feeding (Baldwin and Preston, 1999; Tian *et al.*, 2003; Zavala *et al.*, 2004). Constitutive defences, such as a basal level of defence compound or thorns, on the other hand, are always present in the plant and serve to ward off potential attackers, giving the plant a baseline defence (Howe and Jander, 2008). Trade-offs exist between constitutive and induced defences (Kempel et al., 2011), resulting in extensive variability among plant species defensive compounds and strategies, and contributing to the generation and maintenance of species diversity (Agrawal *et al.*, 2010). For induced defences to be beneficial to plants they have to be rapid, reversible and informed as to which attacker is present. Induced defences that do not affect the attacking herbivore are a cost without benefit to the plant (Karban et al., 1999). It is therefore not surprising that there has been

selection pressure on plants during the arms race, to correctly identify when a specific attacker is present.

Another characteristic of induced defences that plants have evolved is the systemic response. Where, in an effort to decrease the chance that a herbivore will move to an undefended part of the plant, upon perception of attack, plant hormones orchestrate a defence response and prime distal tissues for synthesis of defence compounds not yet attacked by the herbivore (Pearce and Ryan, 2003).

1.4.1 Plant perception of attack

Plants do not possess the adaptive immune system found in vertebrate animals. The adaptive immune system is a relatively recent evolutionary development. Adaptive immunity involves the mobile cells that use immunoglobulin and T-cell receptors. Genes encoding these receptors are re-arranged throughout an individual's lifetime, allowing for the 'real time' development of resistance through perception of attackers (Boller and Felix, 2009). Conversely, plants only have the evolutionarily ancient innate immunity, where receptors present in the germ line are used in perceiving an attacking organism (Howe and Jander, 2007; Boller and Felix, 2009), though these receptors are still subject to change through novel mutations, selection of favorable genotypes, or horizonal gene transfer events that act in large time-scales.

Innate immunity in plants can be separated into two forms of immunity that represent different stages of herbivore-plant interaction within the arms race. The terminology used to characterize how the plant perceives attack has been adopted from studies of plant-microbe interaction (Howe and Jander, 2008). First, there is the basal level of immunity, where molecular patterns associated with plant damage (DAMPs) or the attacking herbivore (HAMPs) are recognized by plant pattern recognition receptors (PRRs) within the plasma membranes of plant cells. HAMPs and DAMPs represent evolutionarily conserved molecular moieties that are recognized by the plant, triggering a cascade of signalling events leading to a defence program (Figure 1.1). DAMPs are generated from feeding site damage; however, the signals generated following their recognition can be delivered to undamaged parts of the plant in a systemic manner (Tör *et al.*, 2009).

The most extensively researched HAMPs are fatty acid conjugates, found in Lepidopteran, Dipteran and Orthopteran species (Yoshinaga et al., 2007), and inceptins, which are proteolytic products of plant-derived chloroplastic ATP synthase present in *Spodoptera frugiperda* larvae oral secretions following plant feeding and then used by the plant to perceive attack (Schmelz et al., 2006). The plant- and herbivore-derived molecules that induce a response in plants are often referred to as elicitors. To avoid this basal level of innate immunity, some herbivores have evolved a mechanism to suppress plant defences induced during basal immunity via the secretion of different molecules termed effectors, found in saliva, feces or oviposition fluids. These compounds may mimic plant hormones and/or mask the perception of HAMPs (Felton and Tumlinson, 2008). One example of a herbivore derived effector is glucose oxidase, identified in saliva of several noctuid species (Bede et al., 2006). In this way the plant becomes susceptible to the herbivore and cannot adequately defend itself against it. More recently it has been determined that certain plants can also detect a herbivore through what is called effector-triggered immunity. The plant recognizes specific molecular patterns of the attacking herbivore via intracellular pattern recognition receptors or the manipulations of plant cell targets by effectors, and initiates a defence cascade regardless of the presence of effectors that may be present (Böhm et al., 2014).

As the defence cascade starts upon perception of attack, it triggers early local responses including ion fluxes across the plasma membrane, collapse of membrane integrity at the feeding site, initiation of kinase cascades, and generation of reactive oxygen species (Maffei *et al.*, 2007; Wu *et al.,* 2007). Downstream of early responses are hormone cascades, that orchestrate the ensuing defence response. Studies of plant-pathogen interactions show that, despite recognition of pathogens being highly specific in many cases, plants have a common downstream signalling mechanism (Katagiri and Tsuda, 2010) leading to the initiation of the specific defences for each attacker. It has been hypothesized that this paradigm holds true for plant-insect interactions as well (Erb *et al.*, 2012). Through the action of hormone signalling and cross-talk therein, the transcriptional profile of the plant changes from one of growth to defence through the activation or de-repression of defence-associated genes (Sanabria, *et al.*, 2010). These genes can take part in the further regulation of defences by acting as transcription factors or in biosynthetic

pathways as enzymes required for the production of defence compounds, or act as defence proteins themselves by inhibiting herbivore digestion.

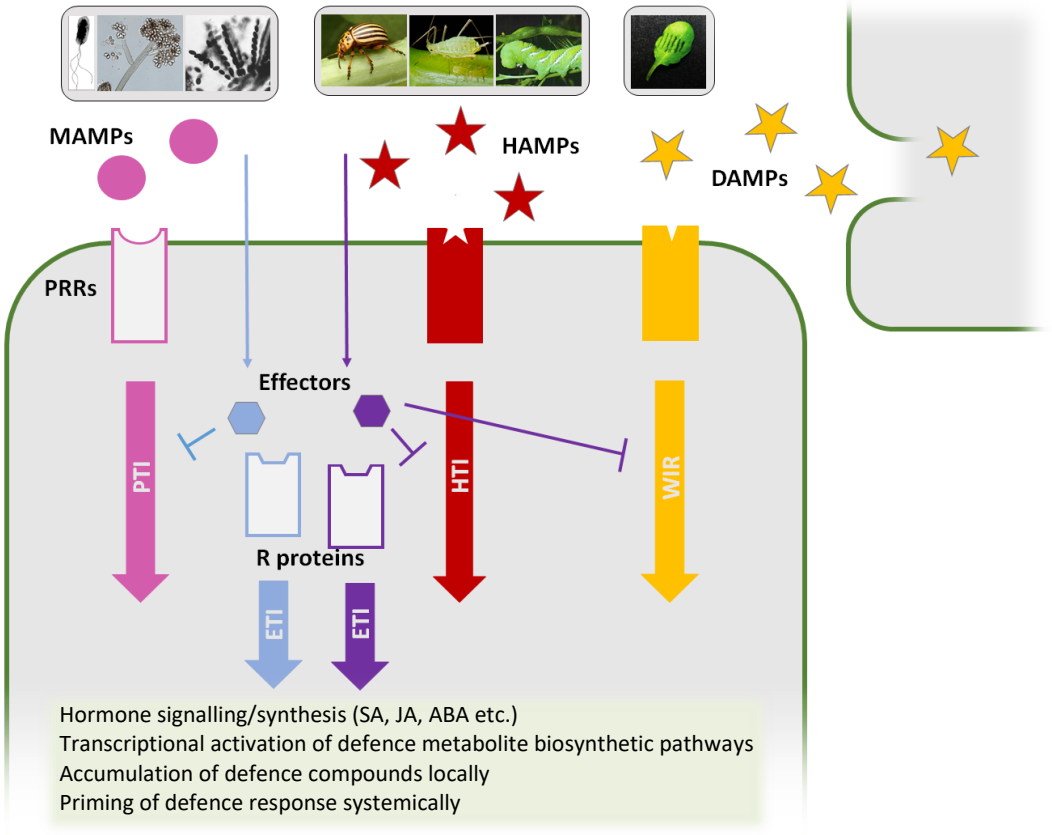


Figure 1.1 Schematic of plant perception of attack and induced defence. The defence responses are initiated by the recognition of conserved microbe/pathogen, herbivore, and/or damage associated molecular patterns (M/H/DAMPs) by pattern recognition receptors (PRRs) within the plasma membrane. This induces P/HAMP-triggered immunity (P/HTI) and/or wound induced response (WIR) that restricts the propagation of attacking pathogens or increases the defences against herbivores. Some strains of pathogens and herbivores have evolved effectors, that lead to the suppression of P/HTI, leading to plant susceptibility. Recognition of effectors (or their activity) by plant resistance proteins (R proteins) leads to effector-triggered immunity (ETI) and plant resistance. Figure modified from Erb *et al.* (2012).

1.4.2 Hormone Signalling in plants

Plant hormones orchestrate plant defence responses downstream of perception of elicitors or effectors and other early molecular recognition events of microbes and herbivores, such as Mitogen Activated Protein Kinase (MAPK) cascades (Pieterse et al., 2012). The main hormones required for the regulation of defences against herbivores incurring various types of tissue damage, in addition to those of wounding, are represented by the jasmonate family of signalling compounds (Howe and Jander, 2008). This is evident from results of studies using mutants compromised in one or more elements of the jasmonate pathway, where they are more susceptible to a wide range of arthropod herbivory including that from: caterpillars (Lepidoptera); beetles (Coleoptera); thrips (Thysanoptera); leafhoppers (Homoptera); spider mites (Acari); fungal gnats (Diptera); and mired bugs (Heteroptera) (Bostock, 2005; Howe, 2004; Kessler and Baldwin, 2002). Jasmonates play a pivotal role in switching the plant from a growth to defence program, allowing the plant to reallocate energy and metabolic resources (Pauwels et al., 2009). In general, jasmonates promote defensive and reproductive processes while inhibiting those of growth and photosynthesis (Turner et al., 2002). Jasmonic acid (JA) is synthesized upon perception of attack and induces expression of defence genes through JA-mediated transcription factors as discussed in detail in Fürstenberg-Hägg et al. (2013). Salicylic acid (SA) is also a very important hormone involved in the response to herbivory, and generally acts in an antagonistic way to JA responses and is itself important in induction of defences against biotrophic pathogens and has been hypothesized to be used by aphids and other phloem feeders to supress JA responses (Howe and Jander, 2008; Smith et al., 2009). However, there are many other hormones that play roles in modulating the defence response driven by JA and its most active form JA-Ile. Notable hormones include ethylene, abscisic acid (ABA) and gibberellins (van Loon et al., 2006; Ton et al., 2009; Daviere and Achard, 2013 respectively). The complexity added to the response to herbivory by hormone cross-talk allows the plant to modulate its immune response in a way that can be tailored to the attacking arthropod, promoting efficient utilization of resources (Wu and Baldwin, 2010).

1.4.3 Plant defence secondary metabolites

Jasmonates and modulating hormones direct defence gene activation followed by the synthesis of induced defence compounds (both direct and indirect). Focusing on direct defences, both constitutive and induced, there are a plethora of mostly low molecular weight molecules that exert repellent, antinutritive or toxic effects on herbivores. These defenses target key biological processes specific to herbivores, including the nervous, digestive and endocrine systems (Rosenthal and Berenbaum, 1992). Bioactive specialized defence compounds can repel or intoxicate insects, whereas defence proteins are usually deployed to interferer with digestion (Fürstenberg-Hägg et al., 2013). Under the continuing selection pressure of the arms race the same compound can act both as a repellant to a generalist herbivore and an attractant or oviposition cue for a specialist herbivore, depending on the interacting species (Remco and Van Poecke, 2007). Some of the most studied defence compounds are listed below, but do not represent a comprehensive list.

1.4.3.1 Defence chemicals

Alkaloids are widely distributed, found in 20% of all vascular plants consisting of more than 15,000 different compounds (Fürstenberg-Hägg et al., 2013). They are prevalent in the Leguminosae spp. (legumes), Liliaceae spp. (lilies), Solanaceae spp. (nightshade plants) and Amaryllidaceae sp. (Amaryllis), mostly in aboveground plant tissues. Alkaloids are well recognized for their effects on mammals, though they are thought to have evolved as defences against insect herbivores and include caffeine, nicotine, morphine, and cocaine (Ziegler and Facchini, 2008).

Another example of small molecule defence chemical are glucosinolates. These sulphur- and nitrogen-containing defence compounds are found only in Brassicaceae and Capparales plant families. There are at least 120 different structures known and they can be divided into different groups based on the amino acid precursor of the side chain (Hopkins et al., 2009). Depending on the type, they can be most dominant in the roots or the shoots of plants. The toxic effect of glucosinolates is attributed to their breakdown products that are usually derived through the reaction carried out by myrosinases (Fürstenberg-Hägg et al., 2013). Glucosinolates are a good example of defence compounds

that are effective against generalists, but function as feeding and/or oviposition attractants for specialists feeding on Brassicaceae (Bradburne and Mithen, 2019).

1.4.3.2 Defence proteins

In addition to toxic chemicals, plants also produce anti-digestive proteins that interfere with the ability of herbivores to digest plant material by limiting the rate of enzymatic conversion of ingested plant tissue. Anti-nutritive proteins on the other hand, limit food utilization by physically altering its availability and/or chemical identity (Duffey and Stout, 1996). There are five major classes of defence proteins: protease inhibitors (PIs), α-amylase inhibitors, lectins, chitinases and polyphenol oxidases (Fürstenberg-Hägg et al., 2013).

First characterised in solanaceous plants, PIs are now one of the best studied direct defences (Kessler and Baldwin, 2002). They are expressed in seeds, tubers and in vegetative tissues and can be induced following wounding and/or herbivore attack. There are 13 different PI families, known to target all main protease families (Rawlings *et al.*, 2006). The effectiveness of PIs in a defensive role relies on: 1) their enzymatic affinity (specificity) for protease found within the attacking herbivore's midgut; and 2) the ability of the attacker to alter its protease profile and increase expression of proteases insensitive to the PIs within plant material ingested (Koiwa et al., 1997). PIs not only delay the digestion of nutritious plant material, they also prevent the degradation of other antinutritional or toxic proteins ingested during feeding, giving the toxins time to exert their effect on the attacker (Amirhusin *et al.*, 2004).

Plants can also regulate the activity of the papain C1A family of proteases through inhibition of the activation step involving cleavage of the N-terminal pro-peptide. C1A N-terminal pro-peptides, 130-160 amino acids long, are involved in the inhibition of their cognate enzymes and participate in subcellular localization and proper folding of the mature protease (Demidyuk et al., 2010). These cysteine protease pro-peptides have also been shown to confer resistance of *Arabidopsis* to spider mites when expressing pro-peptide fragments of the *HvPap-1* gene from barley (Santamaria et al., 2015a). This study, among others, demonstrated the ability of C1A pro-peptides from different species to

inhibit exogenous C1A peptidases and suggests a role in plants as defenses against herbivores through interference with peptidases within the gut.

The activity of α-amylase inhibitors are directed against α-amylase enzymes of animals and microorganisms involved in starch breakdown, seldom affecting plant amylases (Fürstenberg-Hägg et al., 2013). For example, α-amylase inhibitors derived from wheat can inhibit *Tenebrio obscurus* (mealworm), *Tribolium* spp. (flour beetles), *Sitophilus* spp. (wheat weevils) and *Oryzaephilus* spp. (grain beetles) in addition to providing protection against *Bruchus pisorum* (pea weevil) in transgenic peas (Morton et al., 2000).

Lectins are very diverse, with an evolving classification system. Thus far, there are six families of lectins, divided based on carbohydrate recognition domain comparisons. Legume lectins and cereal lectins are the only two family members found in plants. Lectins are sugar-binding proteins enriched in storage organs and are though to interact with glycoproteins lining the gut of insects herbivores where they are assumed to inhibit nutrient absorption (Chrispeels and Raikhel, 1991); however, the mechanisms of lectin resistance in herbivores remain poorly understood (Fürstenberg-Hägg et al., 2013).

Chitin is present in abundance in the exoskeleton and peritrophic membrane of insects, mites and fungi. Plants possess chitinases, which are hydrolytic enzymes that break down glycosidic bonds in chitin, and are therefore proposed as defences against organisms containing chitin. This is supported in studies where transgenic plants overexpressing chitinases are resistant against insect herbivores (Fürstenberg-Hägg et al., 2013).

Polyphenol oxidases (PPOs) are ubiquitous copper-containing enzymes that catalyze the oxidation of phenolics to quinones and are induced by biotic and abiotic stresses (Thipyapong et al., 2007). The PPO-generated quinones are highly reactive and may cross-link or alkylate proteins, leading to the brown pigmentation observed in damaged plant tissue (Constabel and Barbehenn, 2008). PPOs also generate reactive oxygen species (Fürstenberg-Hägg et al., 2013). PPO activity has been associated with herbivore and pathogen resistance in plants among many other biological processes.

1.5 Herbivore adaptation

For phytophagous arthropods to utilize plants for food, they must deal with the plant defences they encounter. Ultimately, this comes down to one of three overarching strategies: avoid, overcome, or suppress. These adaptations can be genetic, morphological, physiological or behavioural in nature and will depend on life style and host range of the herbivore. Avoidance is an example of behavioural adaptation, where herbivores will feed on tissues that are not as heavily protected by the plant (Paschold *et al.*, 2007; Shroff *et al.*, 2008). An example of herbivore physiological adaptations can be seen in the diverse array of mouth-part structures used to uptake plant nutrients. Mouthparts evolved for chewing, tearing and snipping, like those observed in leaf-eating beetles (Coleoptera) or caterpillars (Lepidoptera), comprising about two-thirds of all known insect herbivores (Schoonhoven *et al.*, 1998), physically disrupt plant tissue allowing for ingestion and digestion. Another strategy is observed in thrips and spider mites that use tube-like stylets to pierce cells and suck up the liquid content, whereas leafminers develop in and feed on the soft tissue between epidermal cell layers (Howe and Jander, 2007). Aphids, whiteflies and other Hemiptera are phloem feeders and insert their stylet between cells to establish a feeding site in the phloem (Howe and Jander, 2007).

Other mechanisms in response to plant defensive metabolite profiles observed in herbivores include: increasing feeding intensity to compensate for any decreased efficiency of nutrient utilization due to plant defences (Gomez *et al.*, 2012); sequestering plant defence compounds for use against predators by storing them in specialized tissues or in the integument (Kant et al., 2015); target-site insensitivity to phytotoxins (Dobler *et al.*, 2012); and rapidly excreting phytotoxins before they can act on their target (Heidel-Fischer and Vogel, 2015).

1.5.1 Detoxification of xenobiotics

One of the most important mechanisms of overcoming plant defence compounds is detoxification. Current literature suggests that it results from a common set of detoxification-related enzyme families representing three distinct phases of detoxification. In phase I, the xenobiotic is enzymatically modified with the incorporation of a

nucleophilic functional group (a hydroxyl, carboxyl, or amine group), changing its chemical properties to become more polar/water soluble. The metabolite enters phase II, where it is conjugated to endogenous molecules, like glutathione or a sugar, which further increases its hydrophilicity. The third phase consists of excretion of the modified metabolite by cellular transporters. Sometimes, phase III occurs in the absence of phases I and II, where the plant metabolite is excreted before it can enter cells (sometimes referred to as phase 0; Kant et al., 2015).

Phase I is often carried out by cytochrome P450 monooxygenases (P450s) and carboxyl/choline esterases (CCE). Cytochrome P450s (CYPs) are encoded by the CYP gene superfamily and are membrane-bound enzymes involved in the metabolism of a wide array of compounds including vitamins and hormones in addition to their role in modifying xenobiotics (Heidel-Fischer and Vogel, 2015). Acetylcholinesterase (AChE) and carboxylesterases (CarE) are also enzymes involved in phase I detoxification reactions, and have mostly been studied for their role in resistance to insecticides as well as plant host preference (Rane et al., 2016; Xue et al., 2010).

Phase II conjugation of xenobiotics are carried out by transferases like glutathione-S-transferases (GSTs) and UDP-glycosyltransferases (UGTs). As their names suggest, GSTs operate by conjugating reduced glutathione to the electrophilic centers of xenobiotics (Li et al., 2007). Lipophilic xenobiotics are conjugated with sugars by UGTs, rendering them more water-soluble. Currently, there are more than 310 putative UGTs identified and classified in insects (Ahn *et al.,* 2012).

Phase III mechanisms have not been characterized to the same extent as those involved in phases I and II. However, there have been several reports of transporters providing herbivores with resistance to plant allelochemicals. One example is seen in leaf beetles where the selective transport of plant glycosides has been suggested to be important in the evolution of life history strategies and hosts ranges (Kuhn et al., 2004).

Detoxification, is also a major factor in the metabolism and excretion of synthetic pesticides as the mechanisms mentioned above have been observed for these chemicals as well (Li et al., 2007).

1.5.2 Suppression of plant defences

Another major strategy of herbivore adaptation to plant defences is to suppress them. By secreting small molecules, peptides or proteins as effectors into the plant during feeding and/or oviposition, a herbivore can limit the plant's ability to respond to the attack and decrease the amount of defences it has to encounter, eliminating/decreasing the need to avoid or overcome them (Musser *et al.,* 2002; Zarate et al., 2007; Alba et al., 2011). Alternatively, some herbivores can manipulate the allocation of resources within a host plant, and limit its response to attack that way (Clark and Harvell, 1992). Plant defence suppression is characterized by a lowered rate of defence compound production which can affect any level of the defence pathway (up- or down-stream), blocking it or attenuating it (Kant et al., 2015). As with all life history strategies, there are trade-offs in using suppression as a means of adaptation. For example, suppressing plant defences can cost herbivores through creating a beneficial host to competitors (Blaazer et al., 2018). Nevertheless, defence suppression has been shown to coincide with increased herbivore fitness (Kant et al., 2008; Sarmento et al., 2011; Alba et al., 2014), demonstrating it as a viable adaptation mechanism to host plant defences.

1.6 Selecting a plant-herbivore interaction model

1.6.1 *Solanum lycopersicum*

The cultivated tomato, *Solanum lycopersicum* L, (Solanaceae), has been a model organism for the study of plant-herbivore and plant-pathogen interactions for decades. It is a representative of the Solanaceae family, which also includes other food crops like potato, eggplant, peppers, as well as several medicinal plants including *Solanum nigrum* Linn., known for hepatoprotective and antioxidant properties (Sarethy et al., 2014), and some ornamental plants such as *Petunia*. It was in tomato that herbivore-induced systemic defences were first identified (Green and Ryan, 1972). Tomato is an excellent model for interaction studies based on knowledge of signalling pathways involved in the activation of defence genes in response to herbivore attack (Pearce et al., 1991) and direct defences that are constitutive or induced including alkaloids, chitinases, peroxidases, lipoxygenases, PPOs, and PIs (Kant et al., 2004). Additionally, there are a wide range of tools and

resources available for the identification and cloning of genes associated with agriculturally important traits (Gupta et al., 2009) and the genome was sequenced in 2012 by the Tomato Genome Consortium. Additionally, tomato production in Canadian greenhouses accounted for a farm gate value of $565M CAD in 2018, which was more than any other greenhouse vegetable, including cucumbers, lettuce, or peppers (Statistics Canada).

1.6.2 *Tetranychus urticae*

Tetranychus urticae Koch. (Acari: Tetranychidae), also known as the two-spotted spider mite, or red spider mite (depending on phenotype), is a very small herbivore that has become a model organism in the study of plant-herbivore interaction and a representative of the Chelicerate subphylum of Arthropoda (Grbić et al., 2007; Grbić et al., 2011; Van Leeuwen et al., 2010b; Rioja et al., 2017). It is characterized as *two-spotted* because of the visible dark spots in its near-transparent abdomen, produced by the accumulation of digestive cells within the mite midgut caeca (Bensoussan et al., 2018; Figure 1.1). It is referred to as a *spider* mite due to the copious amounts of silk it produces for a variety of reasons including: mobility throughout the colony, a safe place to lay eggs/avoid predators as well as a dispersal mechanism when the population gets too dense (Clotuche et al., 2013; Iwasa and Osakabe, 2015; Figure 1.2a and b). It is one of the most polyphagous herbivores known to exist, with over 1151 plant species recorded as hosts at the time of writing, including 150 crops (Migeon and Dorkeld, 2006-2019). Its extreme generalist nature makes it a great model for studying how generalists adapt to or overcome the diverse plant defences they encounter. It is also important to note however, that while *T. urticae* has the ability to feed on an extreme range of host plants, individual mite populations do not perform equally well on all potential hosts (Fellous et al., 2014; Gotoh et al., 1993; Navajas, 1999). Variation exists between mite populations as to which plants species may be considered favorable to them and different mite populations may adapt to new host plants when there is selection pressure to do so. In order to study the adaptation process, situations can be engineered such that spider mite populations can be adapted to novel hosts and their performance compared to an ancestral strain (Agrawal, 2000; Agrawal et al., 2002; Fry, 1989; Wybouw et al., 2015). In such cases, mites initially show low preference for, and low performance on the new host, suggesting mites were susceptible to the constitutive

and/or induced plant defences. Over time (5-25 generations), mites have a tendency to remain on the new host and their performance increased. Experimentally derived adapted mite populations allow for the determination of the physiological/genetic basis of host adaptation without the confounding effects of host-searching, and predator avoidance behaviour, etc., found in field conditions, by restricting the number of interacting species to two.

Tetranychus urticae has mouthparts adapted for a sucking mode of feeding, using a stylet to pierce through the plant epidermis to feed on individual mesophyll cells (Park and Lee, 2002; Bensoussan et al., 2016). During feeding, stylets transverse the leaf epidermis without damaging it, either in between epidermal pavement cells or through stomatal openings (Bensoussan et al. 2016). Plants experiencing mite herbivory display symptoms of chlorotic spots. As no macroscopic damage can be seen immediately following a feeding event, the chlorotic spots are not likely caused directly by mite feeding; however, chlorotic spot area can be used as a proxy for the amount of mite feeding on a leaf, as it is the cumulative result of both mite feeding and the plant's response to mite herbivory (Bensoussan et al., 2016).

Figure 1.2 Spider mite life cycle. The various life history stages of *T. urticae* are displayed going from left to right: egg, larvae, protonymph, deutonymph, adult male, and adult female. Photo credit: Zoran Culo.

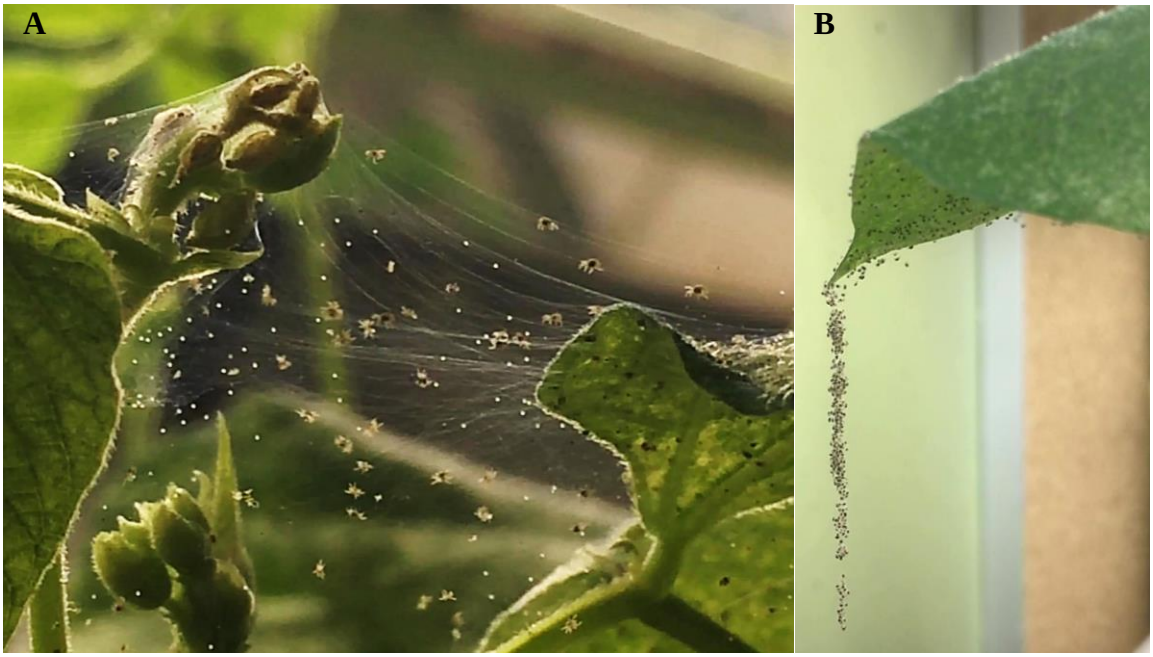


Figure 1.3 Spider mite colony on bean. A, mites using silk to move between plant surfaces and deposit eggs. **B,** over-populated bean leaf with silk string of mostly adult female mites trying to disperse to a new plant.

Since the publication of the mite genome in 2011 by Grbić et al., it has been used in the analysis of reciprocal transcriptional responses between plant and mite simultaneously (Wybouw et al., 2015; Zhurov et al., 2014). Characterization of the both plant and mite responses allows us to dissect the complex response of plants to mites during attack as well as the molecular mechanisms that allow mites to adapt to new plant hosts, providing a real-time view of the state of the arms race during their interaction. The *T. urticae* genome also revealed information regarding its protease profile. C1A cysteine protease genes (29 cathepsins L, 27 cathepsins B) and C13 legumain genes were well represented in addition to two aspartyl protease genes (Grbić et al., 2011). Studies into the active proteases within the mite digestive system, through the analysis of whole mite extracts and feces, have revealed the presence and support the role of cathepsin L, cathepsin B, legumain and aspartyl proteases in the mite digestive system (Carrillo et al., 2011; Nisbet and Billingsley, 2000; Santamaría et al., 2015b), with cathepsin L dominating the protease profile. Additionally, several classes of detoxification enzymes were found to be well represented or expanded in the genome, including CYPs, CCEs, GSTs and ABC transporters (Grbić et

al., 2011). Specifically, 81 CYP genes, 71 genes in the CCE superfamily, and 31 cytosolic GSTs were revealed in the *T. urticae* genome (Grbić et al., 2011).

There are several examples of constitutive defences, both physical and chemical, that act as potent deterrents of mite herbivory. A thick cuticle or wax on the leaf surface of some plant hosts represent physical barriers impeding the penetration of the mite stylet. Toxic or repellent allelochemicals affective against mites include acylsugars (Resende et al., 2002; Salinas et al., 2013), methyl ketones (Antonious et al., 2014; Chatzivasileiadis and Sabelis, 1997), and terpenoids (Bleeker et al., 2012) that accumulate in the trichomes of wild tomato cultivars. Unfortunately, in cultivated tomato varieties, these compounds have been lost (Bleeker et al., 2012), have decreased concentrations (Williams et al., 1980), or have a narrower range of target herbivores (Ghosh et al., 2014). These constitutive defences may be enough to render the plants non-hosts. In such cases, mites would disperse in search of more favorable hosts without incurring damage on the resistant plant (Díaz-Riquelme et al., 2016). Should constitutive defences fail, induced defences may negatively impact mite performance to a point where the plant remains resistant. If both constitutive and induced defences fail, the plant will be susceptible to mite feeding, and will incur damage. Previous studies have determined that *T. urticae* feeding on tomato induces the expression of phenylpropanoid, and terpenoid biosynthetic genes, as well as a wide range of antinutritive enzymes and enzyme inhibitors, including PIs, amino acid catabolizing enzymes, and PPOs (Kant et al., 2004; Martel et al., 2015).

Potential mite elicitors include components from preoral digestion and liquefaction of plant cell contents (Rioja et al., 2017), as well as cellular content leakage into the apoplast due to membrane fragmentation (Tanigoshi and Davis, 1978), during feeding events. These could be recognised as DAMPs by intact plant cells and trigger a defence response. Further from the feeding site, elicitors may also be present in enzymatically active feces or silk depositions (Santamaría et al., 2015b). Spider mite salivary secretions may be a source of elicitors and/or effectors of plant response (Jonckheere et al., 2016; Villarroel et al., 2016).

The extreme polyphagy of *T. urticae* and their ability to develop resistance to pesticides within a short period of time make mites pests in many fields and greenhouses.

Additionally, large populations can develop when mites find themselves in favorable conditions, making them very difficult to control. With warm temperatures, they can develop in less than 7 days from egg to adult on a suitable plant host (Rao et al., 1996), and also have a very high reproductive output (Grbić et al., 2007).

1.7 Research goal

There are many studies into tomato plant-mite interaction reported in the literature. None, however, have simultaneously examined this interaction with respect to *T. urticae* gaining the ability to suppress and/or detoxify tomato defence compounds following adaptation from a previously susceptible state.

There are reports on the ability of various mite species to suppress plant responses. Suppression has been observed indirectly in the case of *T. urticae,* (Alba et al., 2014; Kant et al., 2008) and there is evidence to suggest that effectors targeting SA responses benefit mites (Villarroel et al., 2016); however, the role of detoxification was not assessed in those studies. Elsewhere, several studies into mite response during host feeding have revealed up-regulation of detoxification genes in response to host shift (Wybouw et al., 2015; Zhurov et al., 2014); however, metabolic resistance to plant toxins has not been conclusively demonstrated, with one exception. Wybouw *et al.* (2014) demonstrated that a gene obtained from bacteria through horizontal gene transfer confers resistance of *T. urticae* to cyanide and subsequently gained the ability to feed on cyanide containing plant species. Importantly, none of the studies examining detoxification of phytochemicals by mites assessed the contribution of potential plant defence suppression. Research on *T. urticae* detoxification capabilities has largely focused on their use in the development of resistance to pesticides. In this way the literature, unintentionally, represents a biased view of plant-mite interaction, as the importance of each of these mechanisms of host utilization are only assessed individually. I hypothesize here that both suppression of plant defence responses and detoxification of allelochemicals can be employed concurrently.

The objective of this study was to assess the difference in plant defence response suppression and allelochemical detoxification between tomato-adapted and non-adapted mites. The performance of an experimentally derived tomato-adapted *T. urticae* strain and

the non-adapted, ancestral reference strain were compared. Additionally, aspects of tomato responses to the differentially adapted mite strains are characterized.

Novel findings from this research will determine if both defence response suppression and allelochemical detoxification can act simultaneously or if they may be mutually exclusive. Lack of evidence of either mechanism will not invalidate the hypothesis that they could be used together, as this is only a study of one adaptation event. Should one mechanism dominate the adaptation profile, this could suggest that there are genetic, or physiological constraints to using both mechanisms at once and this would need to be studied in greater detail on an ecological level. However, should both mechanisms be represented, then it will conclusively demonstrate their ability to work simultaneously and potentially synergistically.

2 Materials and Methods

2.1 Plant material and growth conditions

Plant growth chambers were set at 24 °C. The temperature under the lights was closer to 26 °C. Relative humidity was maintained at 60 % under a long-day photoperiod (16:8 h light/dark) using cool-white fluorescent lights (PHILIPS very high output F96T12/CW/VHO/EW). The light intensity inside the chamber was 120-130 μmol m^{-2} s^{-1}. Tomato cultivars used in this study include cv Moneymaker (Halifax Seed Company Inc., cat. No. AIMTOMM, Halifax, NS, Canada), cv Castlemart and def-1 mutant (cv Castlemart background). The def-1 (defenseless-1) mutant is an isogenic mutant line that is deficient in the biosynthesis of JA. The effect of the mutation lies in the octadecanoid pathway between the synthesis of hydroperoxylinolenic acid and 12-oxo-phytodlenoic acid (Howe et al., 1996).

Tomato seeds were germinated in a large petri dish between paper towel layers saturated with water for one week in the growth chamber before planting in 12 cm^3 pots. Bean plants (*Phaseolus vulgaris*, cultivar "California Red Kidney", Stokes, Thorold, ON, Canada), were grown in growth chambers at 25 °C, 60 % relative humidity and with a 16:8 h dark photoperiod and were planted directly in wet soil in 8 cm^3 pots.

Following planting, pots were left covered with a transparent lid for approximately one week before removal of the lid and regular watering. Non-autoclaved, peat–vermiculite growing mix (PRO-MIX® BX MYCORRHIZAE™; Premier Tech Ltd., Rivière-du-Loup, QC, Canada) was used for all planting.

2.2 Mite strains and rearing conditions

The non-adapted, ancestral reference *T. urticae* population (TU) was generated from mites collected from apples in the Vineland region in Ontario, Canada. In other publications, this mite strain is referred to as the 'London strain', which was used for genome sequencing in 2011 (Grbić et al., 2011). The mite colony was reared under laboratory conditions on bean and kept at a high density for more than 8 years.

The *T. urticae* tomato adapted strain (TU-A) was derived by Wybouw et al. (2015) via experimental selection using the non-adapted London reference strain (TU) as the ancestral population. Initially, three independent tomato-selection lines were generated by transferring 200 randomly chosen adult females of the TU strain from bean to 3-week-old potted tomato plants on which they propagated for approximately 30–35 generations to generate the TU-A lines (Wybouw et al., 2015). The lines were shown to perform the same, as measured by total population size on diverse host plant species (bean, cucumber, pepper, tomato) after inoculation of 35 female mites for 10 days, and were combined into one population (Figure 2 in Wybouw et al., 2015). The TU-A population used here has been maintained on tomato (cv Moneymaker) for over 4 years.

2.3 Damage assays

A damage assay was used to verify tomato-adapted status of the TU-A strain, and characterize the performance of non-adapted, TU mites on the non-favorable host, tomato. Four-week-old tomato plants were infested with 100 adult female mites on the terminal-adjacent leaflet of the third emerged leaf (Figure 2.1). Following 24 hours of feeding, the leaflet was cut at the petiole and the adaxial side of the leaflet was scanned using a Canon® CanoScan 8600F model scanner (Canon U.S.A. Inc., Melville, NY, U.S.A) at a resolution of 1200 dpi and a brightness setting of +25. Scanned plants were saved as .jpg files for subsequent analysis as described in Cazaux et al., 2014. Briefly, Adobe Photoshop 5 (Adobe Systems, San Jose, CA) was used for damage quantification in four steps. First, a new layer was added to the picture of the scanned plant and a grid (0.25 mm x 0.25 mm) was overlaid on it. Secondly, red dots of known pixel size (52 pixels) were placed within grid units for which there was damage covering more than half of the grid unit. The next step, after all the damage had been covered by dots, was to calculate the number of dots from the total number of pixels (derived from the histogram tool) divided by the number of pixels per dot (52 pixels/dot). The last part of the process was to calculate area damaged by multiplying the number of dots by the area of one grid unit using the formula:

$$\text{Area damaged } (mm^2) = \text{number of dots} \times 0.25 \text{ mm} \times 0.25 \text{ mm}$$

Three experimental trials were performed using 4 plants/mite strain/trial (n = 12).

2.4 Retention assay

A retention assay was used to characterize the behavioural aspect of adaptation in terms of host acceptability. TU-A and TU lines were assessed for their predisposition to disperse after deposition on a tomato leaflet. Using four-week-old Moneymaker plants, 50 adult female mites were deposited on the terminal-adjacent leaflet of the third emerged leaf (Figure 2.1). Following 24 hours of feeding, the number of mites remaining on the infested leaflet were counted. The results are displayed as % retention on the leaflet. Three experimental trials were performed using 4 plants/mite strain/trial (n = 12).

2.5 Fecundity assay

To characterize the fitness of each mite strain on tomato, and again verify adaptation status of the TU-A strain, a fecundity assay was performed. For this experiment, a terminal-adjacent leaflet of the third emerged leaf was isolated using lanolin (Sigma-Aldrich Co., Cat. No. L7387, St. Louis, MO, U.S.A) such that the 20 adult female mites placed on the leaflet had no choice but to remain there for the duration of the experiment (Figure 2.1). Following 4 days of feeding, the number of eggs on the leaflet were counted, and normalized to the average number of live mites on the leaflet ((# of live mites on day 0 + # of live mites on day 4)/2). This normalization was done to correct for mortality suffered by mites during the experiment as a mechanism to help control for that confounding effect. The results are displayed as number of eggs/mite. Three experimental trials were performed using 4 plants/mite strain/trial (n = 12).

Several other experiments use fecundity as a measure of performance. The normalization procedure was carried out for all of them for the reason specified above.

Figure 2.1 Experimental set-up for damage, retention and fecundity assays. Four-week-old tomato plants are used as a host for mites. Damage and fecundity assays involve infesting the terminal-adjacent leaflet of the 3rd emerged leaf with 100 and 20 adult female mites respectively. The lanolin barrier was used to keep mites on the leaflet they are infested on. The retention assay involved infesting the leaflet with 50 adult female mites and did not include a lanolin barrier, as the intention of the assay was to characterise their dispersal behaviour.

2.6 Transcriptomics

The microarray data set of differentially expressed genes (DEGs) of Moneymaker samples collected after the 24 hour feeding period by 100 TU and TU-A mites taken from their rearing host (Figure 2.2) was obtained from the supplementary material of Wybouw et al. (2015). Analysis was carried out using resources from the Bioconductor project (open source software for bioinformatics; https://bioconductor.org/). Expression measures were calculated using the 'affy' package and included background correction, and normalization. Quality control HTML files of processed microarray data can be found in the supplementary material. Batch effects (between arrays) were removed using the 'ComBat' package. Differential gene expression was performed with the 'limma' package, using p values (no fold change cut off used). The heatmap and expression plots of DEGs and associated clusters were generated using the 'pheatmap', 'amap', 'gplots' and 'ggplot2' packages. Gene ontology (GO) analysis of DEG heatmap clusters was performed using the package 'topGO'. All R scripts used to carry out the analysis can be found in supplementary material. All analyses were performed with the assistance of Dr. Vladimir Zhurov (Department of Biology, Western University, London, ON, Canada) who wrote R scripts for analysis of a similar experiment and were modified for use here.

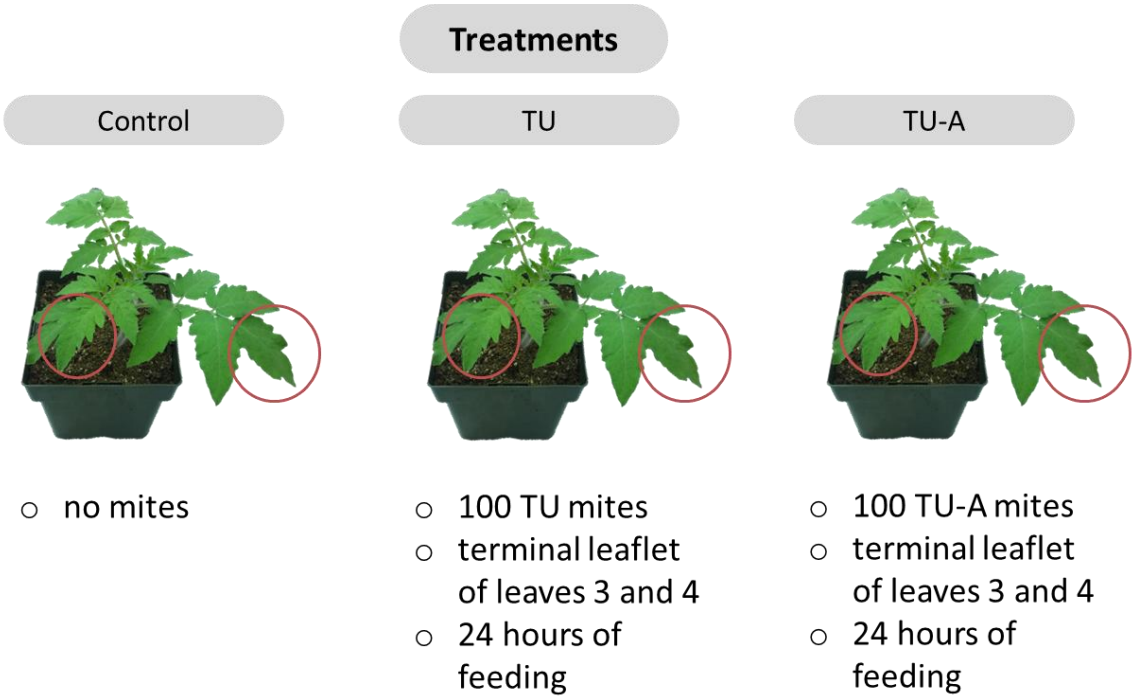


Figure 2.2 Schematic of microarray experimental treatment groups. This experiment was performed by Wybouw et al. (2015) and microarray data can be found in the supplementary material of that work. The same experimental set up was used in RT-qPCR experiment used to validate marker gene expression observed in microarray analysis.

2.7 Suppression assay: Co-infestation experiment

The suppression assay was used to test the ability of non-adapted TU and adapted TU-A mites to suppress tomato defences. In this experiment 100 adult female mites of TU or TU-A strains were applied to one of the terminal-adjacent leaflets of the 3rd emerged leaf of 4-week-old tomato plants. On the opposite terminal-adjacent leaflet, 20 adult female TU mites were applied and allowed to feed and lay eggs for 4 days (Figure 2.3). The leaflets were isolated using lanolin to prevent dispersal of mites. On day 4, the number of eggs laid by the non-adapted females was counted as well as the number of surviving mites. The total number of eggs was then normalized as described above. As the 'inducer' mite treatment occurred on a separate leaflet than the 'receiver' mites assessed for performance, benefits of suppression need to be attributed to a systemic process whereas a lack of observable suppression does not speak to events happening locally at the feeding sites. This experiment was performed 3 times using 6 biological replications/treatment (n = 18).

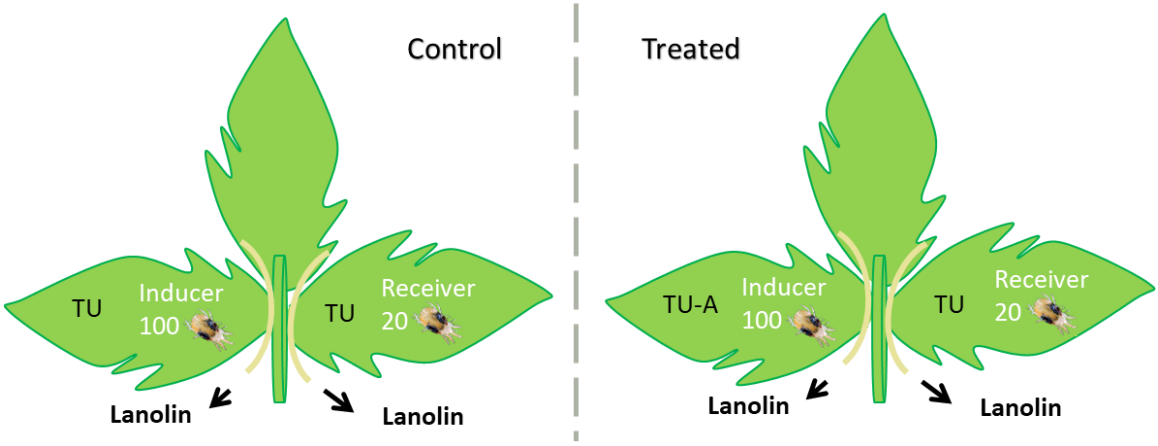


Figure 2.3 Schematic of the suppression assay. Fecundity of 20 TU 'receiver' mites assessed after 4 days of feeding on a leaflet adjacent to one with 100 TU or TU-A 'inducer' mites. Leaflets are isolated using wet cotton and lanolin to prevent mite dispersal. This experiment, like all the others, was performed using whole plants, though the diagram only shows the relevant leaf.

2.8 Quantitative analysis of phytohormones

Quantitative analysis of phytohormones was performed on tomato samples separated into three treatments: a no mite control, TU-infested or TU-A-infested. Using 4-week-old Moneymaker tomato plants, a terminal-adjacent leaflet of the third emerged leaf was infested with 100 adult mites. After 24h of feeding, the infested leaflet was cut at the petiole and immediately frozen in liquid nitrogen. The plant tissue was ground using a mortar and pestle in liquid nitrogen prior to lyophilisation and weighing. Dried samples were then analysed by Dr. Vicent Arbona (Universitat Jaume I, Castelló de la Plana, Spain), using the protocol described in (Durgbanshi et al., 2005). Briefly, plant hormones were quantified by isotopic dilution mass spectrometry of tomato samples. Isotope-labeled standards for JA, JA-Ile, SA and ABA (approximately 0.1 g) were added to plant samples before extraction. Ultra-performance liquid chromatography (UPLC)-electrospray ionization-tandem mass spectrometry analyses were carried out on an Acquity SDS system (Waters, Milford, MA, U.S.A) coupled to a Micromass Quattro LC Triple Quadrupole Mass Spectrometer (Micromass/ Waters, Milford, MA, U.S.A). Quantification was accomplished with an external calibration via calibration curves with known concentrations of plant hormones (Ximénez-Embún et al., 2018).

2.9 Marker gene expression analysis by quantitative RT-PCR

Plant tissue used for this analysis consisted of tomato leaflets that were flash frozen in liquid nitrogen after feeding by 100 female TU, or TU-A mites for 24 hours, or no mites (control). The sample size for marker gene validation of Moneymaker was only 2. The first sample consisted of plant tissue that was pooled from 5 biological replicates of experimental trial 1, and the second sample was derived from pooled tissues of 7 biological replicates of experimental trial 2. The sample size for marker gene validation of Heinz samples was 3, again pooling samples of each trial ($n = 4$ $n = 3$ and $n = 4$, respectively). The RNeasy Plant Mini Kit, including DNase treatment (Qiagen, Cat. No. 74904) was used for total RNA extraction using approximately 40 mg of ground plant tissue. Two micrograms of total RNA was reverse transcribed using the Maxima First Strand cDNA

Synthesis Kit for qRT-PCR (Thermo Fisher Scientific, Cat. No. K1672). Reactions were performed in triplicate for each biological replicate, using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Cat. No. K0222). The qRT-PCR was performed using an Agilent Mx3005P qPCR instrument (Agilent Technologies, Santa Clara, CA). Table 2.1 contains primer sequences and amplification efficiencies (E) used in qPCR reaction. *ACTIN* (*Solyc03g078400.2.1*) was used as the reference gene (Martel et al., 2015) and was found to be transcribed at similar amounts in all samples as indicated by Ct values within ± 1 cycle. Ct values of three technical replicates were averaged to generate a biological replicate Ct value. For plotting, expression values for each target gene (T) was normalized to the reference gene (R). As the traditional ΔΔCt method assumed 100% efficiency of primers, I have incorporated a different formula that accounts for the efficiency of each primer pair. If we take the relative number of fragments at the Ct as 1, then the relative quantity of template in the original sample (RQ) can be calculated using the efficiency (E in %) per gene as follows:

$$RQ = \frac{1}{(1+E)^{Ct}}$$

After calculating the RQ of the target (T) gene, it needs to be normalized for the total amount of cDNA by dividing it by the RQ of the reference gene (R) as discussed in Hellemans et al. (2008). This normalized relative quantity (NRQ) was calculated using the simplified formula below (ER: efficiency of Reference gene (%), ET: efficiency of Target gene (%)):

$$NRQ = \frac{(1+ER)^{CtR}}{(1+ET)^{CtT}}$$

NRQs were Log2-transformed and analyzed via with a fixed factor linear model and ANOVA (Rieu and Powers, 2009) using basic packages in R.

Table 1.1 Primers used in qRT-PCR.

Solyc ID	Description	Forward primer	Reverse primer	Efficiency
Solyc03g078400	Actin	CCTCAGCACATTCCAGCAG	CCACCAAACTTCTCCATCCC	1.02
Solyc09g084480	Proteinase inhibitor I	AAGTGATGGGCCAGAAGTCA	GGGACTGGAGAGCCATTCAA	1.04
Solyc00g071180	Cysteine proteinase inhibitor	TCCATTCCAAAACAAAGTCGAG	TTTCCACCTTCAGTGCCCTC	0.95

2.10 Protease activity in mites

The cysteine proteases activity of cathepsin L, cathepsin B, and legumain-like (legumain) was analysed from mite samples taken from bean (TU) or tomato (TU and TU-A). Each sample consisted of 100 mites, collected directly from the leaf with a pump (Cazaux et al., 2014), frozen in liquid nitrogen, and kept at -80 °C until analysis. The protease activity assays can be found in Santamaría et al., 2015a and are briefly described here. Total proteins were extracted from homogenized mites (2-3 mg) in 150 µL of cold NaCl (150 mM) using an Eppendorf pestle. The mite extracts were centrifuged at 10,000 rpm at 4 °C and supernatants were collected and used for enzymatic assays. The concentration of total protein mite extracts was determined using the Quick Start^{TM} Bradford Protein Assay (Quick start Bradford 1x dye reagent, Bio-Rad, Cat# 500-0205), with Bovine Serum Albumin (Sigma-Aldrich, Cat # A7906) as the standard. Substrates used in the analysis of cathepsin L-, cathepsin B- and legumain-like activities were Z-FR-AMC (N-carbobenzoxyloxy-Phe-Arg-7-amido-4-methylcoumarin, Sigma-Aldrich, Cat # 03-32-1501), Z-RR-AMC (N-carbobenzoxyloxy-Arg-Arg-7-amid o-4-methylcoumarin, Sigma-Aldrich, Cat # C5429), and Z-VAN-AMC (N-carbobenzoxyloxy-Val-Al a-Asn-7-amido-4-methylcoumarin, Bachem, Bubendorf, Switzerland, Cat # I-1840.0050) respectively.

Mite protein extracts were diluted to appropriate concentration in volume of 5 µL per sample (cathepsin L– 1 µg; cathepsin B – 2 µg, legumain-like – 5 µg). Then, 95 µL of appropriate substrate mix (0.1 M citric buffer with appropriate pH (4.5 for legumain and 5.5 for cathepsins), and DTT 0.1 M (Invitrogen, Cat # D1532; final concentration 1 mM) and substrate depending on enzyme tested (final concentration 20 µM) was added to each sample in a 96 well microtiter plate. The plate was then incubated at 30 °C for 1 hour in

the dark. Fluorescence was measured with wavelength of excitation 340 nm and emission 460 nm.

Enzyme activity was calculated using fluorescence data. Quantification was performed with known amounts of AMC (7-amino-4-methylcoumarin, Sigma-Aldrich, Cat # 257370) in a standard reaction with concentration ranges depending on assay (cathepsin L range: 0.781 – 25 µM; cathepsin B range: 0.125 – 10 µM; legumain-like range: 0.01–1 µM). Each sample was run with 3 technical replications, the mean value of which was used in statistical analysis. This experiment was performed twice with 6 biological replications/trial (n = 12).

2.11 Protease % inhibition assay

The ability of plant protease inhibitors to inhibit proteases was assessed *in vivo* against a commercial protease cathepsin L (EC 3.4.22.15; Sigma-Aldrich Cat. No. C6854). The plant samples were collected after feeding by 100 female TU, or TU-A mites for 24 h, or no mites (control) and crude protein extract was used as a source of protease inhibitors to calculate % inhibition (Figure 2.4). Protein isolation and protease activity assays were performed as described above with the additional step of incubating cathepsin L with crude plant protein extract prior to proteases activity detection. Briefly, 20 µg of plant protein extract was preincubated for 10 min at room temperature with 100 ng of cathepsin L. Subsequently, substrates were added at a final concentration of 0.2 mM and incubated for 1 hour at 28 °C. Fluorescence was then measured using an excitation filter of 365 nm and an emission filter of 465 nm. The results are displayed as % inhibition, which is 1 minus the percent of protease activity relative to that in the absence of the inhibitor source (100% cathepsin) using Z-FR-AMC (N-carbobenzoxyloxy-Phe-Arg-7-amido-4-methylcoumarin; Sigma-Aldrich Cat. No. 03-32-1501) substrate susceptible to hydrolysis by cathepsin L activities. This experiment was performed 2 times using 5 - 7 plants/treatment (n = 12).

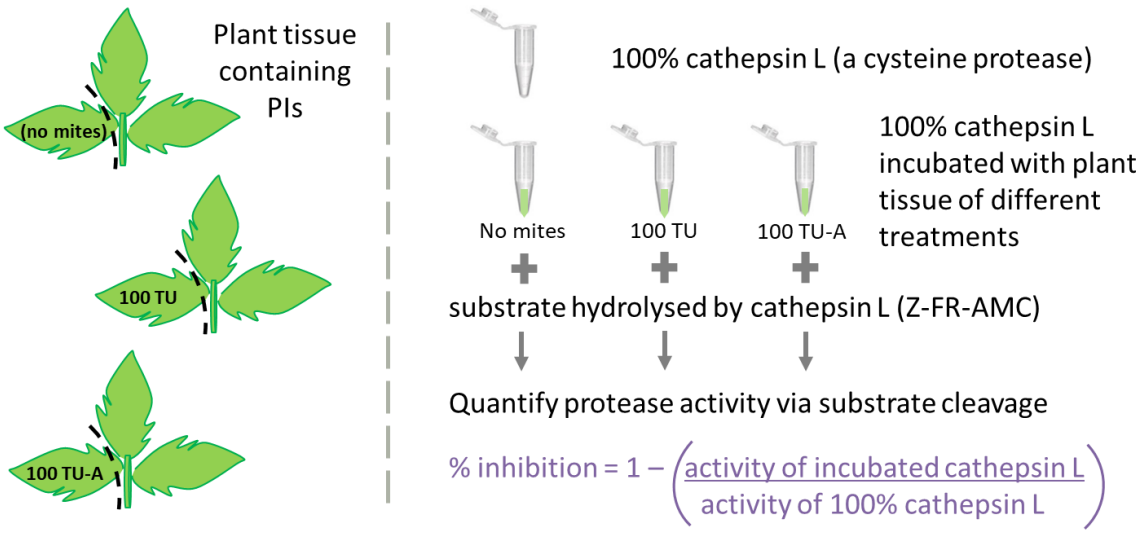


Figure 2.4 Schematic of % inhibition assay. Tomato tissue collection happed at the same time for all samples, following feeding by TU or TU-A mites for 24 hours in infested samples.

2.12 Synthetic PI assay

Using the synthetic cysteine protease inhibitor E-64 (Sigma-Aldrich, Cat # E3132) the effect of protease inhibition was tested on mite cathepsin L activity as well as mite fecundity. The protease activity assay was performed as described as above. Treatment of mites consisted of spraying 2 mL of 10 µM solution of E-64 (or water for controls) onto the third emerged leaflet of Moneymaker tomato plants (4-week-old). Leaves were allowed to dry before the application of 20 adult female mites of either TU or TU-A populations taken from their rearing host. Fecundity was addressed 2 and 4 dpi. The fecundity assay was performed in 3 trials with 6 biological replications/trial (n = 15-18). Cathepsin L activity following E-64 inhibition was performed in 2 trials with 5 biological replications/trial (n = 10). The concentration used was that proposed by the supplier for optimal effect and was found to produce sub-lethal effects on mites in another study (data not shown, manuscript in preparation).

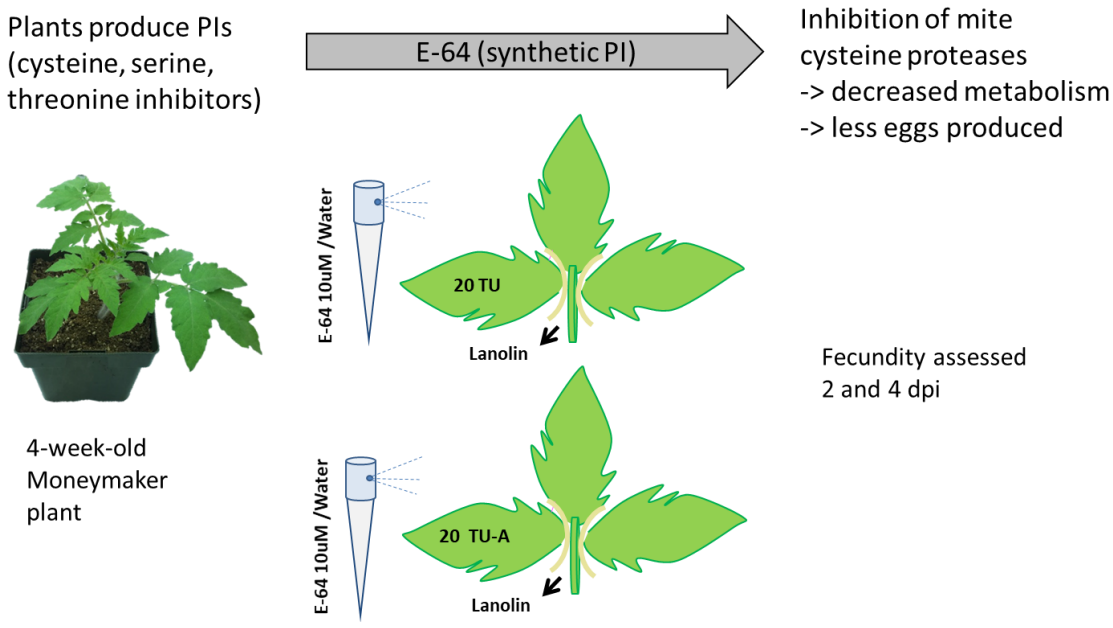


Figure 2.5 Schematic of synthetic PI inhibitor assay. E-64 was used to inhibit cysteine protease inhibitor activity by spraying tomato leaves with 10 µM solution. After the leaflet dried, 20 adult female mites were placed on the leaf of either TU or TU-A populations. Fecundity was used to measure mite performance following treatment with E-64 compared to a control with water 2- and 4-days post inoculation.

2.13 Detoxification enzyme inhibitor assays

Several classes of detoxification enzymes were characterized in terms of their involvement in mite detoxification of plant compounds. Inhibitor assays proceeded by spraying commercially available inhibitors on a terminal-adjacent leaflet of the third emerged leaf of 4-week-old tomato plants, applying mites and measuring their fecundity as a measure of performance following inhibition. The inhibitor compounds used were piperonyl butoxide (PBO; inhibitor of cytochrome P450 monooxygenases), S,S,S tributyl-phosphorotrithioate (DEF; inhibitor of esterases) and diethyl maleate (DEM; inhibitor of Glutathione S-transferases). These inhibitors are well established as commercial insecticide synergists and have been used in studies of pesticide resistance (Snoeck et al., 2017; Van Pottelberge et al., 2009). Concentrations used for the experiment were determined *a priori* through pilot experiments involving dose response curves and checking for any phytotoxicity incurred by the plant in response to the inhibitors. Concentrations were chosen such that the inhibitor treatment caused an approximate increase of mortality of 10% compared to control treatments. To ensure that the inhibitor was showing a slight effect on the mites the activity of the detoxification enzymes were: 1) decreased to a low enough level to ensure survival of most of the mites on a favorable, non-challenging host; but 2) kept high enough that an effect of the inhibition would be seen on performance of mites on a challenging host (tomato), should such an effect exist. Pilot experiment results can be found in Appendix, Figure 1. The concentrations used for the inhibition experiment as determined by the pilot experiments were as follows: 2000 mg/L PBO, 4000 mg/L DEM and 250 mg/L DEF.

Solutions were prepared using dimethyl formamide (0.01% V/V) and an emulsifier (0.0015% V/V) to first dissolve the inhibitors in solution (they are not water soluble) before bringing up the final volume to 10 mL with tap water. The ‘water’ control used in experiments also contained dimethyl formamide and the emulsifier in the same concentrations used in the inhibitor solutions.

Treated Moneymaker leaflets were isolated at the petiole with lanolin and infested with 10 spider mites (TU or TU-A). Following 3 days of feeding, the number of eggs was counted and normalized to the average number of mites/leaflet. Each experiment (control + 3

inhibitors) was performed 3 times using 6 biological replications/treatment (n = 18). To verify that the inhibitors were not affecting mites directly at the concentration used in tomato experiments, the same experiment was performed on the non-challenging host, bean (rearing host of TU and ancestral host of TU-A) and mite fecundity was assessed. It was predicted that inhibition would not affect mite performance on a non-challenging host since there was no difference in fecundity between strains on bean (Appendix, Figure 2), so any effect of inhibitors on tomato would be due to inhibition of detoxification of tomato metabolites. Control experiments on bean plants was performed twice by Dr. Cristina Rioja, Instituto de Ciencias de la Vid y del Vino, Logroño, La Rioja, Spain (n = 9) using the same mite strains, seed batches and chemicals as experiments performed in London, Ontario, Canada.

This experiment was also performed on the Castlemart cultivar and the *def-1* mutant with that background to: 1) assess the importance of mite detoxification of tomato induced responses; and 2) test if the same profile of detoxification classes were involved when using a different tomato cultivar host. This experiment was performed 3 times using 3-6 plants/treatment (n = 12-13).

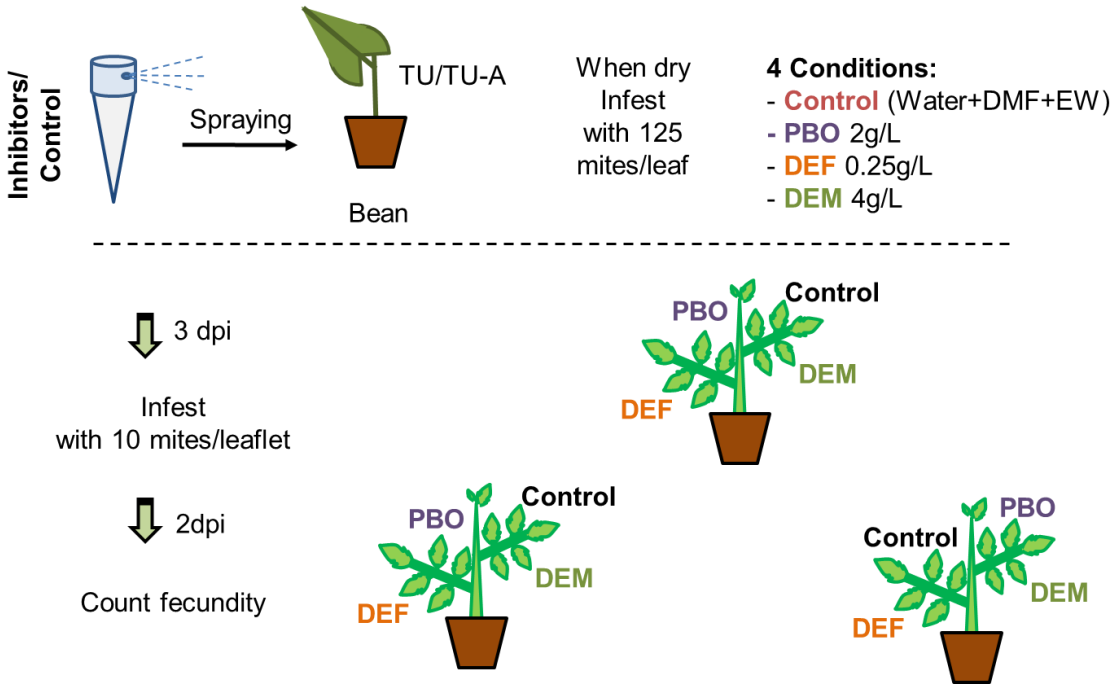


Figure 2.6 Schematic of detoxification assay using inhibitors of different classes of detoxification enzyme.

2.13.1 Detoxification enzyme activity assays

Detoxification enzyme assays of mites following treatment with inhibitors was determined (at general level of enzyme class) in an attempt to validate their decreased activity from inhibition. Esterase activity was measured by following the increase in production of p-nitrophenol (pNP) spectrophotometrically (absorbance at 405 nm) as a result of hydrolysis of p-nitrophenyl acetate (pNPA). Briefly, a 100 mM pNP solution was prepared by dissolving 69.5 mg of pNP in 5 mL of sodium phosphate buffer (pH 7.5) and used to make standard dilutions (10 – 100 µM). A 0.5 mM pNPA (Sigma-Aldrich, Cat # N8130) was used as the substrate for esterases within the mite protein extracts that were prepared as above. Samples were analysed by comparing esterase activities in wells containing 20 µL of pNPA, 80 µL of buffer in addition of 100 µL of mite extract (100 µg/mL) compared to the standard dilution series of pNP.

Glutathione-S-transferases catalyse the conjugation of L-glutathione (GSH) to 1-Chloro-2,4-dinitrobenzene (CDNB) through the thiol group of the glutathione. The formation of GS-DNB conjugate is directly proportional to GST enzyme activity and was used to characterize GST activity in mites spectrophotometrically by measuring absorbance at 340 nm. One unit of GST activity was defined as the amount of enzyme producing 1 mmol of GS-DNB conjugate per minute under the conditions of the assay. Briefly, a 0.1 M sodium phosphate buffer (pH 7.6) and 10 mM GSH (Sigma-Aldrich, Cat # G4251) and CDNB (Sigma-Aldrich, Cat # 237329) solutions were prepared in double distilled autoclaved water and absolute ethanol, respectively. For each sample analysed, 100 µL of GSH and 100 µL of CDNB were added to 100 µL of mite extract (200 µg/mL) per well. Absorbance was read immediately following addition of all solutions to the wells of the plate (no incubation step).

Cytochrome P450 activity was measured by detecting the O-deethylation of 7-ethoxy-4-trifluoromethylcoumarin (7-EFC) by CYP 450s into fluorescent 7-hydroxy-4-trifluoromethylcoumarin (7-HFC) and detected fluorometrically at 510 nm emission, excited at 410 nm. First, mite protein extracts were prepared with the Quick Start™ Bradford Protein Assay (Quick start Bradford 1x dye reagent, Bio-Rad, Cat# 500-0205) and diluted to a final concentration of 200 µg/mL. A reaction mix was prepared containing

5 mg of 7-EFC (Sigma-Aldrich, Cat # T2803) in 500 µL DMSO (0.4 mM final concentration), 152 mg of glucose-6-phosphate (Roche, Cat # 10 127 647 001) in 5 mL buffer (1 mM final concentration), 5 mg of $NADP^+$ (Sigma-Aldrich, Cat # N8035) in 6.54 mL buffer (0.2 mM final concentration) and glucose-6-phosphate dehydrogenase (Sigma-Aldrich, Cat # G6378; 0.014 U/reaction) all added to 3.9 mL of 0.1 M sodium phosphate buffer (pH 7.4). Then, 50 µL of the reaction mixture was added to 50 µL of mite protein extract in each of the sample plate wells and incubated at 37 °C for 30 minutes in the dark while shaking at 200 rpm. Following the incubation step, the reaction was stopped by adding 100 µL of a 1:1 ratio, trizma/acetonitrile mix (trizma buffer (0.05 M, pH 10)). Standards were then added (100 µL/well) to the plate consisting of a standard dilution series of 7-HFC (Sigma-Aldrich, Cat # 368512) ranging from 200 – 2000 µM, where the highest concentration was prepared using 3 µL of 0.2 mM 7-HFC (in DMSO) and 3 mL of sodium phosphate buffer (0.1 M, pH 7.5). Finally, fluorescence of 7-HFC was detected fluorometrically at 510 nm while excited at 410 nm.

All three enzyme activities were run on 96-well plates (clear for esterases and GSTs, opaque for CYPs). Blanks wells (just buffer) were used as a control for all assays. All samples/standard dilutions/blanks were run in triplicate.

2.14 Statistical analysis

ANOVAs were used in hypothesis testing for all experiments. This allowed for the combination of data across trials (of the same experiment) and therefore greater statistical power to detect differences between treatments/groups. Also, using ANOVAs allows for assessing the reproducibility of an experiment, which is also important to the scientific endeavor. In general, depending on the experiment in question, all the analyses consisted of two- or three-way factorial ANOVAs, where experimental trial, treatment, mite strain, and/or plant genotype were all considered categorical main effects. Again, depending on the experiment, an interaction term may also be included to answer certain biological questions. For example, if the difference between treatments in different mite strains were examined, a significant interaction between the main effects of treatment and mite strain would be interpreted as the mite strains responding differently to the treatment(s).

The main effect of trial was used as a reproducibility marker in two ways. If the main effect of trial was found to be significant, this was interpreted as a difference in absolute value of the response variable between trials, (regardless of any effect of other explanatory variables included in the analysis). This is generally to be expected when studying the interaction between two organisms (each bringing their own biological variability to the experiment) and is not reported in the text, but can be found in the supplemental data reporting the analyses. An interaction term between the main effects of trial and treatment was also included in the ANOVAs as a main effect. A significant interaction between trial and treatment indicates that patterns observed in individual trials between treatments was not the same among trials and suggests the experiment may not have been reproducible to the extent that data from all the trials could not be combined for analysis. If there is a significant interaction term, then a more detailed look into the data was warranted to identify: 1) if the pattern between treatments in each trial was different to the extent that the conclusions drawn would be different among trials; or 2) if the different patterns between treatments in each trial were only mildly variable and the same general trends were observed concerning the biological hypothesis in question (effect of treatment was the same direction in each trial, but to statically different magnitudes). Therefore, when an interaction term was found to be significant in the analysis, it is reported in the text with the effect size to be compared to the effect size of treatment. Readers may also be interested in looking at the interaction plots available in the supplementary statistical analyses. If the effect size of the interaction is much less than that of the main effect in question, then the data suggests that the same general trends were observed and the conclusions drawn from the significance value of the main effects can still be considered valid, though conclusions will be drawn with that statistical limitation in mind. Post hoc analyses of differences between means was performed using a Tukey-Kramer test, following identification of significant differences detected with an ANOVA.

All statistical analyses can be found in the supplementary material in the form of R markdown files (HTML). All steps of analysis, from data exploration, hypothesis testing and linear model validation, with associated R scripts, can be found in those files. Model validation included checking residual distribution for normality, as well as plotting residuals against fitted values to check for linearity and equal variance.

3 Results

As described in the Introduction, adaptation of a herbivore to a host plant is characterized by the ability of the herbivore to develop and reproduce having acquired the ability to avoid or overcome host-specific plant defences. It was therefore hypothesized here that TU mites would find tomato a challenging host, and show low acceptance of it as a host due to the constitutive and/or induced tomato defences. It was conversely hypothesized that TU-A mites have overcome some/all of their tomato host's defences and display host acceptance and increased performance relative to TU mites.

It was also hypothesized that the TU-A mite strain would actively suppress aspects of the tomato defence response. This hypothesis was tested through analysis of tomato transcriptome responses following feeding by TU and TU-A mites in a microarray experiment followed up by marker gene analysis using RT-qPCR. Hormone quantification was also performed to test for the suppressive ability of mites downstream of transcriptional responses. Additionally, the suppressive ability was tested at a systemic level using a co-infestation experiment.

The adaptation mechanisms of detoxification was also characterized in TU-A mites using TU mites as a non-adapted reference. Detoxification enzyme inhibitors were used to test for the requirement of TU-A mites using detoxification as a method of adaptation to tomato.

3.1 Damage analysis

Damage analysis was performed to characterize the performance of the two mite strains on the tomato cultivar, Moneymaker, that served as host in subsequent experiments. TU-A mites were expected to inflict much more damage than TU mites due to their adaptation status.

TU-A mites displayed a significantly greater amount of damage to tomato leaflets following 24h of feeding compared to TU mites (Figure 3.1). While the damage from non-adapted, TU mites was near undetectable by the human eye (few, if any chlorotic spots), damage from TU-A mites was obvious and extensive (Figure 3.1B). TU-A mites produced

73.22 mm^2 of damage on Moneymaker plants, whereas TU mites produced only 0.55 mm^2 (Figure 3.1A).

To ascertain if adaptation of TU-A mites was tomato cultivar specific (adapted only to the Moneymaker cultivar they were maintained on or if it was adapted to tomato in general), 100 TU or TU-A mites were left to feed on 3 different tomato cultivars in addition to Moneymaker for 24h prior to sample collection and damage quantification. TU-A mites were found to produce far greater damage than the TU mites on all of the cultivars tested, including Castlemart, Heinz, Microtom, and Moneymaker (Figure 3.2). On Castlemart, Heinz (1706), and Microtom TU-A mites produced around 30-40 mm^2 of damage. Additionally, though TU-A mites produced a lot of damage on all cultivars tested relative to TU mites, they did incur the most damage on Moneymaker plants (62 mm^2; Figure 3.2). TU mites performed poorly on all tomato cultivars producing 1.1 – 1.6 mm^2 damage on Castlemart, Heinz and Moneymaker, and 0.36 mm^2 damage on Microtom (Figure 3.2).

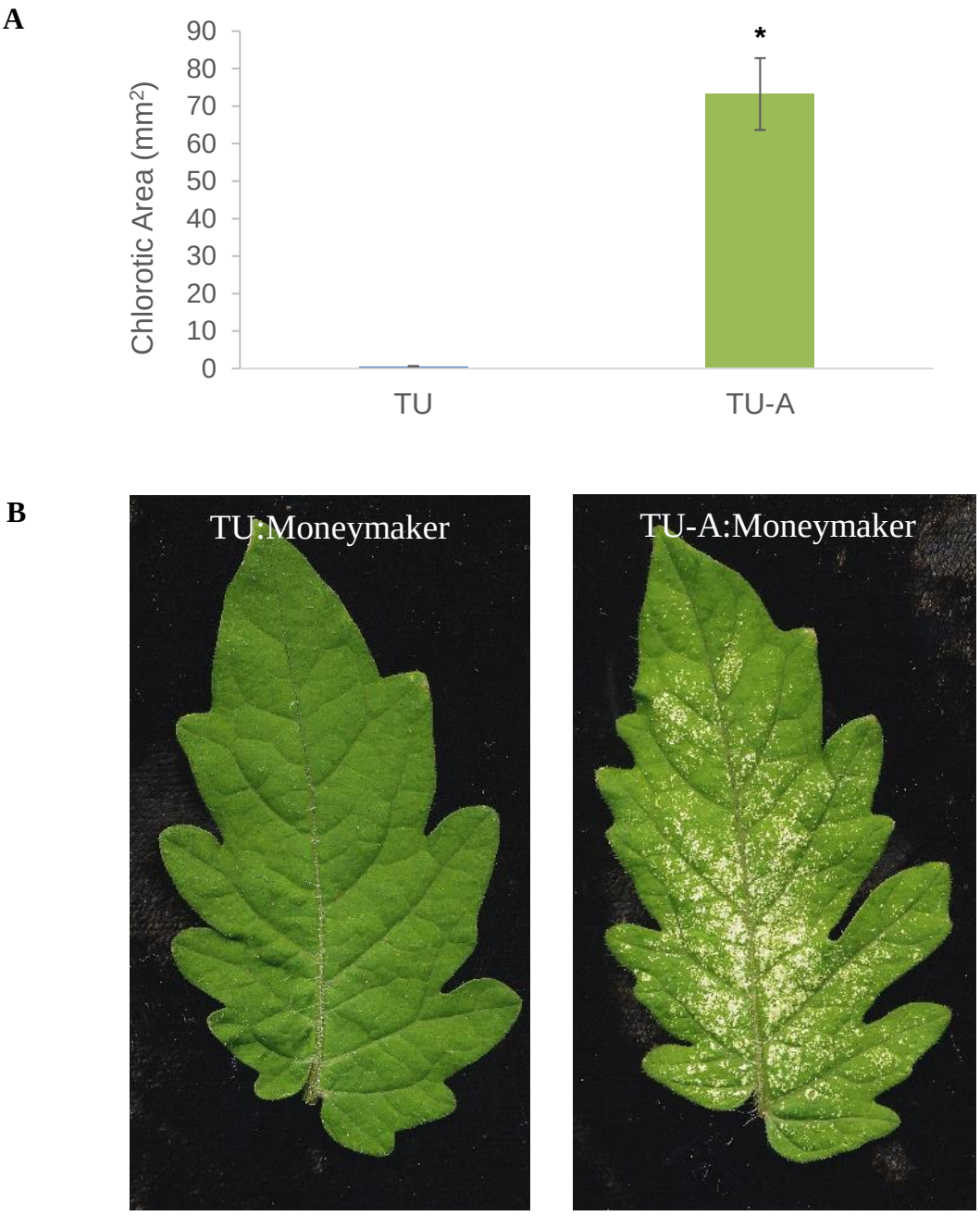


Figure 3.1 Mite damage on tomato cv. Moneymaker. Damage on tomato leaflets after 24h of feeding by 100 adult female spider mites of different adaptation status. **A,** Bar graph shows the mean ± SE chlorotic spot area (mm^2). Asterisk represents a significant difference between mite strains (two-way ANOVA, F = 71.38, p = 1.118e-07, η^2p = 0.80, n = 12/mite strain). **B,** pictures of representative Moneymaker leaflets fed on by TU and TU-A mite strains.

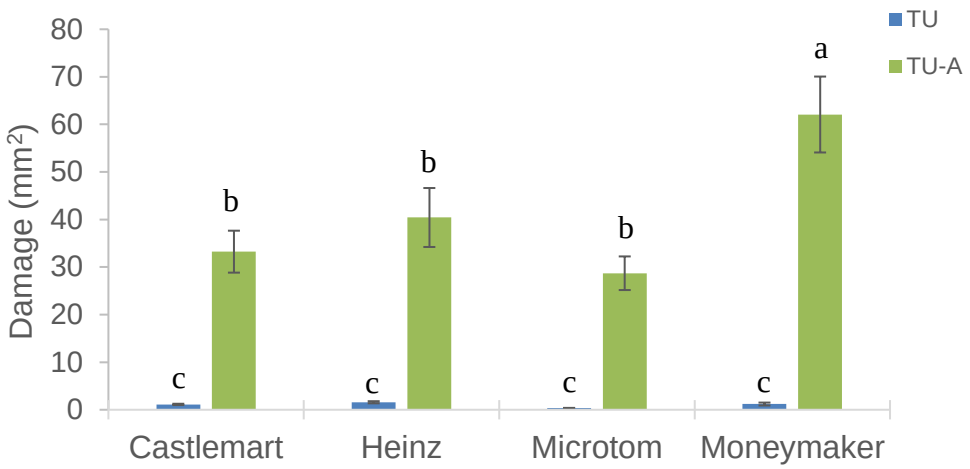


Figure 3.2 Mite damage on multiple tomato cultivars. Damage to tomato leaflets of different cultivars after 24h of feeding from 100 adult spider mites of TU or TU-A strains. Bar graph show the mean ± SE damage (mm^2). Different letters represent significant differences between means (three-way ANOVA, $F_{mite.strain} = 311.85$, $p_{mite.strain} < 2.2e\text{-}16$, $\eta^2p_{mite.strain} = 0.71$; , $F_{plant.genotype} = 10.99$, $p_{plant.genotype} = 1.842e\text{-}06$, $\eta^2p_{plant.genotype} = 0.21$, followed by Tukey-Kramer post hoc test, $p < 0.05$; n = 18/mite strain). There was a significant interaction term between mite strain and trial ($F_{mite.strain:trial} = 21.12$, $p_{mite.strain:trial} = 1.227e\text{-}08$, $\eta^2p_{mite.strain:trial} = 0.25$); however, comparing the effect size of the interaction to that of the main effect of mite strain shows the interaction effect size to be almost 3-fold less than that of the mite strain. Additionally, the interaction plot shown in the supplementary material suggest that this interaction arises due to the variability of damage by TU-A mites between trials, compared to a similar level of damage by TU in each trial. In all trials however, TU-A produced much more damage than TU, so the overall pattern was the same between trials.

3.2 Retention assay

Another feature that is associated with mite host adaptation/acceptance is the tendency of the herbivore to stay on the host-plant it encounters. Therefore, an experiment designed to test the mite's proclivity for staying and feeding on tomato was also used to characterize the adaptation status of TU-A mites. This assay was used to test how readily the mites accepted a new tomato plant as a host by testing if they stayed on the same leaflet they were placed on, or if they dispersed, looking for a new, more hospitable host. It was hypothesized that TU-A mites would remain on the tomato leaflet on which they were placed, whereas TU mites would likely move off the tomato leaflet in search of a more favorable host.

Non-adapted, TU mites dispersed readily on Moneymaker with only ~31% remaining on the leaflet they were deposited onto. In contrast, ~92% of adult female TU-A mites remained on the leaflet they were placed on after 24h (Figure 3.3). This dispersal pattern is consistent with the hypothesis that adapted mites would remain on the tomato leaflet due to their ability to overcome defences encountered.

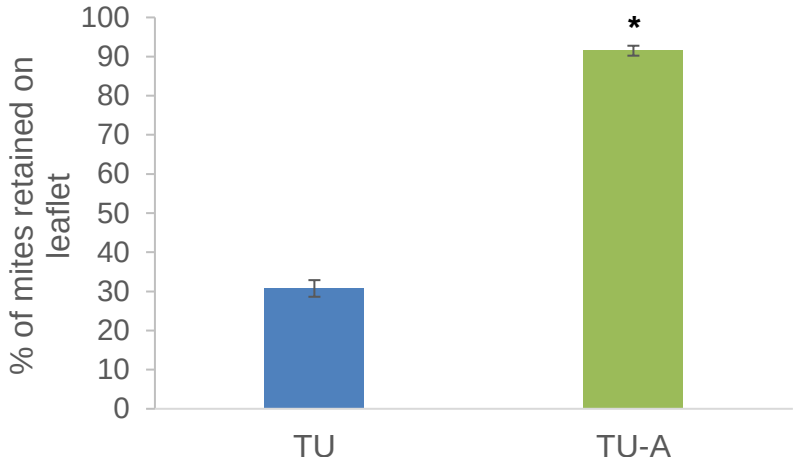


Figure 3.3 Mite retention from tomato leaflet. Bar graph representing mean ± SE % of TU and TU-A mites remaining on Moneymaker leaflets after 24h following inoculation with 50 adult female mites. Asterisk represents a significant difference between mite strains (two-way ANOVA, F = 588.80, p < 2e-16, η^2p = 0.96, n = 18/mite strain).

3.3 Fecundity assay

The best measure of mite performance is fecundity as it relates directly to fitness and therefore adaptation status in a very meaningful way. As previously demonstrated, TU mites disperse when placed on tomato and would not be confined to the leaflet where fecundity was to be measured without a barrier impeding their escape. Lanolin (sheep wool fat) was used for this purpose. A lanolin barrier dispensed by a syringe was placed around the petiole of the infested leaflet and on leaf surfaces of any adjacent tomato leaves that would come into contact with the experimental leaf during the experiment (4 days of mite feeding and egg deposition). As the behavioural response of TU proved to be strong, a small number of mites would try to climb through the lanolin barrier and die within it. Therefore, a normalization of the number of eggs deposited to the average number of mites on the leaflet was performed following counting as described in the Methods section. The results are therefore displayed as the average number of eggs/mite 4 dpi.

Fecundity of the different mite strains was tested by infesting tomato leaflets with 20 adult female mites and counting the eggs on the adaxial and abaxial side of the leaflet following 4 days of feeding along with the number of remaining live mites. As TU-A mites are hypothesized to use tomato effectively as a plant host, deriving nutrients required for development and reproduction, it was hypothesized that TU-A mites would lay more eggs than TU mites and the effect size should be substantial to account for the high population counts found on the tomato plants used to rear and maintain TU-A mites.

TU mites displayed significantly reduced fecundity relative to TU-A mites. TU mites laid an average of ~4 eggs/mite in 4 days. By contrast, TU-A mites laid ~27 eggs/mite (Figure 3.4). It should be noted that normalization was also helpful in accounting for the observed but not quantified increase in the mortality of TU mites (non-lanolin related) when the number of eggs was being counted at the conclusion of the experiment, compared to very little mortality of TU-A mites. It is likely that mortality was due to the toxic effect of tomato metabolites on TU mites, or starvation if they refused to feed.

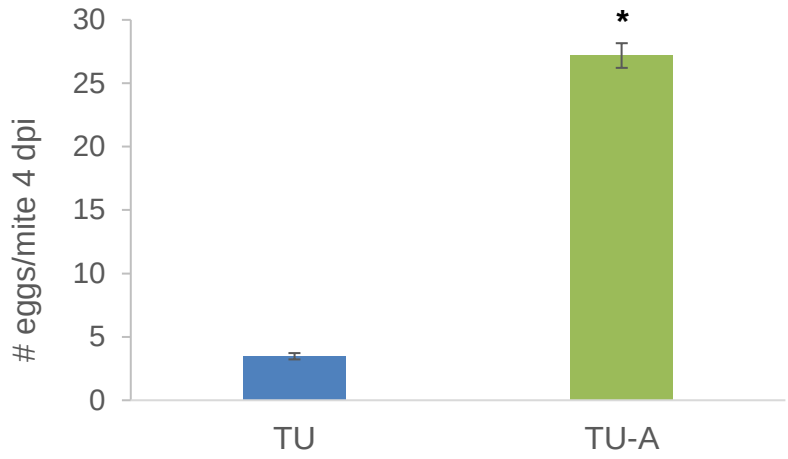


Figure 3.4 Mite fecundity on tomato. Bar graph representing the mean ± SE number of eggs/mite 4 dpi on Moneymaker leaflets for TU and TU-A mites. Asterisk represents a significant difference between mite strains (two-way ANOVA, F = 623.54, p < 2e-16, η^2p = 0.96, n = 16/mite strain).

Results from the damage, dispersal and fecundity assays all support the characterization of TU-A mites as adapted to tomato to the extent that they cause substantial plant damage and produce well enough to colonize the host. This supports the overall hypothesis of this work: TU-A mites use mechanisms (whether genetic or physiologically based) to overcome and/or suppress tomato defences. Further analysis detailed below revealed several such mechanisms at play.

3.4 Tomato transcriptomics

Plant response to mite feeding is an important aspect of studying the interaction between mites and tomatoes and can offer unique insight into the result of herbivore adaptation on plant response to feeding. The response to herbivory and wounding is to a large extent orchestrated by JA and its bioactive conjugate, JA-Ile. This has been conclusively demonstrated with respect to mite induced defences in tomato (Martel et al., 2015). The hormone SA also seems to be involved in the response to spider mites by tomato (Ament et al., 2010; Kant et al., 2004). If TU-A mites are manipulating tomato defences, then induced responses are expected to be lower than those induced by the non-adapted TU mites. It was hypothesized that a lack of induced responses would be evident at the transcriptional level, and an analysis of tomato microarray data should reveal DEGs in biological processes related to defence against herbivory. Biological processes containing genes differentially induced by TU-A mites compared to TU mites that would support a characterization of attenuation of induced defences include: JA biosynthesis, JA response, response to wounding, genes associated with the synthesis of PIs and other tomato defences such as alkaloids and PPOs.

Previous research regarding plant suppression by mites focused the analysis of gene expression induction using only marker gene analysis by RT-qPCR (Alba et al., 2014; Glas et al., 2014; Schimmel et al., 2017). It was not until Wybouw et al. (2015) published their work on tomato response to TU and TU-A mites that plant modulation by adapted mites could be assessed at the level of whole transcriptome response. In the original analysis by Wybouw et al. (2015), global analysis of DEGs was performed in response to both TU and TU-A mites. The analysis presented here narrowed the focus to those genes that were found to be differentially expressed upon feeding by TU mites in the original analysis, as I was interested in how the response to TU mites changes compared to the same set of genes in response to TU-A mites. A heatmap illustrating tomato induced responses to TU and TU-A mites feeding for 24h, filtered by tomato response to TU (Figure 3.5A). Different clusters generated by this analysis revealed differential gene induction based on mite adaptation status (Figure 3.5B), representing 4 patterns of gene induction by both TU and TU-A mites.

Cluster 2 contained 270 DEGs that were most highly induced in TU-treated samples and much less induced in TU-A-treated samples. Gene ontology (GO) analysis of biological processes (BP) revealed that DEGs detected in cluster 2 were enriched in genes involved in many processes related to herbivory recognition and induced defence including: 85 of 430 annotated genes involved in the response to JA; 18 of 268 annotated genes in the response to wounding; 7 of 101 annotated genes in JA biosynthetic processes; 6 of 34 annotated genes in the negative regulation of peptidase activity (PIs) and 4 of 65 annotated genes involved in alkaloid biosynthetic processes. These differential responses to TU-A mites compared to TU mites indicate that some elements within the defence response against non-adapted mites are being manipulated to the potential and hypothesized advantage of adapted mites.

Clusters 4 and 1 included genes that are similarly expressed in response to TU and TU-A mites. Cluster 4 contained 304 genes that are upregulated in response to mite feeding regardless of adaptation state. Biological processes represented in cluster 4 include: 41 of 430 annotated genes in response to JA and 20 of 268 annotated genes in response to wounding. This suggests that some elements within the JA defence response pathway were still employed by the plant, regardless of mite adaptation status. Indeed, in cluster 4 there are also 9 of 225 annotated genes in response to chitin; 8 of 255 annotated genes in the MAPK cascade; 7 of 164 annotated genes in the JA mediated signalling pathway; and 10 of 193 annotated genes in the SA mediated signalling pathway. These genes were upregulated in response to both mite strains and represent early events in the defence response. Additionally, there were 8 of 34 annotated genes in the negative regulation of peptidase activity that were upregulated and could encode PIs encountered by both mite strains.

Cluster 1 contained 107 annotated genes that were downregulated in response to mite feeding by both mite strains. Biological processes represented in cluster 1 include: 4 of 152 annotated genes in chlorophyll biosynthetic process; 4 of 256 annotated genes in chloroplast organization; and 5 of 134 annotated genes in aromatic amino acid family biosynthetic process; and 3 of 150 annotated genes involved in the regulation of meristem growth. These results suggest that many aspects of the transcriptional change from growth

and developmental processes to reallocate resources to the defence response were still occurring in response to TU-A mites.

Cluster 3 contained 115 genes that were downregulated in response to TU mites and less downregulated in response to TU-A mites, and therefore represent genes attenuated in response to TU by TU-A mites. Biological processes represented in this cluster included: 4 of 215 annotated genes in abscisic acid-activated signalling pathway; 1 of 8 annotated genes in the negative regulation of developmental growth; 1 of 5 annotated genes involved in DNA replication, and synthesis of RNA primers; 1 of 5 annotated genes in photosystem stoichiometry adjustment; and 1 of 6 annotated genes in positive regulation of protein complex disassembly. Therefore, many other aspects of the switch from a growth transcriptional program to a defensive one were attenuated in the response to TU-A mites.

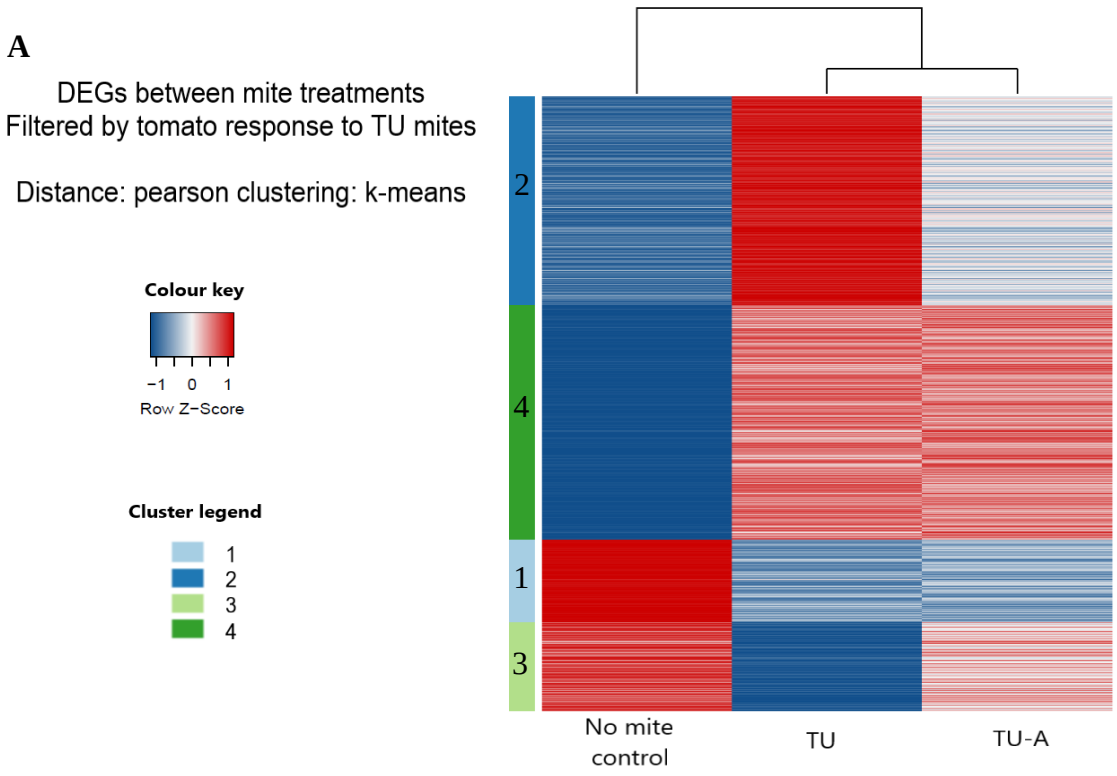
In general, more genes were up-regulated (574) than down-regulated (222). Up-regulated genes were almost split evenly between clusters 2 and 4 (270 and 304, respectively) and similarly so with the down-regulated genes being split between clusters 1 and 3 (107 and 115, respectively).

There are also several genes that were robust in their reproducibility as markers of the JA-dependent induced defence response in tomato including: JA biosynthetic enzymes *lipoxygenase D (LOXD)* and *allene oxide synthase 1 (AOS1); proteinase inhibitors (PI); leucine aminopeptidase (LAP); threonine deaminase;* and *polyphenol oxidases (PPO)* (Martel et al., 2015). Genes of this type were assessed in the list of DEGs in order to characterize their levels of induction under the conditions of this analysis. *Lipoxygenase D (LOXD; Solyc03g122340)* was represented in cluster 2, showing an attenuated induction upon feeding by TU-A mites compared to TU, while another JA biosynthetic enzyme, *allene oxide synthase (AOS; Solyc04g079730)*, was represented in cluster 4, being upregulated to the same degree by both mite strains. Two well studied tomato cysteine PIs were selected as markers for that class of defence protein. *Protease inhibitor I (Solyc09g084480)* was represented in cluster 4 and *Cysteine PI (Solyc00g071180)* was represented in cluster 2. Interestingly, 2 leucine aminopeptidases (of 3 in tomato) were found to be differentially expressed upon mite feeding in this analysis and both were

represented in cluster 4. They were *Leucine aminopeptidase A1 (LapA1; Solyc12g010020)* and *leucine aminopeptidase 2 (lap2; Solyc00g187050). Threonine deaminase (Solyc09g008670)* was represented in cluster 4. The uncharacterized PPOs with locus IDs: *Solyc08g074620* and *Solyc08g074650* were both represented in cluster 2, while *Solyc08g074630* was in cluster 4.

In summary, the transcriptional analysis performed here revealed an attenuation of induced tomato defences by TU-A mites in a way that is hypothesized to benefit them. Further analysis was required to validate the biological relevance of this modulation of plant response with respect to increased mite performance including the relevance of cysteine protease inhibitors and their effect on mites.

A


DEGs between mite treatments
Filtered by tomato response to TU mites
Distance: pearson clustering: k-means
Colour key
−1 0 1
Row Z−Score
Cluster legend
1
2
3
4
2
4
1
3
No mite control
TU
TU-A

B

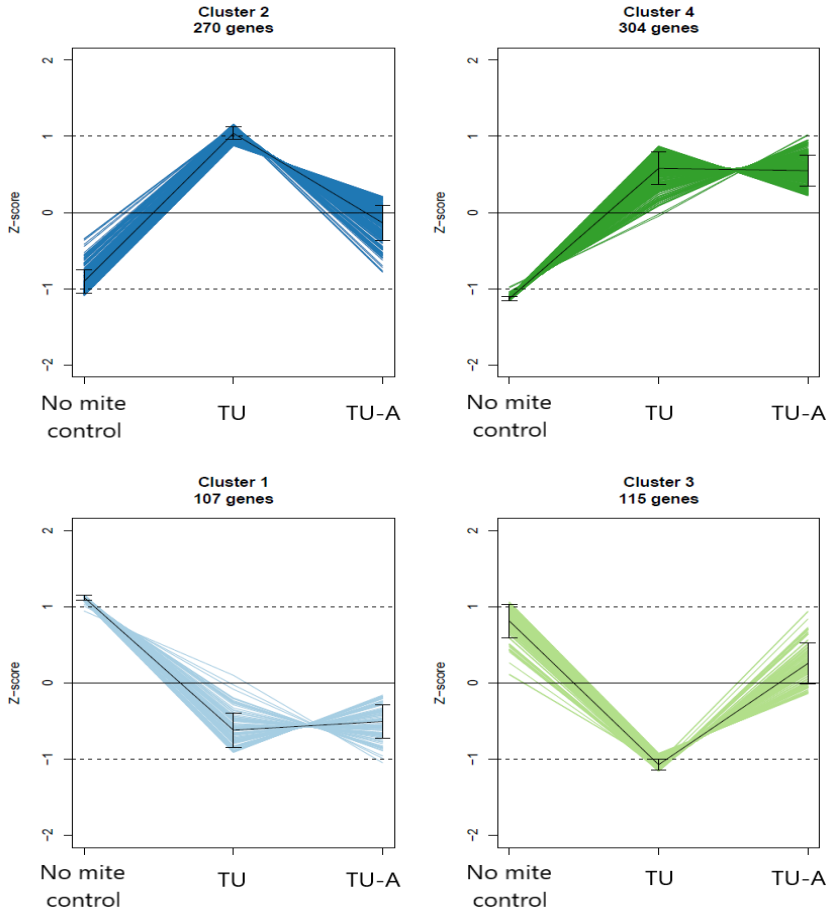

Cluster 2
270 genes
Cluster 4
304 genes
Cluster 1
107 genes
Cluster 3
115 genes
Z-score
No mite control
TU
TU-A

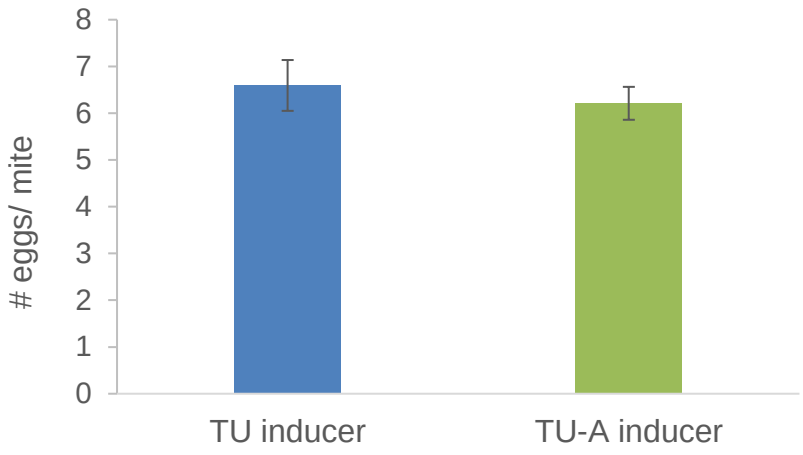
Figure 3.5 Analysis of tomato DEGs in response to mite feeding. A, heatmap illustrating DEGs following 24 hours of feeding by TU and TU-A mites filtered by DEG in response to TU mites. **B,** Expression plots of clusters identified in heatmap. Data represents an analysis of published microarray data (Wybouw et al., 2015).

3.5 Suppression assays

Results from the transcriptional analysis of tomato genes induced by TU and TU-A mites revealed an attenuation, but not complete suppression of tomato defences. To test the biological and functional relevance of this attenuation, a co-infestation experiment was performed. It was hypothezed that there would be an increase in the performance of TU mites when feeding on the same leaf (though different leaflets) as TU-A mites as they would benefit from any suppression of plant defences by TU-A (Glas et al., 2014). Specifically, the prediction was that TU mites would deposit more eggs during 4 days of feeding on a leaf co-infested with TU-A 'inducer' mites compared to the control TU 'inducer' mites.

Contrary to the hypothesis, there was no benefit observed in terms of fecundity of TU mites when co-infesting a leaf with TU-A mites (Figure 3.6), suggesting whatever benefit of transcriptional attenuation TU-A mites exerts is relevant locally, only at the feeding site. Unfortunately, due to the fact that there was no observable phenotypic difference between eggs laid by TU and TU-A mites, a co-infestation experiment where both mite strains feed on the same leaflet is not feasible.

A



B

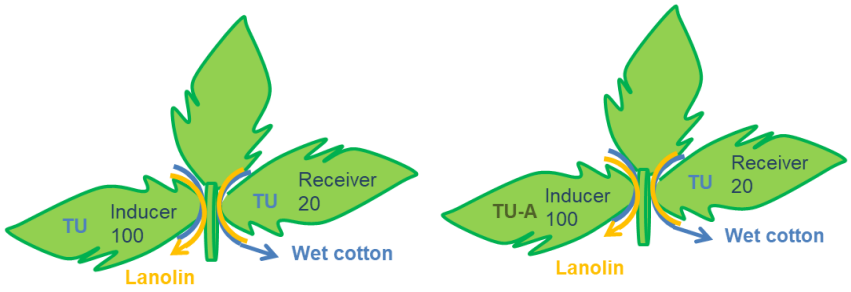


Figure 3.6 Suppression assay. A, Bar graph representing the mean ± SE number of eggs/mite 4 dpi of 'receiver' mites on Moneymaker leaflets. No difference in fecundity was observed between treatments of TU or TU-A 'inducer' mites (n = 20/mite strain). **B,** schematic of assay.

3.6 Quantitative analysis of phytohormones

To further characterize the observed transcriptional attenuation locally, I tested whether transcriptional changes translated into physiological states. Several genes involved in JA biosynthesis and response to JA were found to be attenuated in TU-A infested tomato leaves, so it was predicted that levels of JA and JA-Ile in these leaves would be intermediate between those of non-infested and TU-infested plants. As the only genes associated with SA were those involved in SA biosynthetic processes and the salicylic acid mediated signaling pathway, both represented in cluster 4, it was predicted that there would be similar levels of SA accumulated in response to both TU and TU-A mites, but there would be an increase relative to the non-infested control. As the only involvement of ABA detected in the analysis of DEGs in response to TU did not include ABA biosynthesis, it was hypothesized that not only would ABA levels be the same between mite strains, but there would also be no induction due to mite feeding.

To test these predictions, hormone levels were quantified in tomato leaflets infested with the different mite strains. Leaflets with no mites (but still isolated with lanolin) were taken as a control at the same time as samples collected after feeding by 100 mites for 24 hours. Whole leaflets were sampled for the analysis and a lanolin barrier was used to make sure TU mites remained on the infested leaflet. TU mites induced a dramatic (10x) increase in JA levels, indicating a strong response of tomato tissue to mite herbivory, whereas TU-A mites induced the accumulation of lower amounts of JA (Figure 3.7A). The same pattern, with less magnitude was seen in JA-Ile levels, where TU mites induced more than double the amount of constitutive levels and TU-A again induced intermediary levels (Figure 3.7B). Interestingly, SA and ABA levels did not change upon feeding by TU or TU-A mites (Figure 3.7C, D) with the possible exception of induced SA levels upon TU feeding that were not statistically significant. These results support the interpretation of the transcriptional response being attenuated in tomato fed on by TU-A mites, where the JA defence pathway was manipulated.

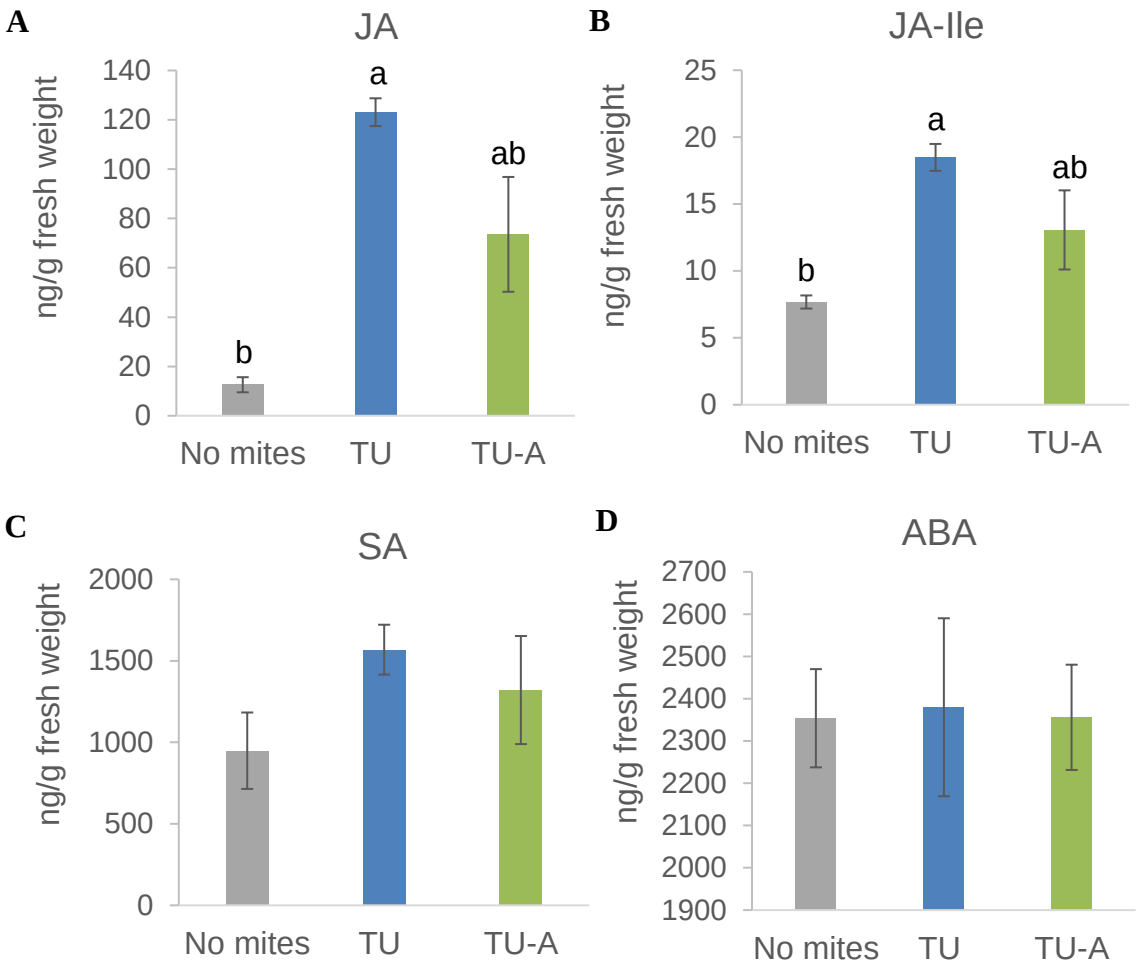


Figure 3.7 Quantitative analysis of Moneymaker phytohormones. Moneymaker leaflet samples were taken after feeding by TU and TA mites for 24 hours. Samples were analyzed using Ultra-performance liquid chromatography (UPLC)-electrospray ionization-tandem mass spectrometry. Bar graphs represent mean ± SE hormone level (ng/g). **A,** JA levels are shown. Different letters represent significant differences between means (two-way ANOVA, F = 7.28, $p = 0.01952$, $\eta^2p = 0.68$, followed by Tukey-Kramer post hoc test, $p < 0.05$, n = 4-6/cultivar). **B,** JA-Ile levels are shown. Different letters represent significant differences between means (two-way ANOVA, F = 5.46, $p = 0.03194$, $\eta^2p = 0.58$, followed by Tukey-Kramer post hoc test, $p < 0.05$, n = 4-6/cultivar). **C and D,** SA and ABA levels are shown, respectively. No significant differences between means were detected.

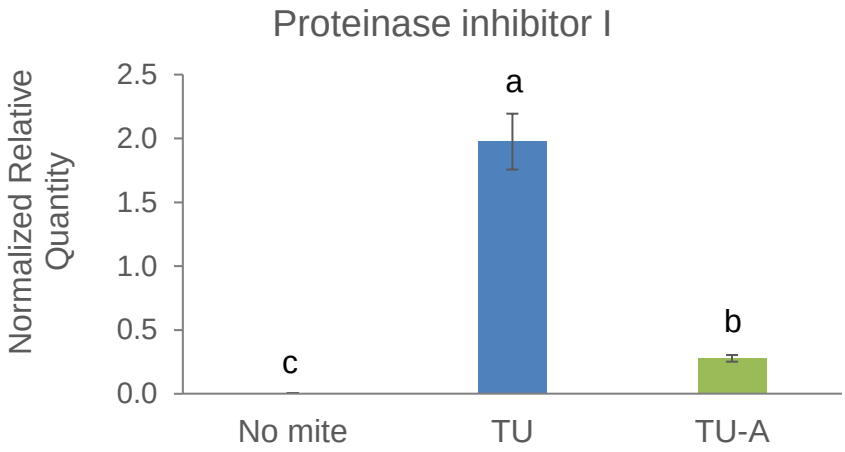
3.7 Protease inhibitor marker gene analysis

Protease inhibitors represent a major defence protein used by tomato against herbivores, and Figure 3.5B displays an attenuation of the expression of genes encoding protease inhibitors in cluster 2 when plants are fed on by TU-A mites. This attenuation of PIs may represent a functional benefit of plant response modulation by TU-A mites. Using an independent set of samples, validation of microarray data of PI induction was performed using RT-qPCR of the two chosen protease inhibitor transcripts identified in section 3.4. *Protease inhibitor I (Solyc09g084480)* and *Cysteine PI (Solyc00g071180)* were assessed for transcript abundance in samples fed on by TU, TU-A or no mites (control). It was predicted that *Protease inhibitor I (Solyc09g084480)* would be induced to similar levels in both TU and TU-A-treated samples and *Cysteine PI (Solyc00g071180)* would be attenuated in TU-A-treated samples.

TU mites induced high levels of expressions of both genes relative to the non-challenged tomato, and TU-A produced a much more attenuated, though still significant induction (Figure 3.8). This suggests that there is more suppression of cysteine proteases than is evident in the microarray data, or conversely, as this experiment was performed ~2 years after the microarray, the TU-A population may have increased their ability to suppress them since the time the microarray was performed.

These results suggest that the increased performance of TU-A mites could be in part due to the decreased amount of cysteine PIs encountered during mite feeding, allowing TU-A mites to metabolize plant contents without peptidases within their guts being inhibited.

A



B

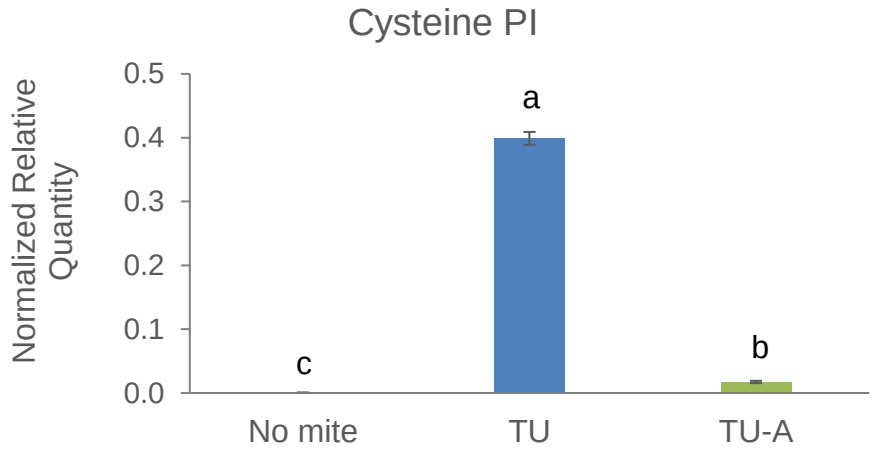


Figure 3.8 Protease inhibitor marker gene analysis. Normalized relative quantity of marker genes in Moneymaker determined by RT-qPCR. Shown are mean NRQs ± SE. *Protease inhibitor I (Solyc09g084480)* and *Cysteine PI (Solyc00g071180)* values were normalized to *Actin (Solyc03g078400)*. **A,** *Protease Inhibitor I* constitutive (No mite) and induced transcriptional activity following feeding by TU and TU-A mites. Different letters represent significant differences between means (one-way ANOVA, F = 175.48, p = 0.00078, η^2p = 0.99, followed by Tukey-Kramer post hoc test, $p < 0.05$, n = 2/treatment). **B,** *Cysteine PI* transcriptional levels. Different letters represent significant differences between means (one-way ANOVA, F = 2699.20, p = 1.309e-05, η^2p = 0.999, followed by Tukey-Kramer post hoc test, $p < 0.0001$, n = 2/treatment).

3.8 Protease % inhibition assay

Gene expression data suggest that TU-A mites transcriptionally suppress PI genes. This is expected to reduce PI activity in tomato and might be a method evolved by TU-A mites to overcome the negative effects PIs may have on their digestive physiology. To test if transcriptional attenuation leads to reduced activity of PIs in tomato plants upon infestation by TU-A mites, the activity of PIs was determined. Inhibitor activity of tomato PIs was determined *in vitro* using % inhibition assays. This assay characterizes the ability of PIs in protein extracts of tomato tissue to inhibit a commercial protease. These assays therefore serve as a proxy for determining the inhibitory affect of tomato PIs against proteases that would be found in the mite gut. Cathepsin L serves as a good representative of biologically important cysteine proteases presumed to act within the mite gut.

It was hypothesized that tomato samples fed on by TU-A mites would have decreased PI activity and thus reduced ability to inhibit the cathepsin L protease activity that was measured in the *in vitro* assay. Contrary to the prediction, Moneymaker samples collected after feeding by TU-A mites had increased PI activity seen as higher inhibitory activity (37.4%) than TU samples (24.9%; Figure 3.9A). Interestingly, TU mites failed to induce PIs, where no mite control samples displayed 19.4% inhibition, although there was a trend of increased inhibition in TU-treated samples. This was unexpected given that they have been shown to induce expression of PI associated genes (Figure 3.5B, and Figure 3.8).

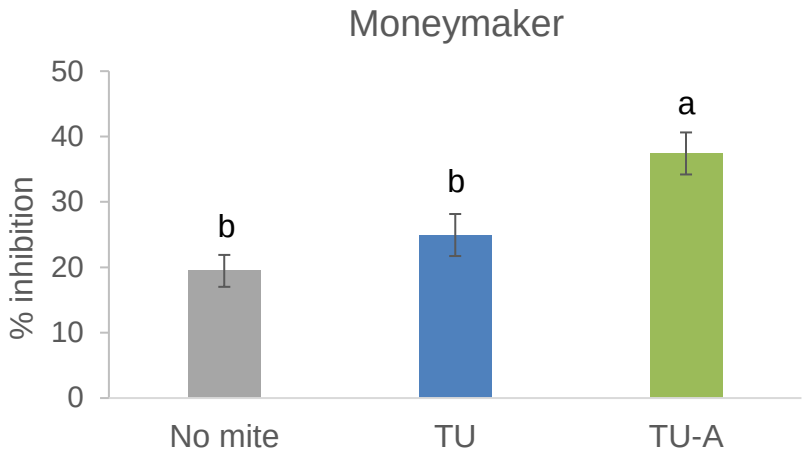
This result was so surprising that the experiment was repeated using a different tomato cultivar, Heinz (1706). The damage assay revealed that while TU-A mites produce far more damage on Heinz than TU mites, in accordance to their adaptation status, the amount of damage they produce was almost halved on Heinz compared to Moneymaker. Given that PIs are highly responsive to wounding, I hypothesized that infesting TU-A mites on Heinz, where it produced less damage, would provide an opportunity to test my original hypothesis again. This assay supported the prediction, where TU mites produced a significant increase of inhibitory activity in Heinz plants, whereas TU-A mites had an intermediate increase that was not statistically different than the control (Figure 3.9B). Additionally, RT-qPCR marker gene analysis of Heinz samples characterizing the

expression of the same PI genes assessed in Moneymaker showed that they were attenuated in Heinz in response to TU-A mites, as they were in Moneymaker (Figure 3.9C).

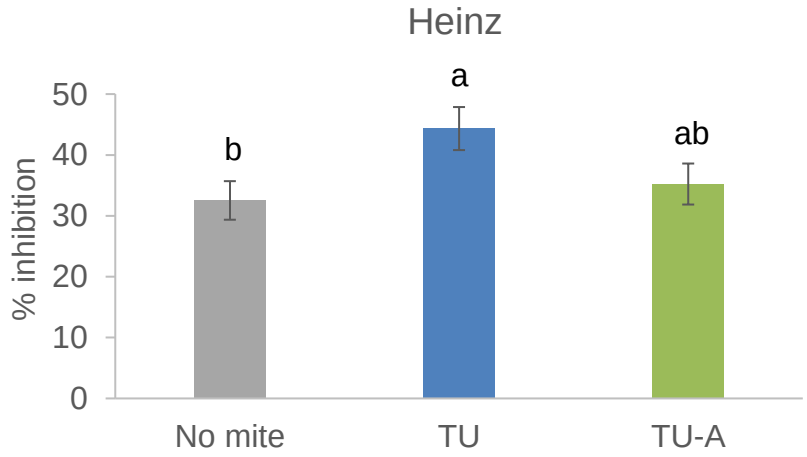
Thus, PI activity in the Moneymaker background did not follow transcriptional changes, indicating that, PI activity in this cultivar may be regulated at the post-transcriptional level or conceivably, there could be unannotated PIs that went undetected in the microarray. Conversely, on Heinz, TU-A mites did suppress tomato PI activity at both the transcriptional and physiological levels. The difference observed in response to TU and TU-A mites by these two tomato cultivars may be due to the genetic or physiological differences between them, or due to the difference in the amount of damage incurred during TU-A mite feeding, representing a confounding effect of wounding on tomato PI activity.

In summary, as TU-A did affect PI activity in Heinz, but not in Moneymaker, it has been demonstrated that attenuation of PI activity may still be one of the strategies used by mites to overcome this defence mechanism; however, the attenuation of their expression is not expected to contribute to their adaption status on Moneymaker.

A


Moneymaker
% inhibition
50
40
30
20
10
0
b
b
a
No mite
TU
TU-A

B


Heinz
% inhibition
50
40
30
20
10
0
b
a
ab
No mite
TU
TU-A

C

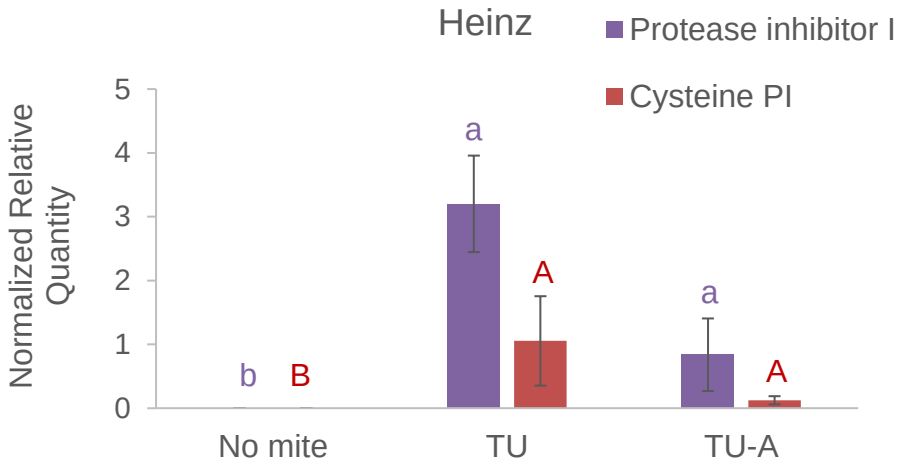

Heinz
Protease inhibitor I
Cysteine PI
Normalized Relative Quantity
5
4
3
2
1
0
b
B
a
A
a
A
No mite
TU
TU-A

Figure 3.9 Percent inhibition of commercial cathepsin L. The ability to inhibit cathepsin L activity in tomato protein extracts was tested using tomato samples that were fed on by no mites, or 100 mites of TU/TU-A strains. Data shown in **A and B** are mean ± SE % inhibition of samples relative to samples of cathepsin L activity that were not incubated with plant extracts (100% cathepsin L). **A,** % inhibition of Moneymaker tomato samples. Different letters represent significant differences between means (two-way ANOVA, $F = 22.00$, $p = 1.536e\text{-}06$, $\eta^2p = 0.60$, followed by Tukey-Kramer post hoc test, $p < 0.001$; n = 12/treatment). **B,** % inhibition of Heinz tomato samples. Different letters represent significant differences between means (two-way ANOVA, $F = 4.32$, $p = 0.025026$, $\eta^2p = 0.26$, followed by Tukey-Kramer post hoc test, $p < 0.001$; n = 12/treatment). **C,** normalized relative quantity of Protease inhibitor I (Solyc09g084480) and Cysteine PI (Solyc00g071180) normalized to Actin (Solyc03g078400). Shown are mean NRQs ± SE. Different letters represent significant differences between means within each gene (uppercase and lower case letters are used to distinguish between genes; one-way ANOVAs, $F_{PI1} = 46.88$, $p_{PI1} = 0.0002176$, $\eta^2p_{PI1} = 0.94$; $F_{CysPI} = 24.37$, $p_{CysPI} = 0.001317$, $\eta^2p_{CysPI} = 0.89$; followed by Tukey-Kramer post hoc test, $p < 0.05$; n = 2-3/treatment).

3.9 Mite protease inhibitor assay

Given the opposing results of PI activity in Moneymaker and Heinz tomato cultivars due to mite feeding, I investigated the importance of cysteine proteases on mite physiology when feeding on tomato. To address this, a fecundity assay was performed after treatment of mites with a synthetic PI, E-64. This pharmacological approach was used because it would remove the possibility of PI activity manipulation by mites. The use of a controlled concentration of E-64 allowed for direct determination of any inhibitory effect. Fecundity was used as the measure of mite performance on tomato with and without PI treatment. Fecundity was measured 2 and 4 dpi to make sure the treatment had enough time to take effect and to test for any possible feedback loop associated with inhibition of mite cysteine proteases, which would represent a limitation to this approach. It was predicted that E-64 treatment would lead to decreased fecundity due to inhibitory effects on cysteine proteases in the mite gut affecting digestion.

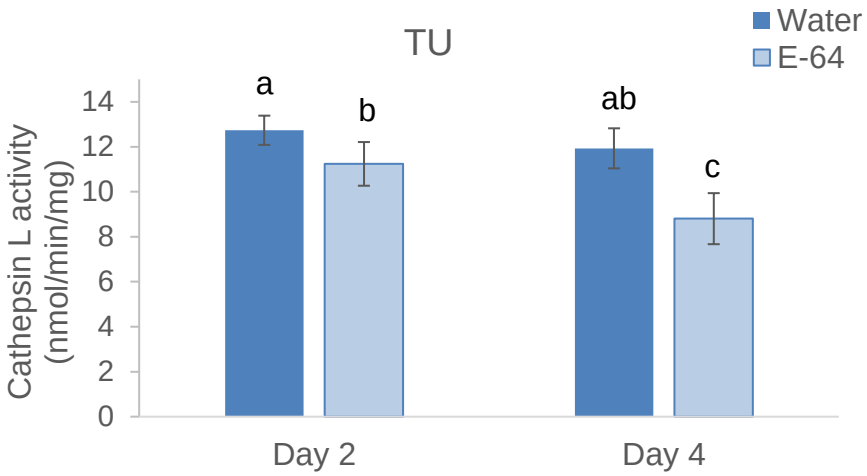
E-64 is an irreversible, potent, and highly selective cysteine protease (and trypsin) inhibitor. The trans-epoxysuccinyl group (active moiety) of E-64 irreversibly binds to an active thiol group in many cysteine proteases, such as papain, actinidase, and cathepsins B, H, and L to form a thioether linkage. E-64 is a very useful cysteine protease inhibitor for use in *in vivo* studies because it has a specific inhibition, is permeable in cells and tissues, and has low toxicity (Sigma-Aldrich, Cat # E3132, product information). When mite protein extracts were incubated with E-64, a greater than 80% reduction in cathepsin L-like activity was observed (Santamaría et al., 2015b). This suggests that E-64 is an appropriate inhibitor of the cathepsin L for use in this study. The leaf spraying application technique of E-64 was chosen because this more accurately reflects the natural interaction, where PIs would be ingested upon plant feeding and interact with digestive content.

Conversely to the % inhibition experiment that determined the effect of tomato PIs on a commercial protease (cathepsin L from human liver), this experiment tests the effect of a commercial PI against endogenous mite proteases. First, an *in vitro* assay to determine cathepsin L activity post treatment with 10 µM of E-64 was performed under the same conditions as the fecundity assay to verify decreased cysteine protease activity in mites due to E-64. As previously mentioned, cysteine proteases have been hypothesized to be

important in mite digestion, and cathepsin L is highly represented among mite cysteine proteases, so it serves as a good marker for this experiment. E-64 was capable of decreasing cathepsin L activity in TU mites both 2- and 4-days post feeding on treated tomato plants (Figure 3.10A). Activity in TU-A mites was decreased only on day 4 post feeding compared to the control, and to a substantial lesser extent than the decrease observed in TU mites (Figure 3.10B). There was a significant interaction between treatment with E-64 and dpi for TU mites (three-way ANOVA, $F_{\text{treatment:dpi interaction}} = 5.0427$, $p_{treatment:dpi} = 0.03134$, $\eta^2 p_{\text{treatment:dpi}} = 0.13$), but the decrease in cathepsin L activity was most pronounced at 4 dpi, suggesting that there was no feedback loop that would result in increased protease levels upon inhibition. Therefore, E-64 was able to inhibit Cathepsin L activity, more in TU than in TU-A mites.

The test for physiological effects of the reduced mite cathepsin L activity following E-64 treatment, was determined with a fecundity assay of TU and TU-A mites following treatment. Contradictory to the hypothesis, E-64 had no effect on TU or TU-A mite fecundity at 2 or 4 dpi on Moneymaker (Figure 3.11). The only difference detected was an increase in number of eggs/mite on day 4 relative to day 2 for TU-A samples (Figure 3.11B), which suggests that the mites were performing well and continuing to lay eggs. There was no increase in number of eggs/mite between days in the TU samples, which supports earlier results indicating tomato is not a favorable host that supports their fitness. Therefore, there was no physiological effect associated with reduced cathepsin L activity on TU and TU-A mites, at the level of decrease achieved by E-64 in this experimental set-up.

A


TU
Water
E-64
Cathepsin L activity (nmol/min/mg)
0
2
4
6
8
10
12
14
a
b
ab
c
Day 2
Day 4

B

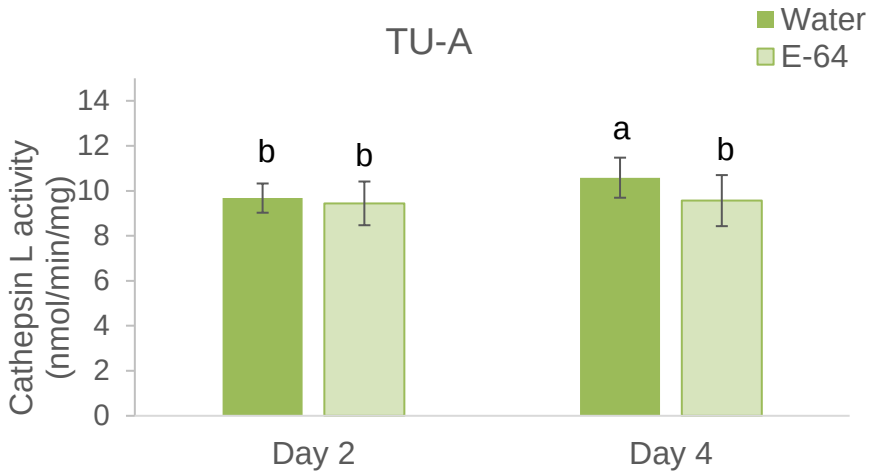

TU-A
Water
E-64
Cathepsin L activity (nmol/min/mg)
0
2
4
6
8
10
12
14
b
b
a
b
Day 2
Day 4

Figure 3.10 E-64 inhibition of cathepsin L activity. Cathepsin L activity in mite samples after feeding on water/E-64 treated Moneymaker leaves for 2 and 4 dpi. Bar graphs depict the mean ± SE cathepsin L activity (nmol/min/mg). **A,** TU mite samples. Different letters represent significant differences between means (three-way ANOVAs, $F_{dpi} = 21.10$, $p_{dpi} = 6.084e\text{-}05$, $\eta 2p_{dpi} = 0.39$; $F_{treatment} = 42.93$, $p_{treatment} = 1.918e\text{-}07$, $\eta 2p_{treatment} = 0.57$; followed by Tukey-Kramer post hoc test, $p < 0.05$; $n = 10$/treatment). **B,** TU-A mite samples. Different letters represent significant differences between means (three-way ANOVAs, $F_{dpi} = 6.39$, $p_{dpi} = 0.016412$, $\eta 2p_{dpi} = 0.16$; $F_{treatment} = 9.48$, $p_{treatment} = 0.004157$, $\eta 2p_{treatment} = 0.22$; followed by Tukey-Kramer post hoc test, $p < 0.05$; $n = 10$/treatment). An interaction was detected between treatment and trial ($F_{treatment:trial} = 11.46$, $p_{treatment:trial} = 0.001847$, $\eta 2p_{treatment:trial} = 0.26$). The effect size was 0.2-fold higher than that of the main effect of treatment and the interaction plot (supplementary material) shows the decrease in activity due to E-64 happened only in one of two trials. Therefore, the statistically significant difference displayed on the graph is very weak. Additionally, the biological relevance of such a small decrease should be considered.

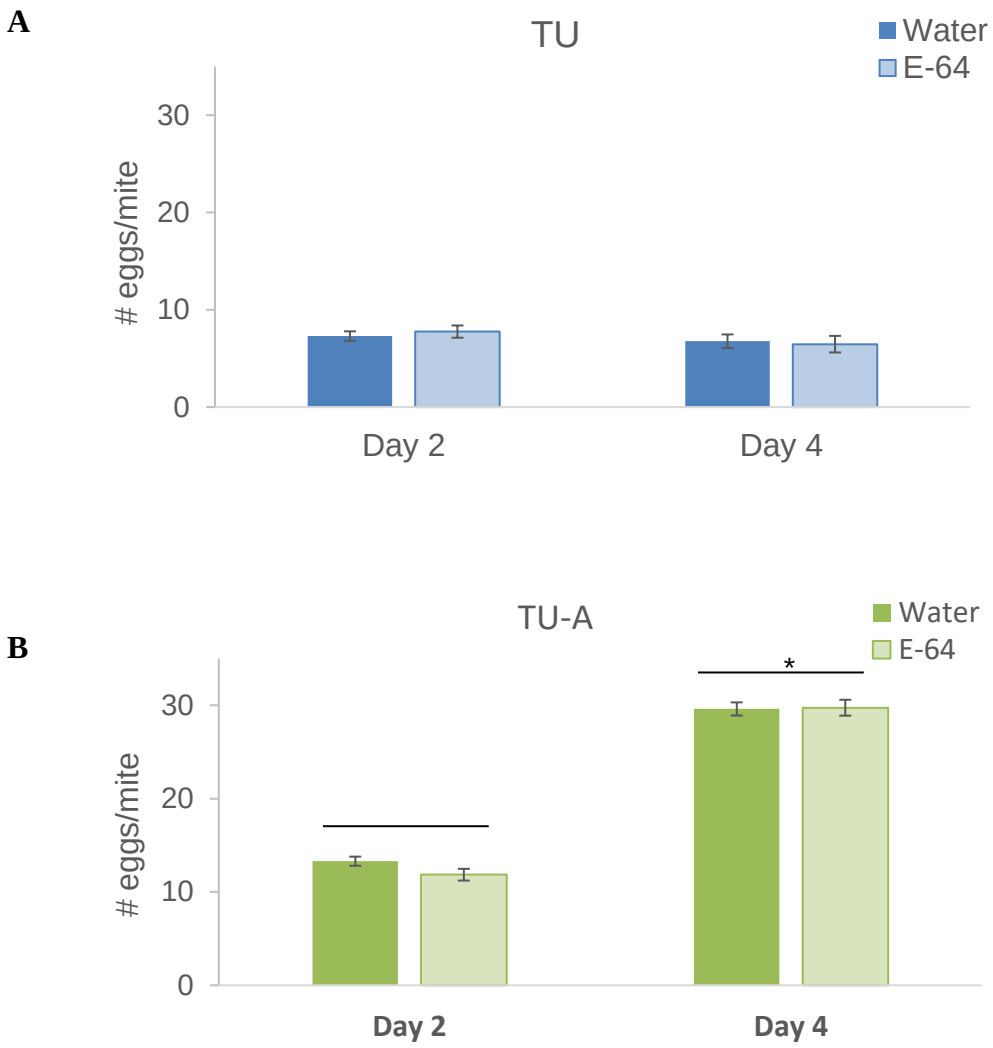


Figure 3.11 E-64 fecundity assay. Bar graphs showing mean ± SE number of eggs/mite mites 2 and 4 dpi following application of 20 female mites to treated leaves. **A,** TU fecundity. No differences between means observed. **B,** TU-A fecundity. Asterisk represents significant difference between days (three-way ANOVA, F = 716.60, $p < 2.2e\text{-}16$, $\eta^2p = 0.92$; n = 18). The effect of treatment was not found to be significant for TU or TU-A mite strains and there was no interaction between treatment and dpi (mites behaved the same to treatment both days). There was an interaction between dpi and trial ($F_{dpi:trial} = 7.69$, $p_{dpi:trial} = 0.001074$, $\eta^2p_{dpi:trial} = 0.21$); however, the effect size was more than 4-fold less than that of dpi and the interaction plot (supplementary material), shows the same general pattern in all three trials, with more eggs laid by day 4 than day 2.

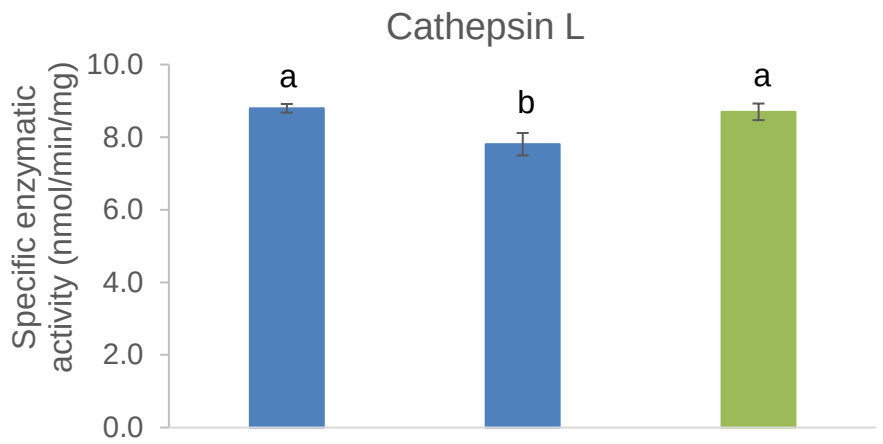
3.10 Mite cysteine protease activity on rearing and experimental hosts

Despite the transcriptional attenuation of some PIs revealed in the microarray analysis and the marker gene RT-qPCR, the % inhibition assay suggested that TU-A mites are indeed encountering PIs against cathepsin L when feeding on Moneymaker. Additionally, the E-64 assay determined that inhibition of cathepsins by E-64 was relatively small and had no effect on mite fecundity, suggesting that both non-adapted and adapted mites are insensitive to inhibition by E-64 to a biologically relevant level (with this method of delivery). The prediction then arises that on Moneymaker, while TU mites are probably not encountering a high level of PIs during ingestion (constitutive levels and not much induced), TU-A mites possess a level of cysteine protease activity high enough to outcompete the high levels of PIs ingested, and therefore serves as the mechanism of overcoming this specific induced tomato defence.

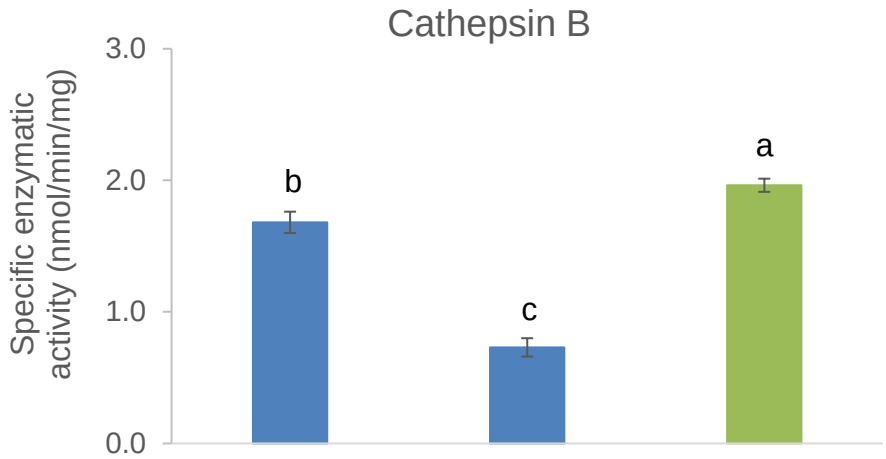
The cathepsin L activities of TU and TU-A mites were comparable when feeding on Moneymaker in the E-64 experiment; however, it is unclear if that level of activity was constitutively expressed in the ancestral non-adapted strain or induced only upon host-shift to tomato. Therefore, cysteine protease activity was measured in TU mites on bean (rearing host) and both TU and TU-A mites on Moneymaker tomato plants. Cysteine protease activity was measured by the activity of cathepsins L- and B-like as well as legumain-like.

Cathepsin L and B activity of TU mites on bean and TU-A mites on Moneymaker were the not statistically different (8.7-8.8 and 1.7-1.9 nmol/min/mg respectively; Figure 3.12A, B), suggesting there was no global change in the activity of these enzyme families during the adaptation process. Interestingly, TU mites on Moneymaker had decreased cathepsin L and B activity compared to their activity on bean (7.8 and 0.7 nmol/min/mg, respectively; Figure 3.12A, B). TU mites on Moneymaker had slightly higher Legumain activity (0.15 nmol/min/mg) than those of TU mite on bean and TU-A mite on Moneymaker, which had the same levels (0.12-0.13 nmol/min/mg; Figure 3.12C). However, the small scale on which the difference was detected, leaves the biological relevance of that statistical difference in question.

A


Cathepsin L
Specific enzymatic activity (nmol/min/mg)
10.0
8.0
6.0
4.0
2.0
0.0
a
b
a

B


Cathepsin B
Specific enzymatic activity (nmol/min/mg)
3.0
2.0
1.0
0.0
b
c
a

C

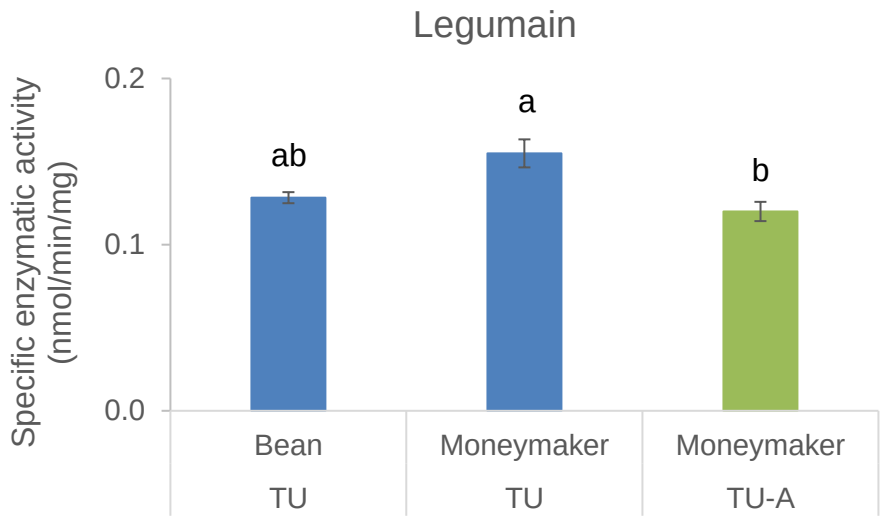

Legumain
Specific enzymatic activity (nmol/min/mg)
0.2
0.1
0.0
ab
a
b
Bean TU
Moneymaker TU
Moneymaker TU-A

Figure 3.12 Mite cysteine protease activity on rearing and experimental hosts. Bar graphs representing mean ± SE specific enzymatic activity (nmol/min/mg) of TU mites on bean and Moneymaker and TU-A on Moneymaker. **A,** cathepsin L activity. Different letters represent significant differences between means (two-way ANOVA, F = 9.012, p = 0.0008607, η^2p = 0.38; followed by Tukey-Kramer post hoc test, $p < 0.01$; n = 12). **B,** cathepsin B activity. Different letters represent significant differences between treatment means (two-way ANOVA, F = 76.91, p = 1.552e-12, η^2p = 0.84; followed by Tukey-Kramer post hoc test, $p < 0.05$; n = 12). **C,** legumain activity. Different letters represent significant differences between treatment means (two-way ANOVA, F = 23.85, p = 6.311e-07, η^2p = 0.61; followed by Tukey-Kramer post hoc test, $p < 0.001$; n = 12). There were significant interactions detected between treatment and trial terms in the cathepsin L and cathepsin B ANOVAs (F = 3.47, p = 0.0439514, η^2p = 0.188; and F = 16.64, p = 1.374e-05, η^2p = 0.53, respectively), however, their effect sizes of the interactions were 2- and 1.6-fold smaller than those of the treatments, respectively. The interaction plot for cathepsin L displayed somewhat different patterns in two trials due to high variability to TU-A on Moneymaker. The interaction plot for cathepsin B shows a similar pattern in both trials (supplementary material).

In summary, with regards to suppression as a mechanism of *T. urticae* adaptation to tomato, there is evidence that modulation occurs at the transcriptional and hormonal levels; however, the biological relevance of that suppression, whatever it may be, is occurring locally where the mite is feeding, and does not include the suppression of cysteine proteases (at least on Moneymaker). It would be surprising if the observed induced defence attenuation has no biological relevance (or benefit) to TU-A mites, as the programs with decreased induction (JA defense pathway related) and decreased down-regulation (growth and photosynthesis) are extremely indicative of a compromised defence response.

3.11 Detoxification enzyme inhibitor assays

Detoxification of toxic plant metabolites is another key adaptation strategy of overcoming plant defence (and xenobiotic compounds in general). This strategy has been implicated in *T. urticae* evolution of pesticide resistance (Alyokhin and Chen, 2017; (Dermauw et al., 2013; Yang et al., 2001) and mite adaptation to cyanogenic plants (Wybouw et al., 2014). In the studies noted above, the overexpression of genes encoding enzymes that metabolize xenobiotics were shown to be associated with the resistance and adaptation, respectively. Therefore, in addition to characterizing the ability of TU-A mites to suppress tomato defences, the ability of mites to detoxify phytochemicals of Moneymaker was also characterized. Specifically, I tested the requirement of global esterase, GST and CYP activity for *T. urticae* adaptation to tomato. Again, a pharmacological approach was used that has been well established in studies of mite resistance to pesticides (Khalighi et al., 2016).

Preliminary experiments determined appropriate concentration of inhibitors (Appendix, Figure 1), producing sublethal effects (Appendix, Figure 2). This was done to ensure there would be no confounding effect of inhibitor induced mortality independent of their effect on detoxification enzyme inhibition. Based on these data, the concentration chosen for use on the challenging host (tomato), did not affect mite performance under the same experimental conditions when feeding on a non-challenging (and ancestral) host (bean). Detoxification enzyme inhibitors were then used to test for their involvement in the high performance of TU-A mites. Mites were treated via ingestion through 3 days of feeding on a non-challenging host (bean) sprayed with an inhibitor. S,S,S tributyl-phosphorotrithioate

(DEF) was used to inhibit esterases, DEM was used to inhibit GSTs and PBO was used to inhibit CYPs. Following treatment, mite performance was measured via fecundity after 2 days of feeding on non-treated Moneymaker tomato plants in order to quantify any effect on mite performance. The prediction was that each inhibitor class is involved in toxic tomato metabolite metabolism, and therefore a decrease in TU-A fecundity would be observed upon their inhibition and subsequent encounter with tomato metabolites. As TU performance on tomato is already quite poor, it was hypothesized that little if any effect of inhibitors would be observed, as they are not using them to the extent that they are overcoming toxic metabolites.

Enzyme activity assays were also performed to test whether the knock-down of activity following treatment with the inhibitor could be verified *in vitro*, again something not done in other studies that use these inhibitors on mites. For these experiments, mites were collected after 24h of treatment (as opposed to the 3 days of treatment in the fecundity assay), because this was thought to be the window of time where a decrease would be most prominent (given hypothesized feedback loops). DEF decreased esterase activity to a large extent in both TU and TU-A mites (Figure 3.13A). No decrease in GST activity could be detected following DEM treatment (Figure 3.13B). PBO treatment had a significant, but minimal effect on mite CYP activity in TU and TU-A mites (Figure 3.13C).

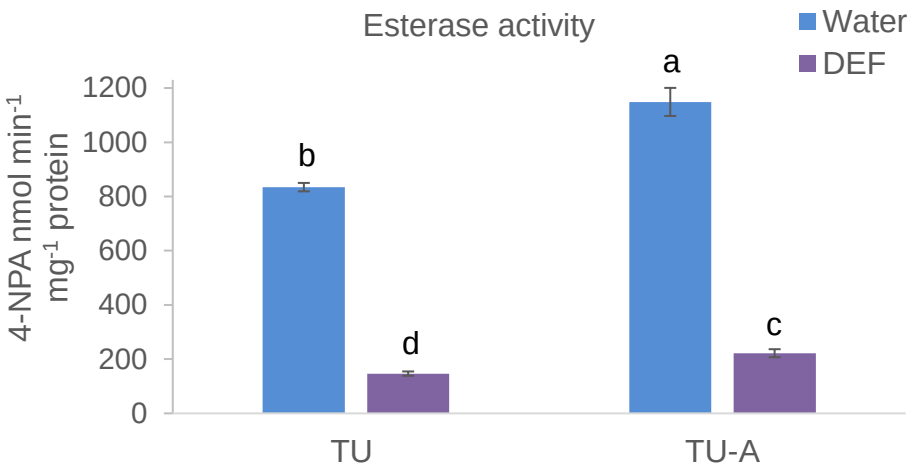
None of the inhibitor treatments affected TU performance on Moneymaker (Figure 3.14A). TU-A mites showed reduction in fecundity following treatment by all 3 inhibitors individually on Moneymaker (Figure 3.14B). As the effect on inhibition of TU-A esterase, GST and CYP enzymes was only observed on tomato and not the non-challenging host bean, it can be concluded that these classes of enzyme are contributing to TU-A performance on tomato by metabolizing otherwise toxic tomato compounds. As before, TU mites laid fewer eggs than TU-A mites on Moneymaker. Treatment with inhibitors did not decrease the fecundity of TU-A mites to the level of TU mites.

In addition to determining the importance of detoxification of tomato compounds derived from the tomato cultivar TU-A mites were reared on, TU-A mites were also presented with a different tomato cultivar defence profile with and without inhibitor treatment. The tomato

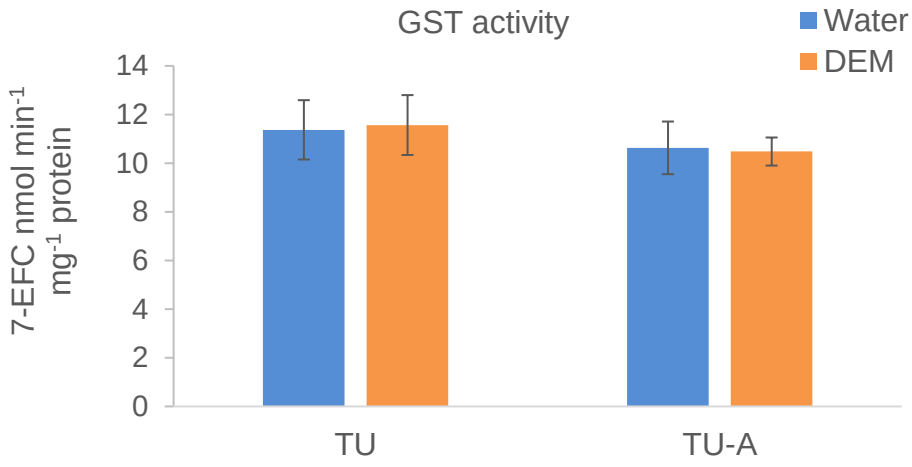
cultivar used to test this was Castlemart. One benefit of using Castlemart is the existence of a mutant in this genetic background, *defenseless-1* (*def-1*), that is devoid of JA accumulation and induced JA-regulated defences upon wounding, including the accumulation of wound-induced proteinase inhibitors (WIPI) transcripts and elevated proteinase inhibitors in response to herbivory (Li et al., 2002). Therefore, it was possible to characterize the involvement of the detoxification enzyme classes that were involved in detoxifying Moneymaker metabolites in the detoxification of both constitutive and induced tomato defences of a different tomato cultivar. Again, the prediction was that TU-A mites would be compromised in their fecundity following treatment with inhibitors of each class tested.

TU mites did not display a decrease in fecundity of inhibitor treatments compared to the control, with the exception of DEF treatment on the Castlemart genotype (Figure 3.15A). Also, the only inhibitor to have an effect on TU-A mites was DEF and only on *def-1*; however, there was a trend of decreased fecundity of DEM- and PBO-treated mites (Figure 3.15B). The fact that esterase inhibition only affected TU-A performance on *def-1* mutant suggests that they are susceptible to induced defences relative to the constitutive ones. Also, that DEM and PBO inhibitor treatment only affected mite performance on Moneymaker suggests that they are using esterases and CYPs to detoxify plant compounds that are potentially unique in quantity or quality compared to those of Castlemart.

A


Esterase activity
Water
DEF
4-NPA nmol min-1 mg-1 protein
1200
1000
800
600
400
200
0
b
d
a
c
TU
TU-A

B


GST activity
Water
DEM
7-EFC nmol min-1 mg-1 protein
14
12
10
8
6
4
2
0
TU
TU-A

C

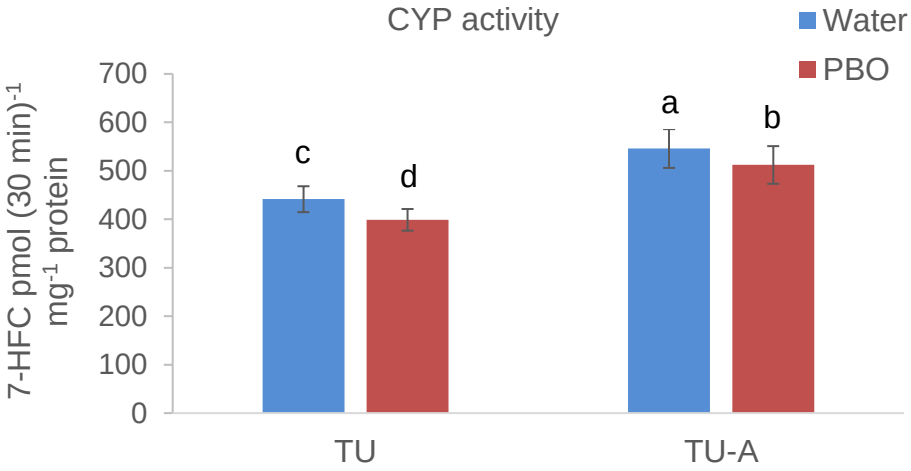

CYP activity
Water
PBO
7-HFC pmol (30 min)-1 mg-1 protein
700
600
500
400
300
200
100
0
c
d
a
b
TU
TU-A

Figure 3.13 Detoxification enzyme inhibitor activity following inhibition. Bar graphs showing the mean ± SE enzyme activity of TU and TU-A mites following treatment with water or enzyme inhibitor on Bean after 24h. **A,** effect of DEF (250 mg/L) on esterase activity of TU and TU-A mites. Difference between esterase activity detected between mite strains (two-way ANOVA, $F_{mite.strain} = 112.16$, $p_{mite.strain} = 2.336e\text{-}08$, $\eta^2p_{mite.strain} = 0.80$). Different letters represent significant differences between means (two-way ANOVA, $F_{treatment} = 2488.71$, $p_{treatment} < 2.2e\text{-}16$, $\eta^2p_{treatment} = 0.99$). Different letters represent significant differences between means as determined by Tukey-Kramer post hoc test, $p < 0.05$; n = 5/mite strain. **B,** no effect of DEM (4000 mg/L) was observed on GST activity in TU or TU-A mites. **C,** effect of PBO (2000 mg/L) activity on CYP activity. Difference between CYP activity detected between mite strains (three-way ANOVA, $F_{mite.strain} = 166.61$, $p_{mite.strain} < 2.2e\text{-}16$, $\eta^2p_{mite.strain} = 0.69$). CYP activity was reduced by PBO in TU and TU-A mites (three-way ANOVA, $F_{treatment} = 27.92$, $p_{treatment} = 1.226e\text{-}06$, $\eta^2p_{treatment} = 0.27$). Different letters represent significant differences between means as determined by Tukey-Kramer post hoc test, $p < 0.05$; n = 19-24/mite strain). An interaction between mite strain and trial was detected (three-way ANOVA, $F_{mite.strain:trial} = 17.19$, $p_{mite.strain:trial} = 7.392e\text{-}07$, $\eta^2p_{mite.strain:trial} = 0.32$; however, the effect size was 2-fold smaller than those of the corresponding main effects and the interaction plot showed no difference in pattern between trials.

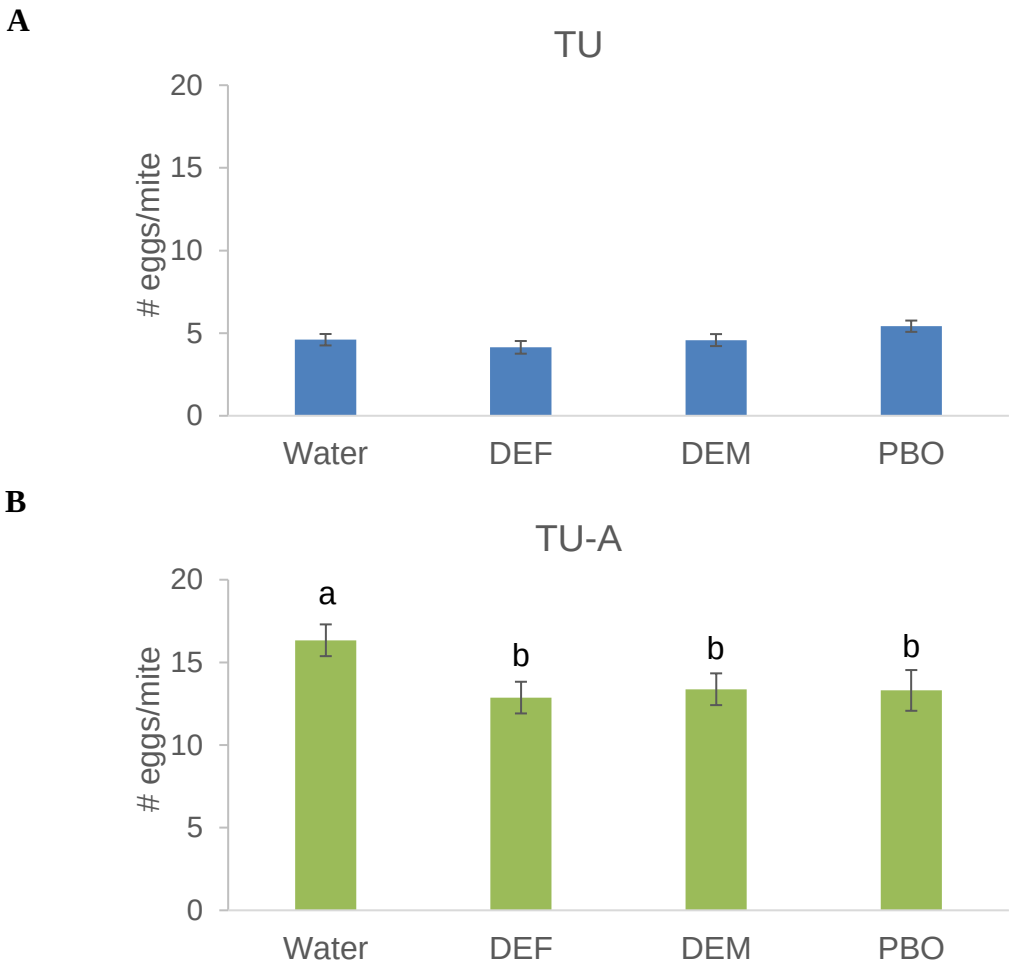
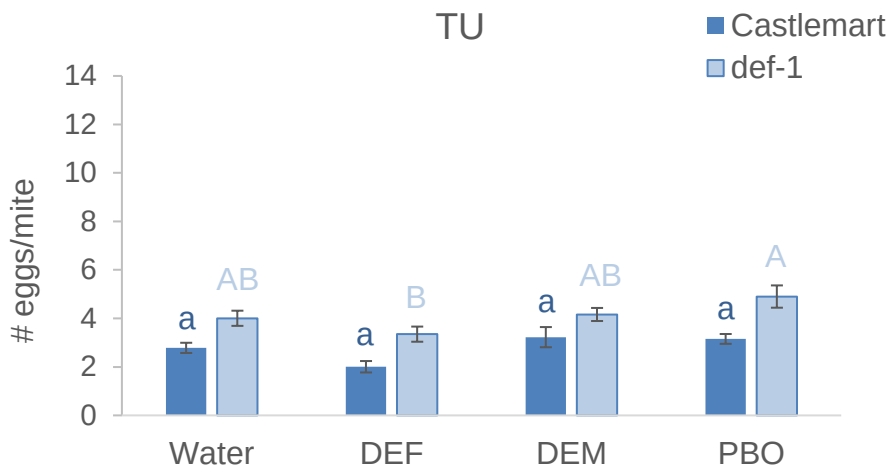


Figure 3.14 Detoxification inhibitor assay: Moneymaker. Bar graphs showing the mean ± SE number of eggs/mite of TU and TU-A following treatment with water or enzyme inhibitor and feeding on Moneymaker. Concentrations of DEF, DEM and PBO were 250 mg/L, 4000 mg/L and 2000 mg/L PBO respectively. **A,** no effect of inhibitors was observed on fecundity of TU mites. **B,** effect of inhibitors on TU-A fecundity. Different letters represent significant differences between means (ANOVA, F = 4.83, p = 0.004445, η^2p = 0.19; followed by Tukey-Kramer post hoc test, $p < 0.05$; n = 18/mite strain). TU-A mites laid more eggs than TU mites (three-way ANOVA, F= 400.90, p < 2.2e-16, η^2p = 0.76). TU-A mites laid more eggs than TU mites (three-way ANOVA, $F_{mite.strain}$ = 546.48, $p_{mite.strain}$ < 2.2e-16, $\eta^2p_{mite.strain}$ = 0.82).

A


TU
Castlemart
def-1
eggs/mite
14
12
10
8
6
4
2
0
a
AB
a
B
a
AB
a
A
Water
DEF
DEM
PBO

B

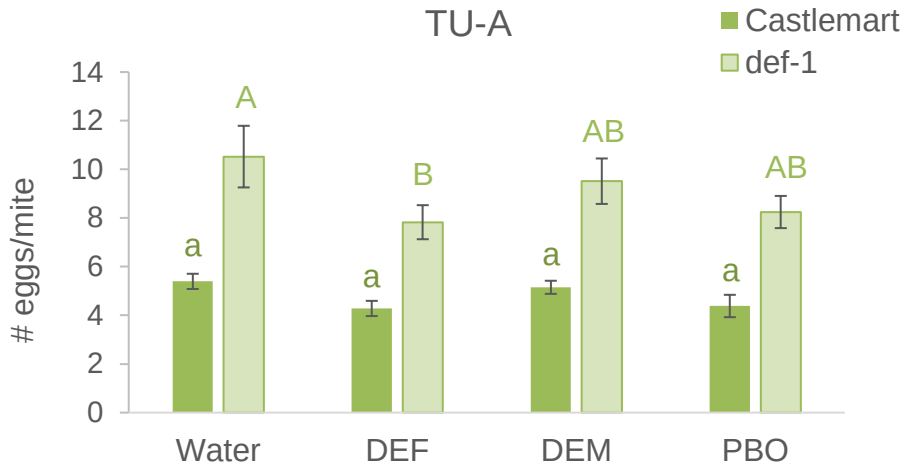

TU-A
Castlemart
def-1
eggs/mite
14
12
10
8
6
4
2
0
a
A
a
B
a
AB
a
AB
Water
DEF
DEM
PBO

Figure 3.15 Detoxification inhibitor assay: Castlemart and *def-1*. Bar graphs showing the mean ± SE number of eggs/mite of TU and TU-A mites following treatment with water or enzyme inhibitor. **A,** effect of inhibitors on fecundity of TU mites on Castlemart and def-1. There was a difference in number of eggs/mite laid by TU mites between plant genotypes (three-way ANOVA, $F_{plant.Genotype} = 48.65$, $p_{pant.Genotype} = 6.523e\text{-}10$, $\eta^2p_{plant.Genotype} = 0.37$). Different letters represent significant differences between means within each genotype (uppercase and lowercase letters used to distinguish between plant genotypes; three-way ANOVA, $F_{treatment} = 8.42$, $p_{treatment} = 5.869e\text{-}05$, $\eta^2p_{treatment} = 0.23$, followed by Tukey-Kramer post hoc test, $p < 0.05$, n = 12-13/mite strain). An interaction between treatment and trial was detected ($F_{treatment:trial} = 2.37$, $p_{treatment:trial} = 0.03651$, $\eta^2p_{treatment:trial} = 0.14$); however, the effect size was 1.6-fold smaller than those of the corresponding main effects and the interaction plot showed little difference in pattern between trials. **B,** effect of inhibitors on TU-A fecundity on Castlemart and def-1. There was a difference in number of eggs/mite laid by TU-A mites between plant genotypes (three-way ANOVA, $F_{plant.genotype} = 78.06$, $p_{plant.genotype} = 3.409e\text{-}13$, $\eta^2p_{plant.genotype} = 0.51$). Different letters represent significant differences between means within each genotype (uppercase and lowercase letters used to distinguish between plant genotypes; three-way ANOVA, F treatment = 3.64, $p_{treatment} = 0.0165769$, $\eta^2p_{treatment} = 0.13$, followed by Tukey-Kramer post hoc test, $p < 0.05$, n = 12-13/mite strain). An interaction between plant genotype and trial was detected ($F_{plant.genotype:trial} = 4.45$, $p_{plant.genotype:trial} = 0.0150106$, $\eta^2p_{plant.genotype:trial} = 0.11$); however, the effect size was 4.6-fold smaller than those of the corresponding main effects and the interaction plot showed little difference in pattern between trials.

4 Discussion

Thus far, avenues of research have focused either exclusively on detoxification as a mechanism of overcoming toxic plant compounds or on suppression of plant defence pathways as a means to adaptation. This study characterized both mechanisms in parallel within one species of generalist herbivore during one adaptation event.

4.1 Tomato damage positively correlated with mite adaptation status

It is of no surprise that tomato leaf tissue experiences more damage from mites that are adapted versus mites that are not (Figure 3.1A). The difference in damage is visually striking (Figure 3.1B) and exemplifies why pest adaptation is such a concern for producers in agriculture. A small population of non-adapted mites will not produce enough damage to be of consequence economically, and thus represents a threshold of pest status that is acceptable.

When TU-A mites were used to infest a selection of tomato cultivars, it was demonstrated that they outperform TU mites every time (Figure 3.2). This supports previous findings in that adaptation to one host will provide protection against those defences that are shared by other hosts (Fellous et al., 2014), in this case, different tomato cultivars. Additionally, though TU-A mites produced a lot of damage on all cultivars tested relative to TU mites, they did cause the most damage on Moneymaker plants (Figure 3.2), suggesting that their adaptation, while not cultivar specific per se, is to some extent shaped by the cultivar-specific secondary compounds they were exposed to during adaptation. This suggests that the ability of TU-A mites to overcome Moneymaker defences required it to adapt to different components of the defence program, some of which may be common to all tomato cultivars, but present in different quantities.

4.2 Mite performance improves dramatically through adaptation

Both fecundity and retention assays support the adaptation status of TU-A mites (Figure 3.3 and 3.4). Adaptation therefore leads to both behavioural and physiological changes to

mite populations. Both their tendency to stay on a tomato leaf and feed, and deposition of large quantities of eggs on the leaf are advantages of adaptation. TU-A mites appear to have gained the ability to utilize tomato tissue in a way that bypasses the defences such that they are no longer distasteful to them (which would lead to a feeding deterrent effect) and they are no longer effective against (which would lead to the low fecundity shown by non-adapted mites).

4.3 Tomato transcriptional response and suppression assay point to a localised attenuation of tomato defences

Blaazer et al. (2018) suggest the ability to suppress host plant defenses may be a mechanism that mites use as generalists, providing them the ability to readily utilize a novel host plant. They argue that detoxification may only be effective on a narrow range of similar hosts, while suppression may allow for utilization of a wider range of hosts, as it would operate by targeting conserved components in the plant defence pathway. The data presented here suggest that the ability to modulate a plants hosts' defence response (at the transcriptional level), occurs over time and generations, where any suppressive ability of TU was not detectable at the transcriptional level. Although there was no other tomato transcriptional profile in response to other non-adapted arthropods to compare the TU response with (to compare changes in the magnitude and composition of response), the fact that tomato was such an unfavorable host to them suggests that the host response to TU mite feeding was not compromised in any significant way.

The difference between the TU-A, induced vs TU, induced tomato responses was one that can best be described as attenuated. The heatmap and cluster analyses highlight DEGs that were differentially expressed upon feeding by TU and TU-A mites (filtered by response to TU). The analysis of genes in clusters 2 and 4 of the heatmap (Figure 3.5) illustrate that despite some aspects of early responses (response to JA and wounding) being suppressed by TU-A mites, much of the early responses of the JA/SA signalling pathways remain upregulated in response to both TU and TU-A mites. Similarly, analysis of clusters 1 and 3 illustrate that while many of the genes involved in growth and photosynthesis that are down-regulated upon TU feeding are still downregulated in response to TU-A, though there are other genes that are significantly less downregulated in response to TU-A involved in

these biological processes. This may mean the plant was compromised in its resource allocation response to attack, where the plant has not sufficiently switched from a growth mode to a defence mode (Clark and Harvell, 1992; Zhou et al., 2015). Overall, this suggests that while the plant is still recognizing TU-A mites as they feed and do attempt to switch from growth/development to defence, aspects of that defence response are compromised by TU-A mites.

When JA and JA-Ile levels in TU-A infested tomatoes were measured, they were intermediate between those of control and TU-treated plants, which verified microarray data (Figure 3.7). At the same time, SA levels did not increase significantly after mite attack, contrary to previous findings (Alba et al., 2014; Ament et al., 2004; Kant et al., 2004, 2008, Martel et al., 2015). Marker gene analysis of selected PI genes induced greatly upon TU feeding also verify microarray data, where expression in TU-treated samples were induced more than two-fold compared to induction by TU-A (Figures 3.8 and 3.9). Additionally, many genes associated with secondary metabolism synthesis were also induced to intermediary levels (Figure 3.5, cluster 2), though the differential down-regulation of these genes were not verified by RT-qPCR.

The biological relevance of the observed suppression of tomato defences by TU-A feeding at the transcriptional level was assessed using a co-infestation experiment where the suppressive capabilities of TU-A was challenged across leaflets. There was no observed suppression of plant defences in a systemic fashion (Figure 3.6). This indicates that the biological effect of TU-A mite attenuated responses was happening locally at the feeding site and not translated systemically, suggesting systemic responses have not been compromised.

It has been demonstrated that *T. urticae* is able to synthesize secreted proteins in their secretory glands and presumably secrete them into plant tissue via the stylet, affecting their interaction with their host plant (Jonckheere et al., 2016; Villarroel el al., 2016). Ectopic expression of prime candidates from an *in silico* prediction of *T. urticae* (and *T. evansi*, a specialist mite species feeding on Solanaceous plants) secretomes resulted in suppression of defences downstream of SA signalling in *Nicotiana benthamiana* (close relative of

tobacco) and improved mite performance (Villarroel et al., 2016). Results from that study demonstrated that both *T. urticae* and *T. evansi* are sensitive to SA facilitated defences, but secreted proteins (via saliva) reduced their negative effects. In my study, sensitivity to SA defences was not assessed; however, if there was a biological relevance to SA pathway attenuation it did not occur downstream of SA signalling, but potentially at its synthesis (Figure 3.7).

In support of localized suppression of tomato defences, Schimmel et al. (2017) observed the phenomenon of hyper-suppression of plant defences by *T. evansi* locally upon the addition of *T. urticae* competitors to the same leaflet. Specifically, hyper-suppression was observed when *T. evansi* mites were allowed to establish on a tomato plant for a few days before the addition of *T. urticae* mites on another isolated section of the leaflet. Both JA and SA defences were shown to be suppressed more strongly than the base level of suppression deployed by *T. evansi* at the local feeding site, resulting in increased reproductive output. This local hyper-suppression of defences coincided with increased expression of candidate *T. evansi* salivary defence-suppressing effector proteins. It was theorised that the competitor-induced overcompensation promoted competitive population growth of *T. evansi* on tomato (Schimmel et al., 2017).

4.4 Plant and commercial cysteine protease inhibitors have little effect on TU-A mites

The analysis of *T. urticae* bodies and faecal extracts by Santamaria et al. (2015b) demonstrated that aspartyl, cathepsin B- and L-like and legumain proteases were prominently represented as digestive proteases in spider mites, with aspartyl and cathepsin L-like proteases being especially active. When taken together with gene expression data, the hypothesis emerged that there was a digestive role for cysteine and aspartyl proteases in *T. urticae* nutritional uptake, making them prime targets for tomato anti-digestive proteins. Cathepsin L was used in this study as a marker for cysteine proteases (cathepsin B-, cathepsin L- and legumain-like).

In this study, microarray and RT-qPCR data indicate an attenuation of tomato PI biosynthesis at the level of gene expression in response to TU-A mites relative to TU

(Figure 3.5 and 3.8, respectively). However, when PI activity was assessed in Moneymaker and Heinz leaf tissue fed on by TU and TU-A mites, compared to a no mite control, no such attenuation was observed in Moneymaker, while it was observed in Heinz (Figure 3.9). TU mites failed to induce cysteine PI activity in Moneymaker after 24h of feeding, as assessed through the % inhibition of cathepsin-L activity, despite high induction of PI genes. These results suggest that post translational regulation of PIs is occurring in Moneymaker such that a small induction of gene expression results in high levels of activity. The post translational modification may be accomplished either by the plant itself or mite effectors present in the ancestral non-adapted strain and either lost or ineffectual in TU-A mites. As supported by the % inhibition/activity of PIs in the Heinz cultivar, it could be that the level of physical wounding to the plant by TU-A mites is enough to overcome whatever suppressive abilities the effectors within the ancestral strain had in the TU-A-treated Moneymaker samples. This supports the hypothesis that mites use suppression of defences as a mechanism of overcoming host-shift, that may not be relied upon to the same extent further down the adaptation timeline, when population numbers are high and damage is extensive.

To further interrogate the role of cysteine protease inhibitors against non-adapted and adapted mites, a commercial inhibitor (E-64) was used to treat mites prior to assessing their fecundity on Moneymaker. In this way, any suppression of PIs by mites was bypassed, and the direct effect of inhibition of cysteine proteases in general, in addition to tryspsin, was characterized. Despite a decrease in cathepsin L-like activity in TU mite samples treated with E-64 (Figure 3.10A), no decrease in mite performance was observed in reproductive output (Figure 3.11A). With respect to TU-A mites, a decrease in cathepsin-L activity was only observed on day 4 post spraying, and its biological relevance was questionable (Figure 3.10B). No change in fecundity was observed in TU-A mites when treated with E-64 (Figure 3.11B). When the concentration of E-64 was increased 5-fold more than the recommended concentration by the supplier, still no effect on TU-A mites was observed (data not shown). The very slight decrease in cathepsin L-like activity observed in TU and TU-A mites upon E-64 treatment could indicate that the levels within mites, in both the ancestral and adapted populations, were up-regulated to such an extent that they were not affected by protease inhibition at the level Moneymaker PIs and the commercial PI E-64

can impart. Protease activities of TU mites on bean and Moneymaker, and TU-A mites on Moneymaker suggest that these high levels of cathepsin L activity are constitutive, and were not induced upon host shift to tomato (Figure 3.12). This is consistent with the work of Santamaria et al., (2015b), where they found no change in cathepsin L, B or legumain activity when mites were reared on bean or tomato. However, there is evidence in *T. urticae* of unspecific increases in cathepsin B and L, legumain and aspartyl protease genes in response to inhibition by the barley PI HvCPI-6, which targets cysteine proteases (Santamaría et al., 2015b). Also, activities of mite proteases change when mites feed on transgenic Arabidopsis plants that over-expressed different types of protease inhibitors (Santamaria et al., 2012). This suggests the increase in proteases, whether constitutive or in response to PIs ingested, is a mechanism of overcoming that particular class of tomato defence protein. However, it has also been demonstrated that proteases can gain mutations that then make them insensitive to inhibitors that once targeted them (Volpicella et al., 2003).

It is unknown if the tomato adaptation of *T. urticae* in this study was at all associated with *de novo* mutation in proteases expressed originally in the mites feeding on Moneymaker, or over-expression of a different repertoire of proteases, representing a more effective set. The study by Wybouw et al. (2015) was performed to determine the reciprocal transcriptional responses of tomato and mites due to mite adaptation. They determined that about ~45% (444 of 994) of the differentially expressed genes between TU and TU-A strains could be attributed to genetic adaptation. They found that TU-A mites both changed their constitutive transcript levels in addition to altering their transcriptional plasticity. Specifically, TU-A mites changed the expression of 16 proteases (GO:0006508 proteolysis) through genetic adaptation and this could have resulted in them being less affected by tomato PIs (Wybouw et al., 2015). Alternatively, it could be that the pH of the mite digestive compartments (3.5–5.5) is not within the optimal or functional range of tomato PIs (Bensoussan et al., 2018; Santamaría et al., 2015b), and therefore they do not have the activity required to produce a negative effect on mites.

4.5 Detoxification by P450s, esterases and GSTs involved in tomato adaptation

A theory postulated by Krieger et al. in 1971, and supported in the literature (Heidel-Fischer and Vogel, 2015), stated that the ability of generalist herbivores to colonize new host species is facilitated by their ability to detoxify a wide range of toxins that different plant families are likely to produce. Following this, the ability of *T. urticae* to feed on such a vast number of different plant species from phylogenetically distant families has also been hypothesized to be facilitated by extensive rearrangement of their xenobiotic metabolism by differential expression and synthesis of enzymes belonging to the three phases of detoxification (Dermauw et al., 2013; Grbić et al., 2011; Wybouw et al., 2014). However, this hypothesis is somewhat vague with regard to the timeframe for xenobiotic metabolism rearrangement and has not been elaborated upon. For example, does this hypothesis apply to the initial host transfer of mites, where they use detoxification to survive long enough to adapt and colonize the plant. Or does it apply to the adaptation process itself, where rearrangement represents an outcome of adaptation, allowing for colonization. The results of this study provide support for the latter. It was observed that the tomato adapted mites used all three classes of detoxification enzymes tested, esterases, GSTs, and CYP in their utilization of Moneymaker as a host (Figure 3.14). Global levels of esterases and CYPs were only slightly higher in TU-A mites compared to TU mites, while global levels of GST activity were the same between TU and TU-A mites (Figure 3.13). The similar global activity of all these enzymes coupled with the fact that inhibition of them affected TU-A mites suggests that the enzymes represented in that global analysis of activity are better tailored to metabolizing tomato defences, as opposed to a general increase in activity of those enzymes to cope with toxic tomato metabolites. Therefore, detoxification does not appear to be employed successfully during the initial host-shift, but rather selection occurs during the adaptation process for detoxification enzymes deployed by the mite tailored to the defences encountered when on tomato. There are reports of allelic variation, gene duplication, overexpression, and sub-functionalization of CYPs as examples of how this modulation can occur (Mao et al., 2006 & 2007; Wen et al., 2006; Bass et al., 2013). For example, Mao et al. identified a CYP gene (*CYP6AE14*) from cotton bollworm (*Helicoverpa armigera*) that allowed it to tolerate gossypol, a cotton metabolite

which is otherwise inhibitory to herbivore growth and development. CYP6AE14 was found to be highly expressed in the bollworm midgut and its expression correlated with larval growth when gossypol is included in the diet.

The decreased performance of TU and TU-A mites (eggs/mite) on Castlemart relative to Moneymaker suggests that Castlemart represents a more challenging host (Figure 3.14 and 3.15), further supported by the results of the damage assay (Figure 3.2). The fact that the decreased performance of TU-A mites in response to inhibition (of esterases) is only observed in the *def-1* mutant (Figure 3.15B) indicates that they are relying on detoxification for defences that are constitutively expressed in tomato (and may be shared with the Moneymaker cultivar). This is consistent with the suppression hypothesis, where it is the induced defences that are inhibited upon attack via effectors. It is reasonable to then hypothesize that mites may make use of an alternative strategy against constitutive defences.

Detoxification enzyme assays revealed that enzyme activity was only severely reduced in DEF treated mites, slightly reduced in PBO treated mites and unaffected in DEM treated mites (Figure 3.13). Therefore, conclusive and very probable biologically relevant decreases in enzyme activity were only verified for DEF treated mites. As an effect on fecundity was observed under each inhibitor condition in TU-A mites on Moneymaker, it is likely that the decrease in activity was biologically relevant for all enzyme inhibitors, but the window to observe that decrease in enzymatic activity may be small due to possible feedback loops associated with their inhibition. The lack of significant effect on TU-A fecundity when mites were treated with any inhibitor on Castlemart and DEM or PBO on *def-1* plants may be due to a lack of sufficient decrease in activity of those enzymes to see an effect. This also may speak to the degree to which mites can tailor their adaptive response to defences of specific plant cultivars. There is much literature on the difference in defensive strategies and traits between plant species (Johnson, 2011), but there is also reason to study the difference between cultivars of the same species, as their metabolite profile will also differentially affect herbivores.

In general, it remains unclear what specific mechanism mites are using in their detoxification of plant toxic compounds. One possibility is that TU-A mites overexpress many genes encoding enzymes that can metabolize (modify, degrade, or detoxify) tomato defence compounds. Another possibility is allelic variation of gene-coding regions of detoxification enzymes allowing for the selection of allozymes with increased metabolic efficiency (Rioja et al., 2017). These are not theoretically mutually exclusive mechanisms and could be happening simultaneously.

This study of *T. urticae* clearly demonstrates the continuum that exists between generalists and specialist herbivores. While the TU-A population is still a generalist in that it could be placed on other hosts and adapt to them, they employ mechanisms used by specialists to adapt to certain host plants when they are selected to do so. It should be mentioned that the ability to utilize different host plant species in the future will most likely be impacted (positively or negatively) by the adaptation to tomato, and the genetic/transcriptomic changes associated with it (Savolainen et al. 2013). Negative genetic correlations in fitness associated with adaptation to new hosts at the cost of losing the ability to develop on an old host can result in host specialization, limiting the potential host range. However, polyphagous herbivores are predicted to develop no or positive correlations, broadening the potential host range they can utilize (Ehrlich & Raven 1964; Gould 1979; Agrawal 2000). Indeed, the ability of any mite population to use new plant hosts will be modulated by the life history of that population and what hosts it has previously been adapted to.

It is important to note that the while I consider TU-A to be an adapted population (genetic change intrinsic to the population compared to the ancestral strain, as supported by Wybouw et al., 2015), there remains the possibility that the increased performance of TU-A mites observed in this study was due to acclimation (physiological level changes due to environment). In other studies, herbivore strains to be compared were often reared on the same host for 2 generations (common garden experiments) to remove any environmental/maternal effects that may be playing a role in their differential performance on a challenging host (Wolf, 2013). As I was only studying the general mechanisms mites can use to overcome plant defences, whether they be genetically or physiologically determined, I did not implement the common garden approach here.

5 Future directions

As there is evidence for both suppression and detoxification in plant host utilization by both generalist and specialists, it remains unresolved whether a generalist's greatest tool for utilizing new hosts it its ability to modulate its detoxification enzyme profile in response to new and different plant compounds so as to propagate on that host long enough for an effector to evolve and target plant responses. Or, conversely, if suppression targeting a conserved portion of the plant response to herbivory is required first in order for there to be enough generations to change its detoxification profile and become 'adapted'. Performing transcriptomic and performance assays of both plant and mite periodically through the adaptation process (every month for 30 generations) would shed a lot of light on how the transcriptional profile of the defence response in tomato and the host shift response in mite change overtime. When this is correlated to performance of herbivore and host, we may have the chance to see if each of these mechanisms are employed to the same extent throughout the adaptation process, or if one allows for the progression of the other. Additionally, the study of reciprocal responses of plant and mite among many different mite populations, having undergone independent adaptation events, would also be extremely helpful in understanding the patterns of use of these mechanisms in naturally derived populations.

As discussed in the Introduction, there are several other mechanisms of adaptation that were not addressed in this study. For example, TU-A mites may have behavioural adaptations that went uncharacterized and unappreciated in this study. Additionally, there could be physiological adaptations, such as increased excretion efficiency of small molecules that could be responsible for some measure of the increased performance on tomato. Indeed, while attenuation of tomato response to feeding was observed at several levels, the lack of systemic attenuation leaves the biological relevance of this attenuation in question. Detoxification, while observed to play a role, was not responsible for the majority of the increased performance of TU-A compared to TU (this may have been better measured by applying all three inhibitors and assessed the cumulative and possible synergistic effects). Therefore, there is much of the adaptation process of TU-A that remains uncharacterized.

The results of this study are summarized in Figure 5.1 and indicate that suppression and detoxification are not mutually exclusive adaptation strategies. While one or the other may be favoured under certain environmental conditions, they should not be considered in isolation. There is every reason to suspect that these processes interact and are dynamic in time. The responses suppressed or not suppressed by the herbivore will result in the selection pressure faced by the detoxification machinery of the herbivore and therefore take part in shaping them. It is also important to note that adaptation may only ever be a journey and not a destination, as there is no evidence to suggest that once a population becomes adapted (performs well by whatever criteria we have set) that their reciprocal responses stop changing and become fixed. As the population remains on one host for an extended period of time, it will continue to fine-tune its adaptation mechanisms, becoming more efficient, as observed in the gradual increase in mite performance through the experimental selection (not a binary characteristic). Whether there is a threshold of adaptation, where the maximum level of nutrients is obtained at the lowest cost to the herbivore remains unknown. Evidence exists suggesting the better a population becomes at utilizing one host, it will loose its capabilities of feeding on different ones (Fellous et al., 2014), leading to specialization and perhaps speciation when coupled with reproductive isolation. An interesting, though potentially extremely long-term, experiment would be to adapt a *T. urticae* population to a challenging host and see if it becomes specialized. In such a case we would expect to observe it lose its ability to feed on other hosts (including its ancestral host), and its polyphagous nature. It could be that other factors present in an ecological setting are required to push a generalist population to be specialized, and that exclusive utilization of one host is not sufficient.

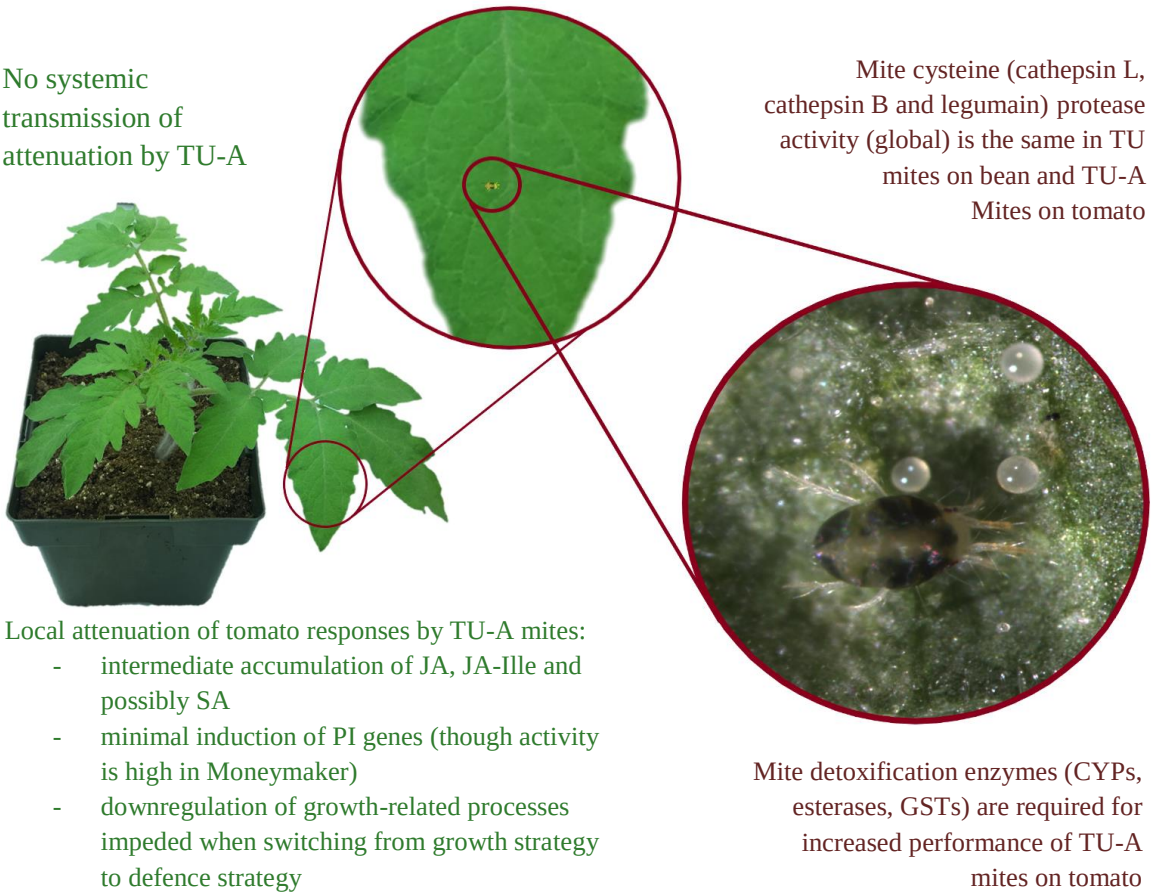


Figure 5.1 Model of the tomato response to adapted spider mites. Tomato responses are in green. Mite responses are in red.

6 Conclusion

As more detailed reports are generated regarding the interaction between plants and herbivores at the molecular through to the ecological level, we are still uncovering more and more complexity within those interactions. The way we engage in agriculture can only be improved upon as we incorporate more of our understanding of these interactions into the control of them in the field. We have been trying to overcome the effects of herbivory on our crops for a fraction of the time that plants have been co-evolving with them, and there is power in the diversity of plant defence compounds that exist in nature. We are not as good at synthesizing compounds as plants, and we have ever decreasing number of new, synthetic compounds being developed. Regulatory bodies are becoming ever more stringent about what compounds are acceptable due to public demand for safer, 'greener' pesticides, with very stringent toxicological and eco-toxicological criteria imposed by regulatory agencies (Marcic, 2012). Regardless, politics largely constrain scientific endeavour at this point, and scientific outreach will be key in the acceptance on any new biotechnologies developed for use in agriculture. There is every reason to believe that economic benefits will come from environmentally sound pest control practices that are more sustainable (Culliney, 2014). We need to focus our attention not just on applied biology, synthetic pesticides, and engineering plants with known defence compounds, but also on the continued interrogation of the basics of plant herbivore interaction at all levels. Despite the great progress that has been made in the field of plant-herbivore interaction, there is still much we do not know about the molecular and genetic mechanisms behind plant defence and herbivore adaptation to those defences.

Works Cited

Agrawal AA, Conner JK, Rasmann S (2010) Evolution After Darwin: The First 150 Years, eds Bell MA, Eanes WF, Futuyma DJ, Levinton JS. Sunderland, MA: Sinauer Associates, pp 242–268.

Agrawal AA (2000) Host–Range Evolution: Adaptation and Trade–Offs in Fitness of Mites on Alternative Hosts. Ecology 81, 500–508.

Agrawal AA, Vala F, Sabelis, MW (2002) Induction of Preference and Performance after Acclimation to Novel Hosts in a Phytophagous Spider Mite: Adaptive Plasticity? The American Naturalist 159, 553–565.

Ahn SJ, Vogel H, Heckel DG (2012) Comparative analysis of the UDP–glycosyltransferase multigene family in insects. Insect biochemistry and molecular biology 42,133–147.

Alba JM, Glas JJ, Schimmel BCJ, Kant MR (2011) Avoidance and suppression of plant defenses by herbivores and pathogens. Journal of plant interactions 6, 221–227.

Alba JM, Schimmel BCJ, Glas JJ, Ataide LMS, Pappas ML, Villarroel CA, Schuurink RC, Sabelis MW, Kant MR (2014) Spider mites suppress tomato defenses downstream of jasmonate and salicylate independently of hormonal crosstalk. The New Phytologist.

Alyokhin A, Chen YH (2017) Adaptation to toxic hosts as a factor in the evolution of insecticide resistance. Current Opinion in Insect Science 21, 33–38.

Ament K, Kant MR, Sabelis MW, Haring MA, Schuurink RC (2004) Jasmonic acid is a key regulator of spider mite–induced volatile terpenoid and methyl salicylate emission in tomato. Plant Physiology 135, 2025–2037.

Ament K, Krasikov V, Allmann S, Rep M, Takken FLW, Schuurink RC (2010). Methyl salicylate production in tomato affects biotic interactions. Plant Journal, 62, 124 134.

Amirhusin B, Shade RE, Koiwa H, Hasegawa PM, Bressan RA, Murdock LL, Zhu Salzman K (2004) Soyacystatin N inhibits proteolysis of wheat alpha–amylase inhibitor and potentiates toxicity against cowpea weevil. Journal of Economic Entomology 97, 2095–100

Antonious GF, Kamminga K, Snyder JC (2014) Wild tomato leaf extracts for spider mite and cowpea aphid control. Journal of Environment and Health Sciences 49, 527 31.

Arbuckle JG, Morton LW, Hobbs J (2015) Understanding Farmer Perspectives on Climate Change Adaptation and Mitigation. Environment and Behavior 47, 205–234.

Baldwin IT, Preston CA (1999) The eco–physiological complexity of plant responses to insect herbivores. Planta 208, 137–145.

Barrett LG, Heil M (2012) Unifying concepts and mechanisms in the specificity of plant enemy interactions. Trends in Plant Science 17, 282–92.

Bass C, Zimmer CT, Riveron JM, Wilding CS, Wondji CS, Kaussmann M, Field LM, Williamson MS, Nauen R (2013) Gene amplification and microsatellite polymorphism underlie a recent insect host shift. Proceedings of the National Academy of Sciences of the United States of America 110, 19460–19465.

Bede JC, Musser RO, Felton GW, Korth KL (2006) Caterpillar herbivory and salivary enzymes decrease transcript levels of Medicago truncatula genes encoding early enzymes in terpenoid biosynthesis. Plant Molecular Biology 60, 519–531.

Bensoussan N, Santamaria ME, Zhurov V, Diaz I, Grbić M, Grbić V (2016) Plant Herbivore Interaction: Dissection of the Cellular Pattern of Tetranychus urticae Feeding on the Host Plant. Frontiers in Plant Science 7, 1–13.

Bensoussan N, Zhurov V, Yamakawa S, O'Neil CH, Suzuki T, Grbić M, Grbić V (2018) The digestive system of the two–spotted spider mite, Tetranychus urticae (Koch), in the context of the mite–plant interaction. Frontiers in Plant Science 9, 1206.

Bernays E, Graham M (1988) On the evolution of host specificity in phytophagous arthropods. Ecology 69, 886–92.

Blaazer CJH, Villacis–Perez EA, Chafi R, Van Leeuwen T, Kant MR, Schimmel BCJ (2018) Why Do Herbivorous Mites Suppress Plant Defenses? Frontiers in Plant Science 9, 1–16.

Bleeker PM, Mirabella R, Diergaarde PJ, VanDoorn A, Tissier A, Kant MR, Prins M, De Vos M, Haring MA, Schuurink RC (2012) Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. Proceedings of the National Academy of Sciences of the United States of America 109, 20124–20129.

Böhm H, Albert I, Fan L, Reinhard A, Nürnberger T (2014) Immune receptor complexes at the plant cell surface. Current Opinion in Plant Biology 20C, 47–54.

Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe associated molecular patterns and danger signals by pattern-recognition receptors. Annual Review of Plant Biology 60, 379–406.

Bostock RM (2005) Signal crosstalk and induced resistance: straddling the line between cost and benefit. Annual Review of Phytopathology 43, 545–580.

Bradburne RP, Mithen R (2019) Glucosinolate Genetics and the Attraction of the Aphid Parasitoid Diaeretiella rapae to Brassica. Proceedings. Biological Sciences 267, 89 95.

Burton I, Lim B (2005) Achieving adequate adaptation in agriculture. Climate Change 70, 191–200.

Campbell BM, Beare DJ, Bennett EM, Hall-Spencer JM, Ingram JSI, Jaramillo F, Ortiz R, Ramankutty N, Sayer JA, Shindell D (2017) Agriculture production as a major driver of the earth system exceeding planetary boundaries. Ecology and Society 22:8.

Carrillo L, Martinez M, Ramessar K, Cambra I, Castañera P, Ortego F, Díaz I (2011) Expression of a barley cystatin gene in maize enhances resistance against phytophagous mites by altering their cysteine–proteases. Plant Cell Reports 30, 101–112.

Cazaux M, Navarro M, Bruinsma KA, Zhurov V, Negrave T, Van Leeuwen T, Grbić V, Grbić M (2014) Application of Two–spotted Spider Mite Tetranychus urticae for Plant–pest Interaction Studies. Journal of Visualized Experiments.

Chatzivasileiadis EA, Sabelis MW (1997) Toxicity of methyl ketones from tomato trichomes to Tetranychus urticae Koch. Experimental & Applied Acarology 21, 473–84.

Chrispeels MJ, Raikhel NV (1991) Lectins, lectin genes, and their role in plant defense. Plant Cell 3, 1–9.

Chung SH, Rosa C, Scully ED, Peiffer M, Tooker JF, Hoover K, Luthe DS, Felton GW (2013) Herbivore exploits orally secreted bacteria to suppress plant defenses. Proceedings of the National Academy of Sciences of the United States of America A. 110, 15728–15733.

Clark CW, Harvell CD (1992) Inducible defenses and the allocation of resources: a minimal model. American Naturalist 139, 521–539.

Clotuche G, Navajas M, Mailleux AC, Hance T (2013) Reaching the Ball or Missing the Flight? Collective Dispersal in the Two–Spotted Spider Mite Tetranychus urticae. PLoS One 8, 1–9.

Consortium TTG (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485, 635–641.

Constabel CP, Barbehenn R (2008) Defensive Roles of Polyphenol Oxidase in Plants. In: Schaller A (eds) Induced Plant Resistance to Herbivory (Springer, Dordrecht) 253 269.

Culliney TW (2014) Crop Losses to Arthropods. Integrated Pest Management Reviews 202–217.

Daviere JM, Achard P (2013) Gibberellin signaling in plants. Development 140, 1147 1151.

Demidyuk IV, Shubin AV, Gasanov EV, Kostrov SV (2010) Propeptides as modulators of functional activity of proteases. Biomolecular Concepts 1, 305–322.

Dermauw W, Osborne EJ, Clark RM, Grbić M, Tirry L, Van Leeuwen T (2013) A burst of ABC genes in the genome of the polyphagous spider mite Tetranychus urticae. Bmc Genomics 14, 317.

Dermauw W, Wybouw N, Rombauts S, Menten B, Vontas J, Grbić M, Clark RM, Feyereisen R, Van Leeuwen T (2013) A link between host plant adaptation and pesticide resistance in the polyphagous spider mite Tetranychus urticae. Proceedings of the National Academy of Sciences of the United States of America 110, E113-22.

Díaz-Riquelme J, Zhurov V, Rioja C, Pérez-Moreno I, Torres-Pérez R, Grimplet J, Carbonell-Bejerano P, Bajda S, Van Leeuwen T, Martínez-Zapater JM, Grbić M, Grbić V (2016) Comparative genome-wide transcriptome analysis of vitis vinifera responses to adapted and non-adapted strains of two-spotted spider mite, tetranyhus urticae. BMC Genomics 17, 1–15.

Dobler S, Dalla S, Wagschal V, Agrawal AA (2012) Community-wide convergent evolution in insect adaptation to toxic cardenolides by substitutions in the Na,K ATPase. Proceedings of the National Academy of Sciences of the USA 109, 13040–13045.

Duffey SS, Stout MJ (1996) Antinutritive defenses and toxic components against insects. Archives of Insect Biochemistry and Physiology 32, 3–37.

Durgbanshi A, Arbona V, Pozo O, Miersch O, Sancho JV, Gómez-Cadenas A (2005) Simultaneous determination of multiple phytohormones in plant extracts by liquid

chromatography–electrospray tandem mass spectrometry. Journal of Agricultural and Food Chemistry 53, 8437–8442.

Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. Evolution 18, 586–608.

Ekström G, Ekbom B (2011) Pest control in agro–ecosystems: an ecological approach. Critical Reviews in Plant Science 30, 74–94.

Erb M, Meldau S, Howe GA (2012) Role of phytohormones in insect-specific plant reactions. Trends in Plant Science 17, 250–259.

Fellous S, Angot G, Orsucci M, Migeon A, Auger P, Olivieri I, Navajas M (2014) Combining experimental evolution and field population assays to study the evolution of host range breadth. Journal of Evolutionary Biology 27, 911–919.

Felton GW, Tumlinson JH (2008) Plant-insect dialogs: complex interactions at the plant insect interface. Current Opinion in Plant Biology 11, 457–463.

Fry JD (1989) Evolutionary adaptation to host plants in a laboratory population of the phytophagous mite Tetranychus urticae Koch. Oecologia 81, 559–65.

Fuller D, Denham M, Arroyo-Kalin M, Lucas L, Stevens C J, Qin L, Allaby RG, Purugganan MD (2014) Convergent evolution and parallelism in plant domestication revealed by an expanding archaeological record. Proceedings of the National Academy of Sciences 111, 6147–6152.

Fürstenberg-Hägg J, Zagrobelny M, Bak S (2013) Plant defense against insect herbivores. International Journal of Molecular Sciences 14, 10242–10297.

Futuyma DJ, Agrawal AA (2009) Macroevolution and the biological diversity of plants and herbivores. Proceedings of the National Academy of Sciences of the United States of America 106, 18054–18061.

Futuyma DJ, Gould F (1979) Associations of plants and insects in deciduous forest. Ecological Monographs 49, 33–50.

Glas JJ, Alba JM, Simoni S, Villarroel CA, Stoops M, Schimmel BC, Schuurink RC, Sabelis M, WKant MR (2014). Defense suppression benefits herbivores that have a monopoly on their feeding site but can backfire within natural communities. BMC Biology, 12, 1–14.

Gomez S, Steinbrenner AD, Osorio S, Schueller M, Ferrieri RA, Fernie AR, Orians CM (2012) From shoots to roots: transport and metabolic changes in tomato after simulated feeding by a specialist lepidopteran. Entomologia Axperimentalis et Applicata 144, 101–111.

Gotoh T, Bruin J, Sabelis MW, Menken SBJ (1993) Host race formation in *Tetranychus urticae*: Genetic differentiation, host plant preference, and mate choice in a tomato and a cucumber strain. Entomologia Experimentalis et Applicata 68, 171–178.

Gould F (1979) Rapid host range evolution in a population of the phytophagous mite Tetranychus urticae Koch. Evolution, 33, 791–802.

Grbić M, Khila A, Lee KZ, Bjelica A, Grbić V, Whistlecraft J, Verdon L, Navajas M, Nagy L (2007) Mity model: Tetranychus urticae, a candidate for chelicerate model organism. BioEssays 29, 489–496.

Grbić M, Van Leeuwen T, Clark RM, Rombauts S, Grbić V, Osborne EJ, Dermauw W, Phuong CTN, Ortego F, Hernández-Crespo P, Diaz I, Martinez M, Navajas M, Sucena E, Magalhães S, Nagy L, Pace RM, Djuranović S, Smagghe G, Iga M, Christiaens O, Veenstra JA, Ewer J, Villalobos RM, Hutter JL, Hudson SD, Velez M, Yi SV, Zeng J, Pires-dasilva A, Roch F, Cazaux M, Navarro M, Zhurov V, Acevedo G, Bjelica A, Fawcett JA, Bonnet E, Martens C, Baele G, Wissler L, Sanchez-Roiguez A, Tirry L, Blais C, Demeestere K, Henz SR, Gregory TR, Mathieu J, Verdon L, Farinelli L, Schmutz J, Lindquist E, Feyereisen R, Van de Peer Y (2011) The genome of Tetranychus urticae reveals herbivorous pest adaptations. Nature 479, 487–492.

Green ATR, Ryan CA (1972) Wound-Induced Proteinase Inhibitor in Plant Leaves: A Possible Defense Mechanism against Insects. Science 175, 776–777.

Gupta V, Mathur S, Solanke AU, Sharma MK, Kumar R, Vyas S, Khurana P, Khurana JP, Tyagi AK, Sharma AK (2009) Genome analysis and genetic enhancement of tomato. Critical reviews in biotechnology 29, 152–181.

Heidel-Fischer HM, Vogel H (2015) Molecular mechanisms of insect adaptation to plant secondary compounds. Current Opinion in Insect Science 8, 8–14.

Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J (2008) qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biology 8, R19.

Hopkins RJ, van Dam NM, van Loon JJA (2009) Role of glucosinolates in insect-plant relationships and multitrophic interactions. Annual Review of Entomology 54, 57 83.

Howe GA (2004). Jasmonates as signals in the wound response. Journal of Plant Growth Regulation 23, 223–237.

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annual Review of Plant Biology 59, 41–66.

Howe GA, Lightner J, Browse J, Ryan CA (1996) An Octadecanoid Pathway Mutant (JL5) of Tomato Is Compromised in Signaling for Defense against Insect Attack. Plant Cell 8, 2067–2077.

Iwasa T, Osakabe M (2015) Effects of combination between web density and size of spider mite on predation by a generalist and a specialist phytoseiid mite. Experimental & Applied Acarology 66, 219–225.

Johnson MTJ (2011) Evolutionary ecology of plant defences against herbivores. Functional Ecology 25, 305–311.

Jonckheere W, Dermauw W, Zhurov V, Wybouw N, Van den Bulcke J, Villarroel CA,

Greenhalgh R, Grbić M, Schuurink RC, Tirry L, Baggerman G, Clark RM, Kant MR, Wanholme B, Menschaert G, Van Leeuwen T (2016) The Salivary Protein Repertoire of the Polyphagous Spider Mite Tetranychus urticae: A Quest for Effectors. Molecular and Cellular Proteomics 15, 3594–3613.

Kant MR, Ament K, Sabelis MW, Haring MA, Schuurink RC (2004) Differential timing of spider mite-induced direct and indirect defenses in tomato plants. Plant Physiology135, 483–495.

Kant MR, Sabelis MW, Haring MA, Schuurink RC (2008) Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defences. Proceedings. Biological Sciences 275, 443–452.

Kant MR, Jonckheere W, Knegt B, Lemos F, Liu J, Schimmel BCJ, Villarroel CA, Ataide LMS, Dermauw W, Glas JJ, Egas M, Janssen A, Van Leeuwen T, Schuurink RC, Sabelis MW, Alba JM (2015) Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. Annals of Botany 115, 1051.

Karban R, Agrawal AA, Thaler JS, Adler LS (1999) Induced plant responses and information content about risk of herbivory. Trends in Ecology & Evolution 14, 443–447.

Katagiri F, Tsuda K (2010) Understanding the plant immune system. Molecular Plant Microbe Interactions *23*, 1531–1536.

Kempel A, Schädler M, Chrobock T, Fischer M, van Kleunen M (2011) Tradeoffs associated with constitutive and induced plant resistance against herbivory. Proceedings of the National Academy of Sciences of the United States of America 108, 5685–5689.

Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: the emerging molecular analysis. Annual Review of Plant Biology 53, 299–328.

Khalighi M, Dermauw W, Wybouw N, Bajda S, Osakabe M, Tirry L, Van Leeuwen T (2016) Molecular analysis of cyenopyrafen resistance in the two-spotted spider mite Tetranychus urticae. Pest Management Science 72, 103–112.

Koiwa H, Bressan RA, Hasegawa PM (1997) Regulation of protease inhibitors and plant defense. Trends in Plant Science 2, 379–384.

Krieger RI, Feeny PP, Wilkinson CF (1971) Detoxication enzymes in the guts of caterpillars: an evolutionary answer to plant defenses? Science (New York, N.Y.) 172, 579–81.

Kuhn J, Pettersson EM, Feld BK, Burse A, Termonia A, Pasteels JM, Boland W (2004) Selective transport systems mediate sequestration of plant glucosides in leaf beetles: A molecular basis for adaptation and evolution. Proceedings of the National Academy of Sciences of the United States of America 101, 13808–13813.

Labandeira C (2007) The origin of herbivory on land: Initial patterns of plant tissue consumption by arthropods. Insect Science 14, 259–275.

Larsen CS (2006) The agricultural revolution as environmental catastrophe: Implications for health and lifestyle in the Holocene. Quaternary International: the journal of the International Union for Quaternary Research 150, 12–20.

Li C, Williams MM, Loh YT, Lee G I, Howe GA (2002) Resistance of cultivated tomato to cell content-feeding herbivores is regulated by the octadecanoid-signaling pathway. Plant Physiology 130, 494–503.

Li X, Schuler MA, Berenbaum MR (2007) Molecular Mechanisms of Metabolic Resistance to Synthetic and Natural Xenobiotics. Annual Review of Entomology 52, 231–253.

Maffei ME, Mithofer A, Boland W (2007). Before gene expression: early events in plant insect interaction. Trends in Plant Science 12, 310–316.

Marcic D (2012) Acaricides in modern management of plant–feeding mites. Journal of Pest Science 85, 395–408.

Mao W, Rupasinghe S, Zangerl AR, Schuler MA, Berenbaum MR (2006) Remarkable substrate–specificity of CYP6AB3 in Depressaria pastinacella, a highly specialized caterpillar. Insect Molecular Biology 15, 169–179.

Mao W, Rupasinghe SG, Zangerl AR, Berenbaum MR, Schuler MA (2007) Allelic variation in the Depressaria pastinacella CYP6AB3 protein enhances metabolism of plant allelochemicals by atering a proximal surface residue and potential interactions with cytochrome P450 reductase. Journal of Chemical Biology 282, 10544–10552.

Martel C, Zhurov V, Navarro M, Martinez M, Cazaux M, Auger P, Migeon A, Santamaria ME, Wybouw N, Diaz I, Van Leeuwen T, Navajas M, Grbić M, Grbić V (2015) Tomato Whole Genome Transcriptional Response to Tetranychus urticae Identifies Divergence of Spider Mite–Induced Responses Between Tomato and Arabidopsis. Molecular plant-microbe interactions: MPMI 28, 343–361.

Migeon A, Dorkeld F (2006–2019) Spider Mites Web: a comprehensive database for the Tetranychidae. http://www.montpellier.inra.fr/CBGP/spmweb. Accessed January, 2019.

Miller JR, Strickler KL (1984) Finding and accepting host plants. Chemical Ecology of Insects (eds. W.J. Bell & R.T. Card´e) (Chapman and Hall, London) pp. 127–147.

Mitchell C, Brennan RM, Graham J, Karley AJ (2016) Plant Defense against Herbivorous Pests: Exploiting Resistance and Tolerance Traits for Sustainable Crop Protection. Frontiers in Plant Science *7*, 1–8.

Mithöfer A, Boland W (2012) Plant Defense Against Herbivores: Chemical Aspects. Annual Review of Plant Biology 63, 431–450.

Moreira X, Abdala-Roberts L, Gols R, Francisco M (2018) Plant domestication decreases both constitutive and induced chemical defences by direct selection against defensive traits. Scientific Reports 8, 1–11.

Morton RL, Schroeder HE, Bateman KS, Chrispeels MJ, Armstrong E, Higgings TJV (2000) Bean α-amylase inhibitor 1 in transgenic peas (Pisum sativum) provides complete protection from pea weevil (Bruchus pisorum) under field conditions. Proceedings of the National Academy of Sciences of the United States of America 97, 3820–3825.

Musser RO, Hum-Musser SM, Ervin G, Murphy JB, Felton GW (2002) Caterpillar saliva beats plant defences. Nature 416, 599–600.

Navajas M (1999) Host plant associations in the spider mite Tetranychus urticae (Acari: Tetranychidae): insights from molecular phylogeography. Pages 163–174 in: Ecology and Evolution of the Acari: Proceedings of the 3rd Symposium of the European Association of Acarologists 1–July 5, 1996, Amsterdam, The Netherlands, J Bruin, L P S van der Geest, and M W Sabelis, eds. Springer Netherlands, Dordrecht, The Netherlands.

Nisbet AJ, Billingsley PF (2000) A comparative survey of the hydrolytic enzymes of ectoparasitic and free-living mites. International Journal for Parasitology 30, 19 27.

Park YL, Lee J H (2002) Leafcell and tissue damage ofcucumber caused by twospotted spider mite (Acari: Tetranychidae). Journal of economic entomology 95, 952–957.

Parry MAJ, Hawkesford MJ (2010) Food security: increasing yield and improving resource use efficiency. The Proceedings of the Nutrition Society 69, 592–600.

Paschold A, Halitschke R, Baldwin IT (2007) Co(i)-ordinating defenses: NaCOI1 mediates herbivore-induced resistance in Nicotiana attenuata and reveals the role of herbivore movement in avoiding defenses. Plant Journal 51, 79–91.

Pauwels L, Inzé D, Goossens A (2009) Jasmonate–inducible gene: what does it mean? Trends in Plant Science 14, 87–91.

Pearce G, Ryan CA (2003) Systemic signaling in tomato plants for defense against herbivores: Isolation and characterization of three novel defense–signaling

glycopeptide hormones coded in a single precursor gene. Journal of Chemical Biology 278, 30044–30050.

Pearce G, Strydom D, Johnson S, Ryan CA (1991) A Polypeptide from Tomato Leaves Induces Wound–Inducible Proteinase Inhibitor. Science 253, 895–898.

Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM (2012) Hormonal Modulation of Plant Immunity. Annual Review of Cell and Developmental Biology 28, 489–521.

Rane R V, Walsh TK, Pearce SL, Jermiin LS, Gordon KH, Richards S, Oakeshott JG (2016) Are feeding preferences and insecticide resistance associated with the size of detoxifying enzyme families in insect herbivores? Current Opinion in Insect Science13, 70–76.

Rao PP, Praslicka J, Sutakova G (1996) Effect of temperature and rearing method on development and fecundity of Tetranychus urticae (Acarina, Tetranychidae) Biologia 51:509–516

Rawlings ND, Morton FR, Barrett AJ (2006) MEROPS: the peptidase database. Nucleic Acids Research 34, D270–D272.

Resende JT, Maluf WR, Cardoso M, Nelson DL, Faria MV (2002) Inheritance of acyl sugar contents in tomatoes derived from an interspecific cross with the wild tomato Lycopersicon pennellii and their effect on spider mite repellence. Genetics and Molecular Research: GMR 1, 106–16.

Rieu I, Powers SJ (2009) Real-Time Quantitative RT-PCR: Design, Calculations, and Statistics. Plant Cell 21, 1031–1033

Rioja C, Zhurov V, Bruinsma K, Grbić M, Grbić V (2017) Plant-Herbivore Interactions: A Case of an Extreme Generalist, the Two–Spotted Spider Mite *Tetranychus urticae*. Molecular Plant-Microbe Interactions 30, 935–945.

Rosenthal GA, Berenbaum MR (1992) Herbivores: Their Interactions with Secondary Plant Metabolites. Ecological and Evolutionary Processes. San Diego, CA: Academic.

Salinas M, Capel C, Alba J M, Mora B, Cuartero J, Fernandez-Munoz R, Lozano R, Capel J (2013) Genetic mapping of two QTL from the wild tomato *Solanum pimpinellifolium L.* controlling resistance against two–spotted spider mite (Tetranychus urticae Koch) TAG. Theoretical and applied genetics 126, 83–92.

Sanabria NM, Huang J, Dubery IA (2010) Self/nonself perception in plants in innate immunity and denfense. Self/Nonself 1, 40–54.

Santamaria ME, Cambra I, Martinez M, Pozancos C, González–Melendi P, Grbić V, Castañera P, Ortego F, Diaz I (2012) Gene pyramiding of peptidase inhibitors enhances plant resistance to the spider mite tetranychus urticae. PLoS One 7, 2–11.

Santamaria ME, Martínez M, Cambra I, Grbić V, Diaz I (2013) Understanding plant defence responses against herbivore attacks: An essential first step towards the development of sustainable resistance against pests. Transgenic Research 22, 697 708.

Santamaria ME, Arnaiz A, Diaz–mendoza M, Martinez M, Diaz I (2015a) Inhibitory Properties of Cysteine Protease Pro–Peptides from Barley Confer Resistance to Spider Mite Feeding. PLoS One 10, e0128323.

Santamaría ME, González–Cabrera J, Martínez M, Grbić V, Castañera P, Díaz l, Ortego F (2015b) Digestive proteases in bodies and faeces of the two–spotted spider mite, Tetranychus urticae. Journal Insect Physiology 78, 69–77.

Sarethy, I. P., Kashyap, A., Bahal, U., Sejwal, N., & Gabrani, R. (2014). Study of liquid culture system for micropropagation of the medicinal plant Solanum nigrum L. and its effect on antioxidant property. Acta Physiologiae Plantarum, 36(11), 2863–2870.

Sarmento RA, Lemos F, Bleeker PM, Schuurink RC, Pallini A, Oliveira MGA, Lima ER, Kant M, Sabelis MW, Janssen A (2011) A herbivore that manipulates plant defence. Ecology Letters 14, 229–236.

Savolainen O, Lascoux M, Merila J (2013) Ecological genomics of local adaptation. Nature Reviews Genetics, 14, 807–820.

Schimmel BCJ, Ataide LMS, Chafi R, Villarroel CA, Alba JM, Schuurink RC, Kant MR (2017) Overcompensation of herbivore reproduction through hyper–suppression of plant defenses in response to competition. The New Phytologist 214, 1688–701.

Schmelz E A, Carroll MJ, LeClere S, Phipps SM, Meredith J, Chourey PS, Alborn HT, Teal PEA (2006) Fragments of ATP synthase mediate plant perception of insect attack. Proceedings of the National Academy of Sciences of the United States of America 103, 8894–8899.

Schoonhoven LM, Jermey T, van Loon, J.J.A. (1998). Insect-Plant Biology: From Physiology to Evolution. London: Chapman and Hall.

Shroff R, Vergara F, Muck A, Svatoš A, Gershenzon J (2008) Nonuniform distribution of glucosinolates in Arabidopsis thaliana leaves has important consequences for plant defense. Proceedings of the National Academy of Sciences of the USA 105, 6196 6201.

Smith, BD (2002) Documenting plant domestication: The consilience of biological and archaeological approaches. Proceedings of the National Academy of Sciences of the USA 98, 1324–1326.

Smith JL, Moraes CM De, Mescher MC (2009) Jasmonate– and salicylate–mediated plant defense responses to insect herbivores, pathogens and parasitic plants. Pest Management Science 65, 497–503.

Snoeck S, Greenhalgh R, Tirry L, Clark RM, Van Leeuwen, T., & Dermauw, W. (2017) The effect of insecticide synergist treatment on genome-wide gene expression in a polyphagous pest. Scientific Reports *7*, 1–12.

Statistics Canada (2019) Table 32-10-0456-01 Production and value of greenhouse vegetables. Retrieved from: https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=3210045601&pickMembers%5B0%5D=1.1&pickMembers%5B1%5D=4.3

Stout MJ (2013) Reevaluating the conceptual framework for applied research on host–plant resistance. Insect Science 20, 263–272.

Tanigoshi LK, Davis RW (1978) An ultrastructural study of Tetranychus mcdanieli feeding injury to the leaves of 'Red Delicious' apple (Acari: Tetranychidae) International Journal of Acarology 4, 47–51.

Taylor G (2008) Evolution's Edge: The coming collapse and transformation of our world. New Society Publishers. Gabriola Island, BC: New Society Publishers, pp 104 105.

Thipyapong P, Stout MJ, Attajarusit J (2007) Functional Analysis of Polyphenol Oxidases by Antisense/Sense Technology. Molecules 12, 1569–1595.

Tian D, Traw MB, Chen JQ, Kreitman M, Bergelson J (2003) Fitness costs of R-gene mediated resistance in Arabidopsis thaliana. Nature 423, 74–77.

Ton J, Flors V, Mauch-Mani B (2009) The multifaceted role of ABA in disease resistance. Trends in Plant Science 14, 310–317.

Tör M, Lotze MT, Holton N (2009). Receptor-mediated signalling in plants: molecular patterns and programmes. Journal of Experimental Botany 60, 3645–3654.

Turner JG, Ellis C, Devoto A (2002) The Jasmonate Signal Pathway. Plant Cell 153–165.

United Nations, Department of Economic and Social Affairs, Population Division (2019) World Population Prospects 2019: Ten Key Findings.

Van Leeuwen T, Witters J, Nauen R, Duso C, Tirry L (2010a) The control of eriophyoid mites: State of the art and future challenges. Experimental & Applied Acarology 51, 205–224.

Van Leeuwen T, Vontas J, Tsagkarakou A, Dermauw W, Tirry L (2010b) Acaricide resistance mechanisms in the two–spotted spider mite Tetranychus urticae and other important Acari : A review. Insect Biochemistry and Molecular Biology*40*, 563–572.

van Loon LC, Geraats BPJ, Linthorst HJM (2006) Ethylene as a modulator of disease resistance in plants. Trends in Plant Science 11, 184–191.

Van Poecke RMP (2007) Arabidopsis-Insect Interactions. The Arabidopsis Book (pp. 1–34). American Society of Plant Biologists.

Van Pottelberge S, Van Leeuwen T, Nauen R, Tirry L (2009) Resistance mechanisms to mitochondrial electron transport inhibitors in a field-collected strain of Tetranychus urticae Koch (Acari: Tetranychidae). Bulletin of Entomological Research 99, 23–31.

Villarroel CA, Jonckheere W, Alba JM, Glas JJ, Dermauw W, Haring MA, Van Leeuwen T, Schuurink RC, Kant MR (2016) Salivary proteins of spider mites suppress defenses in Nicotiana benthamiana and promote mite reproduction. Plant Journal 86, 119–131.

Volpicella M, Ceci LR, Cordewener J, America T, Gallerani R, Bode W, Jongsma MA, Beekwilder J (2003) Properties of purified gut trypsin from Helicoverpa zea, adapted to proteinase inhibitors. European Journal of Biochemistry 19, 10–19.

Walia S, Saha S, Tripathi V, Sharma KK (2017) Phytochemical biopesticides: some recent developments. Phytochemistry Reviews: proceedings of the Phytochemical Society of Europe 16, 989–1007.

Wen Z, Rupasinghe S, Niu G, Berenbaum MR, Schuler MA (2006) CYP6B1 and CYP6B3 of the black swallowtail (Papilio polyxenes): adaptive evolution through subfunctionalization. Molecular Biology and Evolution 23, 2434–2443.

Wheat CW, Vogel H, Wittstock U, Braby MF, Underwood D, Mitchell-Olds T (2007) The genetic basis of a plant–insect coevolutionary key innovation. Proceedings of the National Academy of Sciences of the United States of America, 104, 20427–20431.

Williams WG, Kennedy GG, Yamamoto RT, Thacker JD, Bordner J (1980) 2 Tridecanone: A naturally occurring insecticide from the wild tomato *Lycopersicon hirsutum f.glabratum.* Science 207, 888–9.

Wolf JBW (2013) Principles of transcriptome analysis and gene expression quantification: an RNA-seq tutorial. Molecular Ecology Resources 13, 559–572.

Wu J, Baldwin IT (2010) New Insights into Plant Responses to the Attack from Insect Herbivores. Annual Review of Genetics 44, 1–24.

Wu JQ, Hettenhausen C, Meldae S, Baldwin IT (2007). Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of *Nicotiana attenuata*. Plant Cell 19, 1096–1122.

Wybouw N, Dermauw W, Tirry L, Stevens C, Grbić M, Feyereisen R, Van Leeuwen T (2014) A gene horizontally transferred from bacteria protects arthropods from host plant cyanide poisoning. Elife 3, e02365.

Wybouw N, Zhurov V, Martel C, Bruinsma KA, Hendrickx F, Grbić V, Van Leeuwen T, Grbić V, Van Leeuwen T (2015) Adaptation of a polyphagous herbivore to a novel host plant extensively shapes the transcriptome of herbivore and host. Molecular Ecology 24, 4647–4663.

Ximénez-Embún MG, González-Guzmán M, Arbona V, Gómez-Cadenas A, Ortego F, Castañera P (2018) Plant-mediated effects of water deficit on the performance of tetranychus evansi on tomato drought-adapted accessions. Frontiers in Plant Science 871, 1–15.

Xue M, Pang YH, Li QL, Liu TX (2010) Effects of four host plants on susceptibility of Spodoptera litura (Lepidoptera: Noctuidae) larvae to five insecticides and activities of detoxification esterases. Pest Management Science 66, 1273–1279.

Yang X, Margolies DC, Zhu KY, Buschman LL (2001) Host plant-induced changes in detoxification enzymes and susceptibility to pesticides in the two-spotted spider mite (Acari: Tetranychidae). Journal of Economic Entomology 94, 381–387.

Yoshinaga N, Aboshi T, Ishikawa C (2007) Fatty Acid Amides, Previously Identified in Caterpillars, Found in the Cricket Teleogryllus taiwanemma and Fruit Fly *Drosophila melanogaster* Larvae. Journal of Chemical Ecology 33, 1376–1381.

Zarate SI, Kempema LA, Walling LL (2007) Silverleaf Whitefly Induces Salicylic Acid Defenses and Suppresses Effectual Jasmonic Acid Defenses. Plant Physiology143, 866–875.

Zavala JA, Patankar AG, Gase K, Baldwin IT (2004) Constitutive and inducible trypsin proteinase inhibitor production incurs large fitness costs in *Nicotiana attenuata*. Proceedings of the National Academy of Sciences of the United States of America 101, 1607–1612.

Zhou S, Lou Y, Tzin V, Jander G (2015) Alteration of plant primary metabolism in response to insect herbivory. Plant Physiology 169, 01405.

Zhu F, Poelman EH, Dicke M (2014) Insect herbivore–associated organisms affect plant responses to herbivory. Journal of Physiology 204, 315–321.

Zhurov V, Navarro M, Bruinsma KA, Arbona V, Santamaria ME, Cazaux M, Wybouw N, Osborne EJ, Ens C, Rioja C, Vermeirssen V, Rubio-Somoza I, Krishna P, Diaz I, Schmid M, Gómez-Cadenas A, Van de Peer Y, Grbić M, Clark RM, Van Leeuwen T, Grbić V (2014) Reciprocal Responses in the Interaction between Arabidopsis and the Cell-Content-Feeding Chelicerate Herbivore Spider Mite. Plant Physiology 164, 384–399.

Ziegler J, Facchini PJ (2008) Alkaloid biosynthesis: metabolism and trafficking. Annual Review of Plant Biology 59: 735–769.

Appendices

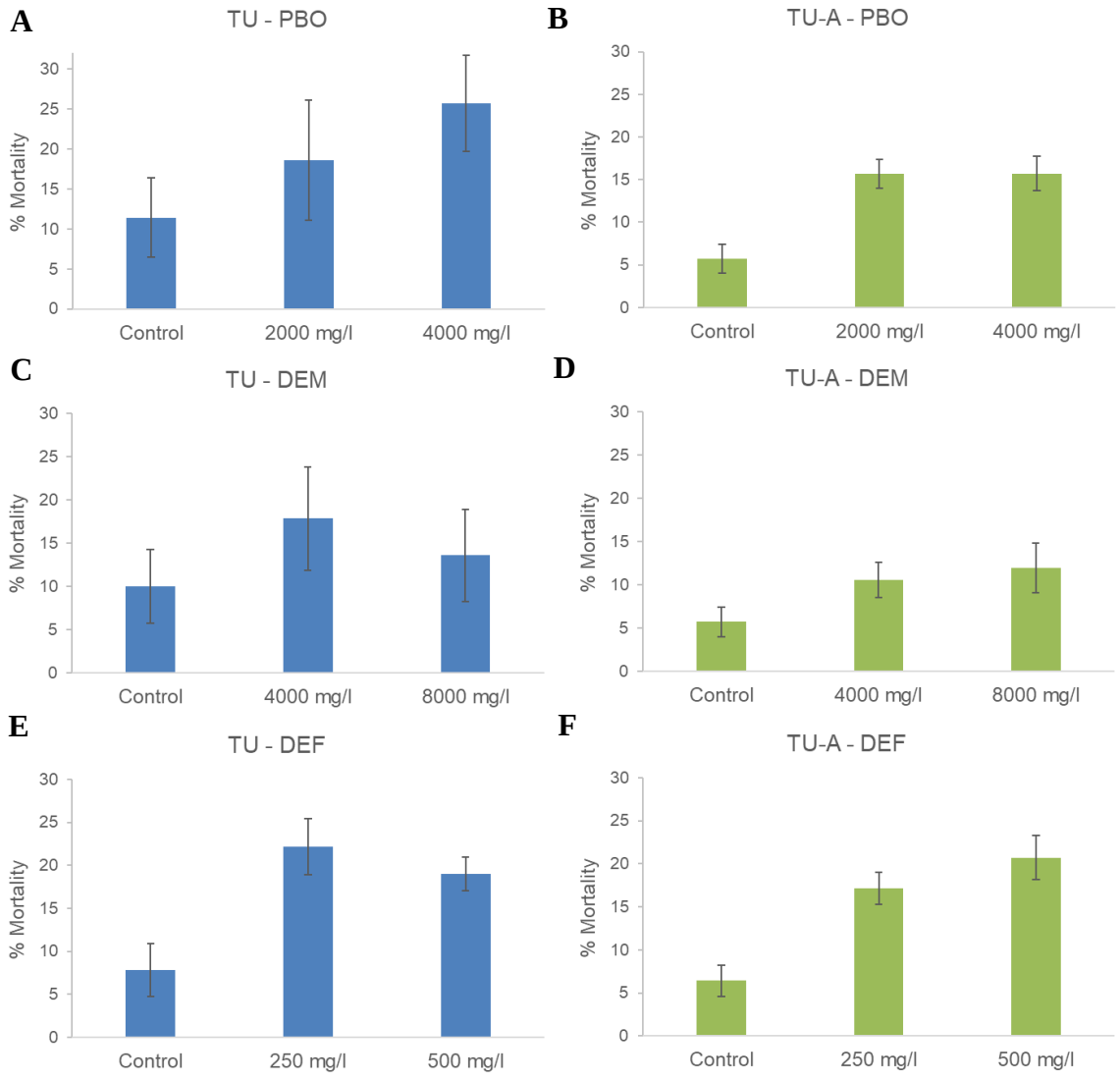


Figure 1 Detoxification enzyme inhibitor assay pilot experiments. Bar graphs showing mean ± SE % Mortality of TU and TU-A mites following treatment with different inhibitors at increasing concentrations. **A and B,** PBO treatment. **C and D,** DEM treatment. **E and F,** DEF treatment. Control was water with dimethyl formamide and emulsifier in the same concentration that was used for dissolving inhibitors.

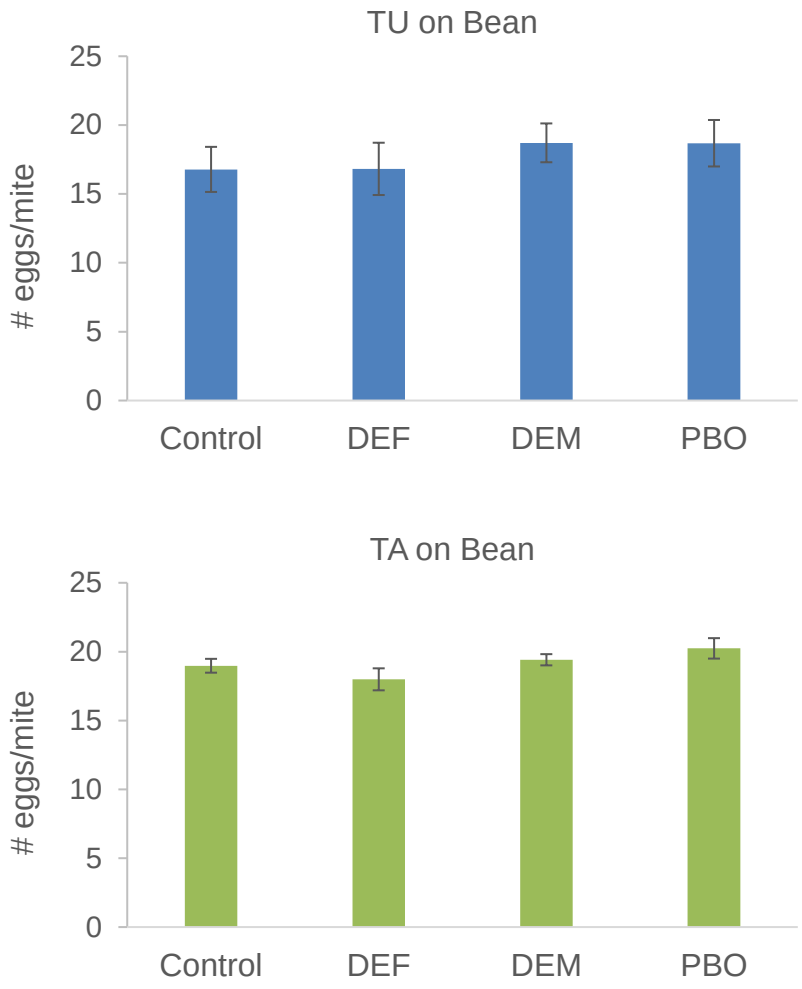


Figure 2 Inhibitor assay control: fecundity on non-challenging host. Bar graphs representing fecundity after treatment with water or inhibitors of TU and TU-A mites on bean. The lack of inhibitor effect on bean means these enzymes are not contributing to mite performance on this non-challenging host. Inhibitors are not having an effect on mite performance in the absence of challenge (metabolites that require detoxification). There is also a lack of difference between mite strains on bean, suggesting there was no cost to TU-A mites in their adaptation to tomato, as they can still use their ancestral host without consequence. Control was water with dimethyl formamide and emulsifier in the same concentration that was used for dissolving inhibitors.

Curriculum Vitae

ACADEMIC TRAINING

Doctor of Philosophy Candidate (Biology) **Sep 2014 – (Aug 2019)**
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PhD Thesis: Host Adaptation Mechanisms of Tetranychus urticae

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MSc Thesis: Arabidopsis thaliana-Spider Mite Interaction: Plant Perception, Signalling, and Response

Bachelor of Science Honors **Sep 2006 – April 2011** University of Western Ontario, London, Ontario, Canada
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RESEARCH EXPERIENCE

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Work Study Student **2010 – 2011**
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TEACHING EXPERIENCE

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PEER REVIEWED PUBLICATIONS

Rioja, C.*, Zhurov, V.*, **Bruinsma, K.A.**, Grbić, M., Grbić, M. (2017) Plant-herbivore interactions: a case of an extreme generalist, the two-spotted spider mite, Tetranychus urticae. Molecular Plant-Microbe Interactions. 30: 935-945. (PhD work)

Wybouw, N., Zhurov, V., Martel, C., **Bruinsma, K.A.**, Hendrickx, F., Grbić, V., and Van Leeuwen, T. (2015). Adaptation of a polyphagous herbivore to a novel host plant extensively shapes the transcriptome of herbivore and host. Molecular Ecology. 24: 4647-4663. (PhD work)

Zhurov, V., Navarro, M.*, **Bruinsma, K.A*.**, Arbona, V., Santamaria, M.E., Cazaux, M., Wybouw, N.,Osborne, E.J., Ens, C., Rioja, C., Vermeirssen, V., Rubio-Somoza, I., Krishna, P., Diaz, I., Schmid, M., Gomez-Cadenas, A., Van der Peer, Y., Grbić, M., Clark, R.M., Van Leeuwen, T., Grbić, V. (2014) Reciprocal responses in the interaction between *Arabidopsis* and the cell content feeding chelicerate herbivore *Tetranychus urticae*. Plant Physiology. 164: 384-399. * co-second authors (MSc work)

Cazaux, M.*, Navarro, M.*, **Bruinsma, K.A.***, Zhurov, V.*, Negrave, T., Van Leeuwen, T., Grbić, V., and Grbić, M. (2014). Application of two-spotted spider mite *Tetranychus urticae* for plant-pest interaction studies. Journal of Visual Experiments. * co-first authors (MSc work)

MANUSCRIPTS IN PREPARATION

Bruinsma, K.A.,*Rioja, C.*, Zhurov, V., Martel, C., Estrella Santamaría, M., Arbona, V., M., Grbić, M., Grbić, V. Mechanisms of spider mite adaptation to tomato. * co-first authors

Salehipourshirazi, G., **Bruinsma, K.A.**, Ratlamwala, H., and Grbić, V. *Tetranychus urticae* adaptation to *Arabidopsis* indole glucosinolates.

Salehipourshirazi, G., **Bruinsma, K.A.**, Grbić, V. Testing effectiveness of novel bio-pesticide against spider mite, *Tetranychus urticae*.

Rioja, C., Zhurov, V., **Bruinsma, K.A.**, Grbić, M., Grbić, V. Early *Arabidopsis* Responses to *Tetranychus urticae*: Local vs. Systemic Responses.

CONFERENCES AND PRESENTATIONS

Conference Presentation: Host-adaptations in Tetranychidae mites
10th Annual Spider Mite Meeting **Oct 15 – 18, 2018**
Logroño, La Rioja, Spain

Contributing author - conference Presentation: Early *Arabidopsis* Responses to *Tetranychus urticae*: Local vs. Systemic Responses
9th Annual Spider Mite Meeting **Oct 23 – 25, 2017**
Logroño, La Rioja, Spain

Conference Presentation: Adaptation mechanisms of spider mite to Arabidopsis
8th Annual Spider Mite Meeting **Oct 17 – 21, 2016**
Logroño, La Rioja, Spain

Contributing author - conference Presentation: Adaptation mechanisms of spider mite to Tomato
8th Annual Spider Mite Meeting **Oct 17 – 21, 2016**
Logroño, La Rioja, Spain

Poster Presentation: Attenuation of tomato induced transcriptional response upon herbivory by adapted two-spotted spider mites.
Canadian Society of Plant Biologists Eastern Regional Meeting **Nov 20 – 21, 2015**
University of Toronto, St. George Campus
Toronto, ON, Canada

Poster Presentation: Arabidopsis immune response to spider mite feeding: Perception, Signalling and Response.
Sustainability and Environmental Research Showcase **Mar 6, 2014**
University of Western Ontario,
London, ON, Canada

Conference Presentation: Arabidopsis response to spider mite feeding: Perception, Signalling & Response.
Canadian Society of Plant Biologists Eastern Regional Meeting **Dec 6 – 7, 2013**
University of Toronto - Mississauga
Mississauga, ON, Canada

Conference Presentation: Arabidopsis immune response to spider mite feeding: Perception, Signalling and Response
Biology Graduate Research Forum **Oct 19, 2013**
University of Western Ontario
London, ON, Canada

Poster Presentation: PEP peptides and associated receptors in Arabidopsis defence response to spider mite feeding – Awarded 2nd place
Canadian Society of Plant Biologists Eastern Regional Meeting **Nov 30 – Dec 1, 2012**
Wilfrid Laurier University
Waterloo, ON, Canada

SCHOLARSHIPS AND AWARDS

Ontario Graduate Scholarship -$15 000 (x3) **2015/16 & 2016/17 & 2017/18**

Dean's Honor List (Undergraduate) **2009, 2010, 2011**

Dr. Donna Green Mathematics and Science Award (Secondary school) **2005**

Ontario Scholar Award (Secondary school) **2005**

PROFESSIONAL MEMBERSHIPS

Canadian Society of Plant Biologists **2012 – present**

Society of Biology Graduate Students – University of Western Ontario **2012 – present**