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Adaptation Mechanisms of Two-Spotted Spider Mite, Tetranychus Urticae, to Arabidopsis Indole Glucosinolates

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Abstract

The two-spotted spider mite *Tetranychus urticae* Koch is a key agricultural pest that causes significant yield losses in a wide range of economically important crops. Rapid development of resistance to several classes of pesticides in *T. urticae* necessitates introduction of alternative management strategies to control this pest. Indole glucosinolates (IGs) are secondary metabolites found in Brassicaceae plants (including *Arabidopsis thaliana*) that have been shown to be effective against *T. urticae* and could be potential candidates to control spider mites. However, a laboratory population selected on IG-containing Arabidopsis was able to evolve adaptation to this plant. The overall objective of this thesis was to identify the mechanism of adaptation of two-spotted spider mites to Arabidopsis and IGs. Similar expression of marker genes and levels of plant defense-related metabolites after feeding of IG-adapted and non-adapted adult spider mites indicated that plant suppression is not the strategy used by spider mites to adapt to Arabidopsis and IGs. On the other hand, higher activity of P450 monooxygenases in IG-adapted mites and the negative effect of inhibitors of these detoxification enzymes on fecundity of adapted spider mites suggested that spider mites use detoxification to overcome the effect of IGs. HPLC-mediated detection of conjugates of IG breakdown products in adapted compared to non-adapted mites supports the involvement of detoxification in the adaptation of *T. urticae* to IGs. In addition, RNA-seq analysis showed induction of detoxification enzyme genes upon mite feeding on IGs. Upregulation of genes associated with growth, development and fecundity in adapted spider mites suggests that *T. urticae* neutralizes the negative effect of IGs. Genes that were differentially upregulated in adapted compared to non-adapted spider mites likely capture gene sets associated with the adaptation to IGs, suggesting that these genes can be further used in manipulation of *T. urticae* to avoid development of adaptation or to succumb it.

Keywords

Tetranychus urticae, adaptation, indole glucosinolates, *Arabidopsis thaliana*

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Abbreviations

1mIMG	1-methoxy indole-3-ylmethyl glucosinolate
4hIMG	4-hydroxy indole-3-ylmethyl glucosinolate
4mIMG	4-methoxy indole-3-ylmethyl glucosinolate
7-HEC	7-ethoxy-4-trifluoromethylcoumarin
7-HFC	7-hydroxy-4-trifluoromethylcoumarin
ABC	ATP-binding cassette transporters
AGs	Aliphatic glucosinolates
ANOVA	Analysis of variance
AOS	allene oxide suynthase
BGs	Benzenic glucosinolates
CDNB	1-chloro-2,4-dinitrobenzene
Col-0	Columbia-0
CPM	count per million
DEM	diethyl maleate
DEF	S,S,S tributyl-phosphorotrithioate
DEGs	differentially expressed genes
Et	ethylene
GBD	galactose-binding domain-like
GRR	gross reproductive rate
GSH	glutathione
GSS	glucosinolate sulfatase
GST	glutathione-S-transferase
HPLC	high performance liquid chromatography
I3C	indole-3-carbinols
IAN	indole acetonitriles
IGs	indole glucosinolates
IMG	indole-3-ylmethyl glucosinolate
IMI	indole-3-ylmethyl isothiocyanate
JA	Jasmonic acid
JA-Ile	JA-isoleucine
MS	mass spectrometry

NRQ normalized relative quantity
NSP nitrile-specifier protein
P450s P450 monooxygenases
PBO piperonyl butoxide
pNP p-nitrophenol
pNPA p-nitrophenyl acetate
RNA Ribonucleic acid
RNAi RNA interference
SA salicylic acid
SCN- thiocyanate ion
SLC solute carrier
TCPPE trichlorophenylpropynyl ether
TOF time of flight
tRNA transfer RNA
UGTs uridine-diphosphate-glycosyltransferases
UPLC ultra-performance liquid chromatography

Chapter 1
Introduction

1.1 Herbivore adaptation to host plants

The control of pests that attack our crops is a costly challenge that needs to be overcome in order to meet the global rising demand for food and fiber (Popp *et al.*, 2013). Arthropod pests including insects and mites mediate an average of 35% of potential pre-harvest crop yield loss worldwide (Oerke, 2006). Populations of these pests are regularly exposed to potentially harmful compounds in their environment called xenobiotics (e.g. pesticides and plant defensive metabolites). Survival of arthropod pests depends on their ability to develop tolerance or resistance to xenobiotics (Popp *et al.*, 2013; Van Leeuwen and Dermauw, 2016). Also, pest adaptation to xenobiotics is of great importance to agriculture, the environment and human health since it could lead to more frequent application of pesticides. Recently, it has been suggested that the ability to adapt to various host plants could lead to rapid development of pesticide resistance in arthropod pests (Dermauw *et al.*, 2013; Van Leeuwen and Dermauw, 2016). However, only a few studies have investigated the molecular mechanisms of herbivore adaptation to host plants (Feyereisen, 2005; Després *et al.*, 2007; Díaz-Riquelme *et al.*, 2016; Van Leeuwen and Dermauw, 2016).

Understanding the mechanism of herbivore adaptation to plant hosts will enable development of novel strategies for pest control aimed at preventing herbivore adaptation to host plants and potentially blocking the cross-resistance between phytotoxins and pesticides (Dermauw *et al.*, 2013; Díaz-Riquelme *et al.*, 2016). In this thesis two-spotted spider mites were used to investigate mechanisms that enable this pest to evolve its adaptation to new plant hosts. *Arabidopsis thaliana* was used a model host plant to study the reciprocal responses in this plant-pest interaction. The availability of genome-wide transcriptomic platform for both interacting organisms (Halkier and Gershenzon, 2006; Sønderby *et al.*, 2010 a; Grbić *et al.*, 2011) provides valuable tools for molecular and biochemical analyses of plant-spider mite interactions.

1.2 Two-spotted spider mites

1.2.1 Biology and effect on host plant

The two-spotted spider mite, *Tetranychus urticae* Koch. (Acari: Tetranychidae), is a cosmopolitan generalist plant feeder and an important agricultural pest (van de Vrie *et al.*, 1972; Jeppson *et al.*, 1975; Migeon *et al.*, 2010). The ability of *T. urticae* to evade a wide variety of plant defenses has made it one of the most polyphagous herbivores (Grbić *et al.*, 2011; Attia *et al.*, 2013; Van Leeuwen and Dermauw, 2016). Two-spotted spider mites feed on about 1200 plant species belonging to more than 140 different plant families, from which more than 150 species are economically important (Migeon *et al.*, 2010) including field crops (e.g. corn, cotton and soybean), horticultural crops (e.g. apple, pear and peach), greenhouse crops (e.g. strawberries, tomato and cucumber) and ornamentals (e.g. rose and gerbera) (van de Vrie *et al.*, 1972; Jeppson *et al.*, 1975; Park and Lee, 2005; Migeon *et al.*, 2010; Meena *et al.*, 2013; Scott *et al.*, 2013; Warabieda, 2015; Liu *et al.*, 2016).

Two-spotted spider mites belong to the subclass Acari from the class Arachnida. The members of this subclass are unusual arachnids as they are the only group that includes plant-feeding mite species (Hoy, 2011). The phytophagous species of mites occur in five families: Tetranychidae, Tenuipalpidae, Eriophyidae, Tarsonemidae and Tuckerillidae (Hoy, 2011). Members of the Tetranychidae family are referred to as spider mites since they make silk fibers from silk glands at their mouthpart palps (Alberti and Crooker, 1985). Spider mites produce silk webs for a variety of different functions, including protection from natural enemies and environmental conditions, pheromone communication, dispersal and colony establishment (van de Vrie *et al.*, 1972; Gerson, 1985).

The life cycle of *T. urticae* have five developmental stages: egg, larva, protonymph, deutonymph, and adults (Alberti and Crooker, 1985). Adult spider mites are sexually dimorphic, so that females are larger with a rounded posterior compared to smaller males with a more tapered posterior end (Hoy, 2011). Eggs are deposited singly as translucent spheres that are 0.1 mm in diameter (Tehri, 2014). It takes from three days at 30º C to 13 days at 15º C (Abd-El-Wahed and El-Halawany, 2012) for eggs to hatch into larvae. The six-legged larvae develop into eight-legged nymphs, protonymphs and then deutonymphs,

which are all active stages that feed on the host plant (Crooker, 1985; Hoy, 2011). Each of the developmental stages is followed by an inactive period of quiescence (Crooker, 1985; Hoy, 2011). The developmental time of each stage is dependent on temperature and humidity (Herbert, 1981; Abd-El-Wahed and El-Halawany, 2012). The life cycle of *T. urticae* from egg to adult can be completed in 30-34 days at 15º C or can be as short as six to seven days at high temperatures of about 30º C (Abd-El-Wahed and El-Halawany, 2012). Males have a shorter developmental time compared to females (Mitchell, 1973) and wait around a female deutonymph in the quiescence state to mate immediately after the adult female emerges (Crooker, 1985). Eggs produced by a fertilized female can develop into offspring of both sexes with a ratio of 3:1 to 4:1 female:male (Overmeer and Harrison, 1969; Modarres Najafabadi, 2012), while unfertilized females can produce eggs through arrhenotoky, resulting in haploid males (Crooker, 1985; Tehri, 2014). Oviposition begins as early as one or two days after maturity and continues for almost the whole life span of females, during which about 100 eggs might be laid by each female (Modarres Najafabadi, 2012; Tehri, 2014). The haplodiploidy genetic system and arrhenotoky that enable a single female to develop a new colony and also the ability of females to lay a large number of eggs per day can cause a potential outbreak of spider mites in a short time after their occurrence in a new environment (Meena *et al.*, 2013; Tehri, 2014).

Spider mites cause damage on host plants during feeding as they penetrate the plant tissue using specialized piercing-sucking, stylet-like mouthparts. They pass their stylets through the epidermal cells and empty chlorophyll and other contents of parenchyma cells (Tomczyk and Kropczynska, 1985; Park and Lee, 2002; Bensoussan *et al.*, 2016). The resulting damage and induction of plant wound/herbivory responses lead to discoloration of the feeding area, forming whitish to yellowish spots on the upper surface of the leaf (Park and Lee, 2002; Bensoussan *et al.*, 2016). Spider mites penetrate their stylets into the leaf through a stomatal opening or in between epidermal cells mediating minimum damage to epidermal cells (Bensoussan *et al.*, 2016). When the population is large, the chlorotic spots may join and become brownish, leading to defoliation and plant death. Spider mite feeding at lower intensity affects plants by reducing photosynthesis and transpiration rate (Sances *et al.*, 1979; Park and Lee, 2002), which in turn leads to a decrease in the amount of harvestable material (Sances *et al.*, 1979; Park and Lee, 2005; Warabieda, 2015).

1.2.2 Control of spider mites and pesticide resistance

Natural populations of *T. urticae* usually occur at low densities due to natural enemies, diseases and limited nutrients in the host plants (van de Vrie *et al.*, 1972; Roush and Hoy, 1978). In modern agricultural systems occurrence of *T. urticae,* as an agricultural pest happens due to the following reasons: 1) spider mites are hosted by high quality plants and are not limited by nutrients, water, diseases, pests or weed competition as a result of fertilizer and pesticide application (van de Vrie *et al.*, 1972); 2) application of general pesticides affects non-target arthropods including spider mite natural enemies and competitors; and 3) monoculture of crops reduces population of natural enemies by eliminating their shelter (Roush and Hoy, 1978) . Although changes in agroecosystem and biological characteristics of spider mites (e.g. high fecundity and short generation time) contributed in the pest status of *T. urticae*, the fast growth of *T. urticae* population in agroecosystems results from its ability to develop quick resistance to pesticides (Cranham and Helle, 1985).

Resistance happens when a previously susceptible population becomes less susceptible to a particular pesticide (Agrawal *et al.*, 2002; Després *et al.*, 2007). Resistance of *T. urticae* to pesticides is well-documented (Van Leeuwen *et al.*, 2010; Grbić *et al.*, 2011) so that *T. urticae* is identified as the most resistant plant pest: to date, the resistance of *T. urticae* to over 95 pesticide active ingredients has been reported in over 60 countries (DARP, 2015). Spider mites can develop tolerance to a new pesticide after a few applications while full resistance can be obtained within two to four years (Sato *et al.*, 2005; Van Leeuwen *et al.*, 2009; Grbić *et al.*, 2011). The reason behind this rapid development of resistance in spider mites is their high reproduction rate, short life cycle and, as a result, too many generations per year (Van Leeuwen *et al.*, 2009). In addition to these factors, development of pesticide resistance is accelerated in *T. urticae* by arrhenotoky inbreeding and high mutation rate (Cranham and Helle, 1985; Van Leeuwen *et al.*, 2009).

1.2.3 Association between plant adaptation and insecticide resistance

Plant secondary metabolites are defensive compounds (which are not involved in plant growth and development compared to primary metabolites) produced in plants constitutively or might be induced by herbivore feeding (Després *et al.*, 2007; Heidel-Fischer and Vogel, 2015; Kant *et al.*, 2015). To neutralize these metabolites, a defensive response is activated in herbivores (Després *et al.*, 2007; Alba *et al.*, 2011; Van Leeuwen and Dermauw, 2016). Counter-adaptation to plant secondary metabolites can be achieved if herbivores are in contact with plant metabolites consistently (Després *et al.*, 2007; Li *et al.*, 2007; Simon *et al.*, 2015; Van Leeuwen and Dermauw, 2016). It has been shown that the resulting activation of a herbivore's defensive response could cause development of tolerance or resistance to pesticides in herbivores, including *T. urticae* (Grbić *et al.*, 2011; Dermauw *et al.*, 2013; Van Leeuwen and Dermauw, 2016). Castle *et al.* compared the effect of three different host plants on susceptibility of the whitefly, *Bemisia tabaci,* to insecticides and indicated that both field collected and greenhouse-reared populations of whiteflies on *Brassica* species, including broccoli and kale were more tolerant to insecticides compared to those on the less challenging host plant, cantaloupe (Castle *et al.*, 2009). Similarly, larvae of the tobacco caterpillar, *Spodoptera litura*, reared on an artificial diet were more susceptible to two insecticides, cypermethrin and profenophos, compared to those reared on cauliflower (Karuppaiah *et al.*, 2016). Comparable results were reported for *T. urticae*: a population of two-spotted spider mites that were selected for resistance to a variety of cucumber were more resistant to organophosphate pesticides compared to the susceptible population from which it originated (Gould *et al.*, 1982). When a susceptible population of *T. urticae* was transferred from beans to a challenging host plant (i.e., tomato), after five generations the adapted population showed a transcriptional profile resembling that of multipesticide resistant populations of *T. urticae*. Also, adapted spider mites were less susceptible to pesticides with different modes of action, suggesting a link between pesticide resistance and host plant adaptation in *T. urticae* (Dermauw *et al.*, 2013).

It has long been suggested, and supported through molecular analyses, that the enormous *T. urticae* host plant range leads to the development of resistance to pesticides (Dermauw

et al., 2013; Van Leeuwen and Dermauw, 2016). Therefore, determination of the mechanism of spider mite adaptation to plant secondary metabolites also provides insights into the potential further development of resistance to pesticides. Using this knowledge, adaptation of spider mites to tolerant host plants or resistance to pesticides can be avoided. Two-spotted spider mites cannot increase their populations on the model plant *A. thaliana* (Arabidopsis) like that on a less challenging host plant such as bean. It has recently been shown that a group of defensive metabolites in Arabidopsis, called glucosinolates are responsible for the poor performance of *T. urticae* on this plant (Zhurov *et al.*, 2013). A strain of spider mites selected on Arabidopsis showed better performance on the wild type compared to mutants devoid of glucosinolates, suggesting a partial adaptation of spider mites to glucosinolates (Ratlamwala, 2014 c). To investigate the mechanism of adaptation of spider mites to glucosinolates, background on glucosinolate biochemistry, activation and the potential mechanisms of herbivore adaptation to glucosinolates is necessary.

1.3 Glucosinolates

Glucosinolates are plant secondary metabolites that are characteristic of the order Brassicales and are primarily found in the Brassicaceae, including the model plant Arabidopsis and agriculturally important plants such as cabbage, broccoli and canola (Agerbirk and Olsen, 2012). These compounds, known for their negative influence on plant pests and pathogens, affect specialist and generalist herbivores differently so that they usually stimulate specialists whereas they most often act as a repellent to generalists (Lankau, 2007; Hopkins *et al.*, 2009; Müller *et al.*, 2010). The insecticidal effect of glucosinolates breakdown products is reported repeatedly for both specialists and generalists (Lichtenstein *et al.*, 1962; Seo and Tang, 1982; Agrawal and Kurashige, 2003; Beekweelder *et al.*, 2008; Hopkins *et al.*, 2009; Müller *et al.*, 2010; Bohinc *et al.*, 2012; Schramm *et al.*, 2012). Besides their agricultural importance, glucosinolates have been of interest for their cancer-prevention potential in different model animals through the activation of phase II detoxification enzymes (Zhang *et al.*, 1992; Holst and Williamson, 2004; Keum *et al.*, 2004). A wide variety of glucosinolates are identified as having different structures and functions (Halkier and Gershenzon, 2006; Hopkins *et al.*, 2009; Bohinc *et al.*, 2012).

1.3.1 Structure, classification and biosynthesis of glucosinolates

Glucosinolates have a common core structure containing an oxime moiety with a sulfur-linked thioglucose group and an oxygen-linked sulfate group attached to a variable amino-acid derived side chain (Halkier and Gershenzon, 2006; Clarke, 2010; Sønderby *et al.*, 2010 a). Based on the structure of the side chain and the amino acid precursors, glucosinolates are divided into three classes: (1) aliphatic glucosinolates (AGs) derived from methionine, leucine, isoleucine or valine; (2) benzenic glucosinolated (BGs) also called aromatic glucosinolates derived from tyrosine or phenylalanine; and (3) indole glucosinolates (IGs) derived from tryptophan (Halkier and Gershenzon, 2006; Clarke, 2010; Sønderby *et al.*, 2010 a).

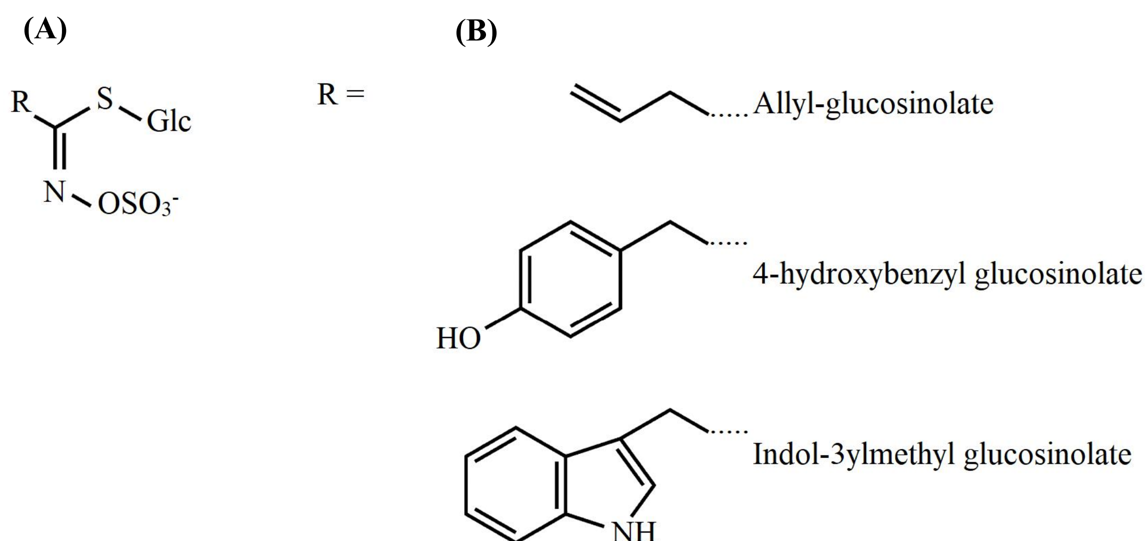


Figure 1-1 General chemical structure of glucosinolates (A) and examples of side chains (R) of major glucosinolates present in Arabidopsis (B) (adapted from Halkier and Gershenzon, 2006).

The biosynthesis of glucosinolates includes three phases: (a) amino acid chain elongation in which methylene groups are added into the side chain; (b) formation of glucosinolate core structure from the amino acid moiety; and (c) modifications of the side chain via hydroxylation, O-methylation, glycosylation, desaturation or acylation (Halkier and Gershenzon, 2006). The diversity of more than 130 glucosinolates identified up to date is mediated through the side chain elongation followed by the side chain modification phase

(Clarke, 2010; Agerbirk and Olsen, 2012). Diversion of IGs from AGs and BGs biosynthesis pathways occurs in the core structure formation phase where amino acid conversion to aldoximes is catalyzed by cytochrome P450 genes belonging to the CYP79 family. CYP79B2 and CYP79B3 use tryptophan (the precursor amino acid for biosynthesis of IGs) as a substrate, CYP79F1 and CYP79F2 convert methionine (the precursor for AGs) and CYP 79A2 metabolizes phenylalanine (the precursor for BGs) (Sønderby *et al.*, 2010 a). A *cyp79B2/cyp79B3* double knockout is completely devoid of IGs and so provides a useful tool in IGs studies (Halkier and Gershenzon, 2006).

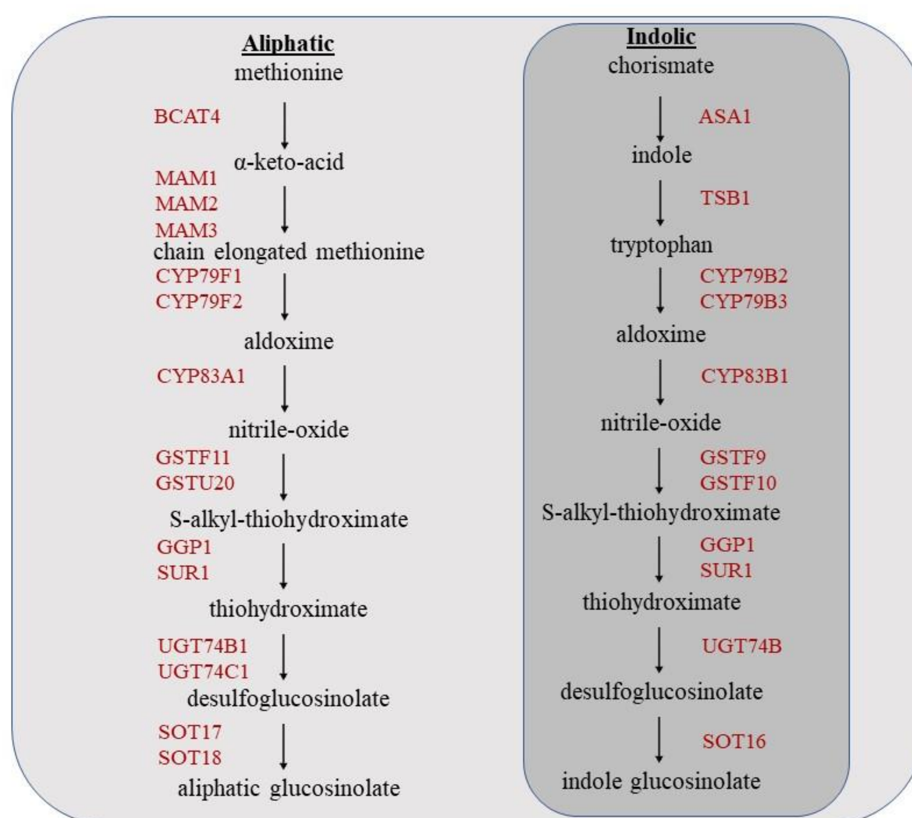


Figure 1-2 Biosynthesis pathway of aliphatic and indolic glucosinolates in Arabidopsis and enzymes involved in each step. Homologous enzymes catalyze similar steps in each pathway, except for GGP1 and SUR1 that are shared between the two pathways. ASA1, anthranilate synthase α1; BCAT4, branched chain amino acid transferase 4; CYP, cytochrome P450; GGP1, gamma glutamyl peptidase; GST, glutathione-Stransferase; MAM1-3, methylthioalkylmalate synthase1-3; SOT16-18, sulphotransferase 16-18; SUR1, C-S lyase; TSB1, tryptophan synthase β chain 1; UGT, UDP-glucosyl transferase (adapted from Sønderby *et al.*, 2010).

1.3.2 Glucosinolate breakdown

Intact glucosinolates are not toxic but their enzymatic hydrolysis results in the production of an array of physiologically active breakdown products (Agerbirk *et al.*, 2009; Hopkins *et al.*, 2009; Winde and Wittstock, 2011; Wittstock, 2011). The hydrolysis of glucosinolates is catalyzed by a group of endogenous β-glucosidases named myrosinase or thioglucoside glucohydrolases (TGGs) (Wittstock, 2011). To avoid the toxic effect of glucosinolates, in plants, myrosinases are stored separately from glucosinolates in plant tissue and are only mixed with a substrate upon tissue damage (Bones and Rossiter, 1996; Andréasson *et al.*, 2001). Myrosinases catalyze the cleavage of glucosinolate molecules to an unstable aglucone, which can be rearranged to isothiocyanates or be converted into nitriles, epithionitriles or organic thiocyanates depending on structure, pH and presence of specifier proteins (Uda *et al.*, 1986; Wittstock, 2011). Under neutral conditions, the bond between sulfur and glucose is cleaved, and the aglycone moiety gives rise to sulfate and produces isothiocyanates. However, at lower pH, a sulfur loss from the molecule leads to formation of nitriles. The presence of Fe^{2+} or thiol compounds and epithio-specifier proteins increases the likelihood of nitrile formation (Uda *et al.*, 1986; Lambrix *et al.*, 2001; Burow and Wittstock, 2009). Epithionitrile formation requires the same conditions as those for nitriles plus the presence of terminal unsaturation of the R group. Another product, organic thiocyanate, is sometimes produced. Thiocyanate production is controlled by the presence of a specifier R group (Uda *et al.*, 1986; Lambrix *et al.*, 2001; Burow and Wittstock, 2009; Wittstock, 2011). Indole and 4-hydroxybenzyl glucosinolate yield thiocyanate (Agerbirk *et al.*, 2009). The formation of thiocyanate from IGs occurs over a wide pH range, whereas 4-hydroxybenzyl glucosinolates yield thiocyanate only at a more basic pH (Agerbirk *et al.*, 2009). Glucosinolates with β-hydroxylated side chains spontaneously cyclize to yield the oxazolidine-2-thiones (Wittstock, 2011).

Among breakdown products of glucosinolates, isothiocyanates have been most frequently shown to be toxic to both generalist and specialist insects (Lichtenstein *et al.*, 1962; Seo and Tang, 1982; Li *et al.*, 2000; Agrawal and Kurashige, 2003). The toxicity of isothiocyanates results from their electrophilic nature, which leads to their interaction with proteins (Kawakishi and Kaneko, 1987; Hanschen *et al.*, 2012). As isothiocyanates are

relatively lipophilic, they can cross cellular membranes and reach the intracellular environment where they react nonspecifically and irreversibly with nucleophiles such as glutathione (GSH) and/or proteins through interacting with sulfhydryl groups, disulfide bonds, and amines (Kawakishi and Kaneko, 1987). Also, cyanide inactivates certain enzyme systems, especially those involved in cellular respiration such as cytochrome oxidase (Johnson, 1987). Although reactions of isothiocyanates with amino acids and proteins leading to inactivation of enzymes are demonstrated *in vitro* (Kawakishi and Kaneko, 1987), their mode of action in insects is not studied *in vivo*. Toxicity of organic thiocyanates and simple nitriles, likely mediated by the cyano group, is smaller than isothiocyanates and has occasionally been reported to be effective against insect herbivores (Wadleigh and Yu, 1988; Peterson *et al.*, 1998), whereas to date the toxicity of epithionitriles on insects has not been documented.

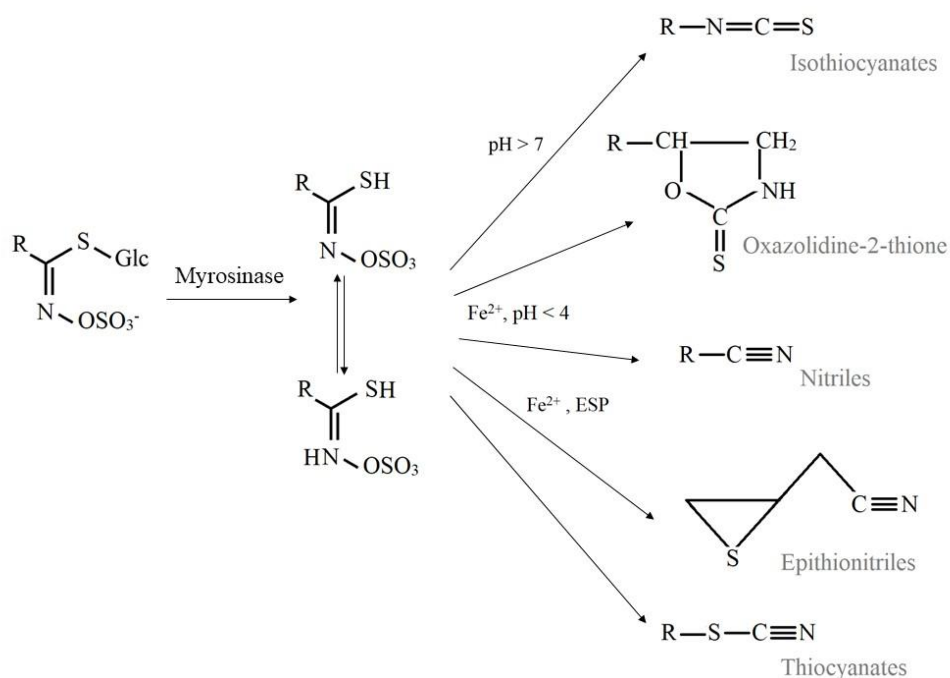


Figure 1-3 Hydrolysis of glucosinolates by myrosinases. Rearrangement and conversion of the unstable aglucone to the corresponding breakdown products affected by presence of specifier proteins, pH or presence of specific ions. ESP, epithiospecifier proteins (adapted from Halkier and Gershenzon, 2006).

1.3.3 Spatial and temporal distribution of glucosinolates and myrosinases

Since the distribution of glucosinolates in an individual plant relative to myrosinases significantly affects hydrolysis to bioactive products, determination of the accumulation sites of the glucosinolates in the plant is of great importance. Glucosinolates and myrosinases can be found in various plant organs and in all developmental stages (Bridges *et al.*, 2002; Andréasson and Jørgensen, 2003; Brown *et al.*, 2003). However, composition and concentration of glucosinolates varies significantly between plant organs and stages (Kliebenstein *et al.*, 2001; Brown *et al.*, 2003; Van Dam *et al.*, 2009). In Arabidopsis seedlings, the highest concentration of glucosinolates is demonstrated in the cotyledons (Van Dam *et al.*, 2009). The level of glucosinolates in vegetative plants is higher in roots compared to leaves (Van Dam *et al.*, 2009). Brown *et al*. showed that, in the Columbia ecotype of Arabidopsis, the highest concentration of glucosinolates can be observed in reproductive organs, followed by young leaves, while the lowest concentration of glucosinolates could be found in senescing rosette leaves (Brown *et al.*, 2003). The differences in glucosinolate level among stages is suggested to be due to adaptive allocation patterns to provide the optimal protection for the most valuable plant organs against herbivory (Van Dam *et al.*, 1996; Brown *et al.*, 2003).

Distribution of the myrosinase-glucosinolate system in plant tissues is still unclear although it has been investigated for a long time (Thangstad *et al.*, 1990; Bones and Rossiter, 1996; Andréasson *et al.*, 2001; Bridges *et al.*, 2002; Müller, 2009). To avoid the toxic effect of glucosinolate hydrolysis products in an intact plant, myrosinases should be kept separated from glucosinolates (Bones and Rossiter, 1996; Müller, 2009). Nevertheless, rapid and direct contact of glucosinolates and myrosinases should be allowed upon occurrence of damage to plant tissue. Myrosinases are synthesized at the rough endoplasmic reticulum and stored in vacuoles of single cells referred to as myrosin cells (Thangstad *et al.*, 1990; Andréasson *et al.*, 2001; Bridges *et al.*, 2002; Andréasson and Jørgensen, 2003; Carter, 2004; Wittstock, 2011). Myrosin cells are specialized idioblast cells that are scattered within different tissues at low frequencies of 2-5% (Thangstad *et al.*, 1990; Andréasson and Jørgensen, 2003). In the Arabidopsis, myrosin cells are found

in the phloem parenchyma in leaves, stem, inflorescences, and in guard cells (Andréasson *et al.*, 2001; Husebye *et al.*, 2002). These cells are localized separately but directly neighboring glucosinolate-containing cells (Husebye *et al.*, 2002; Andréasson and Jørgensen, 2003).

Although glucosinolates can be found throughout the tissue, they accumulate in vacuoles of specialized sulfur-rich cells called S-cells (Koroleva *et al.*, 2000; Bridges *et al.*, 2002; Andréasson and Jørgensen, 2003). Koroleva *et al.* described these cells for the first time in Arabidopsis flower stalks, where they were situated between vascular bundles and the endodermis (Koroleva *et al.*, 2000). Glucosinolates were estimated to account for 84% of the S-cell sulfur content with a high concentration of aliphatic and indole glucosinolates (more than 100 mM) (Koroleva *et al.*, 2000). Glucosinolate-containing S-cells were later found in the epidermis, along the midvein of rosette leaves, the midvein and lateral veins of cauline leaves, outer margin of mature rosette leaves and all main veins of pedicle and siliques of Arabidopsis (Shroff *et al.*, 2008; Koroleva *et al.*, 2010). Glucosinolate biosynthesis occurs in the vascular tissues suggesting the involvement of transport processes (Du and Halkier, 1998; Reintanz *et al.*, 2001; Chen *et al.*, 2003; Redovniković *et al.*, 2008). Biosynthetic enzymes, CYP79F2 and CYP79F1, are demonstrated to be localized in the endoplasmic reticulum, while side-chain elongation is suggested to be localized in chloroplasts (Reintanz *et al.*, 2001; Falk *et al.*, 2004; Textor *et al.*, 2007). In some cases, myrosinases are detected in S-cells that are believed to be localized in a subcellular compartment different to that of glucosinolates (Koroleva and Cramer, 2011).

1.3.4 Effect of glucosinolates on plant-herbivore interaction

Although the effect of glucosinolates and their breakdown products is documented on a wide range of herbivores (Hopkins *et al.*, 2009; Winde and Wittstock, 2011), this effect depends on the class of glucosinolate and the type of herbivore (Halkier and Gershenzon, 2006; Hopkins *et al.*, 2009; Badenes-Perez *et al.*, 2013). Using transgenic plants devoid of one or more classes of glucosinolates, it has been demonstrated that the growth and development of chewing herbivores are affected by isothiocyanates derived from AGs, BGs and/or IGs (Beekweelder *et al.*, 2008; Schlaeppi *et al.*, 2008; Müller *et al.*, 2010; Sarosh *et al.*, 2010). All three classes of glucosinolates (AGs, BGs and IGs) affect two

generalist caterpillars, the African cotton leafworm, *Spodoptera littoralis* (Lepidoptera) and the beet armyworm, *Spodoptera exigua* (Lepidoptera) but to different extents, so that the effects of AGs and BGs are stronger than that of IGs (Schlaeppi *et al.*, 2008; Müller *et al.*, 2010). Both AGs (Beekweelder *et al.*, 2008) and IGs (Schlaeppi *et al.*, 2008) affect the cabbage moth, *Mamestra brassicae* (Lepidoptera) while the larvae of the cabbage looper, *Trichoplusia ni* (Lepidoptera) and the tobacco hornworm, *Manduca sexta* (Lepidoptera) are only affected by AGs. Despite the documents indicating the negative effect of glucosinolates on specialists, these herbivores can usually avoid the formation of isothiocyanates and dismantle the effect of plant glucosinolates (Müller *et al.*, 2001; Ratzka *et al.*, 2002). Some specialists even use glucosinolates as feeding or oviposition stimulants (Hilker and Meiners, 2002; Marazzi and Städler, 2004; Barker *et al.*, 2006; Badenes-Perez *et al.*, 2013).

Compared to chewing herbivores, arthropods with piercing sucking mouthparts do not mediate as much tissue damage during feeding. Therefore, it is believed that they can avoid the hydrolysis and, consequently, the negative effect of glucosinolates. However, IGs are less stable than other glucosinolates and can be hydrolyzed independent of myrosinases (Agerbirk *et al.*, 2009). Therefore, compared to AGs, IGs are suggested to be more involved in deterring the herbivory of sucking herbivores (Kim and Jander, 2007; Kim *et al.*, 2008; De Vos and Jander, 2009). Kim *et al*. showed that although the generalist green peach aphid, *Myzus persicae* (Hemiptera), takes up intact glucosinolates while feeding on Arabidopsis, IGs are broken down in the aphid body (Kim *et al.*, 2008). Hydrolysis products act as antifeedants while unchanged AGs are excreted in the honeydew with no significant negative effect on the aphid (Kim and Jander, 2007; Kim *et al.*, 2008). Also, an IG-induced reduction in performance of *T. urticae* was reported (Zhurov *et al.*, 2013) which demonstrates a shorter developmental time and lower mortality of *T. urticae* larvae on an IG-devoid genotype of Arabidopsis (Zhurov *et al.*, 2013).

Different responses to various types of glucosinolates due to the different feeding behaviors of chewing and sucking herbivores reflect the specific distribution and induction of classes of glucosinolates (Bones and Rossiter, 1996; Siemens and Mitchell-Olds, 1996; Kliebenstein *et al.*, 2001; Shroff *et al.*, 2008). The effectiveness of the glucosinolate

breakdown products has been shown in various bioassays using artificial diets supplemented with the breakdown products as well as by comparing performance of herbivores on wild-type and glucosinolate-deficient transgenic plants (Li *et al.*, 2000; Kim and Jander, 2007; Müller *et al.*, 2010; Schramm *et al.*, 2012). Although numerous studies have been performed on the biosynthesis and biological activities of IGs in recent years, the modes of action of the toxic IGs breakdown products in herbivores remain to be identified. Also, our understanding of metabolic counter-adaptations of plants and herbivores, specifically neutralizing the glucosinolate-based defense of plants by herbivores, is very limited.

1.3.5 Indole glucosinolates and their breakdown products

IGs are the second most abundant glucosinolates in nature after AGs. The biosynthesis and function of IGs have been of interest in recent years due to their abundance in the model plant Arabidopsis. The four most common IGs in Brassicaceae plants are indole-3-ylmethyl glucosinolate (IMG), 1-methoxyIMG (1mIMG), 4-hydroxy IMG (4hIMG) and 4-methoxy IMG (4mIMG) [(Figure 1-4 (Fahey *et al.*, 2001; Kliebenstein *et al.*, 2001; Windsor *et al.*, 2005; Agerbirk and Olsen, 2012)]. These different groups of IGs are not distributed equally in different parts of plants (Windsor *et al.*, 2005; Shroff *et al.*, 2008; Van Dam *et al.*, 2009). IMG is generally predominant in Arabidopsis leaves while 4hIMG and IMG are the main IGs in seeds (Bennett *et al.*, 2004; Windsor *et al.*, 2005).

As in the case of other glucosinolates, IGs are degraded when they become in contact with myrosinases upon plant tissue disruption. Enzymatic hydrolysis of IGs leads to formation of an unstable aglucone (indole-3-ylacetothiohydroximate-O-sulfonate in the case of IMG), which in turn gives rise to the corresponding isothiocyanates and nitriles (Agerbirk *et al.*, 2009). The prerequisites for rearrangement of the aglucone to isothiocyanates or conversion to nitriles are the same as those for other glucosinolates (Agerbirk *et al.*, 2009). Formation of epithionitriles and organic thiocyanates are not reported for IGs (Agerbirk *et al.*, 2009).

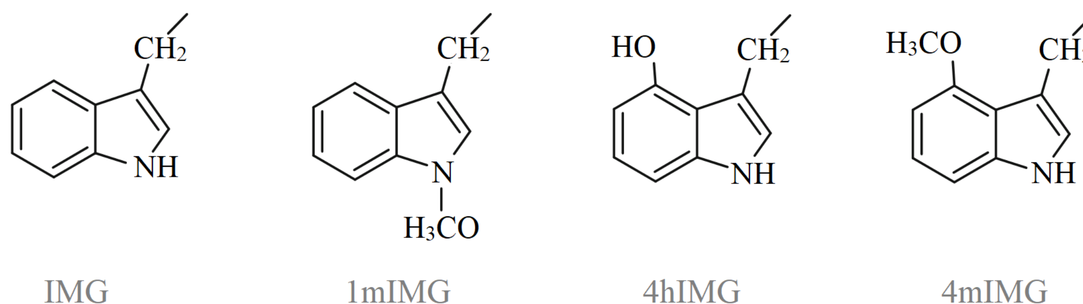


Figure 1-4 Structure of the R-group of the four common indole glucosinolates in Arabidopsis: indole-3-ylmethyl glucosinolate (IMG), 1-methoxyIMG (1mIMG), 4-hydroxy IMG (4hIMG) and 4-methoxy IMG (4mIMG) (adapted from Agerbirk *et al.*, 2009).

The breakdown of IGs in plants is partially different from that of AGs since the IG-derived isothiocyanates are unstable and react with nucleophiles with loss of a thiocyanate ion (SCN^-) (Agerbirk *et al.*, 2009; Wittstock, 2011). The IMG-derived isothiocyanate, indole-3-ylmethyl isothiocyanate (IMI), has not been detected during IG hydrolysis due to its high reactivity (Bryan Hanley and Parsley, 1990; Agerbirk *et al.*, 2009). The two main downstream products of IMI are ascorbigen and indole-3-carbinols (I3C), which may in turn react with nucleophiles (Tsagkarakou *et al.*, 1998; Burow and Wittstock, 2009). Ascorbigen can be formed as a result of the immediate reaction of IMI or the slower reaction of I3C with ascorbate (Agerbirk *et al.*, 1998). In leaf homogenates, ascorbigen is seven times more concentrated than I3C, while in absence of ascorbic acid, the main end product of IGs hydrolysis is I3C (Agerbirk *et al.*, 1998, 2009; Buskov *et al.*, 2000). Pedras *et al.*, showed that in turnip roots the main hydrolysis products of isotopically labeled IMG were indole-3-carboxaldehyde and indole-3-carboxylate, both of which can be formed through oxidation of I3C or oxidative chain shortening of IAN (Pedras *et al.*, 2002; Agerbirk *et al.*, 2009). In neutral conditions, I3C is slowly converted to 3,3′-diindolylmethane (DIM) (Agerbirk *et al.*, 2009). I3C reaction with the thiols, cysteine and glutathione may lead to production of S-indol-3-ylmethylcysteine and S-indol-3-ylmethylglutathione adducts independent of myrosinase activity (Buskov *et al.*, 2000; Staub *et al.*, 2002). Formation of ethers and reaction of I3C with amino acids and peptides are also reported (Agerbirk *et al.*, 1998; Buskov *et al.*, 2000; Kim *et al.*, 2008). Unlike isothiocyanates, nitriles derived from IGs are relatively stable metabolites. The IMG-derive

nitrile, indole-3-acetonitrile (IAN), can also be produced independent of myrosinases e.g. via non-specific nitrilases (Vorwerk *et al.*, 2001; Pedras *et al.*, 2002).

Although the negative effect of IGs on spider mite performance is reported (Zhurov *et al.*, 2013), the mode of action of IGs or their breakdown products on spider mites is not well understood. Moreover, the mechanism of *T. urticae* adaptation to Arabidopsis remains to be studied. Investigation of the mechanism of *T. urticae* adaptation to IGs can be guided by a background on the general strategies that insect herbivores employ to dismantle the plant defense. The documented evidence of adaptation of chewing insects to glucosinolates (mainly AGs) might provide insight into the potential mechanisms of *T. urticae* adaptation to IGs (Ratzka *et al.*, 2002; Wittstock *et al.*, 2004; Winde and Wittstock, 2011; Schramm *et al.*, 2012).

1.4 Adaptation of herbivores to glucosinolates

The selection pressure that chemical plant defenses impose on herbivores leads to the evolution of counter-adaptations through which herbivores dismantle plant defenses (Karban and Agrawal, 2002; Després *et al.*, 2007; Alba *et al.*, 2011; Van Leeuwen and Dermauw, 2016), e.g. cope with deterrent compounds or neutralize phytotoxins produced by plants (Alba *et al.*, 2011). As suggested by Karban and Agrawal (Karban and Agrawal, 2002) herbivores might bypass plant defenses using three different strategies. The first and the most aggressive one is manipulation of plant defense. The second strategy is development of morphological or physiological changes in herbivores, which help them to better overcome plant defenses. The third and the least aggressive strategy is the behavioral mechanism when herbivore’s feeding or oviposition choices of host plant results in an increase in the herbivore performance. These strategies are explained in more detail in the following sections.

1.4.1 Mechanisms of adaptation of arthropods to plant defense

1.4.1.1 Suppression of plant defense

To hamper herbivore feeding, plants employ different defense strategies, which can be constitutive and/or induced by the presence or feeding of herbivores (Walling, 2000; Wu and Baldwin, 2010; Kant *et al.*, 2015). Constitutive defenses include morphological characteristics of plants and/or secondary metabolites that are synthesized by plants even in the absence of herbivore attack (Strauss *et al.*, 2002; Alba *et al.*, 2011). Induced defenses in plants may occur after sensing the presence of herbivores and through morphological changes, synthesis of toxins and antifeedants, hypersensitive responses or resource allocation (Kessler and Baldwin, 2002; Gómez *et al.*, 2012).

The defensive plant response occurs in three different steps: signaling, phytohormones and biosynthesis of secondary metabolites. The first step of plant defense against herbivores is signaling (Kessler and Baldwin, 2002; Zhu-Salzman *et al.*, 2005; Wu and Baldwin, 2010; Kant *et al.*, 2015), which happens through sensing the presence or feeding of herbivores by herbivore elicitors or their molecular patterns (Mithöfer *et al.*, 2005; Howe and Jander, 2008; Wu and Baldwin, 2010). The sensing of herbivore attack may lead to the biosynthesis of phytohormones that act as intermediates between the perception of herbivory and activation of plant defense (e.g. synthesis of secondary metabolites). The three most important phytohormones involved in induced plant defense against pathogens and herbivores are salicylic acid (SA), Jasmonic acid (JA) and ethylene (Et). JA, and specifically its bioactive form JA-isoleucine (JA-Il), plays a prominent role in plant defensive response to herbivores (Howe and Jander, 2008; Wu and Baldwin, 2010). It has been shown that any interference with the biosynthesis of JA leads to defense deficiencies in plants and as a result to improvement of herbivore performance. For example, in the whitefly, *B. tabaci*, suppression of tomato JA-based defenses through salivary compounds of whiteflies leads to an increase in the whitefly's survival and fecundity (Su *et al.*, 2015). Also, the feeding of *S. exigua* larvae with impaired salivary secretions resulted in less induction of JA defense in Arabidopsis compared to intact larvae (Weech *et al.*, 2008). In addition, the aster leafhopper, *Macrosteles quadrilineatus* (Hemiptera), uses an effector

produced by a vectored phytoplasma to suppress JA defenses, which in turn increases the fecundity of the leafhopper (Sugio *et al.*, 2011).

Suppression of plant defenses is well-documented in plant-pathogen interactions [e.g., (Kasschau and Carrington, 1998; Voegele and Mendgen, 2003; Abramovitch *et al.*, 2006; Kamoun, 2006)]. Herbivores can also develop the means to manipulate plant defensive responses so as to be able to overcome the defense (Ferry *et al.*, 2004; Alba *et al.*, 2011; Kant *et al.*, 2015). This manipulation can occur at any step of the defensive pathway and can block or reduce the level of defensive response (Alba *et al.*, 2011). Therefore, suppression of plant defense is characterized by reduction of biosynthesis of the defensive compound (Kant *et al.*, 2015). Musser *et al*. (2002) suggest that, although insect saliva can be recognized by plants and induce the plant defensive response, the saliva of corn earworm, *Helicoverpa zeae* (Lepidoptera), contains a glucose oxide that catalyzes the oxidation of glucose to D-gluconic acid, and as a result generates hydrogen peroxide, which in turn suppresses biosynthesis of nicotine (a defensive compound in tobacco that is induced in response to herbivory). Treating tomato leaves with *H. zea* saliva resulted in the reduction of adenosine-5′-triphosphate (ATP) levels in tomato leaves related to the high level of ATPase activity in saliva. It has been shown that these ATPase enzymes decreased biosynthesis of terpenoids through interfering with the JA and ethylene pathways in tomato leaves (Bede *et al.*, 2006; Wu *et al.*, 2012). In *S. littoralis*, not only saliva but also their eggs contain compounds that mediate suppression of the JA-pathway in Arabidopsis. The newly hatched larvae of *S. littoralis* take advantage of the locally suppression of this plant defense (Bruessow *et al.*, 2010).

Manipulation of plant defense is called suppression only if it leads to an increase in performance of the herbivore, e.g. by increasing the reproduction or body weight, or by decreasing the developmental time or mortality of the herbivore (Kant *et al.*, 2015). Suppression of Arabidopsis JA response by *B. tabaci* results in the shorter developmental time of the whitefly larvae (Zarate *et al.*, 2006). Also, *B. tabaci* can interfere with the JA response of lima beans. Consequently, it reduces the biosynthesis of (E)-b-ocimene, which in turn decreases the fecundity of *B. tabaci* (Zhang *et al.*, 2009). The larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera) gained more weight through

suppression of the tomato JA defense (Chung *et al.*, 2013). The interference of the Cotton mealybug, *Phenacoccus solenopsis* (Homoptera), with JA defense response resulted in suppression of Gossypol production in cotton, which led to shorter developmental time of the nymphs and weight gain in the adults (Zhang *et al.*, 2011). The green peach aphid, *M. persicae*, showed a higher fecundity after reducing the biosynthesis of indole glucosinolates in Arabidopsis through effector proteins in their saliva (Elzinga *et al.*, 2014).

Mites can also interfere with plant defenses. It has been shown that two species of spider mites, *Tetranychus evansi* and *T. urticae*, are able to suppress the induction of tomato defenses downstream of JA and SA and consequently increase their performance (Kant *et al.*, 2008; Sarmento *et al.*, 2011). Also, the tomato russet mite, *Aculops lycopersici*, showed population growth because of the suppression of the tomato JA defense (Glas *et al.*, 2014).

1.4.1.2 Adaptation of herbivores to xenobiotics

Due to the significant financial impact of pesticide resistance development in herbivores, a large part of the current knowledge of adaptations to plant secondary metabolites has originated from the pesticide resistance studies (Després *et al.*, 2007; Van Leeuwen and Dermauw, 2016). Although the mode of action of plant secondary metabolites is generally different to that of agrochemicals, the mechanism of adaptation to these two groups of xenobiotics is assumed to be similar as plant responses to agrochemicals and plant secondary metabolites greatly overlap (Dermauw *et al.*, 2013).

The adaptation of herbivores to xenobiotics can be achieved through two general mechanisms: pharmacokinetic and pharmacodynamic responses. Pharmacokinetic responses allow herbivores to avoid or minimize exposure to xenobiotics through sequestration, and/or detoxification of xenobiotics. Pharmacodynamic responses make herbivores less sensitive to xenobiotics by affecting interactions between xenobiotics and their target site (Kennedy and Tierney, 2013).

1.4.1.2.1 **Pharmacokinetic responses**

Detoxification

Three groups of detoxification enzymes have been shown to be involved in detoxification of xenobiotics by insects and mites: glutathione-S-transferases (GSTs), cytochrome P450 monooxygenases and esterases (Van Leeuwen *et al.*, 2010; Heckel, 2014; Heidel-Fischer and Vogel, 2015). The detoxification process of xenobiotics can be divided into three phases. In phase I, the xenobiotic is oxidized, hydrolyzed or reduced. In these reactions a nucleophilic functional group (a hydroxyl, carboxyl or amine group) is usually converted to a more hydrophilic side chain. P450s and esterases are the enzymes that are usually involved in phase I reactions. If the products of phase I reactions are not polar enough to be excreted, they will be modified by phase II reactions. In phase II, the polar products resulting from phase I are conjugated with a variety of endogenous molecules such as glutathione, sugar, sulfate, phosphate or amino acid. These conjugations increase the compound's polarity, which facilitates excretion. Enzymes that usually operate in phase II include GSTs and uridine-diphosphate-glycosyltransferases (UGTs). In phase III, metabolites of phase II are transported out of the cell by ATP-binding cassette (ABC) and solute carrier (SLC) family proteins (Heckel, 2014; Heidel-Fischer and Vogel, 2015; Kant *et al.*, 2015).

Adaptations that improve detoxification activity of herbivores are often mediated by gene amplification, upregulation or coding sequence mutations that increase the production of specific detoxification enzymes or enhance their catalytic activities (Després *et al.*, 2007; Li *et al.*, 2007; Brattsten, 2012). P450-mediated adaptations of herbivores to xenobiotics usually result from overexpression, amplification or upregulation by mutation in trans-regulatory loci (Li *et al.*, 2007; Yu *et al.*, 2015). The activity of P450s (expressed by the large family of CYP genes) against organic compounds through enhancing oxidative metabolism is rather nonspecific, which explains the observed cross-resistance to plant secondary metabolites and xenobiotics (Feyereisen, 2012; Schuler, 2012; Dermauw *et al.*, 2013). As one of the first documentations of the role of P450s in the adaptation of insects to xenobiotics, Krieger *et al.* suggested that higher activity of P450s in polyphagous compared to monophagous species is an indicator of the role of these enzymes in

detoxification of plant toxic products (Krieger *et al.*, 1971). Earlier evidence of the involvement of P450s in the adaptation of insects to plant secondary metabolites was shown for the detoxification of furanocoumarin by the black swallowtail butterfly, *Papilio polyxenes* (Lepidoptera) (Berenbaum, 1983). A wide range of plant secondary metabolites, including flavonoids, terpenoids, alkaloids and glucosinolates, can be oxidized by P450s of insects and mites (Després *et al.*, 2007; Feyereisen, 2012). Mao *et al*. (Mao *et al.*, 2007) showed that the larvae of *H. armigera* cannot grow in the presence of gossypol (the secondary metabolite in cotton) without the induction of P450 activities. They also indicated that growth on gossypol is correlated with expression of CYP6AE14 in the midgut of *H. armigera*. Suppression of CYP6AE14 expression using RNAi (ribonucleic acid interference) leads to a delay in larval growth (Mao *et al.*, 2007) and CYP6AE14 dsRNA transgenic cotton impairs the growth of *H. armigera* larvae and decreases plant damage (Mao *et al.*, 2011) consistent with the possibility that CYP6AE14 is required for gossypol detoxification.

Esterases are involved in the detoxification of xenobiotics that contain the ester linkage in their chemical structure (Yu *et al.*, 2015). This detoxification occurs through the hydrolysis of target substances. However, sometimes esterases have very limited catalytic properties and mediate resistance to xenobiotics through sequestration (Van Leeuwen *et al.*, 2009; Kant *et al.*, 2015). In this case, esterases are produced in large quantities, leading to the decreased availability of the xenobiotic, because esterases bind to it (Van Leeuwen *et al.*, 2009). The increase in activity of esterases occurs most commonly as a result of gene amplification and, in some cases, through gene upregulation (Wheelock *et al.*, 2005; Li *et al.*, 2007). The overproduction of esterases mediated by gene amplification is well documented in aphids and mosquitoes that are resistant to organophosphates (Devonshire and Field, 1991; Hemingway *et al.*, 1998). The resistance of the green peach aphid to organophosphate and carbamate pesticides was shown to be correlated to enhanced esterase activity in these aphids resulting from the gene amplification and overexpression of the coding gene (Srigiriraju *et al.*, 2009), whereas, in the mosquito, *Culex pipiens* (Diptera), overproduction of esterases is due to upregulation rather than gene amplification (Raymond, 1987). Although, the involvement of esterase activity in the resistance of insects and mites to several pesticides is well-documented, its role in adaptation to plant

defense is studied in only a few cases and just at the biochemical level (Després *et al.*, 2007; Li *et al.*, 2007). The inhibition of activity of detoxification enzymes in *Papilio glaucus* (Lepidoptera) showed involvement of esterases in the resistance of these insects to their host plants (Lindroth, 1989). In *S. lituralis*, exposure to rutin, a plant secondary metabolite, mediated a significant increase in the carboxylesterase activity of midgut (Ghumare *et al.*, 1989). The survival rate of the gypsy moth, *Lymantria dispar* (Lepidoptera), larvae that were fed on glycosides-containing artificial diet was correlated with esterase activity, suggesting the potential role of esterases in detoxification of glycosides (Lindroth and Weisbrod, 1991).

The GST superfamily is involved in phase II detoxification of various plant xenobiotics through substrate sequestration or by catalyzing the conjugation of glutathione to electrophilic toxic molecules. Conjugation to glutathione increases their solubility of the conjugated substrate, which in turn facilitates the excretion of the xenobiotic molecules from the insect body (Enayati *et al.*, 2005). Increase in GST activity is often induced by ingestion of xenobiotics. It has been shown that increases in enzymatic activity can be a result of gene amplification or more commonly through increases in transcriptional rate (Grant and Hammock, 1992; Ranson *et al.*, 2001). GST activity has been shown to be linked to the resistance of insects to all major classes of pesticides and in a number of cases to plant xenobiotics (Prapanthadara *et al.*, 1993; Huang *et al.*, 1998; VONTAS *et al.*, 2001; Enayati *et al.*, 2005). For example, detoxification of DDT as a result of dehydrochlorination is catalyzed by GSTs (Clark and Shamaan, 1984). Also, detoxification of other organochlorine pesticides mediated by GSTs is shown to be a result of conjugation to glutathione (Enayati *et al.*, 2005). The conjugation of glutathione to organophosphate insecticides results in their detoxification through the O-dealkylation or the O-dearylation of organophosphates (Oppenoorth *et al.*, 1979; Chiang and Sun, 1993). It is suggested that in *B. tabaci* and in the Drosophilid specialist fly, *Scaptomyza nigrita* (Diptera), GSTs are involved in developing tolerance to glucosinolates (Elbaz *et al.*, 2012; Gloss *et al.*, 2014).

Another class of conjugation enzymes involved in phase II detoxification are UGTs. These enzymes can conjugate the xenobiotics with UDP-glucose and convert lipophilic aglycones of the substrate into more hydrophilic glycosides, which can more easily be eliminated

from the insect body (Kant *et al.*, 2015). UGTs have been shown to be involved in the resistance of lepidopteran *Helicoverpa* species to the alkaloid capsaicin and in the detoxification of benzoxazinoids in the *Spodoptera* species (Ahn *et al.*, 2011; Wouters *et al.*, 2014).

Phase III detoxification has been documented in far less detail compared to phases I and II. It is suggested that in *M. sexta*, excretion of nicotine and other alkaloids is mediated by an ABC transporter (Gaertner *et al.*, 1998). Also, an ABC transporter is identified as a protector of lepidopteran nervous tissues by potentially acting as a cardenolide efflux carrier (Petschenka *et al.*, 2013). Downregulation of the major facilitator superfamily (MFS) in *M. sexta* after feeding on tobacco mutants that lack JA and upregulation of the same superfamily in *T. urticae* after being transferred to a more challenging host plant are other evidence of the involvement of transporters in the detoxification of plant metabolites (Govind *et al.*, 2010; De La Paz Celorio-Mancera *et al.*, 2013; Dermauw *et al.*, 2013).

Sequestration

Some herbivores can sequester ingested plant secondary metabolites in specialized tissues or in the integument (Willinger and Dobler, 2001) and subsequently use them for their own defense against their natural enemies (Ode, 2006), to protect themselves against UV light and photoactivated phytotoxins (Carroll *et al.*, 1997) or as pheromones (Nishida, 2002). A variety of mechanisms are necessary to enable the selective import the appropriate plant metabolite and to also transport and store them without negatively interfering with the physiological processes of the herbivore (Després *et al.*, 2007; Kant *et al.*, 2015). Thus, this complex scenario of adaptation does not occur very frequently. However, the successful uptake and reuse of some xenobiotics include pyrrolizidines (Hartmann, 1999), cardenolides (Malcolm and Brower, 1989), iridoid glycosides (Dyer and Deane Bowers, 1996), cyanogenic glycosides (Nahrstedt and Davis, 1986), and glucosinolates (Nishida, 2002). Xenobiotics can be sequestered directly or after being converted to less toxic products [e.g. through oxidation or conjugation (Opitz and Müller, 2009; Kant *et al.*, 2015)]. A group of leaf beetle (chrysomelids) larvae degrade plant phenol glycosides and use the amino acid-conjugated aglycones to secrete as their chemical defense against their natural enemies (Kuhn *et al.*, 2007). The larvae of the monarch butterfly, *Danaus*

plexippus (Lepidoptera), sequester cardenolides, a secondary metabolite of milkweed, and retain it in their bodies until the adult stage to make themselves unpalatable for their predators (Nishida, 2002).

1.4.1.2.2 **Pharmacodynamic responses**

Alteration in the structure of the xenobiotic target site in herbivores resulting from point mutation might reduce the affinity of the xenobiotic to the target site and lead to resistance of the herbivore to that xenobiotic (Van Leeuwen *et al.*, 2010; Feyereisen *et al.*, 2015). Although insensitivity of the pesticide target site is well-documented in the field of herbivore resistance to pesticides (Van Leeuwen *et al.*, 2010; Ffrench-Constant, 2013; Feyereisen *et al.*, 2015), few studies have shown this mechanism of adaptation in herbivore adaptation to phytochemicals. This lack of evidence of target-site resistance to plant metabolites likely arises due to multiple modes of action of phytochemicals and our limited knowledge about them (Després *et al.*, 2007; Kant *et al.*, 2015).

An example of structural modification of a phytochemical target-site is the substitution of amino acids in the target site of ouabain, a secondary metabolite of Apocynaceae plants, that results in resistance in insect orders such as Lepidoptera, Coleoptera, Heteroptera and Diptera to ouabain (Zhang *et al.*, 2009; Zhen *et al.*, 2012). The same substitution is responsible for the insensitivity of the cardenolide target site in some other insect species that are specialized in plants containing this metabolite (Dobler *et al.*, 2012; Dalla *et al.*, 2013). Also, the mechanism of adaptation of the bruchid beetle, *Caryedes brasiliensis* (Coleoptera), to L-canavanine, an amino acid found in leguminous plants, can be considered as an example of insensitivity of target site. L-canavanine acts by incorporating into proteins, resulting in the substitution with L-arginine. Beetles have evolved an arginyl-tRNA (transfer RNA) synthetase that can distinguish L-canavanine from L-arginine and as a result avoid the effect of L-canavanine (Leisinger *et al.*, 2013).

1.4.2 Adaptation to glucosinolates

Although the complexity of the glucosinolate-myrosinase system limits the ability of herbivores to adapt to plants containing glucosinolates, some herbivores can feed and survive on these plants (Fahey *et al.*, 2001; Brown *et al.*, 2003; Heckel, 2014; Pentzold *et*

al., 2014). Herbivores can overcome a two-component activated defense, such as the glucosinolate-myrosinase system, at different stages of the activation process (Pentzold *et al.*, 2014). They could either avoid the activation of the glucosides or decrease toxicity of the hydrolysis products (Pentzold *et al.*, 2014). A potential strategy of preventing the toxic effect of glucosinolates is by adopting feeding strategies that avoid the uptake of myrosinases and thus the hydrolysis of glucosinolates (Francis *et al.*, 2001; Müller *et al.*, 2001; Aliabadi *et al.*, 2002; Nishida, 2002; Beran *et al.*, 2014). Also, herbivores can change the core structure of the glucosinolates and, consequently, avoid their hydrolysis by myrosinases (Ratzka *et al.*, 2002; Falk and Gershenzon, 2007; Malka *et al.*, 2016). If glucosinolates are broken down, toxic hydrolysis products can be either neutralized (Kim *et al.*, 2008; Schramm *et al.*, 2012), or the hydrolysis reaction can be modified by insect to produce less toxic metabolites (Wittstock *et al.*, 2004). These strategies will be discussed in this section, which is divided into general detoxification mechanisms and specialized adaptations.

1.4.2.1 General glucosinolate detoxification mechanisms

Since generalist herbivores feed on a wide range of host plants and therefore are challenged with a diverse range of plant defense metabolites, they need efficient adaptation strategies that are effective against a variety of different xenobiotics (Després *et al.*, 2007; Lankau, 2007; Kant *et al.*, 2015; Van Leeuwen and Dermauw, 2016; Rioja *et al.*, 2017). Many plant secondary metabolites are lipophilic and can undergo a broad range of enzymatic xenobiotic detoxification reactions to increase their polarity for easier excretion (Kant *et al.*, 2015; Van Leeuwen and Dermauw, 2016). The most common break down product that is produced as a result of feeding of generalist chewing herbivores is isothiocyanate formed from AGs and BGs (Yu, 1989; Schramm *et al.*, 2012; Zou *et al.*, 2016). Since isothiocyanates are very reactive, the general detoxification pathway that is reported for them is mostly phase II conjugation reactions (Brown and Hampton, 2011).

The feeding of piercing-sucking herbivores on plants does not mediate severe damage in plant tissue like in the case of chewing insect herbivory. Therefore, it might be expected that piercing-sucking herbivores evade the toxic effects of glucosinolates (Kim and Jander,

2007). However, it has been shown that in green peach aphids, *M. persicae*, IGs are hydrolyzed independently of myrosinases, which results in a strong antifeedant effect on aphids, whereas AGs are excreted intact in their honeydew (Kim and Jander, 2007; Kim *et al.*, 2008). Therefore, it is suggested that IGs are more important for plant defense against piercing-sucking herbivores compared to AGs and BGs (De Vos and Jander, 2009). The general mechanism of detoxification of glucosinolates by chewing and piercing-sucking herbivores is discussed in more detail below.

1.4.2.1.1 **Chewing Insects and Conjugation of Isothiocyanate**

Isothiocyanates that are produced from the breakdown of glucosinolate are lipophilic and very reactive electrophiles (Enayati *et al.*, 2005; Brown and Hampton, 2011). They can enter cells through passive diffusion and their central electrophilic carbon can react with biological nucleophiles of the cell, such as the amine group of lysine residues of proteins and the thiol groups of cysteine and glutathione (Brown and Hampton, 2011). The produced electrophilic metabolites can be detoxified via conjugation with glutathione (Enayati *et al.*, 2005). Glutathione is an important reducing agent and a biological nucleophile in cells and a tripeptide (Glu-Cys-Gly) with a g-peptide bond between the amine of the Cys-side chain and carboxyl group of the Glu-side chain (Habig *et al.*, 1974; Brown and Hampton, 2011). The conjugation of glutathione happens due to the addition of the thiol at the Cys-side chain to the electrophilic center of isothiocyanates (Figure 1-5). This conjugation can occur spontaneously or be catalyzed by GSTs. The formed conjugates then undergo hydrolysis by the mercapturic acid pathway to produce the Gly-Cys- and Cys-conjugates. Cys-conjugates might be further N-acetylated (Habig *et al.*, 1974). The resulting conjugates and derivatives of the mercapturic acid pathway are more polar compared to the original isothiocyanates and as a result can be excreted more easily from the cell. The transporters involved in this process are not currently known. Nitriles formed from glucosinolates hydrolysis are not as electrophilic as isothiocyanates and are metabolized differently. Feeding on glucosinolates also leads to the induction of genes encoding phase I and II detoxification enzymes, including UGTs and cytochrome P450s (Deng *et al.*, 2009; Huang *et al.*, 2011).

The glutathione conjugation of isothiocyanates has been shown in several arthropod species, mollusks and mammals including humans (Wadleigh and Yu, 1988; Kassahun *et al.*, 1997; Janobi *et al.*, 2006; Schramm *et al.*, 2012; Falk *et al.*, 2014; Gloss *et al.*, 2014). Although involvement of insect GSTs in the detoxification of isothiocyanated was first reported almost 30 years ago (Yu, 1989), the identification of the products formed (Schramm *et al.*, 2012) and their function *in vivo* (Zou *et al.*, 2016) have been reported only recently. Analysis of cDNA sequences of the midgut tissue of *S. litura* showed higher expression of eight GSTs (Deng *et al.*, 2009; Huang *et al.*, 2011). RNA-mediated silencing of a GST gene in *S. litura* larvae impaired the consumption of brown mustard leaves, *Brassica juncea*, resulting in decreased bodyweight compared to a control group. It demonstrated the involvement of the encoded enzyme in the detoxification of isothiocyanates.

1.4.2.1.2 **Piercing-Sucking Insects and Indolic Glucosinolates**

Compared to chewing herbivores, piercing-sucking arthropods cause only minimal tissue damage during feeding as they guide their stylets through an apoplast towards the phloem in the case of aphids feeding (Tjallingii and Esch, 1993). Therefore, piercing-sucking insects are expected to avoid the negative effects of glucosinolate hydrolysis by myrosinases. However, in aphids, IGs undergo hydrolysis in the aphid gut independently of myrosinase activity and form nitriles, alcohols and unstable isothiocyanates that can be metabolized further by both phase I and phase II detoxification reactions (Agerbirk *et al.*, 2009). Also, feeding of *M. persicae* induced the biosynthesis of IGs, supporting the defensive role of IGs against aphids (Bodnaryk, 1994; Kim and Jander, 2007; Agerbirk *et al.*, 2009).

The degradation of IGs results in the formation of indole acetonitriles (IAN) or the corresponding isothiocyanates, which are unstable and react with nucleophiles to form conjugates or I3C in aqueous conditions (Figure 1-6). I3C itself undergoes several further alterations, e.g. it can be oxidized to the related aldehyde and carboxylic acid, dimerized, or conjugated with amino acids, glutathione or ascorbate (Agerbirk *et al.*, 2009). In *M. persicae*, there is myrosinase activity in the gut that could be involved in the degradation of indole glucosinolates (Ramsey *et al.*, 2010). The breakdown products detected in the

honeydew include amino acid and glutathione conjugates, which could illustrate active detoxification products. GST-like genes were identified in the gut of *M. persicae* and the pea aphid, *Acyrthosiphon pisum*, a Fabaceae specialist, suggesting that GSTs play a role in the metabolism of IGs (Ramsey *et al.*, 2010). Also, the activity of GSTs is increased in *M. persicae* in response to feeding on glucosinolate- or isothiocyanate-containing diets (Francis *et al.*, 2001). However, some indolic-derived detoxification products of I3C, specifically its conjugates with glutathione and cysteine, resulted in a significant decrease in aphid reproduction (Kim *et al.*, 2008). Our understanding of modes of action and biological targets of the IGs breakdown products, as well as any metabolic counter-adaptations on the part of herbivores, is very limited.

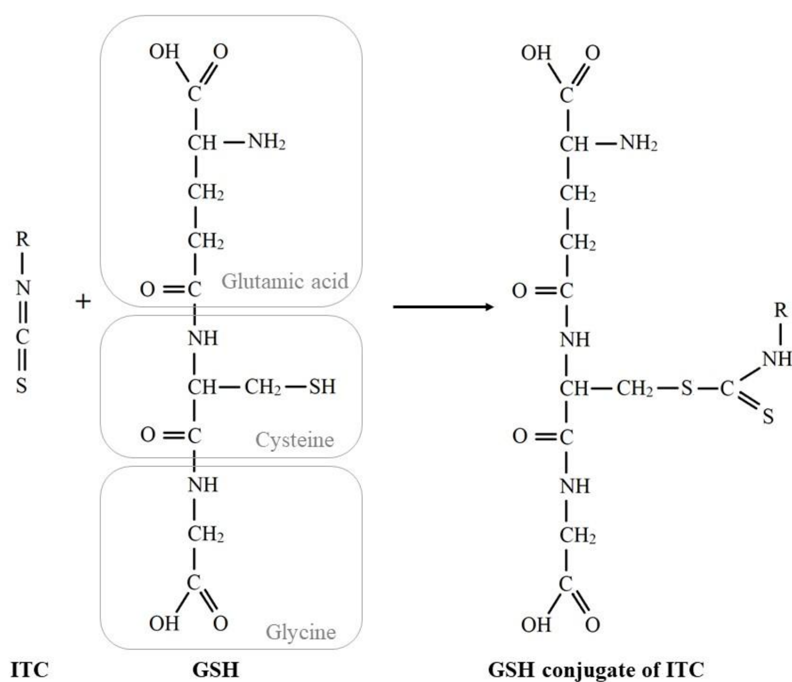


Figure 1-5 Conjugation of glutathione (GSH) to isothiocyanates (ITC) (adapted from Brown and Hampton, 2011; Kim et al., 2008).

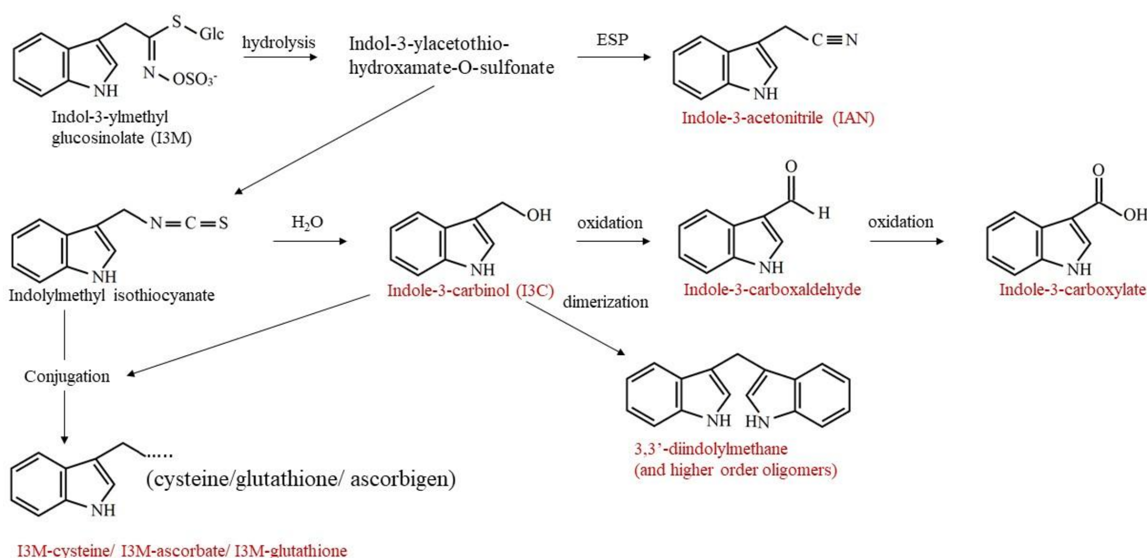


Figure 1-6 Hydrolysis of indole-3-ylmethyl glucosinolate and its breakdown products. (adapted from Kim *et al*., 2008).

1.4.2.2 Specialized detoxification strategies for glucosinolates

Specialized strategies to dismantle plant glucosinolates are expected to be found in oligophagous or monophagous feeders that are adapted to one plant family or species, and so are challenged with a limited range of plant defenses (Müller *et al.*, 2001; Ratzka *et al.*, 2002; Wittstock *et al.*, 2004). However, there is evidence that some polyphagous insects employ some of the strategies found in specialist feeders to neutralize the plant defense (Falk and Gershenzon, 2007; Malka *et al.*, 2016).

Compared to generalists, the performance of Brassicaceae-specialized insects usually is not severely affected after feeding on glucosinolate-containing plants (Li *et al.*, 2000; Harvey *et al.*, 2007; Gols *et al.*, 2008; Müller *et al.*, 2010; van Geem *et al.*, 2014). Some even take advantage of glucosinolates to find food or choose oviposition sites (Hilker and Meiners, 2002; Barker *et al.*, 2006; Badenes-Perez *et al.*, 2013). The adaptation of specialists to glucosinolates allows them to feed successfully on plants containing this

metabolite, which leads to major damage to agriculturally important Brassicaceae plants such as cabbage and broccoli (Ahuja *et al.*, 2009).

Three specialized processes of the metabolism of glucosinolates have been well characterized: 1) redirection of glucosinolate breakdown towards formation of less toxic products via specifier protins; 2) Modification of the core structure of glucosinolates using sulfatases; and 3) sequestration of glucosinolates (Müller *et al.*, 2001; Aliabadi *et al.*, 2002; Francis *et al.*, 2002; Ratzka *et al.*, 2002; Wittstock *et al.*, 2004; Beran *et al.*, 2014). To date, the first strategy is only found in the *Pieris* species (Wheat *et al.*, 2007), while the other two have been reported in some generalist herbivores as well (Falk and Gershenzon, 2007; Malka *et al.*, 2016). These processes are explained in more detail in the following sections.

1.4.2.2.1 **Specifier Proteins**

The adaptation mechanism that enables Pierid butterflies to feed on Brassicaceae plants was the evolution of a nitrile-specifier protein (NSP) in the gut of larvae. This protein allows Pierid larvae to overcome the glucosinolate-myrosinase system of the Brassicaceae plants by redirecting the hydrolysis of glucosinolates to produce nitriles, which are less toxic and reactive compared to noxious isothiocyanates (Wittstock *et al.*, 2004). These nitriles may be excreted either unchanged or after further metabolism. To date, NSP proteins have only been found in Pierid species while generalist insects usually do not harbor any NSP activity (Wheat *et al.*, 2007; Winde and Wittstock, 2011; Edger *et al.*, 2015).

It has been shown that aliphatic nitriles are excreted unchanged in *P. rapae* larval feces, whereas nitriles formed from benzenic glucosinolates may be excreted after further metabolism of the nitrile group and/or the benzenic side chain (Wittstock *et al.*, 2004; Agerbirk *et al.*, 2010; Winde and Wittstock, 2011). In *P. rapae* feces, the glycine and isoserine conjugates of indole-3-carboxylic acid are found, and are thought to be derived from I3M mediated by a nitrilase action rather than NSPs (Vergara *et al.*, 2006).

Although Pierid larvae evidently have an efficient strategy to avoid toxic ITCs, the formation of cyanide (‘cyanide bomb’) during the metabolism of benzenic GLS could also result in toxicity (Stauber *et al.*, 2012). However, *P. rapae* larvae can effectively detoxify

cyanide to nontoxic b-cyanoalanine and thiocyanate using cyanoalanine synthase and rhodanese, respectively (Stauber *et al.*, 2012).

1.4.2.2.2 **Glucosinolate Sulfatases**

Another strategy in evading the negative effect of glucosinolates is to modify them so that they are unrecognizable as substrates for myrosinases. In this strategy, intact glucosinolates are converted to desulfo-glucosinolates by the enzyme, glucosinolate sulfatase (GSS) (Figure 1-7). Plant myrosinases bind the sulfate moiety of glucosinolates in their active site, but do not recognize desulfo-glucosinolates as substrates (Matile, 1980). Moreover, the free sulfate that is released as a result of GSS activity inhibits myrosinase activity (Shikita *et al.*, 1999).

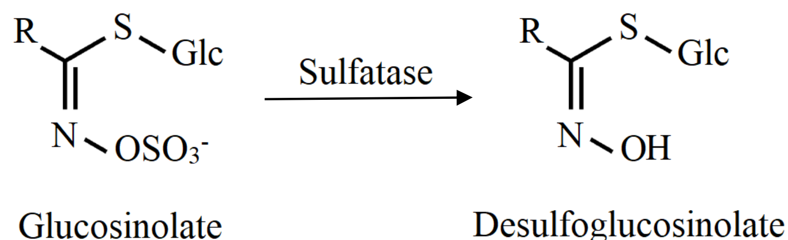


Figure 1-7 Conversion of glucosinolates to desulfo-glucosinolates catalyzed by glucosinolate sulfatase (adapted from Jeschke *et al*., 2015).

The desulfation of glucosinolates has been reported both *in vivo* and *in vitro* in different orders of insects (Ratzka *et al.*, 2002; Falk and Gershenzon, 2007; Wheat *et al.*, 2007; Opitz *et al.*, 2010). However, the only insects shown to elude the glucosinolate-myrosinase system using GSS are the diamondback moth, *P. xylostella* and the desert locust, *Schistocerca gregaria* (Orthoptera) (Falk and Gershenzon, 2007; Wheat *et al.*, 2007). Desulfo-glucosinolates were found in feces of *P. xylostella* (Ratzka *et al.*, 2002). The constitutive expression of GSS gene has been shown only in larval stages of *P. xylostella* that actively feed on glucosinolate-containing plants but not in other stages. As GSS affects glucosinolates with widely different structures, *P. xylostella* larvae are not affected by changes in the glucosinolate content (Li *et al.*, 2000; Sarosh *et al.*, 2010). The polyphagous locust, *S. gregaria*, that feeds on the West African plant, *Schouwia purpurea*, (containing high amounts of glucosinolates) without detrimental effects, was shown to have GSS

activity as well (Mainguet *et al.*, 2000; Falk and Gershenzon, 2007). Almost all the glucosinolate ingested by *S. gregaria* is excreted in feces as desulfo-glucosinolate. Also, upon feeding on glucosinolate, levels of GSS in *S. gregaria* increased 10 times (Falk and Gershenzon, 2007). To date, GSS activity has not been reported in any glucosinolate-specialist arthropod species. No GSS activity was identified in gut extracts of thirteen Pierid species that are specialized on the glucosinolate-containing plants (Wheat *et al.*, 2007).

1.4.2.2.3 **Sequestration**

Some insects have evolved means to sequester intact glucosinolates and efficiently exploit them for their own defense against natural enemies (Müller *et al.*, 2001; Aliabadi *et al.*, 2002; Francis *et al.*, 2002; Beran *et al.*, 2014; Heckel, 2014). The sequestration of glucosinolates has been reported in insect species of different orders, including the piercing-sucking specialist cabbage aphid, *Brevicoryne brassicae* (Hemiptera), (Francis *et al.*, 2001), the chewing specialist feeder turnip sawfly, *A. rosae* (Hymenoptera) (Müller *et al.*, 2001), the striped flea beetle, *Phyllotreta striolata* (Coleoptera) (Beran *et al.*, 2014) and the harlequin cabbage bug *Murgantia histrionica* (Hemiptera) (Aliabadi *et al.*, 2002).

Arthropods that adapt to plant glucosinolates through sequestration should have an efficient system to avoid autotoxicity via: 1) uptaking glucosinolates before being degraded by myrosinases, and/or 2) obtaining a storage site in which breakdown of glucosinolate is prevented until this is required for defense (Müller, 2009). Larvae of the sawfly *A. rosae* store glucosinolates exclusively in their hemolymph and upon the predator attack release it as droplets on the integument (Boevé and Schaffner, 2003; Müller and Wittstock, 2005 a; Opitz *et al.*, 2010). Larvae of *A. rosae* are tolerant to a wide range of glucosinolate and myrosinase concentrations (Abdalsamee *et al.*, 2014). The fact that glucosinolate uptake happens in the front part of the *A. rosae* gut, where the lowest myrosinase activity is observed, suggests that *A. rosae* larvae have a fast glucosinolate uptake mechanism, as well as a strategy to inhibit myrosinases (Müller and Sieling, 2006; Abdalsamee *et al.*, 2014). Different glucosinolates are not sequestered in a similar proportion so that AGs and BGs are found in higher concentrations compared to that in the host plant, while IGs are detected in minute amounts (Müller and Wittstock, 2005 a; Opitz *et al.*, 2010; Abdalsamee and

Müller, 2012). It is suggested that IGs might undergo the spontaneous breakdown before uptake (Kim *et al.*, 2008).

Some herbivores including the specialist aphid, *B. brassicae*, and the striped flea beetle *P. striolata*, harbor their own specific myrosinases that convert glucosinolates into their toxic breakdown products (Beran *et al.*, 2014). However, insect myrosinases, which are different from the Brassicaceae myrosinases, should be kept separately from glucosinolates to avoid autotoxicity (Husebye *et al.*, 2005; Beran *et al.*, 2014). In aphids, the myrosinase is stored in a non-flight muscle, while glucosinolates are sequestered in the hemolymph (Jones *et al.*, 2001; Pontoppidan *et al.*, 2001; Bridges *et al.*, 2002; Francis *et al.*, 2002; Husebye *et al.*, 2005). The processes that are involved in glucosinolate uptake, accumulation and excretion are poorly described in the literature related to glucosinolate-sequestering insects, which necessitates their further study.

1.4.3 Adaptation of *T. urticae* to indole glucosinolates

Numerous studies have been performed on the biosynthesis and biological activities of IGs recently, demonstrating the negative effect of IGs on herbivores, mostly piercing-sucking insects [reviewed in (Halkier and Gershenzon, 2006; Hopkins *et al.*, 2009)]. Two-spotted spider mites, similar to piercing-sucking insects, use their stylets to feed from the plant. Therefore, they are expected to be affected by IGs more than AGs or BGs. It is shown that spider mites cannot increase their population on Arabidopsis. IGs are responsible for the poor performance of *T. urticae* on Arabidopsis, shown by a significant increase in mortality and developmental time of *T. urticae* larvae (Zhurov *et al.*, 2013). However, the modes of action of IGs and their breakdown products that are toxic for *T. urticae* are not known. Since spider mite feeding leads to a minimal disruption of one or two cells around the feeding area (Bensoussan *et al.*, 2016), involvement of myrosinases in IGs toxicity to spider mites is less likely. The mode of action of IGs can be shown using artificial diets supplemented with the breakdown products, as well as by comparing performance of *T. urticae* on wild-type, glucosinolate-deficient and myrosinase-deficient transgenic plants (Agrawal and Kurashige, 2003; Kim *et al.*, 2008).

Not all populations of *T. urticae* perform well on all host plants (Díaz-Riquelme *et al.*, 2016; Rioja *et al.*, 2017). Although adaptation of *T. urticae* to plant secondary metabolites is a well-known phenomenon, only a few studies have been performed on the mechanism of adaptation (Dermauw *et al.*, 2013; Wybouw *et al.*, 2015; Van Leeuwen and Dermauw, 2016). Selection of a *T. urticae* population on Arabidopsis led to development of a population that performed significantly better on Arabidopsis compared to the original non-adapted population (Ratlamwala, 2014 c). Using an IG-deficient mutant of Arabidopsis and also a population of spider mites that were selected on these transgenic plants, it was shown that spider mites have potentially developed adaptation to IGs (Ratlamwala, 2014 c). The IGs dose-dependent expression was observed in the number of differentially expressed spider mite (non-adapted) genes that were related to detoxification of xenobiotics (Zhurov *et al.*, 2013). This indicates that *T. urticae* may be able to detoxify IG-associated metabolites. In the current thesis, I investigated the mode of action of IGs and the mechanism of two-spotted spider mite adaptation to IGs.

1.5 Objectives

Studying the interaction between *T. urticae* and plants provides an insight into the capacity and the mechanism of adaptation in spider mites, as well as development of strategies to avoid adaptation to plants and potentially cross-resistance between phytotoxins and pesticides. The recent genome sequencing of *T. urticae* (Grbić *et al.*, 2011), together with the development of functional genomic techniques (e.g. RAN-Seq) and high throughput genomic technologies provide an opportunity to study adaptation of *T. urticae* to Arabidopsis IGs (Després *et al.*, 2007). The overall objective of this project was to determine the mechanism of *T. urticae* resistance to Arabidopsis IGs. It was hypothesized that spider mites could develop adaptation to Arabidopsis IGs through detoxification of IGs breakdown products. Experiments were conducted to elucidate the adaptation mechanism through fulfilling three more specific objectives:

1- **To characterize the effect of IGs on *T. urticae* performance**. To determine the most sensitive parameters of *T. urticae* to IGs, life table experiments, larval development assays and fecundity assays were performed, comparing performance of adapted and non-adapted spider mites on wild type and IGs-devoid genotypes of

Arabidopsis. The sensitive parameter identified could be used as the adaptation readout for following experiments.

2- **To determine the mode of IGs action against *T. urticae***. To investigate whether myrosinases are involved in the negative effect of IGs on spider mites, life table parameters, developmental time of larvae and fecundity of spider mites were compared on wild-type and myrosinase-devoid genotypes of Arabidopsis. It was expected that due to the minimal tissue damage mediated by spider mites during feeding, myrosinases could not be involved in hydrolysis of IGs upon spider mite feeding. Also, the breakdown products of Arabidopsis IGs were detected in IG-adapted and non-adapted spider mites after feeding on Arabidopsis using HPLC-MS to determine the IGs break down products that were differentially modified in IG-adapted and non-adapted spider mites and potentially were responsible for the negative effect of IGs on spider mites.

3- **To determine the mechanism of adaptation of *T. urticae* to IGs-related metabolites.** To investigate whether adaptation of spider mites to IGs is due to suppression of plant defense, expression of IGs-related marker genes and levels of Arabidopsis defense-related metabolites after feeding of IG-adapted and non-adapted adult spider mites was measured. Moreover, to test involvement of detoxification in adaptation of spider mites to IGs, activity of spider mite detoxification enzymes was compared in adapted and non-adapted spider mites upon feeding on bean, wild-type and IG-devoid genotypes of Arabidopsis. Also, the influence of detoxification enzyme inhibition on fecundity of IG-adapted and non-adapted spider mites was assessed. Furthermore, potential IGs modifications in spider mites body were investigated by detection of the known IGs breakdown products and their synthesized conjugates in adapted and non-adapted spider mites using HPLC-MS. Finally, RNA-seq analysis of adapted and non-adapted spider mites after 24 h feeding on bean, wild-type and IG-devoid genotypes of Arabidopsis was performed to identify the genes involved in adaptation of spider mites to IGs.

Chapter 2
Methods

2.1 Live materials used for experiments

Plants. Four genotypes of *Arabidopsis thaliana* (Arabidopsis) were used for experiments: a wild type Columbia-0 (Col-0), *cyp79b2 cyp79b3* double mutant which lacks IGs, *tgg1 tgg2* double mutant that lacks TGG1 and TGG2 myrosinases and *atr1-D* in which IGs over-accumulate. Seeds for Col-0 were acquired from P. Morandini (University of Milan) and *atr-1D* from J. Bender (Brown University), while those for *cyp79b2 cyp79b3* and *tgg1 tgg2* were obtained from the Arabidopsis Biological Resource Center. Plants were reared in a growth chamber at 22°C with a relative humidity of 55% and a short-day photoperiod (10 h light: 14 h dark) using cool-white fluorescent lights. Plants were grown from seed with a light intensity of 120 μE m^{-2} sec^{-1}. Seeds were stratified for three days at 4°C in the dark before being sewn into 2.5 cm x 2.5 cm pots filled with moist soil. After sowing seeds, the tray of pots was covered with a transparent lid for approximately one week, after which the lid was removed, and plants were watered regularly. Whole plants or detached rosette leaves of five to six-weeks old plants were used for experiments (

Figure 2-1).

Figure 2-1 Size and stage of plants used for experiments. Five to six-week old plants of different Arabidopsis genotypes were employed for experiments.

Spider mites. The London strain of *Tetranychus urticae* (Koch) was originally collected from apple orchards near London, Ontario, Canada in 2006, and was reared on bean plants (*Phaseolus vulgaris*, cultivar “California Red Kidney”, Stokes, Thorold, Ontario, Canada) in growth chambers at 24 °C, 60 % relative humidity and with a 16 h light: 8 h dark photoperiod for more than 200 generations.

Three independent populations of the London strain spider mites were experimentally adapted to and reared on Col-0 (referred to as Col-adapted) and three populations on *cyp79b2 cyp79b3* (referred to as cyp-adapted) for more than 25 generations by Huzefa Ratlamwala (Ratlamwala, 2014). Populations selected on each plant genotype were pooled together after they displayed no significant difference in their performance on their host plant genotypes. Col- and cyp-adapted spider mites were kept on Col-0 and *cyp 79b2 cyp79b3* plants, respectively, at 24 °C, 60 % relative humidity and with a 16 h light: 8 h dark photoperiod. To remove any maternal effects of selected spider mites and to obtain enough mites of selected *T. urticae* for experiments, selected spider mites were reared on bean leaves for two generations and used as the second generation of females (2G spider mites) on beans for each experiment.

2.2 Life table experiment

The set up designed by Kristie Bruinsma (Bruinsma, 2014) was used for the life table experiment (Figure 2-2). The top of a small petri dish (3 cm diameter) was covered with a layer of Parafilm and the petri dish was filled with water using a syringe. The hole made by the syringe was expanded in the Parafilm so that the petiole of an Arabidopsis detached leaf could be fit into the hole. After putting a leaf in the water-filled petri plate (through the hole made in the Parafilm) a lid was put on the leaf. The lid was a petri plate of the same size as the water-filled petri plate with a hole in center for ventilation and a 0.1 mm mesh sieve glued over the hole to prevent spider mite escape. Using a strip of Parafilm, the set up was sealed so that even larvae of spider mites could not escape. For beans, leaves of about 7 cm diameter were cut and put in petri dishes with wet filter paper beneath and wet napkins around the leaves. Three holes of one cm diameter were made in the petri plate lid for ventilation and petri plates were sealed using Parafilm.

Spider mite adult females from London strain, Col-adapted and cyp-adapted colonies were placed on detached leaves of three genotypes of Arabidopsis, Col-0, *cyp79B2 cyp79B3* and *tgg1 tgg2*, and on bean leaves as controls. One rosette leaf of a five to six-weeks old plant from a desired genotype was put in each set-up. Then a five- to seven-day old female spider mite was put on each leaf and the set-up was sealed. After four hours, females and all eggs laid by them were removed except one egg per each set-up. The day that eggs were laid was reported as day zero and from that date on, each set-up was checked daily. Mortality, progression of spider mite developmental life stages and number of eggs laid by each female were followed every day until death of the last adult. Each treatment had 20 replicates and I repeated the experiment three times.

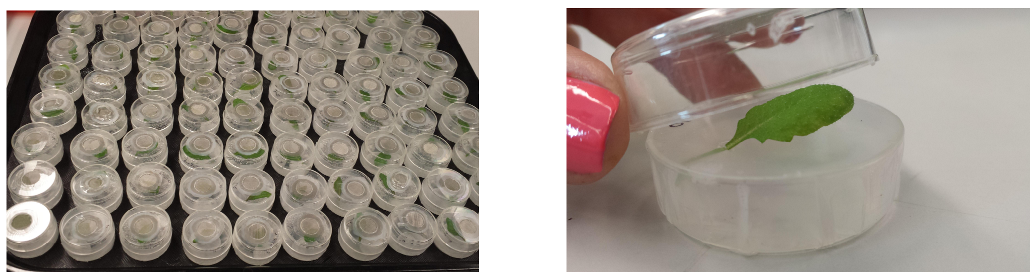


Figure 2-2 Cage-like set-up for maintaining *Tetranychus urticae*.

2.2.1 Calculation of life table parameters

The number of eggs produced per surviving female at each age (x) is shown by (m_x) or individual fecundity. The total number of eggs produced by a cohort is called Gross reproductive rate (GRR), the total number of eggs produced by all females of the experimental population for each treatment:

$$GRR= \sum m_x$$

The number of eggs produced per original individual at each age ($l_x m_x$; where l_x is the proportion of females alive at the beginning of age x) is an important value to consider in population studies. By summing $l_x m_x$ across all ages, the population replacement rate (R_0) can be obtained in units of female/female/generation.

$$R_0= \sum l_x m_x$$

R_0 reflects the number of females produced by living females of the population. R_0 of 1.0 means the population is just replacing itself each generation, $R_0 < 1.0$ indicates the population is declining, and $R_0 > 1.0$ shows the population is increasing.

Besides R_0, the basic reproductive rate, several other population characteristics can be determined from life tables. Two other common features are the cohort generation time (T) and the intrinsic growth rate (r_m). Generation time is defined as the average time between the birth of an individual and the birth of its offspring. Therefore, it can be calculated by summing all the lengths of time to offspring production for the entire cohort divided by the total offspring produced by the survivors (Carey, 1982):

$$T = \frac{\sum l_x m_x x}{\sum l_x m_x} = \frac{\sum l_x m_x x}{R_0}$$

If R_0 remains constant from generation to generation, then it can also be used to predict population size several generations in the future. To predict the population size at any future time, the intrinsic rate of natural increase (r_m: rate of population growth when there is no density-dependent force regulating the population) is calculated by the following equation (Birch, 1948):

$$r_m = \frac{\log_e (R_0)}{T}$$

To statistically analyze the data related to biological parameters of the three strains of spider mites (London strain, cyp- and Col-adapted) on bean and the three Arabidopsis genotypes (Col-0, *cyp79B2 cyp79B3* and *tgg1 tgg2*), incubation time of eggs (time needed for eggs to hatch), developmental time of different stages and longevity of adults were compared using one-way ANOVA analysis followed by Tuckey's test. Also, a one-way ANOVA followed by Tuckey's test was performed between three replicates of the experiments to compare life table parameters of *T. urticae* strains on different host plants. SPSS version 19.0 (IBM Corp., 2010) was employed for statistical analysis of the life table experiment data.

2.3 Spider mite larvae developmental time and mortality

The effect of different genotypes of Arabidopsis on developmental time and mortality of *T. urticae* larvae were investigated with a sample size larger than that in the life table experiment for a robust statistical analysis of larval mortality and development time. To synchronize *T. urticae* larvae, a method introduced by Suzuki *et al.* (2017) was used. A Total of 150 adult spider mite females were placed on a petri plate with its inner wall covered with a strip of wet cotton to limit the females to the bottom of the petri plate. After 24 h, the cotton and female spider mites were removed, leaving eggs at the bottom of the petri plate. Water was added to the petri plate to a level that just covered all eggs. After four days, the water was removed. Eggs were hatched within two hours of water removal. To isolate spider mite larvae in a cage-like system, the same set-up employed for the life table experiment was used. To each set-up, one detached leaf of either Col-0 or *cyp79B2 cyp79B3* plants were added. Ten larvae of London, Col-adapted or cyp-adapted strains of spider mites were transferred to each detached leaf and the set-up was sealed using Parafilm (on a total of ten detached leaves for each treatment). All cages were checked every day and the number of larvae alive as well as number of larvae molted into protonymph were reported. To assure that larvae are exposed to good quality leaves during the experiment, a fresh detached leaf was added to each set-up every other day until the last larvae molted into protonymph or died.

Three replications of the assay were performed using different batches of each Arabidopsis genotype. I analyzed the results using two-way ANOVA followed by Tukey test in SPSS version 19.0 (IBM Corp., 2010) to determine if there were significant differences between the conditions of genotype and/or replication (separate batches of plants) and whether there was an interaction between the experimental conditions. Data from the three replications that did not show significant interactions were pooled. A one-way ANOVA followed by Tukey test was performed to analyze data. The same assay was carried out to compare the larval development and mortality for London strain, Col-adapted and cyp-adapted spider mite larvae on detached leaves of Col-0 and *tgg1 tgg2* genotypes of Arabidopsis. Data were analyzed as described above.

2.4 Fecundity

In the life table experiment, the effect of different Arabidopsis genotypes was observed on reproduction rate of *T. urticae* females. Also, a difference was shown between fecundity of adapted and non-adapted spider mite populations. To ensure that the observed effect on fecundity is not due to different feeding source of the females during their development from egg to adult, a fecundity assay was performed using females that had spent their immature stages on bean. Fecundity of London strain, Col-adapted and cyp-adapted adult females of spider mites on detached leaves of Col-0 and *cyp79B2 cyp79B3* plants. To perform the experiment, a cage-like set-up similar to that described in the life table experiment was used. To synchronize females, 50 individual 1G females (adapted or non-adapted spider mites that were reared on bean detached leaves for 1 generation) were placed on a detached bean leaf for 24 h and then all females were removed leaving only synchronized eggs on the leaf. One to two days after emergence of the adult females, they were used for the experiment. A one- to two-days old 2G female was put on each detached leaf. Every other day, the number of eggs laid by each female were counted and detached leaves were replaced with fresh ones. The total number of eggs laid by each female in six days was recorded.

The experiment included ten replicates for each treatment and each experiment was repeated three times using different batches of plants. Statistical analysis was the same as that for larval development/mortality assay and it was performed using SPSS version 19.0 (IBM Corp., 2010). To investigate the role of myrosinases on the observed effect of IGs on the spider mite reproduction, the fecundity of London strain, Col-adapted and cyp-adapted spider mite larvae was compared on detached leaves of Col-0 and *tgg1 tgg2* genotypes of Arabidopsis.

2.5 Adaptation of *T. urticae* to the Arabidopsis defense

2.5.1 Suppression of plant defense

If tolerance of Col-adapted *T. urticae* to Arabidopsis is due to suppression of plant defense, a reduction in expression of genes involved in biosynthesis of IGs and/or a decrease in level of IGs in plants upon feeding of adapted spider mites on the plant is expected.

2.5.1.1 Expression of genes related to biosynthesis of IGs

To determine whether adaptation of Col-adapted spider mites is due to suppression of plant defense, expression of genes in Col-0 plants associated with biosynthesis of IGs was measured. It has been previously shown that a functional jasmonic acid (JA) biosynthesis pathway is necessary for an effective IGs-related defense response of Arabidopsis to spider mites (Zhurov et al., 2014; Figure 2-3). Also, two enzymes, CYP79B2 and CYP79B3, are required for the biosynthesis of the indole class of glucosinolates (Hull *et al.*, 2000; Mikkelsen *et al.*, 2000; Sønderby *et al.*, 2010 a). Thus, the expression of three genes was measured: *CYP79B2* and *CYP79B3* that are related to biosynthesis of IGs in Col-0 plants and *AOS* (allene oxide suynthase), the JA pathway marker gene (Figure 2-3). Col-0 plants (four to five weeks-old) were infested with 10 adult females of either London strain, cyp-adapted or Col-adapted spider mites. After 24 h, the whole rosettes were cut and immersed in liquid nitrogen for RNA isolation. The experiment was performed in two independent replicates with similar outcomes.

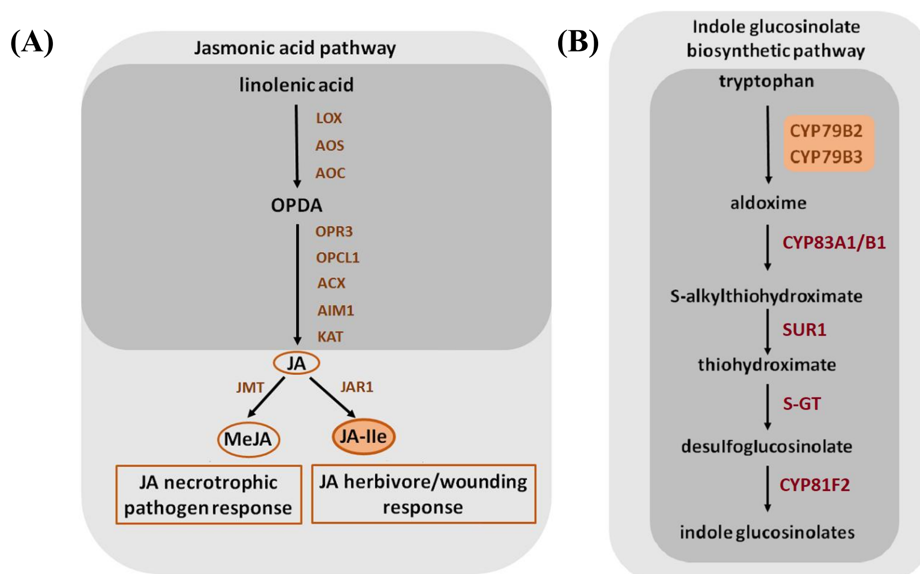


Figure 2-3 Simplified schematic of jasmonic acid (A) and indole glucosinolate (B) biosynthesis pathways in Arabidopsis. Biosynthesis starts by conversion of linolenic acid to (9S,13S)-12-oxo-phytodienoic acid (OPDA) through different steps catalyzed by 13-lipoxygenase (LOX), allene oxide synthase (AOS) and allene oxide cyclase (AOC) enzymes. Jasmonic acid (JA) is generated from OPDA through different steps of reduction and β-oxidations. JA is converted to jasmonic acid-isoleucine (JA-Ile) which is the active form of JA that induces the transcription of genes associated with response to wounding and defense against herbivores including indole glucosinolates biosynthesis genes. Indole glucosinolates are derived from the amino acid tryptophan through conversion of tryptophan to aldoxime by cytochrome P450 (CYP) gene products CYP79B2 and CYP79B3. Aldoximes are then metabolized by CYP83A1 and/or CYP83B, SUR1, S-glucosyltransferases S-GT, and CYP81F2 monooxygenase to form indole glucosinolates.

Using the RNeasy Plant Mini Kit, including DNase treatment (Qiagen, Venlo, Limburg, Netherlands), RNA was extracted from approximately 100 µl of grounded rosette tissue of four- to five-weeks old Col-0 Arabidopsis plants that were treated with Col-adapted, cyp-adapted, London strain or no mites. Two micrograms of total RNA was reverse transcribed using the Maxima First Strand cDNA Synthesis Kit for qRT-PCR (Thermo Fisher Scientific, Waltham, MA). There were three biological replicates for each treatment and reactions were performed in triplicates for each biological replicate, using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Waltham, MA). An Agilent Mx3005P qPCR instrument (Agilent Technologies, Santa Clara, CA) was used to perform qRT-PCR. Primer sequences and amplification efficiencies (E) are listed in Table 2-1. For

Arabidopsis marker gene normalization, a ubiquitin conjugation enzyme, *PEROXIN4* or *PEX4 (AT5G25760)* was used as the reference gene (Czechowski, 2005) which is found to be transcribed at similar amounts in all samples as indicated by Ct values within ± 1 cycle. To obtain a Ct value for each biological replicate, mean Ct values of three technical replicates were used. Expression values were normalized for each target gene (T) relative to the reference gene (R) to calculate the normalized relative quantity (NRQ) using the formula below:

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

Where:
ER = efficiency of reference gene, and
ET = efficiency of target gene.

The Log_2-transformed NRQs were analyzed using 3×2 factorial ANOVA analysis to determine the significance of main effect of spider mite strain and experimental replication (Rieu and Powers, 2009). ANOVA analysis and the following Tukey's HSD test were performed using R.

Table 2-1 List of primer sequences used in qRT-PCR and associated efficiencies

Gene ID	Description	Primers		Efficiency
AT5G25760	PEX4	Forward	GCTCTTATCAAAGGACCTTCGG	0.992
		Reverse	CGAACTTGAGGAGGTTGCAAAG	
AT5G42650	AOS	Forward	AAATCCAACGGCGGAGAACT	0.984
		Reverse	TCGTCGCCAACGGTTGATAA	
AT4G39950	CYP79B2	Forward	GAAAAGAGGTTGTGCGGCTC	0.994
		Reverse	TCTCACTTCACCGTCGGGTA	
AT2G22330	CYP79B3	Forward	TCTACCGATGCTTACGGGATTG	0.973
		Reverse	TACAAGTTCCTTAATGGTTGGTTTG	

2.5.1.2 Plant metabolites related to IGs

The same plant material used for the analysis of plant marker gene expression was used to determine levels of JA and JA-Ile phytohormones, and I3M, the main IGs in Arabidopsis leaves. The level of phytohormones, JA and JA-Ile, were quantified by isotopic dilution mass spectrometry. Isotope-labeled standards were added to plant samples (approximately 0.1 g) before extraction as described by Durgbanshi *et al.* (2005). Ultra-performance liquid chromatography (UPLC)-electrospray ionization-tandem mass spectrometry analyses were carried out on an Acquity SDS system (Waters, Milford, Massachusetts, United States) coupled to a triple quadrupole mass spectrometer (MicroMass, North Carolina, United States). An external calibration was performed for quantification. Levels of Arabidopsis JA and JA-Ile in untreated control and upon feeding of non-, cyp- and Col-adapted mites were compared through one-way ANOVA analysis using R (R Core Team, 2014).

Level of I3M was quantified using an UPLC-electrospray ionization-Quadrupole Time-Of-Flight-mass spectrometry system operated in negative or positive electrospray mode as described previously (Malitsky et al., 2008; Böttcher et al., 2009). Each sample included six rosettes of Col-0 plants (about 50 mg). Metabolites were extracted in 70% (v/v) methanol:water (supplemented with biochanin A) by ultrasonication for 10 min. Myrosinases were deactivated by incubation of samples at 80°C for 15 min in a water bath. Afterward, each sample was centrifuged, and the supernatant was filtered through a 0.2-mm polytetrafluoroethylene membrane filter (GE Healthcare, Little Chalfont, United Kingdom). From each sample, a 10-µL aliquot was directly injected into the UPLC system interfaced to a MicroMass QTOF Premier mass spectrometer. Extraction of mass data was achieved with XCMS software (Smith et al., 2006; Arbona et al., 2010). Relative quantification was performed by assessing the recovery of internal standard and dividing corrected peak areas by actual tissue weight (normalized peak area). An external calibration was performed for quantification. Levels of Arabidopsis I3M, JA and JA-Ile in untreated control and upon feeding of non-, cyp- and Col-adapted mites were compared through one-way ANOVA analysis using R (R Core Team, 2014).

2.5.2 Detoxification of plant metabolites

2.5.2.1 Enzymatic activity assays

To perform the enzymatic activity assays, one or two days-old females of each Col-adapted, cyp-adapted and London strains were placed on bean, Col-0 or *cyp79B2 cyp79B3* plants. After 24 hours, 200 females from each treatment were collected in Eppendorf tubes and kept in -80 ˚C for further experiments. The spider mites were homogenized in liquid nitrogen and the homogenate was centrifuged at 10000 g for five minutes at 4 ˚C. The supernatant (as the enzyme solution) was transferred to another tube and its protein concentration was determined using the Bradford assay (Bradford, 1976). Each sample was diluted to appropriate protein concentrations of 200, 200 and 100 µg/mL to be used for measuring enzymatic activity of glutathione-S-transferases (GSTs), P450 monooxygenases and esterases, respectively.

2.5.2.1.1 **GSTs activity**

The GST activity was assessed spectrophotometrically using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate based on the Habig and Jakoby method (Habig and Jakoby, 1981). GSTs catalyze the conjugation of L-glutathione (GSH) to CDNB through the thiol group of the glutathione.

$$\text{GSH} + \text{CDNB} \xrightarrow{\text{GST}} \text{GS-DNB Conjugate} + \text{HCl}$$

In each well of a 96-well plate, 100 µL of each mite extract, glutathione (4mM) and CDNB (0.4 mM, containing 0.1% (v/v) ethanol) were added. Absorbance of contents of each well was measured at 340 nm at 23 ˚C during a five-minute period at 30 s intervals by Spectramax M2 (Molecular Devices, San Jose, California, United States). Using the extinction coefficient of 9.6 $mM^{-1}cm^{-1}$, the amount of conjugated CDNB formed in five minutes was calculated. The assays were performed in four independent biological and three technical replicates.

The increase in absorbance (ΔA_{340}/min) is directly proportional to the GST activity. The linearity of the reaction was determined by plotting the absorbance values against time. ΔA_{340}/min was calculated in the linear range of the plot using the following equation:

$$\Delta A_{340nm}/min = \frac{A_{340nm}(\text{final read}) - A_{340nm}(\text{initial read})}{\text{Reaction time (min)}}$$

The ΔA_{340}/min of the blank was subtracted from the ΔA_{340}/min of the sample and this rate was used for the calculation of the GST specific activity. Equation for the GST specific activity is as below:

$$\text{GST specific activity} = \frac{(\Delta A_{340}/\text{min}) \times V(\text{mL}) \times \text{dil}}{\varepsilon_{mM} \times V_{sample}(\text{mL})} = \mu\text{mol/mL/min}$$

Where:

ε_{mM} ($mM^{-1}cm^{-1}$): The extinction coefficient for CDNB conjugate at 340 nm: 5.3 mM^{-1} (path length of 0.552 cm)

V: the reaction volume

Dil: the dilution factor of the original sample

V_{Sample}: the volume of the enzyme sample tested

All assays were corrected for occurrence of non-enzymatic conjugation in blank samples in which the enzyme solution was replaced by buffer.

2.5.2.1.2 **P450s activity**

To measure the activity of P450s, 7-ethoxy-4-trifluoromethylcoumarin (7-EFC) was used as the substrate in a fluorometric assay in which the O-deethylation of 7-EFC occurs and is converted into the fluorescent 7-hydroxy-4-trifluoromethylcoumarin (7-HFC) by P450 enzymes (Buters *et al.*, 1993; Figure 9).

Figure 2-4 Conversion of 7-ethoxy-4-trifluoromethylcoumarin to 7-hydroxy-4-trifluoromethylcoumarin catalyzed by P450 monooxygenases.

P450 activity of samples was measured according to the method of Van Leeuwen et al. (Van Leeuwen *et al.*, 2005) which was originally adapted from Buters' method (Buters *et*

al., 1993). In each well of a black 96-well microtitre plate, 50 μL of the enzyme source (mite extract samples diluted to 200 μg/mL) was added to 50 μL of a reaction mixture containing 7-EFC 0.4mM, glucose-6-phosphate 1mM, $NADP^+$ 0.2 mM and glucose-6-phosphate dehydrogenase 0.014 U in sodium phosphate buffer, pH 7.4. The plate was incubated at 37 °C in the dark for 30 min while shaking and the reaction was stopped by adding 100 μL of TRIZMA base buffer (0.05 M, pH 10) and acetonitrile (50+50 by volume) to each well.

To provide a standard curve of absorbance of 7-HFC at each concentration, 7-HFC was diluted in sodium phosphate buffer (0.1 M, pH 7.5) to reach the following concentrations: 0.12, 0.1, 0.08, 0.06, 0.04, 0.02 and 0 μM. For non-enzymatic control, 50 μL sodium phosphate buffer (0.1 M, pH 7.4) was added to 50 μL reaction mixture. A microtitre plate spectrophotometer was used to detect 7-HFC fluorescence at 510 nm while exciting at 410 nm. To determine the specific activity of 7-EFC-O-deethylation, the standard curve of absorbance of different concentrations of 7-HFC was used.

2.5.2.1.3 **Esterase activity**

To measure esterase activity in spider mite extracts, p-nitrophenyl acetate (pNPA) was employed as the substrate to measure the increase in production of p-nitrophenol (pNP) as a result of hydrolysis of pNPA by esterases in spider mite extract samples (Stumpf and Nauen, 2002).

Spider mite extracts were diluted to 100 μg/mL, before adding (to each well of a 96-well plate) 100 μL of each sample to 80 μL of sodium phosphate buffer (0.1 M, pH 7.5) and 20 μL p-nitrophenyl acetate solution prepared in acetone + buffer (10 + 90 by volume) at 40 °C. All samples were normalized by being compared with a blank containing 180 μL buffer and 20 μL pNPA. To provide a standard curve of p-nitrophenol concentration against absorbance, pNP was diluted in sodium phosphate buffer (0.1 M, pH 7.5) to reach to the following concentrations: 25, 20, 15, 10, 5 and 0 nM.

The absorbance of the formed p-NP at 405 nm and 30 °C was measured using a microtitre plate spectrophotometer for five minutes, every 15 sec. The increase in absorbance (ΔA_{405}/min) is directly proportional to the esterase activity. The linearity of the reaction

was determined by plotting the absorbance values against time and calculation of ΔA_{405}/min in the linear range of the plot using the following equation:

$$\Delta A_{405nm}/\min = \frac{A_{405nm}(\text{final read}) - A_{405nm}(\text{initial read})}{\text{Reaction time (min)}}$$

The standard curve of ΔA_{405}/min of different pNA concentrations was used to convert ΔA_{405}/min values of samples into specific esterase activity values (units reported as nmol/min/µg protein).

For all enzymatic activity assays, enzymatic activity of adapted and non-adapted *T. urticae* on different host plants was compared through one-way ANOVA using SPSS (IBM Corp., 2010).

2.5.2.2 Synergism assay

A synergism assay was performed to determine if glutathione-S-transferases (GSTs), P450 monoxygenases (P450s) and esterases are involved in adaptation of Col-adapted spider mites to Arabidopsis. Detached leaves from four to five weeks old Col-0 and *cyp79B2 cyp79B3* plants were dipped in either DEM (diethyl maleate), PBO (piperonyl butoxide) or DEF (S,S,S tributyl-phosphorotrithioate) to inhibit activity of GSTs, P450s and esterases respectively.

To determine the appropriate concentrations of inhibitors to be applied in the assay, the range of inhibitor concentrations used against spider mites in literature was extracted (Khalighi et al 2009; Khalighi et al 2014; Van Pottelberge et al., 2008; Van Pottelberge et al 2009) and the highest concentrations that caused less than 10% mortality in mites and no phytotoxicity on Arabidopsis detached leaves were chosen. To detect phytotoxicity, solutions of different concentrations of DEM (100, 200, 500, 1000 and 2000 mg/L), PBO (30, 100, 500, 1000 and 2000 mg/L) and DEF (10, 20, 100, 200 and 500 mg/L) were made by dissolving inhibitors in acetone (1:1 by volume) and diluting the solutions by distilled water to reach the desired concentration. Total of 20 detached leaves of four- to five-weeks old Col-0 plants was dipped in each solution. After 24 h any deformation or color change of leaves was recorded as phytotoxicity. Bean detached leaves were treated with the highest

concentrations of DEM, PBO and DEF that did not cause any phytotoxicity on Col-0 leaves. Five bean detached leaves were treated with each inhibitor or a solution of water and acetone as the control. Total of 50 spider mite females of London strain was placed on each detached leaf. After 24 h, number of live spider mites was recorded and mortality was calculated using the Abbott's formula (Abbott, 1925):

$$\text{Corrected \%} = (1 - \frac{n_T}{n_C}) \times 100,$$

Where:

n_T = number of inhibitor-treated mites

n_C = number of control (water-treated) mites

To ensure that the applied concentrations of inhibitors are effective on mites, enzymatic activity was determined after treatment of spider mites with inhibitors. To perform the enzymatic activity assay, for each inhibitor three samples of 100 inhibitor-treated female spider mites and three samples of 100 spider mites that were treated with water and acetone (as described below) were used. For each sample, ten detached leaves of Col-0 Arabidopsis were treated with the chosen concentration of each inhibitor or water and each of the detached leaves was placed in a water-containing set-up as described in section 2.2 for the life table experiment. After the solution on leaves dried, ten adult females of Col-adapted spider mites were put on each detached leaf and the set-up was closed and sealed with Parafilm. After 24 h, 100 spider mites were pooled for each sample of mites treated with each inhibitor or water. GST, P450 and esterase activity of DEM-, PBO- and DEF-treated spider mites were measured respectively as described in section 2.5.2.1. Means of enzymatic activity of inhibitor-treated with water-treated samples were compared using t-test in SPSS (IBM Corp., 2010).

Inhibitor solutions of 2000 mg/L, 1000 mg/L and 100 mg/L concentration were made for DEM, PBO and DEF, respectively. Since PBO is not a universal inhibitor of P450s, 15 mg/L of TCPPE was also used to investigate the potential effect of P450 enzymes that have low affinity to PBO on adaptation of spider mites to glucosinolates. Leaves dipped in the water-acetone solution served as controls. Leaves were allowed to dry in the fume hood before a one to two-day-old adult female of London strain or Col-adapted spider mite was

added to each detached leaf. The number of eggs laid by each female per day was recorded for six days. To provide spider mites with fresh food, the leaves were changed every other day. Each experiment included ten replicates of each treatment and was repeated three times to confirm the reproducibility of results. To analyze the data, a Mann-Whitney U test was performed between control and inhibitor-treated treatments using SPSS (IBM Corp., 2010).

2.6 RNA-Seq and transcriptome analysis

To identify genes that are potentially involved in adaptation of spider mites to IGs, differences in the expression of genes in adapted compared to non-adapted spider mites upon feeding on different host plants was determined using RNA-seq and transcriptome analysis.

Sample preparation: beans (one week old), *cyp79B2 cyp* 79B3 and Col-0 plants (four to five-weeks old) were infested with London strain, cyp-adapted and Col-adapted spider mite females. After 24h, three samples of 100 spider mites were collected for each treatment, frozen in liquid nitrogen and stored at -80 ˚C before RNA extraction. Total RNA was extracted from each sample of spider mites using RNeasy Plant Mini Kit, including DNase treatment (Qiagen,Venlo, Limburg, Netherlands) following the guideline of the manufacturer. To avoid DNA contamination, on column DNase I digestion was performed according to manufacturer's protocol. The quality and quantity of the extracted RNA was determined using a Nanodrop ND-2000c spectrophotometer (Thermo Scientific, Waltham, Massachusetts, United States).

RNA-Seq analysis: RNA samples were sequenced at The Center for Applied Genomics (TCAG) sequencing facility operated by The Hospital of Sick Children (Toronto, ON). Strand specific paired-end (2 × 150 bp) sequencing was conducted according to Illumina TruSeq protocol (Illumina, San Diego, CA). The transcriptome sequencing of all 27 libraries was performed on a single sequencing lane on an Illumina HiSeq2500 Genome Analyzer (Illumina, San Diego, CA) platform yielding 8-17 million mapped fragments per library. Quality control measures including filtering high quality reads based on the fastq score and trimming the read lengths were performed at TCAG. Reads were mapped to the

reference *T. urticae* genome [assembly 2009-09-29; Grbic *et al.* (2011)] using STAR aligner (Dobin *et al.*, 2013) v.2.5.2b in a single-pass mode allowing only unique mapping, up to five mismatches per read mapped, a minimum intron size of 20bp, a maximum intron size of 15000 bp and outFilterMatchNminOverLread and outFilterScoreMinOverLread of 0.5. Read counts were generated at the level of gene locus using HTSeq v.0.6.0 in "union" mode (Anders *et al.*, 2015) against *T. urticae* genome annotation version 2016-06. Analysis of differential gene expression was performed using voom/limma workflow for genes that demonstrated expression level of at least 1 count per million (CPM) in at least 3 samples (Law *et al.*, 2014). Additional analysis and figures were performed and generated using R (R Core Team , 2014) and BioConductor (Gentleman *et al.*, 2004).

2.7 Spider mite IGs-related metabolites

To determine IGs-related metabolites in spider mites and consequently the potential conversion of plant IGs by spider mites, London and Col-adapted 2G spider mite females were allowed feeding on either Col-0, *cyp79B2 cyp79B3* or *atr1-D* (a mutant of Col-0 Arabidopsis in which IGs over-accumulate) plants for three days. Then, samples from each mite strain on each plant genotype were collected in Eppendorf tubes and flash frozen in liquid nitrogen. Spider mites collected from different batches of plants were pooled to reach 1000 females for each sample.

Each frozen sample was weighed and grounded using a cold pestle before extraction of mite metabolites in ice-cold methanol (1mL per 100 mg of mite), containing 80 µg/mL sinigrin (Sigma-Aldrich) which served as an internal standard. After ten minutes of sonication, samples were centrifuged for five minutes at 4000 g. Commercially available IMG breakdown products were resolubilized in the extraction solvent as the internal standard. The protocol to synthesize indole-3-carbinol (I3C) conjugates was adapted from Kim *et al*. (2008) with the following modifications. Indole-3-carbinol (5 mg) was mixed with an amino acid, glutathione or ascorbic acid in a 1:1 (w/w) ratio in a 10 mL glass vial, followed by adding 5 mL of 80% methanol (analytical grade). The vial was incubated at 85 °C for 30 min in a water bath, vortexed for 5 min and was then left at room temperature overnight. The mixtures were filtered using a 0.22 um PTFE membrane syringe-tip filter and each mixture was injected into the HPLC-TOF. Conjugates were analyzed as a mixture

and peaks were tentatively identified on the basis matching the peak mass spectrum with the mass spectrum of predicted conjugates also identified by Kim *et al*. (2008).

Samples were analyzed by HPLC-TOF-MS (Agilent, Santa Clara, USA; Dr. Mark Bernard's lab at Western University, London, Canada) consisting of a solvent degasser (Agilent 1260), a binary pump (Agilent 1260), a high-performance autosampler (Agilent 1260) and a temperature-controlled column compartment (Agilent 1290). A 1 µL injection of standards and plant extracts and a 10 µL injection of mite extract was separated on a C18 reversed-phase column, 3 mm x 100 mm, 1.8 µm pore size (ZORBAZ Eclipse Plus C18, Agilent) using a two-solvent gradient (solvent A, water + 0.1% formic acid; solvent B, acetonitrile + 0.1% formic acid) at a flow rate of 0.3 mL/min at a column temperature of 25°C. The 30-min total run consisted of 5% B (2 min), 5–80% B (22 min), 80–100% B (0.01 min), a 3 min hold at 100% B. The column was returned to initial conditions of 100–5% B over 1 min followed by a 4 min post-time held at 5% B to return column to equilibrium. Eluent was monitored by time of flight mass spectrometry (Aglient 6230 TOF-MS). The TOF-MS used electrospray ionization as a source (325°C drying gas temperature at 8 L/min with a pressure of 35 psi and a capillary voltage of 3500 V) operated in the negative polarity (fragmentor at 175 V and skimmer at 65 V), with a maximum m/z of 1700, in high resolution mode.

Data was processed using Agilent's Masshunter Workstation Qualitative Analysis Software (Version B.05.00, September 2011). Peak areas for compounds of interest were generated using extracted ion chromatograms (IMG at 447.0537 m/z; 4hIMG at 463.0487 m/z; 4mIMG and 1mIMG at 477.0643) with a mass tolerance of 0.005 m/z, and the elution order of the intact IGs was predicted based on previously published methods (Bennett *et al.*, 2004; Glauser *et al.*, 2012). Extracted ion chromatograms were smoothed using a Gaussian smoothing function with a function width of 15 points and a gaussian width of five points. Peak areas were normalized to the intensity of the internal standard and a ten-point sinigrin calibration curve was used to determine the concentration of each intact IG in 358 m/z sinigrin equivalents. The normalized concentrations of different compounds were compared in adapted and non-adapted spider mites through one-way ANOVA using SPSS (IBM Corp., 2010).

Chapter 3

Results

3.1 Characterization of the effect of indole glucosinolates on *Tetranuchus urticae* performance

3.1.1 Life table experiment

To characterize the effect of indole glucosinolates (IGs) on *Tetranychus urticae* performance, a life table experiment was conducted by daily recording the survival and the fecundity of London, cyp-adapted and Col-adapted spider mite strains on Col-0, *cyp79B2 cyp79B3* and *tgg1 tgg2* Arabidopsis plants, as well as on bean. Survival, developmental time and rate of population growth of the three spider mite strains on different host plants are compared and described in the three following sub-sections, survivorship curves, biological parameters and life table parameters.

Survivorship curves

Survival of individual spider mites of London, cyp- and Col-adapted spider mite strains were followed on different host plants every day from their egg stage until death of the last adult. Distribution of the age-specific survival rate of adult females is presented in Figure 3-1. Survival curves of London strain, cyp- and Col-adapted spider mites were significantly different on each of the Arabidopsis genotypes including Col-0 (R_2=6.38; P= 0.041), *tgg1 tgg2* (R_2=7.40; P= 0.025) and *cyp79B2 cyp79B3* (R_2=6.91; P= 0.032). However, on bean, no significant difference was observed among the survivorship curves of these mite strains.

At the first half of their lives, mortality of spider mites was faster on Col-0 and *tgg1 tgg2* plants compared to that on bean and *cyp79B2 cyp79B3* so that on the 10th day of their lives, less than 70% of spider mites were alive on Col-0 and *tgg1 tgg2* plants (Figures 3-1 A and B), while at the same time, more that 70% of mites were alive on bean and *cyp79B2 cyp79B3* plants (Figures 3-1 C and D).

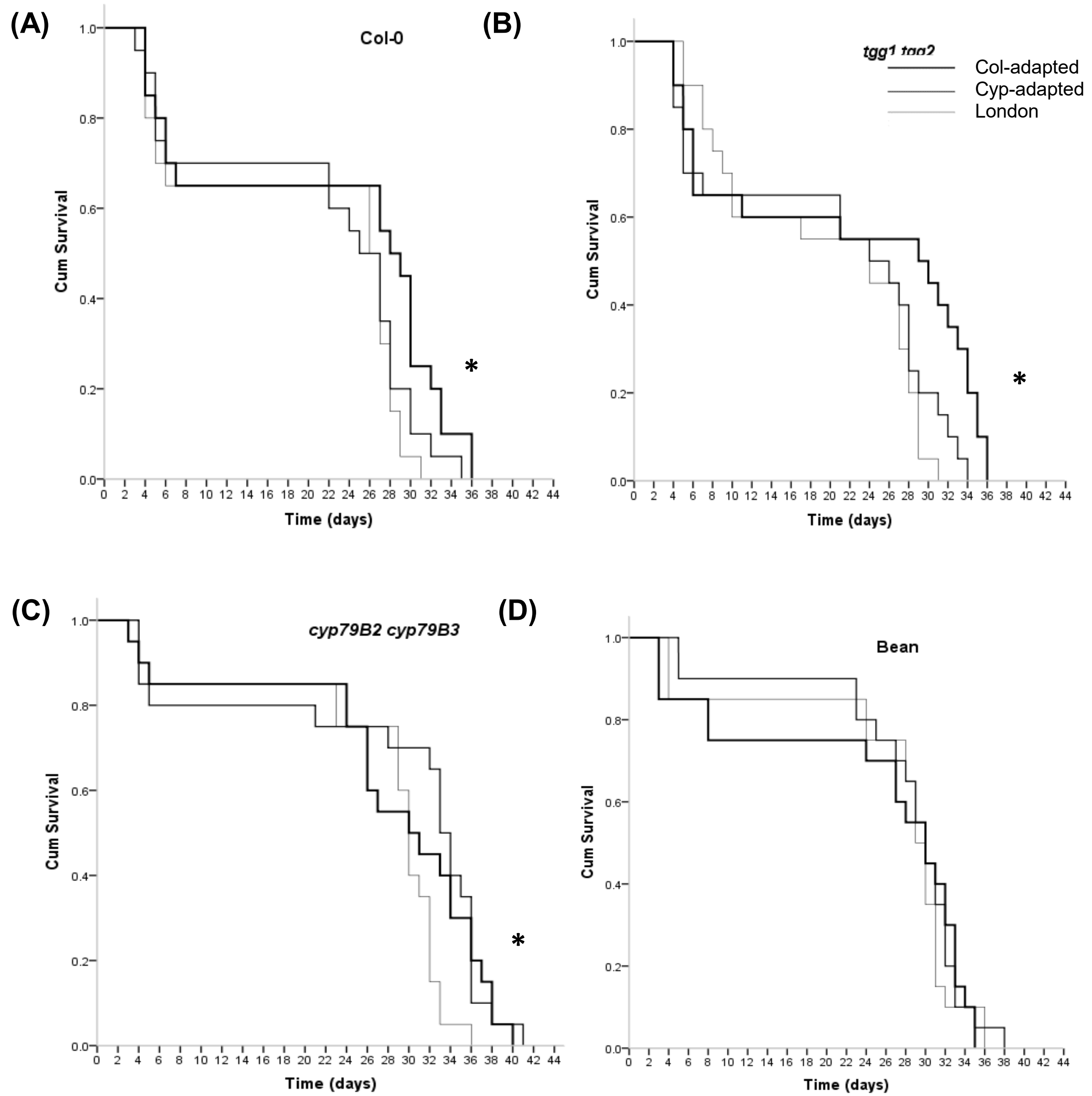


Figure 3-1 Kaplan-Meier estimate of age-specific survival rate (lx) for three strains of *Tetranychus urticae* including London strain, cyp-adapted and Col-adapted on A) Col-0, B) *tgg1 tgg2*, C) *cyp79B2 cyp79B3* and D) bean (one-way ANOVA; * *p*,<0.05).

Table of biological parameters

The developmental time was calculated for egg, larva, protonymph and deutonymph stages of the three *T. urticae* strains on Col-0, *cyp79B2 cyp79B3* and *tgg1 tgg2* Arabidopsis plants and bean for the following reasons: 1) to determine the developmental stage of *T. urticae* that is sensitive to IGs; 2) to determine which stage(s) of Col-adapted spider mites show adaptation to IGs; 3) to investigate involvement of myrosinases in the negative effect of IGs on *T. urticae*.

The mean developmental time of eggs, larvae, protonymphs and deutonymphs, as well as mean longevity of London strain, cyp-adapted and Col-adapted *T. urticae* on bean and different Arabidopsis genotypes including Col-0, *cyp79B2 cyp79B3* and *tgg1 tgg2* is shown in Table 3-1. The incubation time of eggs (the number of days between oviposition and hatching of eggs) was not significantly different between any of the treatments. Developmental time of the London strain, cyp-adapted and Col-adapted larvae did not show any significant difference on bean and *cyp79B2 cyp79B3*, whereas on Col-0, Col-adapted larvae developed to protonymph significantly faster than the London strain ($F_{2,89}$=15.034, P <0.001) and cyp-adapted larvae ($F_{2,89}$=15.034, P =0.012). Also, cyp-adapted larvae had a significantly shorter developmental time compared to London ($F_{2,89}$=15.034, P =0.033). Larvae of all the three strains of *T. urticae* had a significantly shorter developmental time on bean compared to that on Col-0 (London: $F_{3,119}$=35.766, P <0.001; cyp-adapted: $F_{3,119}$=10.298, P <0.001; Col-adapted: $F_{3,119}$=2.384, P =0.046) but just the London strain showed a significant difference in developmental time of larvae on *cyp79B2 cyp79B3* compared to Col-0 plants ($F_{3,119}$=35.766, P <0.001). Among the three spider mite strains, only the London strain showed a significantly shorter larval development time on *tgg1 tgg2* compared to that on Col-0 ($F_{3,119}$=35.766, P <0.001).

Protonymphs of *T. urticae* did not show any significant difference in developmental time on different plant genotypes. On each type of host plant, different strains of *T. urticae* spent comparable amounts of time to develop into the adult. However, each spider mite strain showed a significantly different developmental time on different types of host plant. Deutonymphs of all spider mite strains developed significantly faster on bean compared to

Arabidopsis genotypes but only deutonymphs of the London strain showed a significant difference in developmental time on the three Arabidopsis genotypes, which had a significantly longer developmental time on Col-0 compared to that on *cyp79B2 cyp79B3* ($F_{3,119}$=35.766, *P* <0.001).

Adding up the developmental time of all the pre-adult stages, the premature developmental time was calculated and did not vary significantly among the three strains of *T. urticae* on each of the plant hosts. However, all the three strains showed a significantly longer premature developmental time on Col-0 compared to either bean (London: $F_{3,119}$=26.883, *P* <0.001; cyp-adapted: $F_{3,119}$=20.893, *P* <0.001; Col-adapted: $F_{3,119}$=20.274, *P* =0.046) or *cyp79B2 cyp79B3* (London: $F_{3,119}$=26.883, *P* <0.001; cyp-adapted: $F_{3,119}$=20.893, *P* =0.001; Col-adapted: $F_{3,119}$=20.274, *P* =0.010). The premature developmental time of all the strains was similar on Col-0 and *tgg1 tgg2*.

Longevity (spider mite's age at the time of death) of the three spider mite strains was not significantly different on bean, whereas on *cyp79B2 cyp79B3*, the London strain lived significantly shorter than cyp-adapted spider mites ($F_{2,89}$=4.804, *P* =0.007). On Col-0, the London strain and cyp-adapted spider mites showed a similar longevity, while both lived significantly shorter than Col-adapted spider mites (London: $F_{2,89}$=9.373, *P* <0.001; cyp-adapted: $F_{2,89}$=9.373, *P* =0.006). Comparing the effect of host plant on longevity of each of the spider mite strains, both the London strain and cyp-adapted spider mites lived significantly longer on bean (London: $F_{2,89}$=14.193, *P* =0.003; cyp-adapted: $F_{2,89}$=15.891, *P* =0.005) and *cyp79B2 cyp79B3* (London: $F_{2,89}$=14.193, *P* =0.001; cyp-adapted: $F_{2,89}$=15,891, *P* <0.001) compared to that on Col-0, while their longevity on bean and *cyp79B2 cyp79B3* was not significantly different. Col-adapted spider mites did not show any significant difference in longevity on different types of host plant.

Table 3-1 Developmental time (mean±SEM) of London strain, cyp-adapted and Col-adapted *Tetranychus urticae* at different developmental stages on four host plants, Bean, *cyp79B2 cyp79B3*, *tgg1 tgg2* and Col-0. Data were analyzed using one-way ANOVA, P <0.05, n=20. Different letters in capital orange show a significant difference in each row and different small purple letters indicate a significant difference in each column related to each spider mite strain.

	London	**cyp-adapted**	**Col-adapted**
Egg			
Bean	2.933+0.106 Aa	3.000+0.107 Aa	3.033+0.058 Aa
cyp79B2 cyp79B3	3.000+0.107 Aa	3.000+0.117 Aa	2.967+0.112 Aa
tgg1 tgg2	3.100+0.074 Aa	3.233+0.114 Aa	3.000+0.096 Aa
Col-0	3.033+0.122 Aa	3.100+0.100 Aa	2.967+0.102 Aa
Larva			
Bean	1.633+0.102 Aa	1.567+0.104 Aa	1.567+0.114 Aa
cyp79B2 cyp79B3	2.033+0.089 Ab	1.833+0.108 Aab	1.833+0.112 Aab
tgg1 tgg2	2.167+0.108 Ab	2.167+0.128 Ab	1.767+0.114 Bab
Col-0	3.000+0.083 Ac	2.533+0.171 Bb	2.000+0.117 Cb
Protonymph			
Bean	1.100+0.102 Aa	1.100+0.056 Aa	1.067+0.114 Aa
cyp79B2 cyp79B3	1.200+0.074 Aa	1.200+0.074 Aa	1.133+0.063 Aa
tgg1 tgg2	1.233+0.078 Aa	1.300+0.098 Aa	1.267+0.082 Aa
Col-0	1.067+0.046 Aa	1.233+0.078 Aa	1.133+0.063 Aa
Deutonymph			
Bean	1.267+0.082 Aa	1.133+0.063 Aa	1.067+0.046 Aa
cyp79B2 cyp79B3	1.733+0.106 Ab	1.733+0.095 Ab	1.800+0.121 Ab
tgg1 tgg2	2.300+0.109 Ac	1.733+0.106 Bb	1.833+0.108 Bb
Col-0	2.133+0.115 Ac	2.033+0.089 Ab	1.833+0.128 Ab
Premature			
Bean	6.933+0.197 Aa	6.800+0.139 Aa	6.733+0.126 Aa
cyp79B2 cyp79B3	7.967+0.195 Ab	7.767+0.095 Ab	7.733+0.214 Ab
tgg1 tgg2	8.800+0.194 Abc	8.433+0.233 ABbc	7.867+0.171 Bbc
Col-0	9.233+0.196 Ac	8.900+0.227 Ac	7.933+0.151 Bc
Longevity			
Bean	30.200+0.769 Aa	31.567+0.826 Aa	31.867+0.764 Aa
cyp79B2 cyp79B3	30.600+0.720 Aa	34.200+0.810 Ba	32.567+0.924 ABa
tgg1 tgg2	25.533+0.683 Ab	27.567+0.890 Ab	30.833+0.888 Ba
Col-0	26.867+0.414 Ab	27.667+0.699 Ab	30.233+0.575 Ba

Life table parameters

Daily tracking survival and fecundity of the three strains of *T. urticae* (London strain, cyp-adapted and Col-adapted) on bean and the three genotypes of Arabidopsis (Col-0, *cyp79B2 cyp79B3* and *tgg1 tgg2*), allowed calculation of the life table parameters of *T. urticae* strains, pointing to the effect of IGs on population growth of spider mites and involvement of myrosinases in the effect of IGs on *T. urticae*.

Table 3-2 Life table parameters (mean±SEM) of London strain, cyp-adapted and Col-adapted *Tetranychus urticae* on Bean and the three Arabidopsis genotypes, Col-0, *cyp79B2 cyp79B3* and *tgg1 tgg2*; starting population of 20 eggs. Data were analyzed using one-way ANOVA, P <0.05, n=3. Different capital letters in orange show a significant difference in each row and different small purple letters indicate a significant difference in each column related to each spider mite strain.

	London	**cyp-adapted**	**Col-adapted**
GRR (♀)			
Bean	2118.667±306.44 Aa	2615.333±493.51 Aa	2126.667±295.98 Aa
cyp79B2 cyp79B3	1436.778±213.55 Aa	2150.667±377.14 Aa	1696.000±167.16 Aab
tgg1 tgg2	391.137±21.65 Ab	602.333±49.77 Ab	1074.333±87.72 Bb
Col-0	473.417±34.22 Ab	693.000±81.46 ABb	956.333±78.49 Bb
R_0 (♀/♀)			
Bean	1738.969±310.19 Aa	2219.234±399.25 Aa	1614.037±232.55 Aa
cyp79B2 cyp79B3	1159.561±169.01 Aa	1674.172±267.76 Aa	1273.563±216.83 Aab
tgg1 tgg2	240.381±25.83 Ab	392.556±34.02 Ab	673.617±76.36 Bb
Col-0	311.839±33.09 Ab	461.350±51.23 ABb	644.117±58.73 Bb
r_m (♀/♀/day)			
Bean	0.4546±0.032 Aa	0.4501±0.013 Aa	0.4438±0.011 Aa
cyp79B2 cyp79B3	0.3955±0.011 Aa	0.3866±0.009 Ab	0.3895±0.014 Ab
tgg1 tgg2	0.3523±0.011 Ab	0.3785±0.006 ABb	0.3846±0.009 Bb
Col-0	0.3509±0.009 Ab	0.3752±0.007 ABb	0.3963±0.006 Bb

The life table parameters of the three strains of spider mites on different host plants are listed in Table 3-2, including gross reproductive rate (GRR; the total number of eggs produced by all females of the experimental population for each treatment), net reproductive rate (R_0; population's rate of replacement or the number of females produced by living females of the population) and intrinsic rate of population increase (r_m); the rate of population growth when there is no density-dependent force regulating the population, in other words, number of females produced by living females per day).

On the Col-0 plant, Col-adapted spider mites showed the highest GRR, R_0 and r_m compared to the other two strains of spider mites with a significant difference between GRR, R_0 and r_m for the London strain compared to Col-adapted spider mites (GRR: $F_{2,8}$=12.557, P =0.006; R_0: $F_{2,8}$=11.588, P =0.007; r_m: $F_{2,8}$= 9.524, P =0.011). A similar pattern was observed in spider mites that fed on *tgg1 tgg2* Arabidopsis (GRR: $F_{2,8}$=47.648, P <0.001; R_0: $F_{2,8}$= 18.930, P =0.002; r_m: $F_{2,8}$=9.524, P =0.011). On bean and *cyp79B2 cyp79B3* plants, no significant difference was observed in GRR, R_0 and r_m between the London strain, cyp-adapted and Col-adapted spider mites.

All the three strains of *T. urticae* showed the highest GRR and R_0 on bean while the lowest value of these parameters was on IGs-containing genotypes of Arabidopsis, Col-0 and *tgg1 tgg2*. For the London strain and cyp-adapted spider mites, significantly higher GRR (London: $F_{3,11}$= 19.329, P =0.028; cyp-adapted: $F_{3,11}$=10.546, P =0.045) and R_0 (London: $F_{3,11}$=13.968, P =0.040; cyp-adapted: $F_{3,11}$=16.297, P =0.031) on *cyp79B2 cyp79B3* compared to that on Col-0 was observed, whereas the difference between GRR and R_0 of Col-adapted spider mites on *cyp79B2 cyp79B3* and Col-0 plants was not significant. For all the three strains of *T. urticae*, r_m was significantly higher on bean compared to that on *cyp79B2 cyp79B3* (London: $F_{3,11}$= 6.994, P =0.026; cyp-adapted: $F_{3,11}$= 15.563, P =0.005; Col-adapted: $F_{3,11}$= 6.753, P =0.026) and Col-0 (London: $F_{3,11}$= 6.994, P =0.017; cyp-adapted: $F_{3,11}$= 15.563, P =0.002; Col-adapted: $F_{3,11}$= 6.753, P =0.050) plants. The difference in r_m of any of the spider mite strains was not statistically significant on *cyp79B2 cyp79B3* and Col-0. Neither of the three *T. urticae* strains demonstrated a significant difference in GRR, R_0 and r_m on Col-0 and *tgg1 tgg2* plants.

3.1.2 Larval development/mortality

In order to better understand the effect of IGs on performance of adapted and non-adapted *T. urticae* larvae through employment of larger sample sizes, an assay was performed to determine developmental time and mortality of London, cyp- and Col-adapted strains larvae on Col-0 and *cyp79B2 cyp79B3* genotypes of Arabidopsis (Figure 3-2 A). On *cyp79B2 cyp79B3* plants, the number of days from larva to nymph (larval development time) was not significantly different between the London strain, cyp- and Col-adapted mites. However, on Col-0, the London strain larvae showed a significantly longer developmental time compared to cyp- and Col-adapted larvae ($F_{5,179}$=17.486, P =0.001; $F_{5,179}$=17.486, P <0.001) while no significant difference was observed between developmental time of cyp- and Col-adapted spider mite larvae. There was no significant effect of plant genotype on developmental time of cyp- and Col-adapted larvae, whereas, London strain larvae needed significantly more time to develop to the nymph stage on Col-0 plants compared to that on the *cyp79B2 cyp79B3* genotype ($F_{5,179}$=17.486, P <0.001).

The London strain showed higher mortality compared to cyp- and Col-adapted larvae on both *cyp79B2 cyp79B3* ($F_{5,179}$=46.136, P =0.013; $F_{5,179}$=46.136, P =0.001) and Col-0 plants ($F_{5,179}$=46.136, P <0.001; $F_{5,179}$=46.136, P <0.001; Figure 3-2 B). On either of the Arabidopsis genotypes mortality of cyp-adapted larvae was not significantly different to that of Col-adapted larvae. All three strains of mites, London strain ($F_{5,179}$=46.136, P <0.001), cyp-adapted ($F_{5,179}$=46.136, P <0.001) and Col-adapted ($F_{5,179}$=46.136, P =0.002) demonstrated significantly higher mortality on Col-0 plants compared to that on *cyp79B2 cyp79B3*.

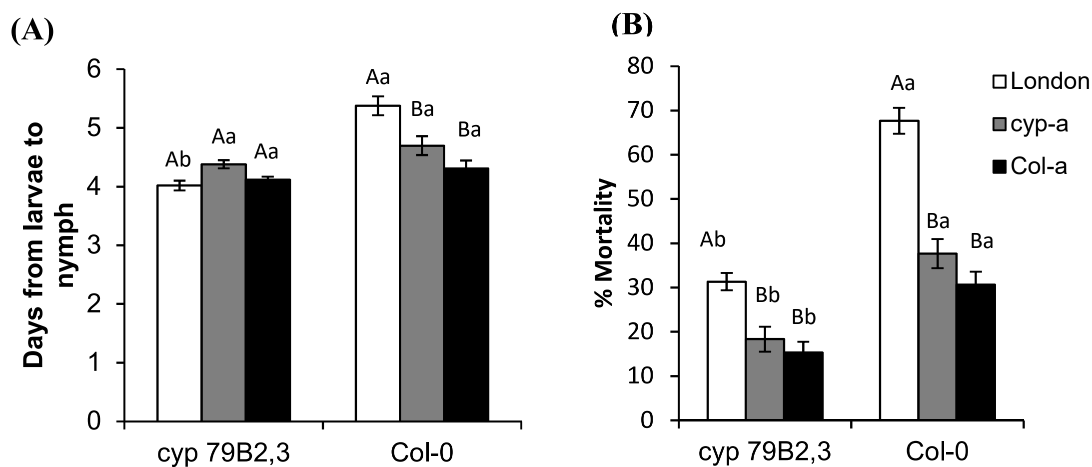


Figure 3-2 Developmental time (A) and mortality (B) (mean±SEM) of London strain, cyp- and Col-adapted *Tetranychus urticae* larvae on two Arabidopsis genotypes, Col-0 and *cyp79B2 cyp79B3* (cyp 79B2,3). Data were analyzed using one-way ANOVA, $P < 0.05$, n=20. Difference in capital letters shows a significant difference among spider mite strains on each plant genotype while different small letters are indicators of significant difference of each spider mite strain on different plant genotypes.

To determine whether myrosinases are involved in the negative effect of IGs on *T. urticae*, the development of *T. urticae* (London strain) larvae was followed on Col-0 and *tgg1 tgg2* plants. The latter Arabidopsis genotype, is a mutant of Col-0 that lacks enzymes necessary for biosynthesis of myrosinases in Arabidopsis. Spider mites completed their larval stages faster on *tgg1 tgg2* compared to that on Col-0 ($t_{1,52}$ =2.773, P =0.008; Figure 3-3 A). However, mortality of spider mite larvae on *tgg1 tgg2* and Col-0 plants was not significantly different (Figure 3-3 B).

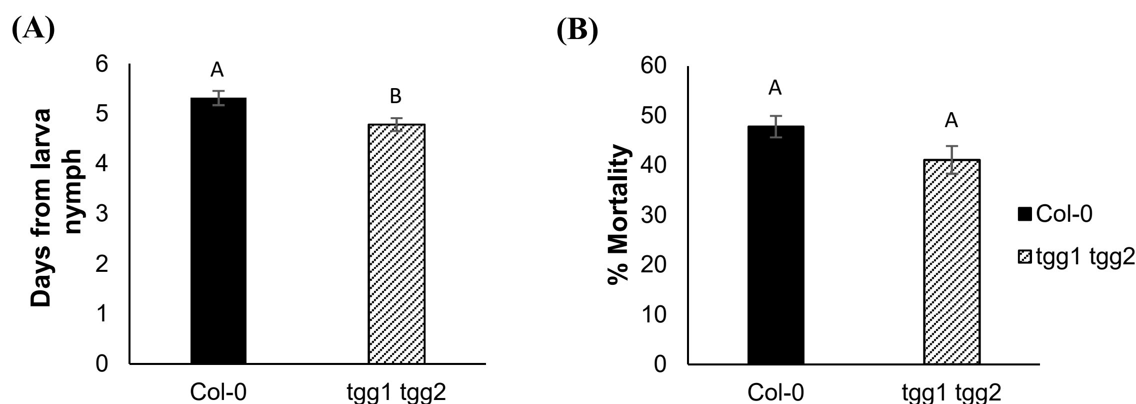


Figure 3-3 Developmental time (A) and mortality (B) (mean±SEM) of London strain *Tetranychus urticae* larvae on two Arabidopsis genotypes, Col-0 and *tgg1 tgg2*. Data were analyzed using one-way ANOVA, P <0.05, n=20. Difference in capital letters shows a significant difference among spider mite strains on each plant genotype.

3.1.3 Fecundity

Fecundity is one of the biological parameters of *T. urticae* that is affected by IGs and is enhanced in IGs-adapted spider mites as shown in life table experiments (section 3-1-1). However, in life table experiments, the premature stages of spider mites fed from different host plants. Therefore, it is not clear if the observed effect was a direct impact of IGs on fecundity or it resulted from different food source or feeding behavior of preadult stages. In order to eliminate the effect of preadult feeding on the fecundity, a six-day fecundity assay was performed using adult females of London strain, cyp- and Col-adapted spider mites that all spent one generation and the preadult stages of the second generation on detached bean leaves before being transferred to Col-0 and *cyp79B2 cyp79B3* detached leaves for the fecundity assay. Col-adapted females laid significantly higher number of eggs in six days compared to London strain and cyp-adapted mites on both Col-0 ($F_{5,119}$=65.975, *P* <0.001; $F_{5,119}$=65.975, *P* <0.001) and *cyp79B2 cyp79B3* ($F_{5,119}$=65.975, *P* <0.001; $F_{5,119}$=65.975, *P* <0.001; Figure 3-4). The number of eggs laid by London strain and cyp-adapted spider mites were not significantly different. A lower fecundity was shown on Col-0 compared to *cyp79B2 cyp79B3* in all the three strains of spider mites, London strain ($F_{5,119}$=65.975, *P* <0.001), cyp-adapted ($F_{5,119}$=65.975, *P* <0.001) and Col-adapted ($F_{5,119}$=65.975, *P* <0.001).

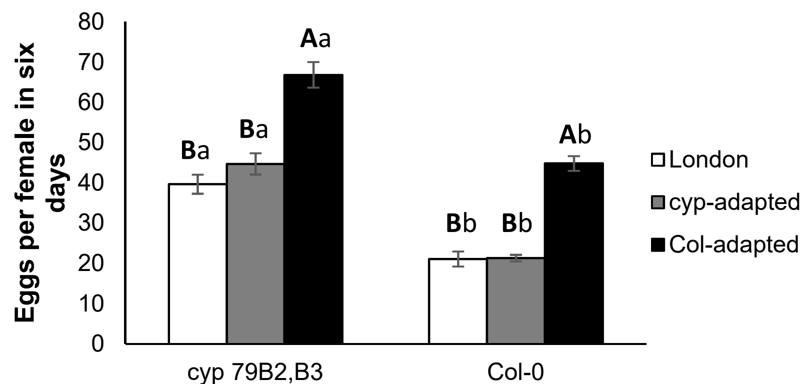


Figure 3-4 Fecundity (mean±SEM) of London strain, cyp- and Col-adapted *Tetranychus urticae* larvae on two Arabidopsis genotypes, Col-0 and *cyp79B2 cyp79B3* (cyp 79B2,B3). Data were analyzed using one-way ANOVA, P <0.05, n=20. Difference in capital letters shows a significant difference among spider mite strains on each plant genotype while different small letters are indicators of significant difference of each spider mite strain on different plant genotypes.

To determine if myrosinase-mediated enzymatic hydrolysis of IGs is necessary for the effect of IGs on *T. urticae* fecundity, the number of eggs laid by females of London strain mites in six days was compared on Col-0 and *tgg1 tgg2* plants. Fecundity of spider mites on *tgg1 tgg2* was not significantly different to that on Col-0 plants ($t_{1,54}$ =0.599, P =0.552; Figure 3-5).

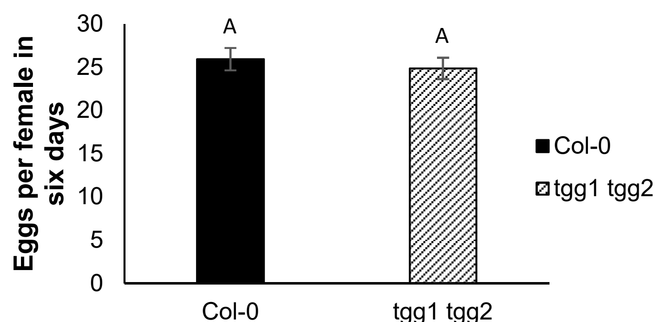


Figure 3-5 Fecundity (mean±SEM) of London strain *Tetranychus urticae* larvae on two Arabidopsis genotypes, Col-0 and *tgg1 tgg2*. Data were analyzed using one-way ANOVA, P <0.05, n=20. Difference in capital letters shows a significant difference among spider mite strains on each plant genotype.

3.2 Adaptation of *T. urticae* to the Arabidopsis defense

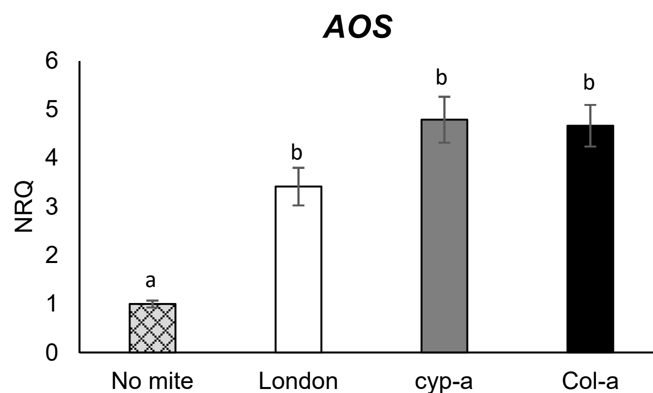
To determine if Col-adapted spider mites can evade Arabidopsis defense through suppression of plant defense (i.e. synthesis of IGs), the following were measured: 1) expression of Arabidopsis genes related to biosynthesis of IGs; and 2) Level of IGs and JA after feeding of Col-adapted spider mites on them.

3.2.1 Suppression of plant defense

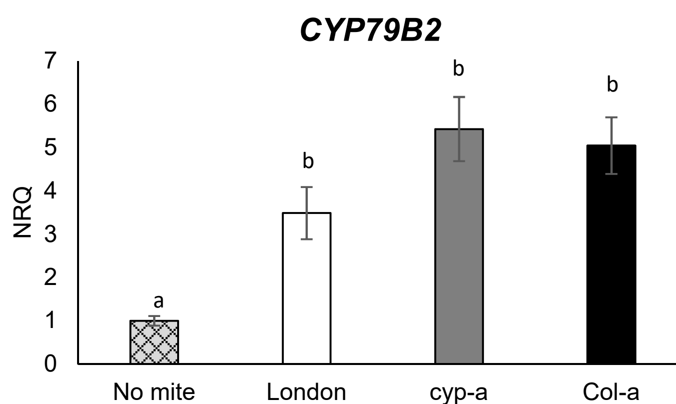
3.2.1.1 Expression of genes related to biosynthesis of IGs

After 24 h feeding of London strain, cyp-adapted and Col-adapted spider mites, the expression levels of two genes related to biosynthesis of IGs in Col-0 plants were measured, including *CYP79B2* and *CYP79B3* that catalyze conversion of tryptophan to the precursor of IGs biosynthesis, indole-3-acetoaldoxime (Sønderby *et al.*, 2010 b). Also, the expression of JA-biosynthesis gene, allene oxide synthase, *AOS* was measured. Feeding of all strains of spider mites induced expression of *AOS* (London: $F_{3,8}= 27.85$, $P = 0.001$; cyp-adapted: $F_{3,8}= 40.90$, $P < 0.001$; Col-adapted: $F_{3,8}= 27.85$, $P < 0.001$; Figure 3-6 A), *CYP79B2* (London: $F_{3,8}= 40.77$, $P < 0.001$; cyp-adapted: $F_{3,8}= 75.08$, $P < 0.001$; Col-adapted: $F_{3,8}= 40.77$, $P < 0.001$; Figure 3-6 B) and *CYP79B3* (London: $F_{3,8}= 71.03$, $P < 0.001$; cyp-adapted: $F_{3,8}= 95.68$, $P < 0.001$; Col-adapted: $F_{3,8}= 71.03$, $P < 0.001$; Figure 3-6 C). However, there was no significant difference in induction of marker genes between the three strains of spider mites.

(A)



(B)



(C)

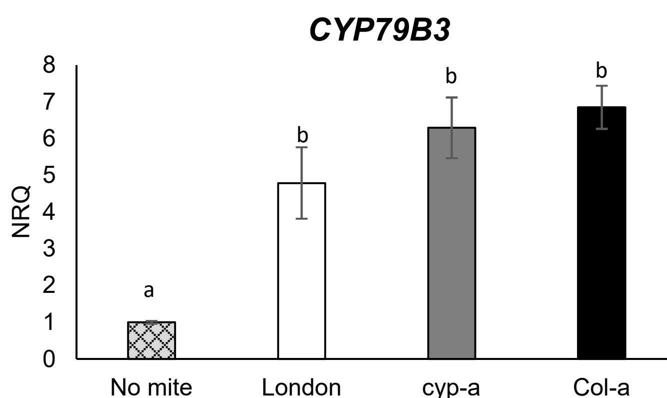


Figure 3-6 Expression levels (mean±SEM) of the indole glucosinolate genes encoding (A) jasmonic acid (JA) biosynthetic enzyme (*AOS*) and enzymes required for the biosynthesis of indole glucosinolates, *CYP79B2* (B) and *CYP79B3* (C), in Col-0 Arabidopsis, in untreated control (No mites) and upon feeding of non-, cyp- and Col-adapted Tetranychus urticae (n = 3). NRQ=Normalized related quantity. Different letters show significant differences in one-way ANOVA (Tukey test).

3.2.1.2 Levels of JA and IGs-related metabolites in mite-infested plants

To investigate whether spider mite feeding can interfere with the synthesis of defensive signaling or toxic metabolites, using HPLC-MS, levels of jasmonic acid (JA), jasmonic acid-isoleucin (JA-Ile), the bioactive form of JA and indole-3-ylmethyl glucosinolate (I3M; the major IG in Arabidopsis) were measured in Col-0 plants infested with Col-, cyp- and non-adapted (London Strain) mites as well as no mites.

Upon feeding of the three different spider mite strains, JA and JA-Ile were induced to the same extent in Col-0 plants: significantly higher levels of JA ($F_{3,11}$= 12.957; London: P =0.002; cyp-adapted: P =0.003; Col-adapted: P =0.032; Figure 3-7 A) and JA-Ile ($F_{3,11}$= 14.358; London: P =0.001; cyp-adapted: P =0.005; Col-adapted: P =0.007; Figure 3-7 B) were observed in plants that were infested with either of the three strains of spider mites compared to non-treated plants. Although not significant, feeding of all strains of spider mites on Col-0 plants led to higher levels of I3M on these plants compared to those with no mite on them. Levels of JA, JA-Ile and I3M were not significantly different between Col-0 plants treated with different strains of mites. (Figure 3-7 C).

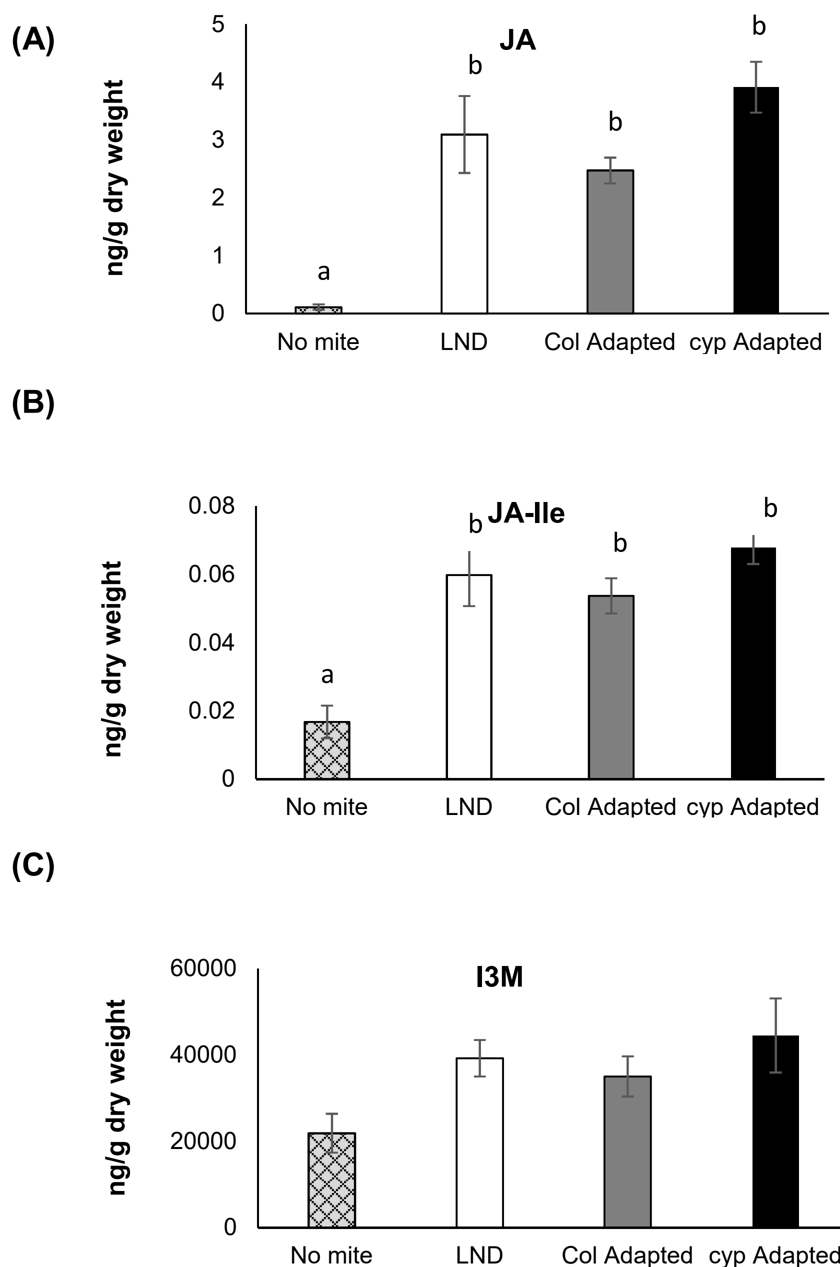


Figure 3-7 Levels of Arabidopsis defense-related metabolites (mean±SEM) in 3-week-old Col-0 plants, in untreated control (No mites) and upon feeding of London strain, cyp- (cyp-a) and Col-adapted (Col-a) *Tetranychus urticae*. A) JA, jasmonic acid; B) JA-Ile, the bioactive jasmonate; C) I3M, indol-3-ylmethyl glucosinolate. Different letters show significant differences in one-way ANOVA (Tukey test). These experiments were done in two independent replicas, each with three biological replicas.

3.2.2 Detoxification as a mechanism of adaptation in spider mites

3.2.2.1 Enzymatic activity assays

To determine if detoxification is a mechanism of adaptation of spider mites to IGs, activities of the three classes of detoxification enzymes including GSTs, P450s and esterases were measured in London strain, cyp-adapted and Col-adapted spider mites after 24 h of feeding on beans, *cyp79B2 cyp79B3* and Col-0 plants.

The activity of GSTs was not statistically different between the three strains of spider mites when fed on beans ($F_{2,11}$=0.010, P=0.990) (Figure 3-8). London strain spider mites did not show any significant difference in GSTs activity upon feeding on beans, *cyp79B2 cyp79B3* or Col-0 plants ($F_{2,11}$=0.476, P=0.636). Although not statistically significant, cyp-adapted and Col-adapted mites had higher activity of GSTs on *cyp79B2 cyp79B3* and Col-0 plants compared to beans (cyp-adapted: $F_{2,11}$=1.765, P=0.226; Col-adapted: $F_{2,11}$=3.902, P=0.060). GSTs activity of Col-adapted spider mites was not statistically different from cyp-adapted or London strain mites (Col-0: $F_{2,11}$=0.535, P=0.603; *cyp79B2 cyp79B3*: $F_{2,11}$=0.713, P=0.516).

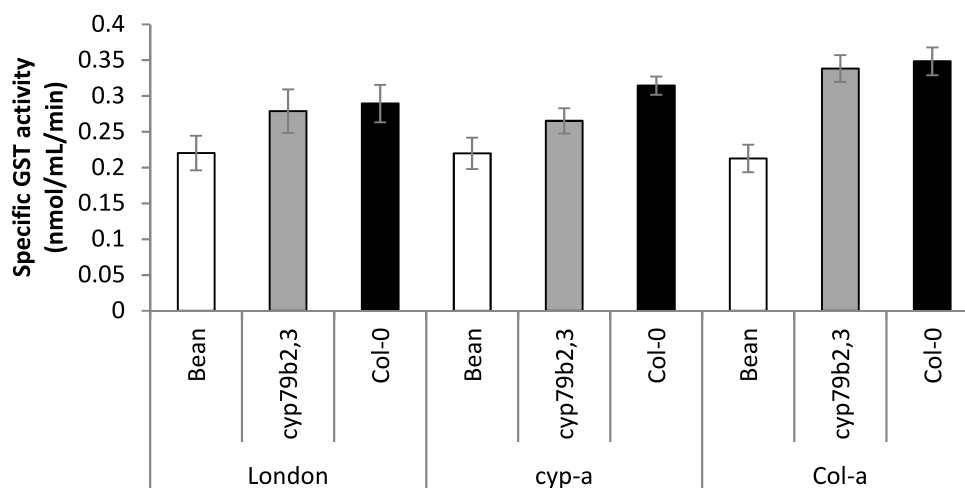


Figure 3-8 Glutathione-S-transferases (GSTs) activity of London strain, cyp- (cyp-a), and Col-adapted (Col-a) *Tetranychus urticae* after 24 h feeding on bean, Col-0 and *cyp79B2 cyp79B3* plants. Data (specific GST activity) were analyzed using one-way ANOVA, P <0.05. Specific GST activity data are presented as mean (nmol per mL per min) ±SEM, n=4.

Esterase activity increased in London strain spider mites upon switching host plant from bean to either *cyp79B2 cyp79B3* or Col-0 plants (*cyp79B2 cyp79B3*: $F_{2,11}$=3.781, P= 0.018; Col-0: $F_{2,11}$=3.781, P=006; Figure 3-9). However, induction of esterase activity was not significantly different between London strain spider mites feeding on *cyp79B2 cyp79B3* or Col-0 ($F_{2,11}$=3.781, P=0.772). A similar increase in cyp-adapted spider mite esterase activity in response to *cyp79B2 cyp79B3* and Col-0 plants was observed ($F_{2,11}$=18.458, P=0.102). There was no statistically significant difference in esterase activity of London strain, cyp- and Col-adapted spider mites feeding on Col-0 plants.

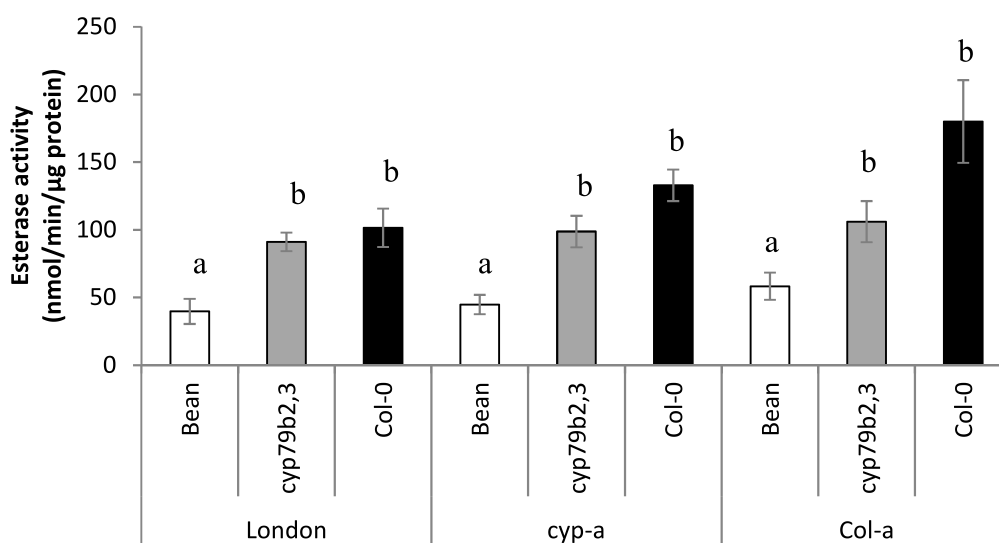


Figure 3-9 Esterase activity of London strain, cyp-, and Col-adapted *Tetranychus urticae* after 24 h feeding on bean, Col-0 and *cyp79B2 cyp79B3* plants. Data (esterase activity) was analyzed using one-way ANOVA, P <0.05. Esterase activity data are presented as mean (nmol per min per µg protein) ±SEM, n=4. Different small letters show a significant difference in esterase activity of each spider mite strain on different host plants.

The activity of P450 enzymes increased in all three strains of spider mites upon changing host plant from beans to Arabidopsis (London strain: $F_{2,11}$=121.638, P<0.001; cyp-a: $F_{2,11}$=1145.492, P<0.001; Col-a: $F_{2,11}$=331.212, P<0.001; Figure 3-10). In all strains of spider mites P450 activity was significantly higher upon feeding on Col-0 compared to that on *cyp79B2 cyp79B3* plants (London strain: $F_{2,11}$=121.638, P<0.001; cyp-a: $F_{2,11}$=1145.492, P<0.001; Col-a: $F_{2,11}$=331.212, P<0.001). On Col-0 plants P450 activity was significantly higher in Col-adapted compared to London strain and cyp-adapted spider

mites ($F_{2,11}$=637.284, P<0.001). Also, significant difference was observed in activity of P450s on Col-0 in comparison with *cyp79B2 cyp79B3* plants ($F_{2,11}$=637.284, P<0.001).

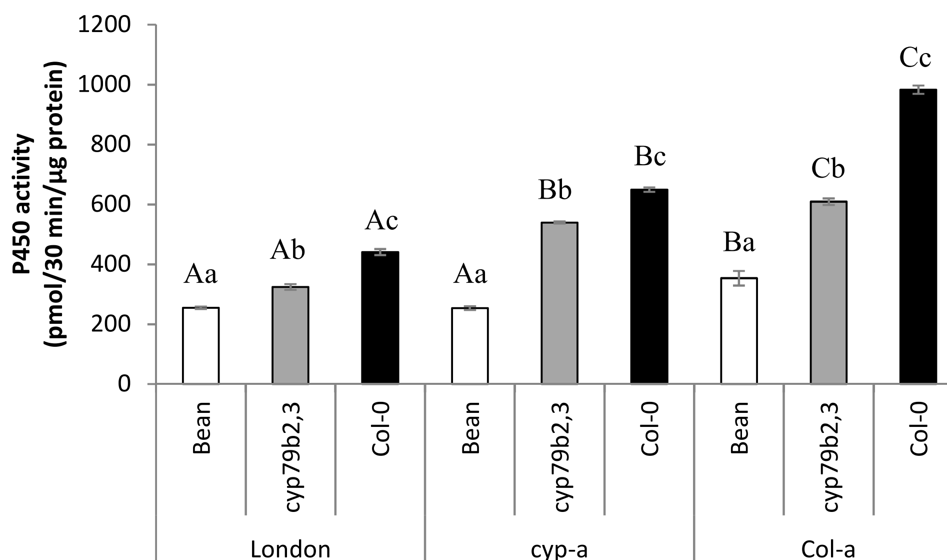


Figure 3-10 P450 activity of London strain, cyp-, and Col-adapted *Tetranychus urticae* after 24 h feeding on bean, Col-0 and *cyp79B2 cyp79B3* plants. Data (P450 activity) were analyzed using one-way ANOVA, P <0.05. P450 activity data are presented as mean (pmol per 30 min per µg protein) ±SEM, n=4. Different small letters show a significant difference in P450 activity of each spider mite strain on different host plants, while different capital letters are representative of significant difference in P450 activity of different spider mite strains on the same host plant.

3.2.2.2 Synergism assays

To determine the role of detoxification enzymes in adaptation of spider mites to IGs, synergism assays were performed by treating London and Col-adapted spider mite strains with DEM, PBO and DEF which are inhibitors of GSTs, P450s, and esterases, respectively. Inhibition of GSTs did not significantly affect fecundity of non- or Col-adapted spider mites on either *cyp79b2 cyp79b3* or Col-0 plants (Figure 3-11 A). Also, on either *cyp79b2 cyp79b3* or Col-0 plants, fecundity of non- and Col-adapted spider mites was not changed

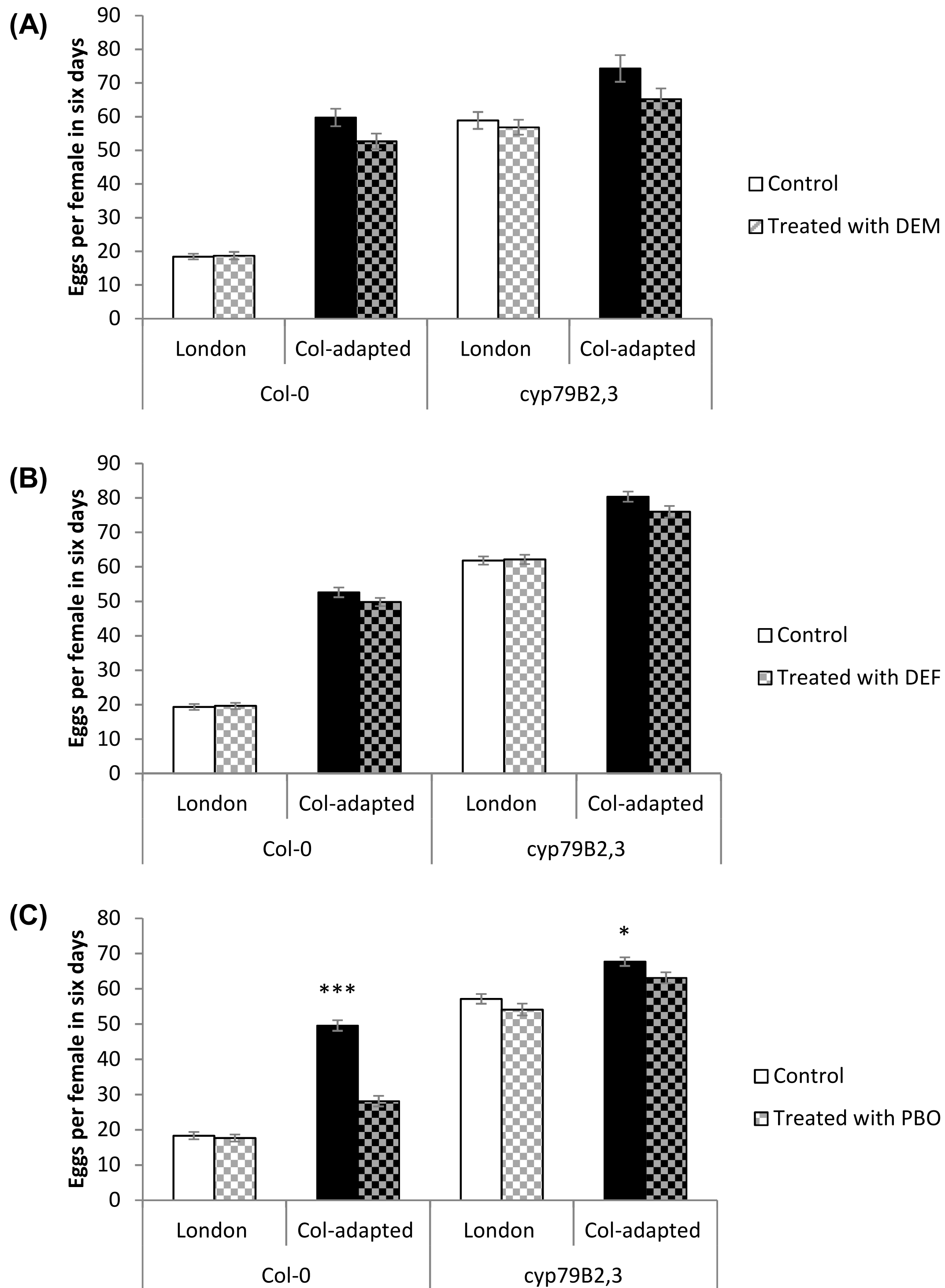


Figure 3-11 Effect of detoxification enzyme inhibitors on fecundity of non- and Col-adapted spider mites upon feeding on Col-0 and *cyp79b2/cyp79b3* plants (n = 30). (A) GSTs (diethyl maleate, DEM), (B) esterases (Tributyl phosphorotrithioat, DEF) and (C) P450s (Piperonyl butoxide, PBO). Error bars are ±SEM (t-test; * $p<0.05$, *** $p<0.001$).

upon treatment of them with esterase inhibitor (DEF; Figure 3-11 B). However, fecundity of Col-adapted spider mites on both *cyp79b2 cyp79b3* and Col-0 plants decreased significantly after being treated by P450 inhibitor (PBO) compared to the same mite strains that were not treated with the inhibitor (Figure 3-11 C). Reduction of performance in Col-adapted mites was not to the same extent on *cyp79B2 cyp79B3* and Col-0 plants so that these mites showed a more significant reduction on Col-0 (U= 305.5, p= 0.032) compared to *cyp79b2 cyp79b3* plants (U= 35.0, p<0.0010). Trichlorophenylpropynyl ether (TCPPE) was also used as an alternative inhibitor of P450s that inhibits a range of P450 enzymes different to that inhibited by PBO. Upon treatment with TCPPE, fecundity of Col-adapted spider mites decreased significantly on both *cyp79b2 cyp79b3* (t= 8.788, p <0.001) and Col-0 (t= 8.386, p < 0.001; Figure 3-12).

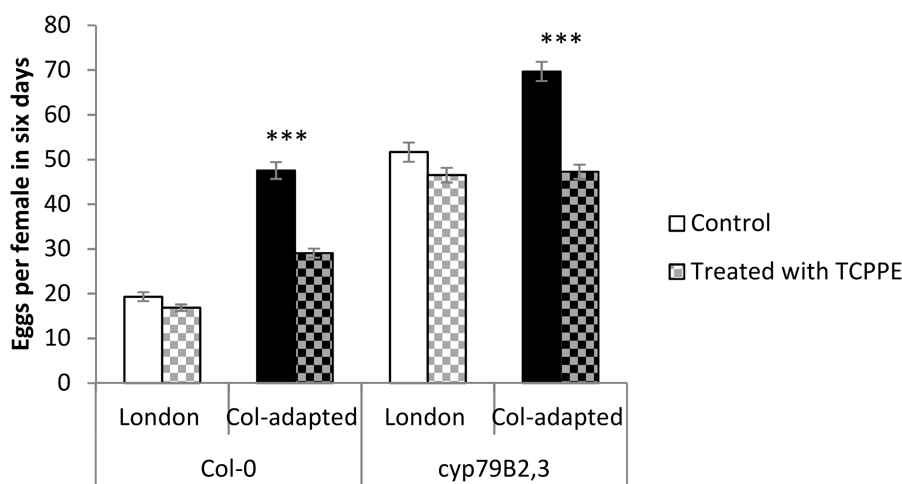


Figure 3-12 Effect of P450s inhibitor (trichlorophenylpropynyl ether, TCPPE) on fecundity of non- and Col-adapted spider mites upon feeding on Col-0 and *cyp79b2/cyp79b3* plants (n = 30). Error bars are ±SEM (t-test; ***p<0.001).

3.3 RNA-Seq and transcriptome analysis

An RNA-Seq study was performed to determine the effect of IGs adaptation on spider mite transcriptomic response as well as to assess the genome-wide effect of feeding on IGs. Samples of three strains of spider mite females including the London strain, cyp-adapted and Col-adapted that fed on either bean, *cyp79B2 cyp79B3* or Col-0 plants for 24 h were collected. RNA was extracted from each sample, and RNA samples were sent for RNA-seq analysis.

Comparative gene expression profiles between adapted and non-adapted spider mites

The overall pattern of expression was considerably different between adapted and non-adapted spider mites. When fed on Col-0 plants, the transcriptomic response was considerably different compared to that on bean and *cyp79B2 cyp79B3*; there were 666 differentially expressed genes (DEGs) between Col-adapted and London strain, 290 DEGs between cyp-adapted and London strain and 230 DEGs between cyp-adapted and Col-adapted spider mites irrespective of host plant (Figure 3-13 A). Also, host plant switch could change expression of genes in spider mites. Shifting from bean to Col-0 plants, 612 DEGs were detected in *T. urticae* compared to 282 DEGs upon shifting from bean to *cyp79B2 cyp79B3* plants. Only five DEGs were detected due to a host shift between Col-0 and *cyp79B2 cyp79B3* (Figure 3-13B). The number of genes upregulated was 3-4 time more than those downregulated as a result of host plant shift (Figure 3-13B).

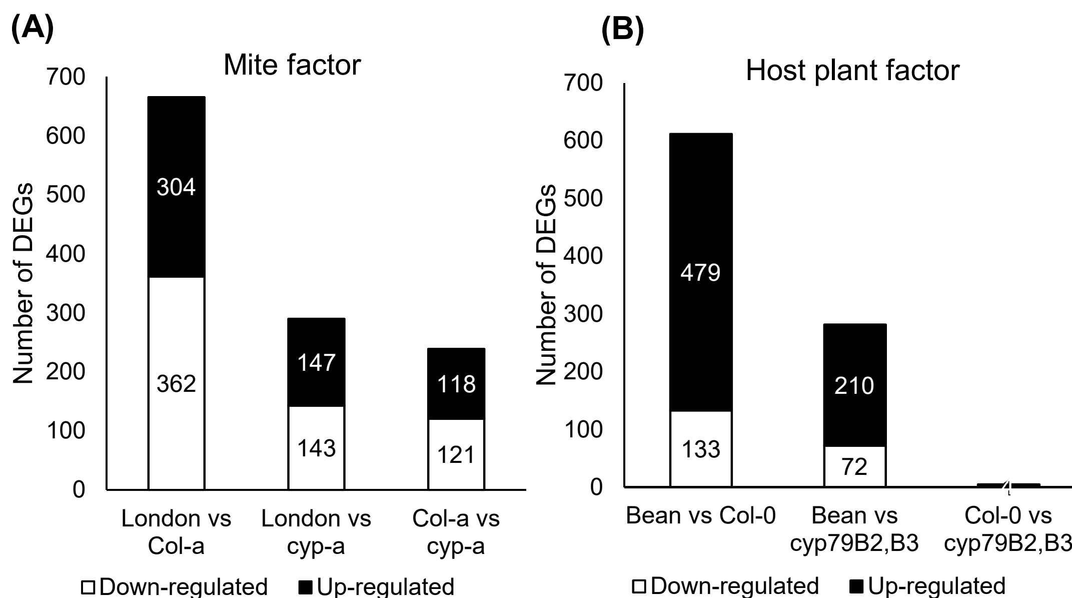


Figure 3-13 Number of differentially expressed genes between (A) different strains of *Tetranychus urticae* including London stain, cyp- and Col-adapted regardless of their host plant; and (B) spider mites that were fed on different genotypes of host plant including bean, *cyp79B2 cyp79B3*, Col-0.

In a principle component analysis, the first three principle components are responsible for 67.4% of the total variation in gene expression (Figure 3-14). The majority of variance in gene expression was due to an interaction between the effect of adaptation status and host plant on spider mite gene expression (PC1, 30.6% of total variation). PC2 which is associated with constitutive gene expression of different strains of spider mite, accounts for 22.5% of the total variation in the gene expression. An obvious separation between expression of genes in mites of different strains on each host plant can be observed (Figure 3-14). Expression of genes in Col- and cyp-adapted spider mites is more similar compared to that between London strain and each of the adapted strains.

Another 14% (PC3) of the total variation in the data is mediated by the effect of host plant on spider mite gene expression (Figure 3-14). Gene expression of mites on Col-0 and *cyp79B2 cyp79B3* plants are more similar compared to that on beans. The difference between mite gene expression on Col-0 and bean is greater than that between *cyp79B2 cyp79B3* and bean.

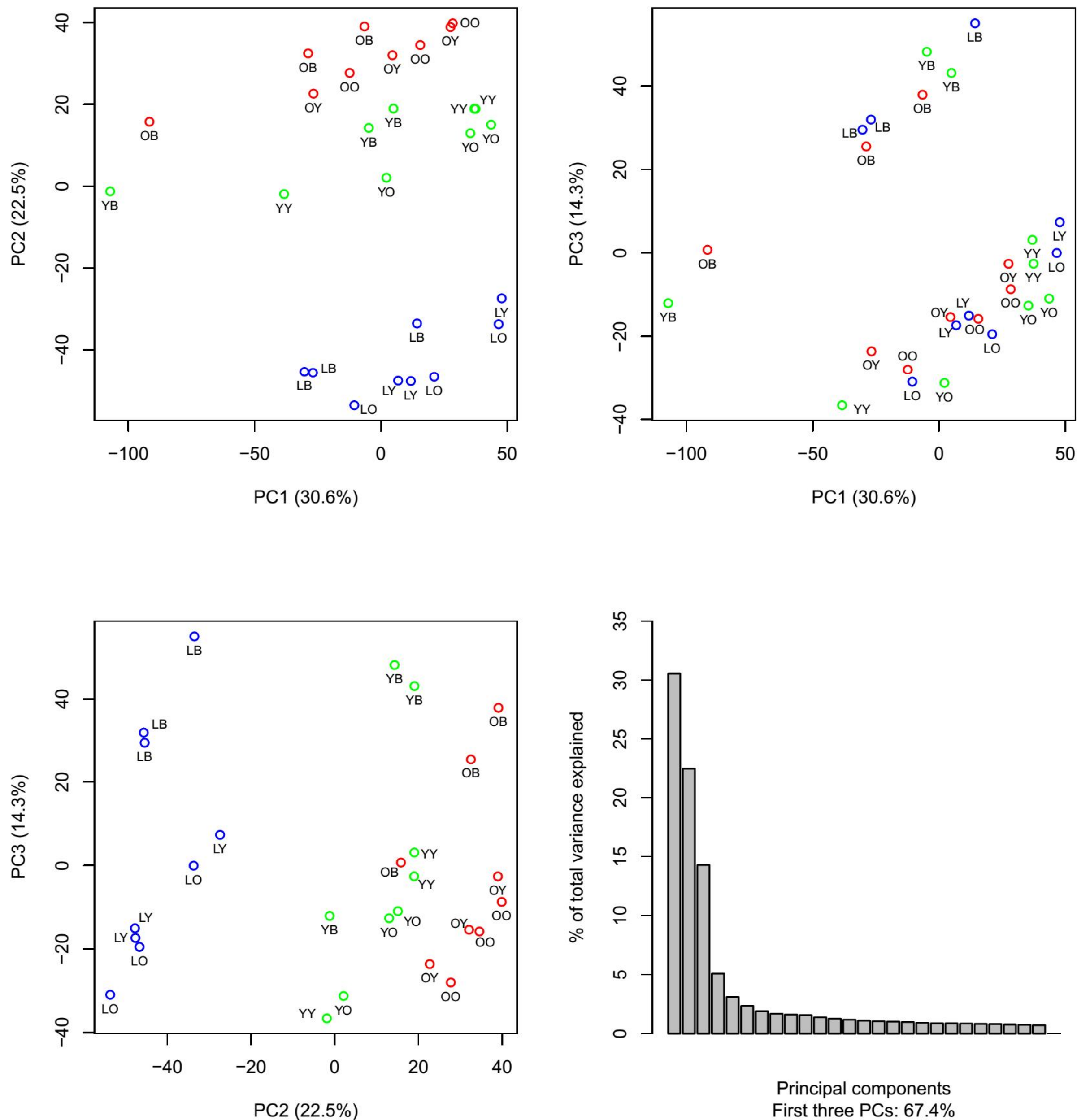


Figure 3-14 Principle component analysis of expression measures for London strain, cyp- and Col-adapted *Tetranychus urticae* on bean, cyp and Col-0 plants. LB- London strain on bean; LY- London strain on *cyp79B2 cyp79B3*; LO- London strain on Col-0; YB- cyp-adapted mite on bean; YY- cyp-adapted mite on *cyp79B2 cyp79B3*; YO- cyp-adapted mite on Col-0; OB- Col-adapted mite on bean; OY- Col-adapted mite on *cyp79B2 cyp79B3*; OO- Col-adapted mite on Col-0.

Regardless of the strain of spider mites, 304 genes were differentially upregulated in mites upon feeding on Col-0 Arabidopsis compared to that on bean. About 30 % of the induced genes on Col-0 were associated with detoxification enzymes including, cytochrome P450 monooxygenases (34 genes), UDP- glycosyltransferase (18 genes), glutathione-S-transferases (15 genes), esterases (12 genes), ABC-transporters (8 genes) and major facilitator superfamily (2 genes). Among the 304 induced genes on Col-0, only 18 genes were differentially upregulated in mites upon feeding on Col-0 compared to that on *cyp79B2 cyp79B3* plants. Compared to the 89 detoxification genes induced in spider mites upon feeding on Col-0 compared to that on bean, only four detoxification genes are differentially upregulated between Col-0 and *cyp79B2 cyp79B3* plants including one gene of each cytochrome P450s, UDP- glycosyltransferase, esterases and major facilitator superfamily.

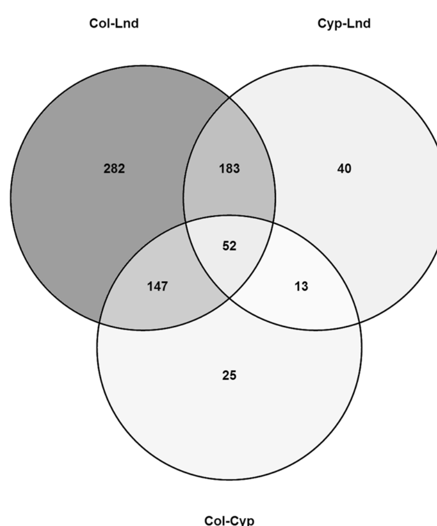


Figure 3-15 Analysis of differentially expressed genes from London strain (Lnd), cyp- (cyp) and Col-adapted (Col) *Tetranychus urticae* upon feeding on Col-0, *cyp79B2 cyp79B3* and bean plants.

The comparison between differentially expressed genes (DEGs) of the three strains of spider mites regardless of the host plant showed 664 DEGs between the London strain and Col-adapted mites, while there was less than half of that between the London strain and cyp-adapted mites (288 DEGs) and between cyp- and Col-adapted mites (237 DEGs;

Figure 3-15). Comparisons of transcriptional responses between different mite-plant combinations is shown in three heat maps related to DEGs between London strain and Col-adapted (Figure 3-16), London strain and cyp-adapted (Figure 3-17) as well as Col- and cyp adapted spider mites (Figure 3-18). A total of 18 comparisons can be observed in each heat map: same mite strain on three host plants, as well as three mite strains compared on the same plant. The dendrogram of heat maps related to DEGs between the London strain and adapted mites shows that regardless of their host plant, the transcriptomic response of Col-adapted and cyp-adapted spider mites are more similar compared to the London strain. Constitutive expression of genes in cyp-adapted mites is more comparable to that in London strain mites rather than Col-adapted mites. There is a subset of DEGs strongly upregulated in Col-adapted mites, while in cyp-adapted and London strain mites, these genes either do not show strong changes in expression or are down-regulated.

In heat maps related to DEGs between adapted and non-adapted mites, gene expression of spider mites on Col-0 and *cyp79B2 cyp79B3* was more similar in comparison with that on bean. However, the heat map of DEGs between cyp- and Col-adapted spider mites (Figure 3-18) shows more similarity in gene expression of mites on bean and *cyp79B2 cyp79B3* compared to that on Col-0 plants.

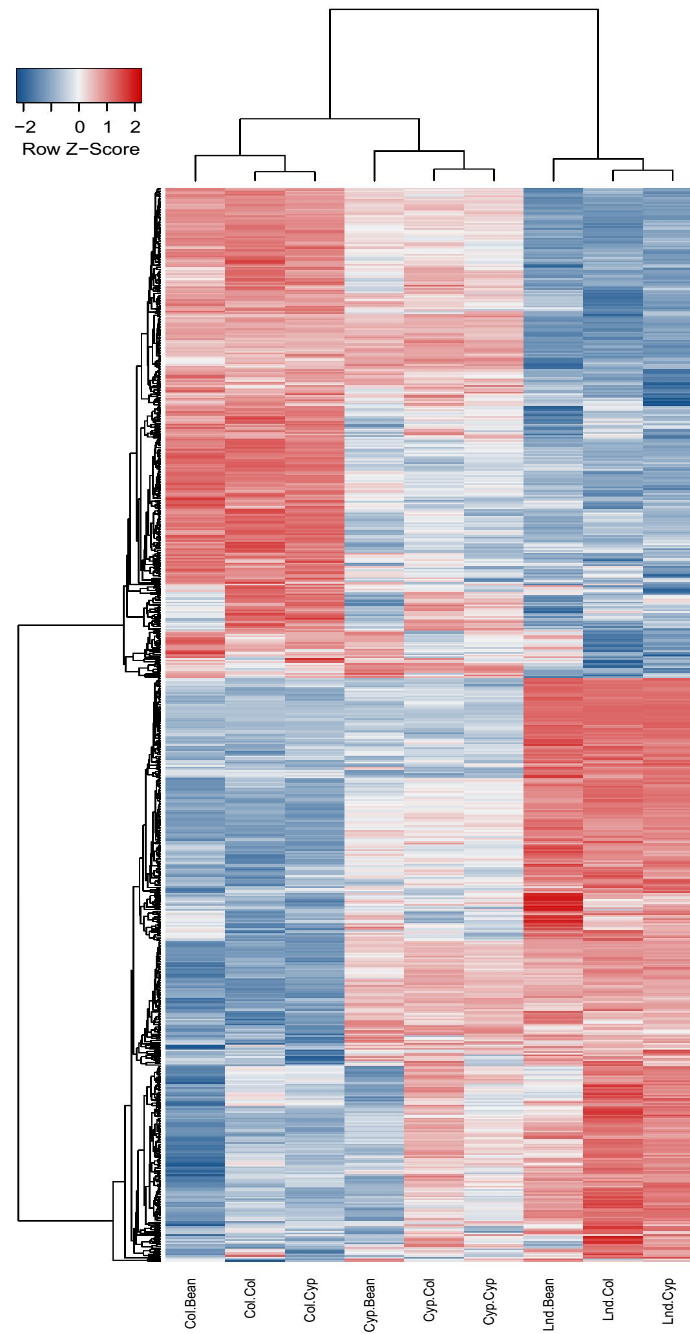


Figure 3-16 Expression of DEGs between Col-adapted and non-adapted (London) *Tetranychus urticae* in different strains of spider mites feeding on bean, *cyp79B2 cyp79B3* and Col-0 plants. Hierarchical clustering analysis of log2 RPKM exhibited by DEGs P, 0.05 detected between Col-adapted and London stain spider mites. The distance metric was Pearson's r, and the clustering method was average distance clustering (FC>2, FDR adjusted p-value <0.05).

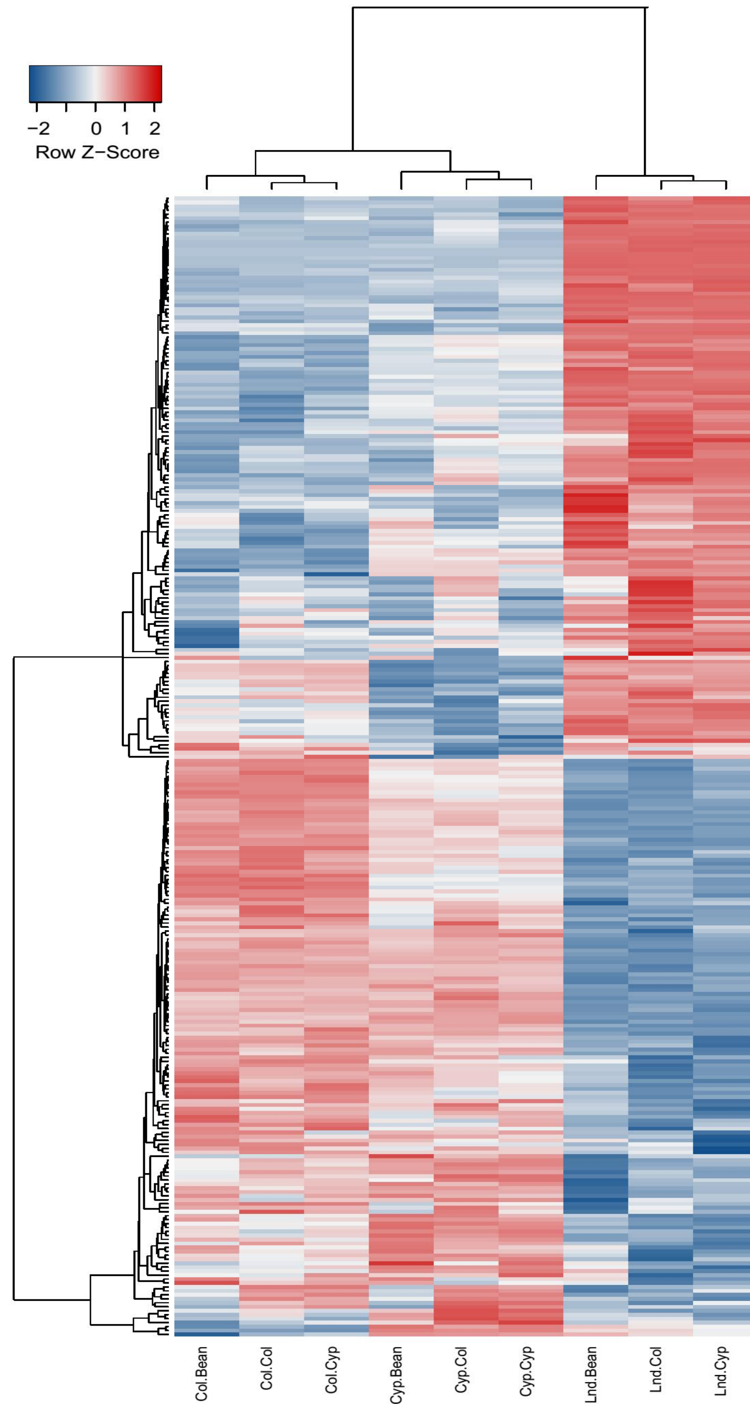


Figure 3-17 Expression of DEGs between cyp-adapted and non-adapted (London) *Tetranychus urticae* in different strains of spider mites feeding on bean, *cyp79B2 cyp79B3* and Col-0 plants. Hierarchical clustering analysis of log2 RPKM exhibited by DEGs P, 0.05 detected between Col-adapted and London stain spider mites. The distance metric was Pearson's r, and the clustering method was average distance clustering (FC>2, FDR adjusted p-value <0.05).

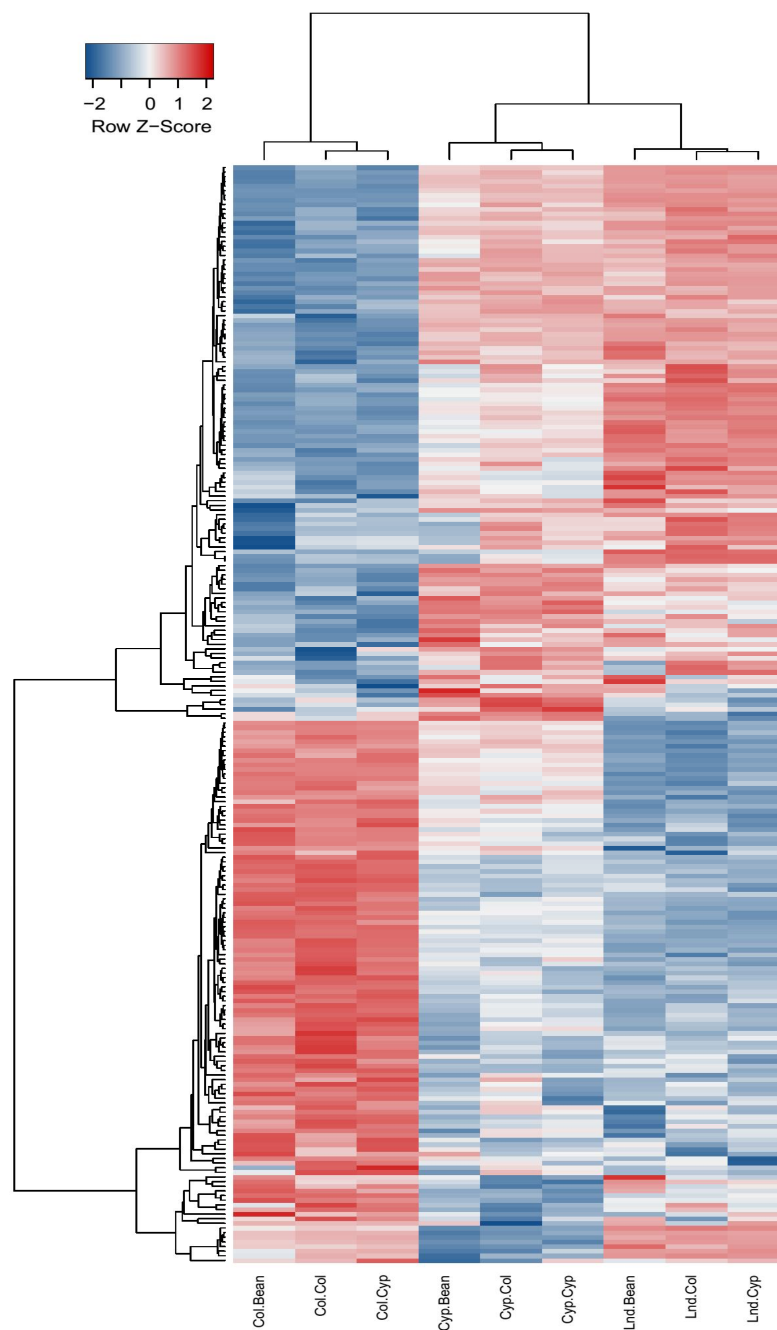


Figure 3-18 Expression of DEGs between Col-adapted and cyp-adapted *Tetranychus urticae* in different strains of spider mites feeding on bean, *cyp79B2 cyp79B3* and Col-0 plants. Hierarchical clustering analysis of log2 RPKM exhibited by DEGs P, 0.05 detected between Col-adapted and London stain spider mites. The distance metric was Pearson's r, and the clustering method was average distance clustering (FC>2, FDR adjusted p-value <0.05).

In all three comparisons, about half of the DEGs were upregulated and the other half downregulated. Analysis of DEGs showed different sets of biological functions. A large fraction of DEGs between the three spider mite strains (30-40%) were not related to any GO term. Genes associated with sulfatase or specifier proteins were not differentially expressed between adapted and non-adapted spider mites. The highest number of up-regulated and down-regulated genes was associated with "Leucin-rich repeat domain, L domain-like" followed by detoxification enzymes. Among the DEGs between Col-adapted and London strain mites, 11.2% of the upregulated genes were associated with detoxification enzymes and transporters including genes that regulate synthesis of P450s, GSTs and esterases, ABC-transporters, UDP-glycosyltransferases and major facilitator superfamily transporters (MFS).

To determine the genes that are potentially responsible for adaptation of Col-adapted spider mites to IGs, constitutive DEGs between cyp- and Col-adapted mites were filtered for genes that were only upregulated in Col-adapted mites. A total of 60 genes were differentially up-regulated in Col-adapted compared to cyp-adapted spider mites, among which 13 genes were related to detoxification of xenobiotics (Table 3-3).

Table 3-3 Genes that are differentially expressed between cyp- and Col-adapted *Tetranychus urticae* and are only upregulated in Col-adapted spider mites.

Gene ID	Log2 Fold changes	Description
tetur11g05200	1.891085898	ABC-transporter; class A
tetur07g03950	2.571112051	acylamino-acid-releasing enzyme
tetur247g00010	1.411177306	ATG4 autophagy related 4 homolog A
tetur09g00350	1.472514057	Cathepsin L
tetur10g01570	1.796227377	cysteine synthase A
tetur20g02620	3.455996648	Galactose-binding domain-like
tetur02g07220	2.402170719	Galactose-binding domain-like
tetur05g05300	1.486363729	Glutathione S-transferase; class mu
tetur17g03650	1.408868333	Major facilitator superfamily domain
tetur02g00510	2.919503242	neprilysin
tetur09g06716	2.673928759	Peptidase M12B, ADAM/reprolysin
tetur07g06390	2.045859474	UDP-glycosyltransferase
tetur21g01400	2.951028151	UDP-glycosyltransferase

3.4 Spider mite metabolomics

To determine the profile of IGs-related metabolites in adapted and non-adapted spider mites, the relative abundance of the four known IGs of Arabidopsis including indol-3-ylmethyl (IMG), 4-Methoxyindol-3-ylmethyl (4mIMG), 1-Methoxyindol-3-ylmethyl (1mIMG) and 4-hydroxyindol-3-ylmethyl (4hIMG) glucosinlates were determined in London and Col-adapted spider mite strains that were fed on *atr1-D*, Col-0 or *cyp79B2 cyp79B3* genotypes of Arabidopsis using high performance liquid chromatography (HPLC) - time of flight mass spectrometer (TOF-MS).

Among the four Arabidopsis intact IGs, three of them (IMG, 4mIMG and 1mIMG) were detected in both adapted and non-adapted spider mites feeding on *atr-1D* and Col-0 (Figure 3-19 A and B). Higher concentration of intact IGs in spider mites on *atr1-D* compared to those on Col-0 is due to overaccumulation of IGs in this mutant of Col-0. In both adapted and non-adapted spider mites feeding on *cyp79B2 cyp79B3*, no IGs was detected (Figure 19 C) which is consistent with the mutant lacking IGs. Comparing the concentration of intact IGs in adapted and non-adapted *T. urticae*, no significant difference was observed between London and Col-adapted spider mite strains feeding on *atr1-D*, although there is a trend for greater IGs accumulation in London strain. In spider mites feeding on Col-0, there was no significant differences between London and Col-adapted mites, although the difference between levels of 4mIMG approaches significance ($p = 0.0568$).

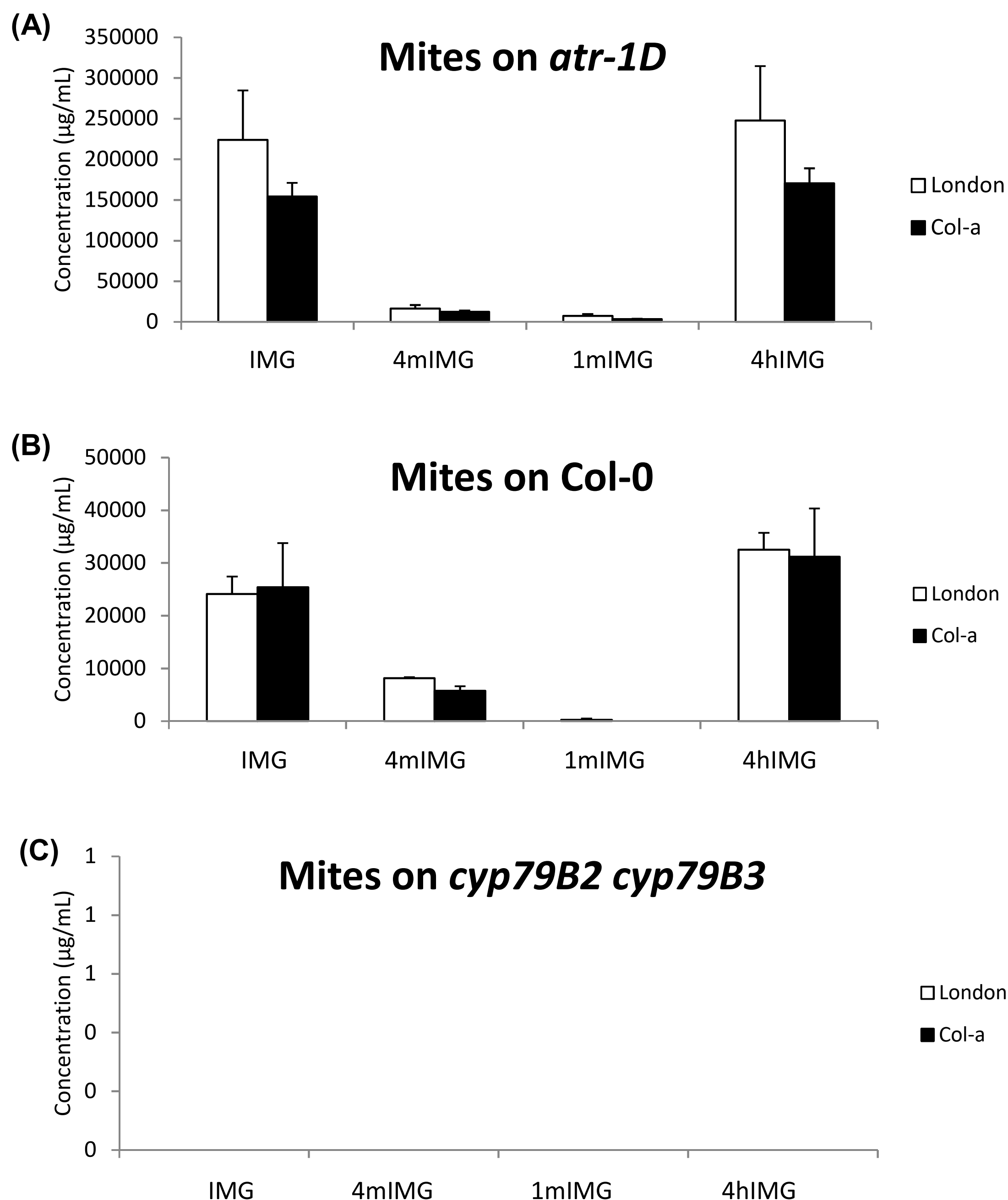


Figure 3-19 Level of indole glucosinolates (IGs; mean±SEM) in Col-adapted (Col-a) and non-adapted (London) *Tetranychus urticae* feeding on different host plants: A) *atr1-D* (over-accumulating IGs), B) Col-0 and C) *cyp79B2 cyp79B3* (devoid of IGs). Error bars are ±SEM (n=3; t-test; no star means no significant difference at $p<0.05$); IMG = indol-3-ylmethyl, 4mIMG = 4-Methoxyindol-3-ylmethyl, 1mIMG = 1-Methoxyindol-3-ylmethyl, 4hIMG = 4-hydroxyindol-3-ylmethyl.

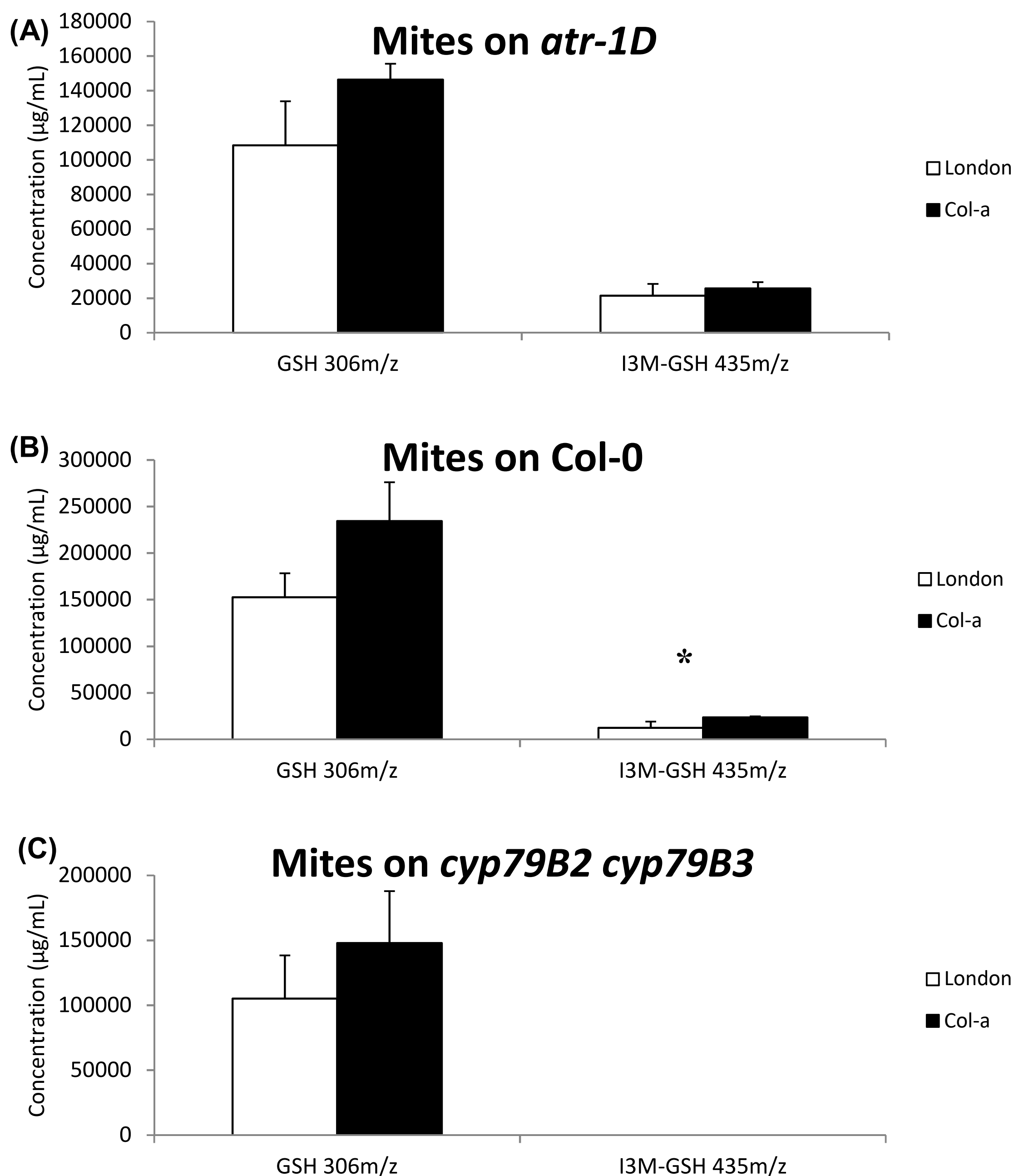


Figure 3-20 Level of the glutathione and the glutathione conjugate of indole-3-ylmethyl (mean±SE) in Col-adapted (Col-a) and non-adapted (London) *Tetranychus urticae* feeding on different host plants: A) *atr1-D* (over-accumulating IGs), B) Col-0 and C) *cyp79B2 cyp79B3* (devoid of IGs). Error bars are ±SEM (n=3; t-test; * $p<0.05$); GSH = glutathione, I3M-GSH = indole-3-ylmethyl glutathione.

To investigate modifications that occur to IGs in spider mites after ingestion and the potential differences in IG modifications in adapted and non-adapted spider mites, evidence for the seven identified IGs breakdown products were measured in spider mites including indole-3-carbinol (I3C), indole-3-acetonitrile, indole-3-carboxaldehyde, methyl indole-3-carboxylate, indole-3-ethanol, indole-3-carboxylic acid and 3,3'-diindolylmethane, as well as their amino acid conjugates [indol-3-ylmethylglutathione (I3M-GSH), indol-3-ylmethylcysteine (I3M-Cys) and indol-3-ylmethyl ascorbate (ascorbigen)] in spider mites that were fed on Col-0, *Atr1-D* and *cyp79B2 cyp79B3*. Of all the breakdown products and synthesized conjugates, only I3M-GSH was detected in mite extracts (Figure 20 A and B). There was no I3M-GSH detected in mites feeding on *cyp79B2 cyp79B3* (Figure 20 C).

No significant difference was observed in the level of IGs or known IG breakdown products between London strain and Col-adapted mites feeding on *Atr1-D* (Figure 20 A). However, I3M-GSH was significantly greater ($p < 0.05$) in Col-adapted mites relative to the London strain feeding on Col-0 (Figure 20 B). There was a trend for greater GSH levels in the Col-0 adapted mites relative to the London population on all three plant genotypes (Figure 20 A, B and C).

Chapter 4
Discussion

Control of two-spotted spider mites (*Tetranychus urticae*), a key pest on many agricultural crops, is problematic due to spider mites' ability to develop resistance to a wide variety of xenobiotics including plant chemicals (Feyereisen, 2005; Després *et al.*, 2007; Díaz-Riquelme *et al.*, 2016; Van Leeuwen and Dermauw, 2016). Indole glucosinolates (IGs) are secondary metabolites found in the Brassicaceae plants [including *Arabidopsis thaliana* (Arabidopsis); (Halkier and Gershenzon, 2006)] that are shown to be effective against *T. urticae* and could be potential candidates to control spider mites (Zhurov *et al.*, 2013). However, a laboratory population selected on IGs-containing Arabidopsis was able to adapt to this plant (Ratlamwala, 2014 b). The overall objective of this thesis was to identify the mechanism of adaptation of two-spotted spider mites to Arabidopsis and IGs. To fulfil this objective, first the mode of action of IGs and stages of spider mites that are sensitive to IGs were identified. Life table experiments, larval development assays and fecundity assays showed that larval mortality and adult fecundity are the most sensitive *T. urticae* parameters to IGs. Between these two parameters, fecundity better showed the IGs-specific adaptation of spider mites to Arabidopsis. Therefore, fecundity was chosen as the readout and adults as the experimental material for the following experiments.

Similar expression of marker genes and levels of plant defense-related metabolites after feeding of IG-adapted and non-adapted adult spider mites suggested that plant defense suppression is not the strategy used by spider mites to adapt to Arabidopsis IGs; whereas, higher activity of P450 monooxygenases in IG-adapted mites and the negative effect of inhibitors of these detoxification enzymes on spider mite fecundity suggests that spider mites use detoxification as a strategy to overcome glucosinolate-dependent Arabidopsis defenses. HPLC-mediated detection of conjugated forms of IGs breakdown products supports the involvement of detoxification in adaptation of *T. urticae* to IGs. RNA-seq analysis, showed induction of a large number of detoxification enzyme genes upon mite feeding on IGs. Genes that were differentially upregulated in adapted compared to non-

adapted spider mites likely capture genes associated with the adaptation to IGs, suggesting that these genes can be further used in manipulation of the *T. urticae* regulatory system to avoid development of adaptation or to succumb it.

4.1 Effect of IGs on *T. urticae* biological parameters

The negative effect of IGs on mortality and developmental time of *T. urticae* larvae was shown previously (Zhurov *et al.*, 2013), however, the effect of IGs on other developmental stages and other biological parameters had not been studied. To determine the developmental stages of *T. urticae* that are sensitive to IGs, experiments were performed to estimate life history parameters (including developmental time of different premature stages and longevity of adults), fecundity and life table parameters of non-adapted spider mites (London strain) on bean and Arabidopsis genotypes, Col-0 and *cyp79B2 cyp79B3*.

Life history parameters

Incubation time of eggs and developmental time of protonymphs were not affected by different host plants. However, developmental time of larvae, deutonymphs and as a result, the total premature developmental time, were significantly shorter on bean compared to that on Col-0 and *cyp79B2 cyp79B3*, suggesting the negative effect of Arabidopsis defense on non-adapted *T. urticae*. Longer developmental time of larvae and deutonymphs as well as shorter longevity of adults on Col-0 compared to those on *cyp79B2 cyp79B3* show that negative effect of Arabidopsis on *T. urticae* was due to the effect of IGs (or any other unknown metabolite that its biosynthesis is ceased or lowered in *cyp79B2 cyp79B3* mutants of Col-0). Therefore, except for the egg and protonymph stages, other developmental stages of *T. urticae* are sensitive to IGs. Also, in a different assay that was performed only on larvae, significantly higher mortality and longer developmental time of London strain larvae was observed on Col-0 compared to that on *cyp79B2 cyp79B3*.

Life table parameters and fecundity

Besides survival, developmental time and longevity, fecundity plays a significant role in population growth of spider mites. Gross reproductive rate (GRR; the total number of eggs produced by all females), basic reproductive rate (R_0; population’s rate of replacement)

and intrinsic growth rate (r_m) are life table parameters that are representative of population growth rate. The highest GRR, R_0 and r_m of London strain spider mites was on bean and the lowest on Col-0 indicating suitability of bean in comparison with Arabidopsis as plant host. The calculated GRR, R_0 and r_m of the London strain on Col-0 were significantly lower than that on *cyp79B2 cyp79B3* which shows the detrimental effect of IGs on reproduction rate and consequently population growth of spider mites. However, in a life table experiment, the observed fecundity might be indirectly influenced by feeding of premature stages from different host plants. To remove the effect of different feeding source of females during their development from eggs to adults, a fecundity assay was conducted using females that were raised on bean. After six days of transferring these bean-grown spider mite females to a different host plant (bean, *cyp79B2 cyp79B3* and Col-0), a significantly lower fecundity of females was observed on Col-0 compared to that on *cyp79B2 cyp79B3* plants. These results revealed the negative effect of IGs on fecundity of *T. urticae* females which confirms the results from the life table parameters.

Consequently, negative effect of IGs can be observed in larva, deutonymph and adult *T. urticae* upon feeding on Col-0 plants. Biological characteristics of *T. urticae* including developmental time, mortality and fecundity are affected by IG which were used as readouts for further studies on IGs effect on *T. urticae*.

4.2 Adaptation of *T. urticae* to IGs

Survivorship curves of adapted (cyp- and Col-adapted) and non-adapted spider mites on different host plants revealed the effect of adaptation on population growth of spider mites regardless of their fecundity. On both Col-0 and *cyp79B2 cyp79B3* plants, survivorship curves of adapted and non-adapted spider mites were significantly different indicating better survival of adapted mites on Arabidopsis compared to non-adapted mites.

The comparison of life history parameters between London, cyp-adapted and Col-adapted *T. urticae* on Col-0 and *cyp79B2 cyp79B3* plants showed no significant difference in developmental time of protonymphs and deutonymphs or incubation time of eggs between adapted and non-adapted spider mites. By contrast, on Col-0, Col-adapted and cyp-adapted larvae were significantly faster in developing to protonymph compared to London strain

larvae. However, developmental time and mortality of larvae did not show the IGs-specific adaptation of Col-0 spider mites, since on Col-0, mortality and developmental time of cyp- and Col-adapted spider mites were not significantly different. This might be due to the very short time mites spend as larvae. Thus, this developmental stage may not be responsive (at least resulting from the applied protocol, recording progression through developmental stages once a day) to host adaptation status.

On Col-0 plants, life table parameters, including GRR, R_0 and r_m, were significantly higher in Col- and cyp-adapted spider mites compared to London strain which shows development of adaptation to Arabidopsis in Col-0 and cyp-adapted mites. This adaptation was not IGs-specific since life table parameters of cyp- and Col-adapted mites was not significantly different. However, in the fecundity assay, in which the effect of preadult food source and survival is removed, Col- adapted spider mites produced significantly more eggs on Col-0 compared to cyp-adapted mites indicating the IGs-specific adaptation of mites to IGs.

Similar life history and life table parameters of the three strains of spider mites on bean demonstrates that there is no fitness cost of adaptation to IGs in adapted mites. It is previously shown that spider mites are able to adapt to a less favorable host plant without presenting an associated fitness cost (Agrawal, 2000). These results do not agree with those reported by Ratlamwala (2014) which suggest a small fitness cost related to adaptation of Col-adapted spider mites on Arabidopsis. This might be due to the difference in time when adaptation-related fitness parameters were measured. While Ratlamwala (2014) checked these parameters after 20 to 25 generations of selection on host plant, mite population used in experiments reported here were maintained on their corresponding hosts for over 50 generations.

4.3 Role of myrosinases in effect of IGs on spider mites

Negative effect of glucosinolates on chewing insects depends on myrosinases-mediated hydrolysis of glucosinolates. In these interactions, the mixing of IGs and myrosinases results from the ingestion of chunks of plant tissue upon feeding of these herbivores. In piercing-sucking insects, despite the minimal damage they impose to the plant tissue during feeding, a myrosinase-independent effect of IGs is expected and has been reported in

aphids. Similarly, IGs negatively affect *T. urticae*, although spider mites feeding damages only a few cells in the feeding area which is less likely to release myrosinases. Life table experiments, larval development assay and fecundity assay were performed to clarify whether myrosinases-depended hydrolysis of IGs is necessary for the effect of IGs on spider mites. No difference was observed in larval mortality, fecundity, longevity or life table parameters of *T. urticae* feeding on Col-0 or *tgg1 tgg2* (the myrosinase-devoid mutant of Col-0). The only parameter of *T. urticae* that was affected by myrosinases was developmental time of larvae which was significantly longer on Col-0 compared to that on *tgg1 tgg2*. These results suggest that myrosinases are involved in, but not required for the negative effect of IGs on spider mites.

4.4 The adaptation strategy: to suppress or to resist plant defense?

4.4.1 Plant defense suppression

Although IGs are present constitutively in plant tissues, over 90% of previous studies showed that herbivore attack to glucosinolate-containing plants induces their greater accumulation (up to 20-fold) of indolic glucosinolates (reviewed in Textor and Gershenzon, 2009). It is indicated that jasmonates increase the expression of genes associated with glucosinolate biosynthesis, including *CYP79B2* and *CYP79B3* that catalyze the oxidation of tryptophan to indol-3-acetaldoxime (Brader, 2001; Mikkelsen *et al.*, 2003; Sasaki-Sekimoto *et al.*, 2005; Halkier and Gershenzon, 2006; Rehrig *et al.*, 2014). In this study, expression of IGs-related genes, as well as induction of indole-3-ylmethyl glucosinolate (I3M), jasmonic acid (JA) and its bioactive form, jasmonic acid-isoleucin (JA-Ile) increased upon herbivory by *T. urticae* supporting a defensive role of IGs in Arabidopsis. Herbivory-induced biosynthesis of IGs was also shown in response to *Myzus persicae* feeding and plant hormone treatment (Kim and Jander, 2007; Agerbirk *et al.*, 2009).

Herbivores can manipulate plant defensive responses and block or reduce the level of the response (Ferry *et al.*, 2004; Alba *et al.*, 2011; Blaazer *et al.*, 2018). Regardless of the step of the defensive pathway that this manipulation occurs, suppression of plant defense is

characterized by reduction of biosynthesis of the defensive compound (Kant *et al.*, 2015). Reduction of aliphatic glucosinolates (AGs) and benzenic glucosinolates (BGs) levels mostly in response to specialist rather than generalist herbivores is shown in previous studies (Birch *et al.*, 1992; Hopkins *et al.*, 1998; Van Dam and Raaijmakers, 2006). Therefore, to investigate whether suppression of plant defense is the mechanism of *T. urticae* adaptation to Arabidopsis IGs, level of the main IG in Arabidosis, indole-3-ylmethyl glucosinolate (I3M), as well as jasmonic acid (JA) and its bioactive form, jasmonic acid-isoleucin (JA-Ile) were measured in spider mite-infested plants. No change in level of the mentioned defensive products upon feeding of adapted and non-adapted spider mites indicates that adaptation in *T. urticae* is obtained through another mechanism rather than plant defense suppression. Also, no change in expression of genes associated with biosynthesis of IGs confirms the metabolomic results, suggesting that Col-adapted spider mites do not suppress the plant defensive response. This strategy is usually effective against AGs and BGs more than IGs (Blaazer *et al.*, 2018). For example, feeding of *Delia floralis* and *D. radicum* decreased level of AGs or BGs of up to 60%, while the level of IGs increased (Birch *et al.*, 1992; Hopkins *et al.*, 1998; Van Dam and Raaijmakers, 2006). Moreover, attack of two specialist herbivores, caused a 50–70% reduction in leaf glucosinolate content (Soler *et al.*, 2005).

4.4.2 Detoxification of IGs-related metabolites

Many arthropods can detoxify plant defensive compounds, using detoxification enzymes including cytochrome P450 monoxygenases (P450s), esterases and glutathione S-transferases (GSTs) (Després *et al.*, 2007; Li *et al.*, 2007). These enzymes are usually induced in the herbivore in response to plant-derived xenobiotics (Després *et al.*, 2007). Among these three detoxification enzyme classes, previous studies have mostly shown involvement of GSTs in detoxification of glucosinolates in insects (Wadleigh and Yu, 1988; Hemming and Lindroth, 2000; Francis *et al.*, 2005; Schramm *et al.*, 2012). In the current study, the three strains of spider mites, London strain, cyp- and Col-adapted, showed similar levels of GSTs activity in the absence of IGs challenge. Activity of GSTs did not increase in non-adapted mites upon switching host plant from bean to glucosinolate-containing plants while small but insignificant increase in GST activity was observed in

adapted mites in response to glucosinolates. On Arabidopsis, GSTs activity of adapted spider mites was not statistically different from non-adapted mites which suggests that GSTs are less likely to be involved in adaptation of spider mites to Arabidopsis. This was confirmed when inhibition of GSTs was tested: it did not affect fecundity of non- or Col-adapted spider mites on Arabidopsis reflecting that the GST enzymes that are inhibited by diethyl maleate (DEM) are not involved in adaptation of Col-adapted spider mites to *Arabidopsis* defense. These results were in contrast with the previous studies indicating the role of GSTs in adaptation of insects to other classes of glucosinolates. For example, GST-mediated conjugation of isothiocyanates (the breakdown product of glucosinolates) with glutathione was shown in generalist caterpillars cabbage moth, *Mamestra brassicae* and cotton bollworm, *Helicoverpa armigera* upon feeding on Arabidopsis (Schramm *et al.*, 2012). Also, GST activity was induced in green peach aphid, *Myzus persicae* in response to increasing glucosinolate concentrations (Francis *et al.*, 2005).

Esterase activity was induced in non-adapted *T. urticae* upon switching host plant from bean to Arabidopsis. However, induction of esterase activity was not significantly different upon feeding on *cyp79B2 cyp79B3* and Col-0, showing that induction of esterase activity is not specific to IGs. Induction of esterases can be due to other defensive metabolites of Arabidopsis which are present in both *cyp79B2 cyp79B3* and Col-0 plants. Similar esterase activity of adapted and non-adapted spider mites on Arabidopsis suggests that esterases are not involved in adaptation of spider mites to IGs. These results were supported as fecundity of adapted spider mites did not change after treatment of Arabidopsis leaves with the esterases inhibitor, tributyl phosphorotrithioat (DEF).

IGs-specific induction of P450s was shown in spider mites upon host plant change to Arabidopsis. Also, higher activity of P450s in Col-adapted compared to cyp- and non-adapted spider mites could be demonstrative of P450s' role in adaptation of Col-adapted mites to Arabidopsis IGs. Furthermore, application of P450s inhibitors, piperonyl butoxide (PBO) and trichlorophenylpropynyl ether (TCPPE) lead to a decrease in fecundity of Col-adapted spider mites on Arabidopsis. It suggested contribution of P450s in adaptation of *T. urticae* to Arabidopsis defense. However, this adaptation did not appear to be IG-s specific,

since reduction of spider mite performance caused by P450s inhibitors occurred on both Col-0 and *cyp79b2 cyp79b3* plants.

The critical role of P450s in metabolism of plant defensive compounds and as a result determination of herbivores' food source is studied in a few cases (Oppenoorth and Van Asperen, 1960; Andersen *et al.*, 1997; Baudry *et al.*, 2003). Involvement of P450s is implicated in the biotransformation of several plant secondary metabolites including furanocoumarins (in swallowtails, *Papilio* spp., corn earworm, *Helicoverpa zea,* and novel orange worm, *Amyelois transitella*), furanochromones (in *Anopheles gambiae*), terpenoids (in *Musca domestica*), sesquiterpenoids (in *Diploptera punctate*) and flavonoid (in *A. mellifera*)(Lindroth, 1989; Seifert and Scott, 2002; Ortelli *et al.*, 2003; Scott and Zhang, 2003; Pan *et al.*, 2004; Després *et al.*, 2007). RNAi silencing of CYP6AE14 transcripts in *Helicoverpa armigera* reduced larval tolerance to the gossypol (Mao *et al.*, 2007). Also, CYP4D and CYP28A subfamily transcripts were induced in cactophilic Drosophila species upon feeding on toxic isoquinoline alkaloids suggesting involvement of P450s in metabolism of these alkaloids (Tijet *et al.*, 2001). The only evidence of involvement of P450s in detoxification of IGs breakdown products is metabolism of indole-3-carbinol in the generalist *H. zea* mediated by CYP6B8 which also metabolizes xanthotoxin, flavone, α-naphthoflavone (α-NF), chlorogenic acid, quercetin and rutin (Li *et al.*, 2004; Rupasinghe *et al.*, 2007).

4.5 Determination of genes associated with the adaptation to IGs

An RNA-sequencing analysis was performed to determine differences in gene expression of adapted and non-adapted spider mites upon feeding on the wild-type Arabidopsis and the IGs-devoid genotype, *cyp79B2 cyp79B3*, as well as that on bean plants. Comparing the effect of host plant on *T. urticae* gene expression, the number of differentially expressed genes (DEGs) when spider mites were shifted from bean to Col-0 was twice more than that when they were shifted from bean to *cyp79B2 cyp79B3*. These results coupled with those reflecting that more than three times the number of DEGs were upregulated (compared to downregulated DEGs), suggest induction of a defensive response to Arabidopsis IGs in

spider mites. A considerable number of genes expressed in spider mites on Arabidopsis are detoxification genes, supporting the defensive role of IGs in Arabidopsis against *T. urticae*. Major detoxification gene families including cytochrome P450 monooxygenases (P450s), glutathione-S-transferases (GSTs), UDP-glycosyltransferases (UGTs), carboxylesterases and ABC transporters (ABCs) have a broad range of substrate specificity and are generally important for generalist herbivores that are exposed to a wide variety of plant defensive compounds (Li *et al.*, 2007; Heidel-Fischer and Vogel, 2015; Stahl *et al.*, 2018). The first indication of glucosinolate-induced expression of detoxification genes was in the generalist *Trichoplusia ni* feeding on Arabidopsis (Herde and Howe, 2014). Similarly, significant changes in gene expression was observed when the generalist herbivore, *Helicoverpa virescens* fed on wild type Arabidopsis compared to that on the glucosinolate-devoid quadruple mutant (*quadGS*) (Schweizer *et al.*, 2017).

The total number of DEGs between the London strain and Col-adapted spider mites was over double that between the London strain and cyp-adapted mites. These results coupled with those indicating better performance of Col-adapted spider mites on Arabidopsis compared to London strain and cyp-adapted mites reflect adaptation of Col-adapted spider mites to IGs. In a similar study of *T. urticae* on tomato, spider mites that were transferred from bean to tomato and were selected on tomato for more than 30 generations showed considerably more expression of detoxification genes compared to non-adapted spider mites (Wybouw *et al.*, 2015). The mechanism of *T. urticae* adaptation to IGs appears to be due to the upregulation of detoxification genes. Induction of detoxification genes, which is common in generalist arthropods, is shown to be often absent in specialist pests (Schweizer *et al.*, 2017). The reason is that specialists usually quickly disarm the two-component defensive system of glucosinolates which prevents production of toxic breakdown compounds. This in turn, avoids induction of general stress signals such as detoxifications enzymes in specialists. Secondly, implication of detoxification enzymes to overcome plant defense is a general mechanism of adaptations usually used by generalist arthropods (Després *et al.*, 2007; Stahl *et al.*, 2018). Thirdly, genes associated with the two most known mechanisms of adaptation to glucosinolates (sulfatases and specifier proteins) were not differentially expressed between adapted and non-adapted spider mites or upon host plant switch from bean to any of Arabidopsis genotypes. Expression of sulfatases and

specifier genes is exhibited in specialist insects or those that are adapted to glucosinolates (Ratzka *et al.*, 2002; Schweizer *et al.*, 2017).

Constitutive expression of genes in Col-adapted mites is more comparable to that in cyp- rather than non-adapted mites which is representative of similar transcriptional alterations of cyp- and Col-adapted spider mites caused by their long-term exposure to Arabidopsis (Figure 3-14). Similarly, upon host plant challenge, the transcriptomic response of Col-adapted and cyp-adapted spider mites to Arabidopsis defense are more similar compared to London strain. It reflects different expression of genes in cyp- and Col-adapted compared to non-adapted spider mites in absence of response to a host plant shift challenge (Figures 3-16, 3-17, 3-18). DEGs between adapted and non-adapted mites show that irrespective of mite strain, gene expression of spider mites on Col-0 and *cyp79B2 cyp79B3* plants was more similar in comparison with that on bean. This is not surprising as Col-0 and *cyp79B2 cyp79B3* are two genotypes of one species, both more distant from bean, belonging to a different plant family. However, cyp- and Col-adapted spider mites showed more similarity in gene expression on bean and *cyp79B2 cyp79B3* compared to that on Col-0 plants.

A large proportion of genes differentially upregulated between Col- and non-adapted spider mites are associated with detoxification enzymes including P450s (subfamilies 392A13 and CYP392E2; 8 genes), GSTs (from beta and mu classes; 10 genes), carboxylesterases (6 genes), ABC transformers (6 genes), MFSs (5 genes) and UGTs (12 genes). Among these genes no genes related to P450s and carboxylesterases, only two UGTs, as well as one of each ABC transporters, GSTs and MFSs were differentially upregulated in Col-adapted compared to cyp-adapted spider mites (Table 3-3). These results support those from inhibitor assay suggesting that P450s are involved in spider mite response to Arabidopsis defenses, but this response is not specific to IGs. Also, upregulation of other detoxification genes in Col- compared to cyp-adapted mites represents contribution of detoxification in IGs-adaptation of Col-adapted spider mites.

A recently suggested mode of action of glucosinolates against insects is impairment of the insect growth and development through inhibition of cathepsin B and L (Agnihotri *et al.*,

2018). A differentially upregulation of cathepsin L gene (Table 3-3) might be reflective of Col-adapted mite strategy to compensate the depleted cathepsin L in spider mite body and recover its normal development. The other gene that is differentially upregulated in Col-adapted *T. urticae* is associated with neprilysin, a protein that regulates sexual activity and fecundity in insects and mammals (Head *et al.*, 1993; Pinto *et al.*, 1999; Carpentier *et al.*, 2004; Sitnik *et al.*, 2014). Upregulation of this gene might be an adaptation strategy of spider mites to compensate the IGs-mediated decrease in fitness by increasing fecundity. This might be the explanation for the observed significantly higher reproduction of Col-adapted compared to cyp-adapted spider mites on *cyp79B2 cyp79B3* plants (Figure 3-4). The two galactose-binding domain-like (GBD) genes, differentially upregulated in Col-adapted spider mites, are carbohydrate-binding proteins that have a high affinity for binding to glycan residues (Kawsar *et al.*, 2009). Presence of an N-glycan structure in myrosinases (Liebminger *et al.*, 2012) leads to the hypothesis that these proteins might be involved in inhibition of myrosinases, preventing the enzymatic breakdown of glucosinolates within the gut.

4.6 Modifications of IG-related metabolites in spider mites

The levels of the four known IGs of Arabidopsis including indol-3-ylmethyl (IMG), 4-methoxyindol-3-ylmethyl (4mIMG), 1-methoxyindol-3-ylmethyl (1mIMG) and 4-hydroxyindol-3-ylmethyl (4hIMG) glucosinolates were measured in adapted and non-adapted spider mites fed on IGs-containing genotypes of Arabidopsis to determine the profile of IGs-related metabolites in spider mites. The level of the three Arabidopsis intact IGs detected in spider mites (IMG, 4mIMG and 1mIMG) was not significantly different between adapted and non-adapted spider mites. As the level of IGs is not higher in adapted compared to non-adapted spider mites, sequestration of intact IGs does not appear to be the adaptation mechanism used by adapted *T. urticae* to overcome Arabidopsis defense. Sequestration of glucosinolates after feeding is an adaptation mechanism employed by insects of different orders, mostly specialist feeders on cabbage family including turnip sawfly, *A. rosae* [Hymenoptera; (Müller *et al.*, 2001)], harlequin cabbage bug, *Murgantia histrionica* [Hemiptera; (Aliabadi *et al.*, 2002)] and the cabbage aphid, *Brevicoryne brassicae* [Hemiptera; (Francis *et al.*, 2001)]. Arthropods that sequester glucosinolates

should have efficient machineries for fast uptake of glucosinolates and inhibition of myrosinases to avoid breakdown of glucosinolates (Müller, 2009). However, it does not appear to be an efficient strategy for IGs, which can undergo spontaneous breakdown even before being ingested (Kim *et al.*, 2008). In various insects, sequestration of AGs and BGs but not IGs is indicated (Müller *et al.*, 2001; Müller and Wittstock, 2005 b; Opitz *et al.*, 2010; Abdalsamee and Müller, 2012).

Furthermore, potential IGs modifications in adapted and non-adapted spider mites body were investigated. Among the identified IGs breakdown products and their synthesized conjugates, only glutathione-conjugated form of I3C (I3M-GSH) was significantly higher in concentration in Col-adapted relative to the non-adapted spider mites feeding on Col-0. It suggests detoxification through glutathione (GSH) conjugation as an adaptation strategy used by Col-adapted spider. However, previous results from enzymatic activity and inhibitor assays in this study did not show involvement of GSTs in *T. urticae* adaptation to IGs. In insects, the midgut cells conditions (pH 7-10 and high concentration of GSH) facilitate spontaneous and non-enzymatic conjugation of GSH with isothiocyanates (Herde and Howe, 2014). The observed trend of greater GSH levels in the Col-0 adapted relative to non-adapted mites might be a strategy used by adapted mites to enhance the non-enzymatic conjugation of GSH with I3C. However, differentially expression of ten GST genes in Col- compared to non-adapted spider mites and one GST gene in Col- compared to cyp-adapted mites reflect the involvement of GSTs together with non-enzymatic conjugation of GSH in detoxification of IGs. In that case, the reason that effect of GST activity contribution in spider mite adaptation was not supported in inhibitor assays might be that the type of GST involved in IGs adaptation of spider mites could not be inhibited by diethyl maleate or detected by the substrate used in enzymatic activity assay.

Although involvement of GSTs in detoxification of glucosinolate breakdown products was first reported about 30 years ago (Yu, 1989), demonstration of product formation and their function *in vivo* have occurred recently (Schramm *et al.*, 2012; Zou *et al.*, 2016). Feeding on glucosinolates resulted in general induction of genes encoding detoxification enzymes, including GSTs, UGTs and cytochrome P450s. Analysis of cDNA sequences of the midgut tissue of the common cutworm, *Spodoptera lituralis* (Lepidoptera) led to the identification

of eight GSTs (Deng *et al.*, 2009; Huang *et al.*, 2011). One GST gene, encoding cytosolic GST epsilon 1 (SlGSTE1), was upregulated when larvae fed on a glucosinolate-containing host plant or artificial diets containing I3C and isothiocyantes (Zou *et al.*, 2016). RNAi-mediated silencing of SlGSTE1 lead to decreased larval consumption of host plant which in turn caused body weight reduction in these larvae compared to a control group, suggesting involvement of the encoded enzyme in detoxification of glucosinolate breakdown products (Zou *et al.*, 2016). Also, in *M. persicae*, amino acid and GSH conjugates of IGs breakdown products was detected in the honeydew, suggesting detoxification of the products by *M. persicae*. An analysis of the cDNA of *M. persicae* identified between 14 and 21 GST-like genes, of which eight belonged to the delta class (Ramsey *et al.*, 2010).

In summary, this study suggests that Col-adapted *T. urticae* employs a combination of general adaptation strategies to overcome the IGs-mediated Arabidopsis defense: 1) detoxification of IGs breakdown products through GSH conjugation; 2) detoxification of glucosinolates (more likely AGs and BGs) and/or other secondary metabolites common to Col-0and *cyp79B2 cyp79B3* plants by P450s; 3) increasing fecundity of adapted mites through upregulation of neprilysins to compensate the negative effect of IGs on spider mite fecundity; 4) decreasing IGs-induced developmental abnormalities through upregulation of cathepsin in spider mites, an essential protein for growth and development that might be inhibited as a result of glucosinolate activity; and 5) inhibition of myrosinases through GBDs binding to the N-glycan residue of myrosinases. Further studies are necessary to assess the integrity of each of these proposed strategies.

Chapter 5
Conclusion

The objective of this thesis was to identify the mode of action of indole glucosinolaes (IGs) on *Tetranychus urticae*, as well as the mechanism of adaptation of two-spotted spider mites to Arabidopsis and IGs. Based on my results, enzymatic hydrolysis of IGs is not required for the detrimental effect of IGs on spider mites, however, the IG effects are enhanced in the presence of the glucosinolate-degrading myrosinases. Also, data suggest that spider mites develop adaptation through detoxification of IGs rather than the suppression of plant defense.

Life table experiments, larval development assays and fecundity assays showed that fecundity is a better indicative of the IG-specific adaptation of spider mites to Arabidopsis compared to the other life history parameters, including developmental time and mortality. Therefore, fecundity was chosen as the readout for experiments. The only parameter of *T. urticae* that was affected by myrosinases was the developmental time of larvae while other biological parameters of spider mites were not affected. These results suggested that myrosinases are involved in, but not required for the negative effect of IGs on spider mites. The only known breakdown product of IGs that I was able to detect in spider mites upon feeding on Arabidopsis was indole-3-ylmethyl carbinol (I3C). Thus, I3C may be the IG breakdown product responsible for the effect of IGs on spider mites.

Similar expression of marker genes and levels of plant defense-related metabolites after feeding of spider mites indicated that plant suppression is not the strategy used by spider mites to overcome IG-dependent Arabidopsis defense. Consistently, enzymatic activity and inhibitor assays suggested the involvement of P450 detoxification enzymes in the adaptation of spider mites to Arabidopsis defense. The detection of I3C glutathione conjugates in the spider mite body extract further supported the involvement of detoxification in the adaptation of *T. urticae* to IGs. The detoxification-dependent adaptation of spider mites to IGs was also supported by RNA-seq analysis which identified the induction of a large number of detoxification enzyme genes upon mite feeding on IGs.

These enzymes include cytochrome P450 monooxygenases (P450s), glutathione-S-transferases (GSTs), UDP-glycosyltransferases (UGTs), carboxylesterases and ABC transporters (ABCs). RNA-seq analysis exhibited that expression of P450 genes in adapted mites in response to Arabidopsis metabolites is not specific to IGs. These results support those from the inhibitor assay suggesting that P450s are involved in *T. urticae* response to Arabidopsis defense, but this response is not specific to IGs.

In spite of similar GST activity of adapted and non-adapted spider mites, higher concentrations of IGs glutathione (GSH) conjugates coupled with the differentially upregulation of GST genes in IG-adapted spider mites suggested the contribution of GST enzymes and/or GSH conjugation in IGs detoxification as an adaptation strategy used by spider mites. Spontaneous and non-enzymatic conjugation of GSH in the spider mite body might be the underlying reason for detection of higher GSH conjugates of IGs despite the similar GST activity in adapted compared to non-adapted spider mites. The observed trend of greater GSH levels in the Col-0 adapted- relative to non-adapted mites might be a strategy used by adapted mites to enhance the non-enzymatic conjugation of GSH with I3C.

The differentially upregulated genes in IG-adapted compared to non-adapted spider mites are likely to contribute in adaptation of these mites to IGs. The observed differential upregulation of cathepsin L gene in IG-adapted spider mites might be a strategy for IG-adapted mites to compensate the IG-mediated depletion of cathepsin L activity (which has a critical role in mites' growth and development) in the spider mite body and consequently recover the normal development of mites. The other mechanism of IGs adaptation could be through an upregulation of neprisylin that is suggested to enhance mite fecundity. Also, spider mites might adapt to IGs through upregulation of galactose-binding domain-like (GBD) genes that may be involved in the inhibition of myrosinase-mediated hydrolysis of glucosinolates. Follow up studies should test the involvement of each of these genes in spider mite adaptation to IGs by assessing the effect of RNAi-mediated silencing of these genes on performance of adapted spider mites.

It is suggested that the non-enzymatic hydrolysis of IGs in spider mites, enhanced by myrosinase activity, lead to negative effect of IGs on spider mites. Further studies are required to investigate the potential presence of myrosinase-like enzymes in spider mite bodies. I3C was the only known breakdown product of IGs detected in spider mites upon feeding on Arabidopsis. Nevertheless, non-targeted analysis of modifications of Arabidopsis metabolites in spider mites should be performed followed by the assessment of the effect of these metabolites on the spider mite performance using artificial diet.

This study suggests that IG-adapted *T. urticae* employs a combination of several strategies to overcome the IGs-mediated Arabidopsis defense: 1) the detoxification of IGs breakdown products through GSH conjugation; 2) the detoxification of other classes of glucosinolates and/or other Arabidopsis secondary metabolites through enhancement of P450s activity; 3) fitness improvement through increased fecundity of adapted mites mediated by the upregulation of neprilysins; 4) the prevention of developmental abnormalities mediated by cathepsin-inhibitory action of glucosinolates through upregulation of cathepsin L in adapted mites; and 5) the inhibition of myrosinases through GBDs binding to the N-glycan residue of myrosinases.

Genes that were differentially upregulated in adapted compared to non-adapted spider mites should be assessed for their involvement in adaptation of spider mites to IGs. This could be conducted by investigating the effect of silencing of those genes on performance of IG-adapted mites. Resulting candidate genes can be further used in manipulation of *T. urticae* to prevent its adaptation to IGs or to succumb it. Several detoxification genes were differentially upregulated in adapted compared to non-adapted spider mites (e.g. UGTs). However, the activity of enzymes related to these genes in adapted and non-adapted spider mites was investigated only for CYPs, GSTs and esterases in this study . Further enzymatic activity and enzyme inhibition studies are needed to assess involvement of these enzymes in detoxification of IGs in spider mites.

The ability to become adapted to a new host plant in a short time, determines the pest status of spider mites on plants that were previously tolerant to spider mites. Understanding the mode of action and mechanism of spider mite adaptation to IGs is necessary for successful

prevention or interference with mite adaptation to IGs. On the other hand, rapid development of resistance to several classes of pesticides in *T. urticae* necessitates introduction of alternative management strategies to control this pest. The negative effect of IGs against *T. urticae* candidates them as a potential strategy to control spider mites. Knowing the mechanism of adaptation/resistance of spider mites to IGs, development of IGs resistance to spider mites could be prevented. Application of detoxification enzymes inhibitors and/or RNAi interfering with genes involved in IGs detoxification in parallel with IGs could be used as pesticide resistance management strategies to prevent or postpone development of IGs resistance in spider mites

References

Abbott, W. S., 1925: A Method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*., **18**, 265–267

Abd-El-Wahed, N. M.; El-Halawany, A. S., 2012: Effect of temperature degrees on the biology and life table parameters of *Tetranychus urticae* Koch on two pear varieties. *Egypt Academic Journal of Biologal Sciences*., **4**, 103–109

Abdalsamee, M. K.; Müller, C., 2012: Effects of indole glucosinolates on performance and sequestration by the sawfly *Athalia rosae* and consequences of feeding on the plant defense system. *Journal of Chemical Ecology*., **38**, 1366–1375

Abdalsamee, M. K.; Giampà, M.; Niehaus, K.; Müller, C., 2014: Rapid incorporation of glucosinolates as a strategy used by a herbivore to prevent activation by myrosinases. *Insect Biochemistry and Molecular Biology*., **52**, 115–123

Abramovitch, R. B.; Anderson, J. C.; Martin, G. B., 2006: Bacterial elicitation and evasion of plant innate immunity. *Nature Reviews Molecular Cell Biology*.

Agerbirk, N.; Olsen, C. E.; Sørensen, H., 1998: Initial and final products, nitriles, and ascorbigens produced in myrosinase-catalyzed hydrolysis of indole glucosinolates. *Journal of Agricultural and Food Chemistry*., **46**, 1563–1571

Agerbirk, N.; De Vos, M.; Kim, J. H.; Jander, G., 2009: Indole glucosinolate breakdown and its biological effects. *Phytochemistry Reviews*., **8**, 101–120

Agerbirk, N.; Olsen, C. E.; Poulsen, E.; Jacobsen, N.; Hansen, P. R., 2010: Complex metabolism of aromatic glucosinolates in *Pieris rapae* caterpillars involving nitrile formation, hydroxylation, demethylation, sulfation, and host plant dependent carboxylic acid formation. *Insect Biochemistry and Molecular Biology*., **40**, 126–137

Agerbirk, N.; Olsen, C. E., 2012: Glucosinolate structures in evolution. *Phytochemistry*.

Agnihotri, A. R.; Hulagabali, C. V.; Adhav, A. S.; Joshi, R. S., 2018: Mechanistic insight in potential dual role of sinigrin against *Helicoverpa armigera*. *Phytochemistry*., **145**, 121–127

Agrawal, A. A., 2000: Host-range evolution: Adaptation and trade-offs in fitness of mites on alternative hosts. *Ecology*., **81**, 500–508

Agrawal, A. A.; Vala, F.; Sabelis, M. W., 2002: Induction of preference and performance after acclimation to novel hosts in a phytophagous spider mite: Adaptive plasticity? *The American Naturalist*., **159**, 553–565

Agrawal, A. A.; Kurashige, N. S., 2003: A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae*. *Journal of Chemical Ecology*., **29**, 1403–1415

Ahn, S. J.; Badenes-Pérez, F. R.; Reichelt, M.; Svatoš, A.; Schneider, B.; Gershenzon, J.; Heckel, D. G., 2011: Metabolic detoxification of capsaicin by UDP-glycosyltransferase in three Helicoverpa species. *Archives of Insect Biochemistry and Physiology*., **78**, 104–118

Ahuja, I.; Rohloff, J.; Bones, A. M., 2009: Defence mechanisms of Brassicaceae: Implications for plant-insect interactions and potential for integrated pest management. *Sustainable Agriculture*., Vol. 2pp. 623–670

Alba, J. M.; Glas, J. J.; Schimmel, B. C. J.; Kant, M. R., 2011: Avoidance and suppression of plant

defenses by herbivores and pathogens. *Journal of Plant Interactions*.

Alberti, G.; Crooker, A. R., 1985: Internal anatomy. In: Helle, W. & M. W. Sabelis (eds.), *Spider Mites: their Biology, Natural Enemies and Control*. Elsevier Press, New York, pp. 29–62

Aliabadi, A.; Renwick, J. A. A.; Whitman, D. W., 2002: Sequestration of glucosinolates by harlequin bug *Murgantia histrionica*. *Journal of Chemical Ecology*., **28**, 1749–1762

Anders, S.; Pyl, P. T.; Huber, W., 2015: HTSeq-A Python framework to work with high-throughput sequencing data. *Bioinformatics*., **31**, 166–169

Andersen, J. F.; Walding, J. K.; Evans, P. H.; Bowers, W. S.; Feyereisen, R., 1997: Substrate specificity for the epoxidation of terpenoids and active site topology of house fly cytochrome P450 6A1. *Chemical Research in Toxicology*., **10**, 156–164

Andréasson, E.; Bolt Jørgensen, L.; Höglund, a S.; Rask, L.; Meijer, J., 2001: Different myrosinase and idioblast distribution in Arabidopsis and *Brassica napus*. *Plant physiology*., **127**, 1750–1763

Andréasson, E.; Jørgensen, L. B., 2003: Localization of plant myrosinases and glucosinolates. *Recent Advances in Phytochemistry*., **37**, 79–99

Attia, S.; Grissa, K. L.; Lognay, G.; Bitume, E.; Hance, T.; Mailleux, A. C., 2013: A review of the major biological approaches to control the worldwide pest *Tetranychus urticae* (Acari: Tetranychidae) with special reference to natural pesticides. *Journal of Pest Science*., **86**, 361–386

Badenes-Perez, F. R.; Reichelt, M.; Gershenzon, J.; Heckel, D. G., 2013: Interaction of glucosinolate content of *Arabidopsis thaliana* mutant lines and feeding and oviposition by generalist and specialist lepidopterans. *Phytochemistry*., **86**, 36–43

Barker, A. M.; Molotsane, R.; Müller, C.; Schaffner, U.; Städler, E., 2006: Chemosensory and behavioural responses of the turnip sawfly, *Athalia rosae*, to glucosinolates and isothiocyanates. *Chemoecology*., **16**, 209–218

Baudry, J.; Li, W.; Pan, L.; Berenbaum, M. R.; Schuler, M. A., 2003: Molecular docking of substrates and inhibitors in the catalytic site of CYP6B1, an insect cytochrome P450 monooxygenase. *Protein Engineering Design and Selection*., **16**, 577–587

Bede, J. C.; Musser, R. O.; Felton, G. W.; Korth, K. L., 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

Beekweelder, J.; van Leeuwen, W.; van Dam, N. M.; Bertossi, M.; Grandi, V.; Mizzi, L.; Soloviev, M.; Szabados, L.; Molthoff, J. W.; Schipper, B.; Verbocht, H.; de Vos, R. C. H.; Morandini, P.; Aarts, M. G. M.; Bovy, A., 2008: The impact of the absence of aliphatic glucosinolates on insect herbivory in Arabidopsis. *PLoS ONE*., **3**, e2068

Bennett, R. N.; Mellon, F. A.; Kroon, P. A., 2004: Screening crucifer seeds as sources of specific intact glucosinolates using ion-pair high-performance liquid chromatography negative ion electrospray mass spectrometry. *Journal of Agricultural and Food Chemistry*., **52**, 428–438

Bensoussan, N.; Santamaria, M. E.; 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**

Beran, F.; Pauchet, Y.; Kunert, G.; Reichelt, M.; Wielsch, N.; Vogel, H.; Reinecke, A.; Svato, A.; Mewis, I.; Schmid, D.; Ramasamy, S.; Ulrichs, C.; Hansson, B. S.; Gershenzon, J.; Heckel, D. G., 2014: *Phyllotreta striolata* flea beetles use host plant defense compounds to create their

own glucosinolate-myrosinase system. *Proceedings of the National Academy of Sciences*., **111**, 7349–7354

Berenbaum, M. R., 1983: Coumarins and caterpillars: A case for coevolution. *BioScience*., **33**, 194–195

Birch, A. N. E.; Wynne Griffiths, D.; Hopkins, R. J.; Macfarlane Smith, W. H.; McKinlay, R. G., 1992: Glucosinolate responses of swede, kale, forage and oilseed rape to root damage by turnip root fly (Delia floralis) larvae. *Journal of the Science of Food and Agriculture*., **60**, 1–9

Birch, L. C., 1948: The intrinsic rate of natural increase of an insect population. *The Journal of Animal Ecology*., **17**, 15

Blaazer, C. J. H.; Villacis-Perez, E. A.; Chafi, R.; Van Leeuwen, T.; Kant, M. R.; Schimmel, B. C. J., 2018: Why do herbivorous mites suppress plant defenses? *Frontiers in Plant Science*., **9**, 1–16

Bodnaryk, R. P., 1994: Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. *Phytochemistry*., **35**, 301–305

Boevé, J. L.; Schaffner, U., 2003: Why does the larval integument of some sawfly species disrupt so easily? The harmful hemolymph hypothesis. *Oecologia*., **134**, 104–111

Bohinc, T.; Ban, S. G.; Ban, D.; Trdan, S., 2012: Glucosinolates in plant protection strategies: A review. *Archives of Biological Sciences*.

Bones, A. M.; Rossiter, J. T., 1996: The myrosinase-glucosinolate system, its organisation and biochemistry. *Physiologia Plantarum*., **97**, 194–208

Brader, G., 2001: Jasmonate-dependent induction of indole glucosinolates in Arabidopsis by culture filtrates of the nonspecific pathogen *Erwinia carotovora*. *Plant Physiology*., **126**, 849–860

Bradford, M. M., 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. *Analytical Biochemistry*., **72**, 248–254

Brattsten, L. B., 2012: Metabolic defenses against plant allelochemicals. *Herbivores: Their Interactions with Secondary Plant Metabolites Ecological and Evolutionary Processes: Second Edition*., Vol. 2pp. 175–242

Bridges, M.; Jones, A. M. E.; Bones, A. M.; Hodgson, C.; Cole, R.; Bartlet, E.; Wallsgrove, R.; Karapapa, V. K.; Watts, N.; Rossiter, J. T., 2002: Spatial organization of the glucosinolate-myrosinase system in brassica specialist aphids is similar to that of the host plant. *Proceedings of Biological sciences / The Royal Society*., **269**, 187–191

Brown, K. K.; Hampton, M. B., 2011: Biological targets of isothiocyanates. *Biochimica et Biophysica Acta - General Subjects*.

Brown, P. D.; Tokuhisa, J. G.; Reichelt, M.; Gershenzon, J., 2003: Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. *Phytochemistry*., **62**, 471–481

Bruessow, F.; Gouhier-Darimont, C.; Buchala, A.; Metraux, J. P.; Reymond, P., 2010: Insect eggs suppress plant defence against chewing herbivores. *Plant Journal*., **62**, 876–885

Bruinsma, K. A., 2014: *Arabidopsis thaliana*-spider mite interaction: plant perception, signalling and response. University of Western Ontario

Bryan Hanley, A.; Parsley, K. R., 1990: Identification of 1-methoxyindolyl-3-methyl

isothiocyanate as an indole glucosinolate breakdown product. *Phytochemistry*., **29**, 769–771

Burow, M.; Wittstock, U., 2009: Regulation and function of specifier proteins in plants. *Phytochemistry Reviews*.

Buskov, S.; Hansen, L. B.; Olsen, C. E.; Sørensen, J. C.; Sørensen, H.; Sørensen, S., 2000: Determination of ascorbigens in autolysates of various *Brassica* species using supercritical chromatography. *Journal of Agricultural and Food Chemistry*., **48**, 2693–2701

Buters, J. T. M.; Schiller, C. D.; Chou, R. C., 1993: A highly sensitive tool for the assay of cytochrome P450 enzyme activity in rat, dog and man. *Biochemical Pharmacology*., **46**, 1577–1584

Carey, J., 1982: Demography of the two-spotted spider mite, *Tetranychus urticae* Koch. *Oecologia*., 389–395

Carpentier, M.; Guillemette, C.; Bailey, J. L.; Boileau, G.; Jeannotte, L.; DesGroseillers, L.; Charron, J., 2004: Reduced fertility in male mice deficient in the zinc metallopeptidase NL1. *Molecular and Cellular Biology*., **24**, 4428–4437

Carroll, M.; Hanlon, A.; Hanlon, T.; Zangerl, A. R.; Berenbaum, M. R., 1997: Behavioral effects of carotenoid sequestration by the parsnip webworm, *Depressaria pastinacella*. *Journal of Chemical Ecology*., **23**, 2707–2719

Carter, C., 2004: The vegetative vacuole proteome of *Arabidopsis thaliana* reveals predicted and unexpected proteins. *THE PLANT CELL ONLINE*., **16**, 3285–3303

Castle, S. J.; Prabhaker, N.; Henneberry, T. J.; Toscano, N. C., 2009: Host plant influence on susceptibility of *bemisia tabaci* (Hemiptera: Aleyrodidae) to insecticides. *Bulletin of Entomological Research*., **99**, 263–273

Chen, S.; Glawischnig, E.; Jørgensen, K.; Naur, P.; Jørgensen, B.; Olsen, C. E.; Hansen, C. H.; Rasmussen, H.; Pickett, J. A.; Halkier, B. A., 2003: CYP79F1 and CYP79F2 have distinct functions in the biosynthesis of aliphatic glucosinolates in Arabidopsis. *Plant Journal*., **33**, 923–937

Chiang, F. M.; Sun, C. N., 1993: Glutathione transferase isozymes of diamondback moth larvae and their role in the degradation of some organophosphorus insecticides. *Pesticide Biochemistry and Physiology*., **45**, 7–14

Chung, S. H.; Rosa, C.; Scully, E. D.; Peiffer, M.; Tooker, J. F.; Hoover, K.; Luthe, D. S.; Felton, G. W., 2013: Herbivore exploits orally secreted bacteria to suppress plant defenses. *Proceedings of the National Academy of Sciences*., **110**, 15728–15733

Clark, A. G.; Shamaan, N. A., 1984: Evidence that DDT-dehydrochlorinase from the house fly is a glutathione S-transferase. *Pesticide Biochemistry and Physiology*., **22**, 249–261

Clarke, D. B., 2010: Glucosinolates, structures and analysis in food. *Analytical Methods*., **2**, 310

Cranham, J. E.; Helle, W., 1985: Pesiticide resistance in Tetranychidae. In: Helle, W. & M. W. Sabelis (eds.), *Spider Mites, Their Biology Natural Enemies and Control*. Elsevier Press, New York, pp. 405–421

Crooker, A. R., 1985: Embryonic and juvenile development. In: Helle, W. & M. W. Sabelis (eds.), *Spider Mites, Their Biology Natural Enemies and Control*. Elsevier Press, New York, pp. 149–163

Czechowski, T., 2005: Genome-Wide Identification and Testing of Superior Reference Genes for Transcript Normalization in Arabidopsis. *Plant Physiology*., **139**, 5–17

Dalla, S.; Swarts, H. G. P.; Koenderink, J. B.; Dobler, S., 2013: Amino acid substitutions of Na,K-ATPase conferring decreased sensitivity to cardenolides in insects compared to mammals. *Insect Biochemistry and Molecular Biology*., **43**, 1109–1115

DARP, 2015: Database of Arthropod Resistance to pesticides: www.pesticideresistance.org

De La Paz Celorio-Mancera, M.; Wheat, C. W.; Vogel, H.; Söderlind, L.; Janz, N.; Nylin, S., 2013: Mechanisms of macroevolution: Polyphagous plasticity in butterfly larvae revealed by RNA-Seq. *Molecular Ecology*., **22**, 4884–4895

De Vos, M.; Jander, G., 2009: *Myzus persicae* (green peach aphid) salivary components induce defence responses in *Arabidopsis thaliana*. *Plant, Cell and Environment*., **32**, 1548–1560

Deng, H.; Huang, Y.; Feng, Q.; Zheng, S., 2009: Two epsilon glutathione S-transferase cDNAs from the common cutworm, *Spodoptera litura*: Characterization and developmental and induced expression by insecticides. *Journal of Insect Physiology*., **55**, 1174–1183

Dermauw, W.; Wybouw, N.; Rombauts, S.; Menten, B.; Vontas, J.; Grbic, M.; Clark, R. M.; 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

Després, L.; David, J. P.; Gallet, C., 2007: The evolutionary ecology of insect resistance to plant chemicals. *Trends in Ecology & Evolution*., **22**, 298–307

Devonshire, A. L.; Field, L. M., 1991: Gene amplification and insecticide resistance. *Annu. Rev. Entomol*., **36**, 1–23

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, J. M.; Grbic, M.; Grbic, 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**

Dobin, A.; Davis, C. A.; Schlesinger, F.; Drenkow, J.; Zaleski, C.; Jha, S.; Batut, P.; Chaisson, M.; Gingeras, T. R., 2013: STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics*., **29**, 15–21

Dobler, S.; Dalla, S.; Wagschal, V.; Agrawal, A. A., 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*., **109**, 13040–13045

Du, L.; Halkier, B. A., 1998: Biosynthesis of glucosinolates in the developing silique walls and seeds of *Sinapis alba*. *Phytochemistry*., **48**, 1145–1150

Durgbanshi, a.; Arbona, V.; Pozo, O.; Miersch, O.; Sancho, J. V.; Gomez-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

Dyer, L. A.; Deane Bowers, M., 1996: The importance of sequestered iridoid glycosides as a defense against an ant predator. *Journal of Chemical Ecology*., **22**, 1527–1539

Edger, P. P.; Heidel-Fischer, H. M.; Bekaert, M.; Rota, J.; Glöckner, G.; Platts, A. E.; Heckel, D. G.; Der, J. P.; Wafula, E. K.; Tang, M.; Hofberger, J. A.; Smithson, A.; Hall, J. C.; Blanchette, M.; Bureau, T. E.; Wright, S. I.; dePamphilis, C. W.; Eric Schranz, M.; Barker, M. S. et al., 2015: The butterfly plant arms-race escalated by gene and genome duplications. *Proceedings of the National Academy of Sciences*., **112**, 8362–8366

Elbaz, M.; Halon, E.; Malka, O.; Malitsky, S.; Blum, E.; Aharoni, A.; Morin, S., 2012: Asymmetric adaptation to indolic and aliphatic glucosinolates in the B and Q sibling species of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Molecular Ecology*., **21**, 4533–4546

Elzinga, D. A.; De Vos, M.; Jander, G., 2014: Suppression of plant defenses by a *Myzus persicae* (green peach aphid) salivary effector protein. *Molecular plant-microbe interactions : MPMI*., **27**, 747–756

Enayati, A. A.; Ranson, H.; Hemingway, J., 2005: Insect glutathione transferases and insecticide resistance. *Insect Molecular Biology*., **14**, 3–8

Fahey, J. W.; Zalcmann, a T.; Talalay, P., 2001: The chemical diversity and distribution of glucosinolates and isothiocyanates amoung plants. *Phytochemistry*., **56**, 5–51

Falk, K. L.; Vogel, C.; Textor, S.; Bartram, S.; Hick, A.; Pickett, J. A.; Gershenzon, J., 2004: Glucosinolate biosynthesis: Demonstration and characterization of the condensing enzyme of the chain elongation cycle in *Eruca sativa*. *Phytochemistry*., **65**, 1073–1084

Falk, K. L.; Gershenzon, J., 2007: The desert locust, *Schistocerca gregaria*, detoxifies the glucosinolates of *Schouwia purpurea* by desulfation. *Journal of Chemical Ecology*., **33**, 1542–1555

Falk, K. L.; Kästner, J.; Bodenhausen, N.; Schramm, K.; Paetz, C.; Vassão, D. G.; Reichelt, M.; Von Knorre, D.; Bergelson, J.; Erb, M.; Gershenzon, J.; Meldau, S., 2014: The role of glucosinolates and the jasmonic acid pathway in resistance of *Arabidopsis thaliana* against molluscan herbivores. *Molecular Ecology*., **23**, 1188–1203

Ferry, N.; Edwards, M. G.; Gatehouse, J. A.; Gatehouse, A. M. R., 2004: Plant-insect interactions: Molecular approaches to insect resistance. *Current Opinion in Biotechnology*.

Feyereisen, R., 2005: Insect Cytochrome P450. *Comprehensive Molecular Insect Science*., pp. 1–77

Feyereisen, R., 2012: Insect CYP genes and P450 enzymes. *Insect Molecular Biology and Biochemistry*., pp. 236–316

Feyereisen, R.; Dermauw, W.; Van Leeuwen, T., 2015: Genotype to phenotype, the molecular and physiological dimensions of resistance in arthropods. *Pesticide Biochemistry and Physiology*., **121**, 61–77

Ffrench-Constant, R. H., 2013: The molecular genetics of insecticide resistance. *Genetics*., **194**, 807-815

Francis, F.; Lognay, G.; Wathelet, J. P.; Haubruge, E., 2001: Effects of allelochemicals from first (Brassicaceae) and second (*Myzus persicae* and *Brevicoryne brassicae*) trophic levels on *Adalia bipunctata*. *Journal of Chemical Ecology*., **27**, 243–256

Francis, F.; Lognay, G.; Wathelet, J. P.; Haubruge, E., 2002: Characterisation of aphid myrosinase and degradation studies of glucosinolates. *Archives of Insect Biochemistry and Physiology*., **50**, 173–182

Francis, F.; Vanhaelen, N.; Haubruge, E., 2005: Glutathione S-transferases in the adaptation to plant secondary metabolites in the *Myzus persicae* aphid. *Archives of Insect Biochemistry and Physiology*., **58**, 166–174

Gaertner, L. S.; Murray, C. L.; Morris, C. E., 1998: Transepithelial transport of nicotine and vinblastine in isolated malpighian tubules of the tobacco hornworm (*Manduca sexta*) suggests a P-glycoprotein-like mechanism. *The Journal of experimental biology*., **201**, 2637–2645

Gentleman, V. J.; Carey, D. M.; Bates, B.; Bolstad, M.; Dettling, S.; Dudoit, B.; Ellis, L.; Gautier, Y. G.; Others, A., 2004: Bioconductor: Open software development for computational biology and bioinformatics R. *Genome Biology*., R80

Gerson, U., 1985: Webbing. In: Helle, W. & M. W. Sabelis (eds.), *Spider Mites: their Biology, Natural Enemies and Control*. Elsevier Press, New York, pp. 223–232

Ghumare, S. S.; Mukherjee, S. N.; Sharma, R. N., 1989: Effect of rutin on the neonate sensitivity, dietary utilization and mid-gut carboxylesterase activity of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *Proceedings: Animal Sciences*., **98**, 399–404

Glas, J. J.; Alba, J. M.; Simoni, S.; Villarroel, C. A.; Stoops, M.; Schimmel, B. C. J.; Schuurink, R. C.; Sabelis, M. W.; Kant, M. R., 2014: Defense suppression benefits herbivores that have a monopoly on their feeding site but can backfire within natural communities. *BMC Medicine*., **12**, 98-111

Glauser, G.; Schweizer, F.; Turlings, T. C. J.; Reymond, P., 2012: Rapid profiling of intact glucosinolates in arabidopsis leaves by UHPLC-QTOFMS using a charged surface hybrid column. *Phytochemical Analysis*., **23**, 520–528

Gloss, A. D.; Vassao, D. G.; Hailey, A. L.; Nelson Dittrich, A. C.; Schramm, K.; Reichelt, M.; Rast, T. J.; Weichsel, A.; Cravens, M. G.; Gershenzon, J.; Montfort, W. R.; Whiteman, N. K., 2014: Evolution in an ancient detoxification pathway is coupled with a transition to herbivory in the drosophilidae. *Molecular Biology and Evolution*., **31**, 2441–2456

Gols, R.; Wagenaar, R.; Bukovinszky, T.; Van Dam, N. M.; Dicke, M.; Bullock, J. M.; Harvey, J. A., 2008: Genetic variation in defense chemistry in wild cabbages affects herbivores and their endoparasitoids. *Ecology*., **89**, 1616–1626

Gómez, S.; Steinbrenner, A. D.; Osorio, S.; Schueller, M.; Ferrieri, R. A.; Fernie, A. R.; Orians, C. M., 2012: From shoots to roots: Transport and metabolic changes in tomato after simulated feeding by a specialist lepidopteran. *Entomologia Experimentalis et Applicata*., **144**, 101–111

Gould, F.; Carroll, C. R.; Futuyma, D. J., 1982: Cross resistance to pesticides and plant defenses: a study of the two-spotted spider mite. *Entomologia Experimentalis et Applicata*., **31**, 175–180

Govind, G.; Mittapalli, O.; Griebel, T.; Allmann, S.; Böcker, S.; Baldwin, I. T., 2010: Unbiased transcriptional comparisons of generalist and specialist herbivores feeding on progressively defenseless Nicotiana attenuata plants. *PLoS ONE*., **5**, e8735

Grant, D. F.; Hammock, B. D., 1992: Genetic and molecular evidence for a trans-acting regulatory locus controlling glutathione S-transferase-2 expression in *Aedes aegypti*. *MGG Molecular & General Genetics*., **234**, 169–176

Grbić, M.; Van Leeuwen, T.; Clark, R. M.; Rombauts, S.; Rouzé, P.; Grbić, V.; Osborne, E. J.; Dermauw, W.; Ngoc, T.; Cao, P.; Ortego, F.; Hernández-Crespo, P.; Diaz, I.; Martinez, M.; Navajas, M.; Sucena, É.; Magalhães, S.; Nagy, L.; Pace, R. M. et al., 2011: The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature*., **479**, 487–492

Habig, W. H.; Pabst, M. J.; Jakoby, W. B., 1974: Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*., **249**, 7130–7139

Habig, W. H.; Jakoby, W. B., 1981: Assays for Differentiation of Glutathione-S-Transferases. *Methods in Enzymology*., **77**, 398–405

Halkier, B. A.; Gershenzon, J., 2006: Biology and biochemistry of glucosinolates. *Annual review of plant biology*., **57**, 303–333

Hanschen, F. S.; Brüggemann, N.; Brodehl, A.; Mewis, I.; Schreiner, M.; Rohn, S.; Kroh, L. W., 2012: Characterization of products from the reaction of glucosinolate-derived isothiocyanates with cysteine and lysine derivatives formed in either model systems or broccoli sprouts. *Journal of Agricultural and Food Chemistry*., **60**, 7735–7745

Hartmann, T., 1999: Chemical ecology of pyrrolizidine alkaloids. *Planta*.

Harvey, J. A.; Gols, R.; Wagenaar, R.; Bezemer, T. M., 2007: Development of an insect herbivore and its pupal parasitoid reflect differences in direct plant defense. *Journal of Chemical Ecology*., **33**, 1556–1569

Head, J. R.; Macdonald, P. C.; Casey, M. L., 1993: Cellular localization of membrane metalloendopeptidase (enkephalinase) in human endometrium during the ovarian cycle. *Journal of Clinical Endocrinology and Metabolism*., **76**, 769–776

Heckel, D. G., 2014: Insect Detoxification and Sequestration Strategies. *Annual Plant Reviews: Insect-Plant Interactions*., Vol. 47pp. 77–114

Heidel-Fischer, H. M.; Vogel, H., 2015: Molecular mechanisms of insect adaptation to plant secondary compounds. *Current Opinion in Insect Science*., **8**, 8–14

Hemingway, J.; Hawkes, N.; Prapanthadara, L.; Jayawardenal, K. G.; Ranson, H., 1998: The role of gene splicing, gene amplification and regulation in mosquito insecticide resistance. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*., **353**, 1695–1699

Hemming, J. D. C.; Lindroth, R. L., 2000: Effects of phenolic glycosides and protein on gypsy moth (Lepidoptera: Lymantriidae) and forest tent caterpillar (Lepidoptera: Lasiocampidae) performance and detoxication activities. *Environmental Entomology*., **29**, 1108–1115

Herbert, H. J., 1981: Biology, life tables, and innate capacity for increase of the two-spotted spider mite, *Tetranychus urticae* (acarina: Tetranychidae). *The Canadian Entomologist*., **113**, 371–378

Herde, M.; Howe, G. A., 2014: Host plant-specific remodeling of midgut physiology in the generalist insect herbivore *Trichoplusia ni*. *Insect Biochemistry and Molecular Biology*., **50**, 58–67

Hilker, M.; Meiners, T., 2002: Induction of plant responses to oviposition and feeding by herbivorous arthropods: A comparison. *Entomologia Experimentalis et Applicata*., Vol. 104pp. 181–192

Holst, B.; Williamson, G., 2004: A critical review of the bioavailability of glucosinolates and related compounds. *Natural product reports*., **21**, 425–447

Hopkins, R. J.; Griffiths, D. W.; Birch, A. N. E.; Mckinlay, R. G., 1998: Influence of increasing herbivore pressure on modification of glucosinolate content of Swedes (*Brassica napus* spp. rapifera). *Journal of Chemical Ecology*., **24**, 2003–2019

Hopkins, R. J.; van Dam, N. M.; van Loon, J. J. a, 2009: Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annual review of entomology*., **54**, 57–83

Howe, G. A.; Jander, G., 2008: Plant immunity to insect herbivores. *Annual Review of Plant Biology*., **59**, 41–66

Hoy, M. A., 2011: *Agricultural Acarology: Introduction to Integrated Mite Management*. CRC Press

Huang, H. S.; Hu, N. T.; Yao, Y. E.; Wu, C. Y.; Chiang, S. W.; Sun, C. N., 1998: Molecular cloning

and heterologous expression of a glutathione S-transferase involved in insecticide resistance from the diamondback moth, *Plutella xylostella*. *Insect biochemistry and molecular biology*., **28**, 651–658

Huang, Y.; Xu, Z.; Lin, X.; Feng, Q.; Zheng, S., 2011: Structure and expression of glutathione S-transferase genes from the midgut of the Common cutworm, *Spodoptera litura* (Noctuidae) and their response to xenobiotic compounds and bacteria. *Journal of Insect Physiology*., **57**, 1033–1044

Hull, A. K.; Vij, R.; Celenza, J. L., 2000: Arabidopsis cytochrome P450s that catalyze the first step of tryptophan-dependent indole-3-acetic acid biosynthesis. *Proceedings of the National Academy of Sciences*., **97**, 2379–2384

Husebye, H.; Chadchawan, S.; Winge, P.; Thangstad, O. P.; Bones, A. M., 2002: Guard cell- and phloem idioblast-specific expression of thioglucoside glucohydrolase 1 (myrosinase) in Arabidopsis. *Plant physiology*., **128**, 1180–1188

Husebye, H.; Arzt, S.; Burmeister, W. P.; Härtel, F. V.; Brandt, A.; Rossiter, J. T.; Bones, A. M., 2005: Crystal structure at 1.1 Å resolution of an insect myrosinase from *Brevicoryne brassicae* shows its close relationship to β-glucosidases. *Insect Biochemistry and Molecular Biology*., **35**, 1311–1320

Janobi, A. A. Al; Mithen, R. F.; Gasper, A. V.; Shaw, P. N.; Middleton, R. J.; Ortori, C. A.; Barrett, D. A., 2006: Quantitative measurement of sulforaphane, iberin and their mercapturic acid pathway metabolites in human plasma and urine using liquid chromatography-tandem electrospray ionisation mass spectrometry. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*., **844**, 223–234

Jeppson, L. R.; Keifer, H. H.; Baker, E. W., 1975: Mites injurious to economic plants. *Florida Entomologist*.

Johnson, P. J., 1987: Encyclopedia of Science and Technology. McGraw-Hill, New York

Jones, A. M. E.; Bridges, M.; Bones, A. M.; Cole, R.; Rossiter, J. T., 2001: Purification and characterisation of a non-plant myrosinase from the cabbage aphid *Brevicoryne brassicae* (L.). *Insect Biochemistry and Molecular Biology*., **31**, 1–5

Kamoun, S., 2006: A catalogue of the effector secretome of plant pathogenic oomycetes. *Annual Review of Phytopathology*., **44**, 41–60

Kant, M. R.; Sabelis, M. W.; Haring, M. A.; Schuurink, R. C., 2008: Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defences. *Proceedings of the Royal Society B: Biological Sciences*., **275**, 443–452

Kant, M. R.; Jonckheere, W.; Knegt, B.; Lemos, F.; Liu, J.; Schimmel, B. C. J.; Villarroel, C. A.; Ataide, L. M. S.; Dermauw, W.; Glas, J. J.; Egas, M.; Janssen, A.; Van Leeuwen, T.; Schuurink, R. C.; Sabelis, M. W.; Alba, J. M., 2015: Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. *Annals of Botany*.

Karban, R.; Agrawal, A. A., 2002: Herbivore offense. *Annual Review of Ecology and Systematics*., **33**, 641–664

Karuppaiah, V.; Srivastava, C.; Subramanian, S., 2016: Effect of host plants on insecticide susceptibility and detoxification enzymes activity in *Spodoptera litura* Fabricius (Noctuidae: Lepidoptera). *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*., **86**, 715–721

Kassahun, K.; Davis, M.; Hu, P.; Martin, B.; Baillie, T., 1997: Biotransformation of the naturally occurring isothiocyanate sulforaphane in the rat: Identification of phase I metabolites and glutathione conjugates. *Chemical Research in Toxicology*., **10**, 1228–1233

Kasschau, K. D.; Carrington, J. C., 1998: A counterdefensive strategy of plant viruses: suppression of posttranscriptional gene silencing. *Cell*., **95**, 461–470

Kawakishi, S.; Kaneko, T., 1987: Interaction of Proteins with Allyl Isothiocyanate. *Journal of Agricultural and Food Chemistry*., **35**, 85–88

Kawsar, S. M. A.; Takeuchi, T.; Kasai, K. ichi; Fujii, Y.; Matsumoto, R.; Yasumitsu, H.; Ozeki, Y., 2009: Glycan-binding profile of a D-galactose binding lectin purified from the annelid, Perinereis nuntia ver. vallata. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*., **152**, 382–389

Kennedy, C.; Tierney, K., 2013: Xenobiotic protection/resistance mechanisms in organisms. In: Law, E. A. (ed.), *Environmental Toxicology*. Springer, New York, pp. 689–721

Kessler, A.; Baldwin, I. T., 2002: Plant responses to insect herbivory: the emerging molecular analysis. *Annu Rev Plant Biol*., **53**, 299–328

Keum, Y. S.; Jeong, W. S.; Tony Kong, A. N., 2004: Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*.

Kim, J. H.; Jander, G., 2007: *Myzus persicae* (green peach aphid) feeding on Arabidopsis induces the formation of a deterrent indole glucosinolate. *Plant Journal*., **49**, 1008–1019

Kim, J. H.; Lee, B. W.; Schroeder, F. C.; Jander, G., 2008: Identification of indole glucosinolate breakdown products with antifeedant effects on *Myzus persicae* (green peach aphid). *Plant Journal*., **54**, 1015–1026

Kliebenstein, D. J.; Kroymann, J.; Brown, P.; Figuth, A.; Pedersen, D.; Gershenzon, J.; Mitchell-Olds, T., 2001: Genetic control of natural variation in Arabidopsis glucosinolate accumulation. *Plant physiology*., **126**, 811–825

Koroleva, O. a; Davies, a; Deeken, R.; Thorpe, M. R.; Tomos, a D.; Hedrich, R., 2000: Identification of a new glucosinolate-rich cell type in Arabidopsis flower stalk. *Plant physiology*., **124**, 599–608

Koroleva, O. a.; Gibson, T. M.; Cramer, R.; Stain, C., 2010: Glucosinolate-accumulating S-cells in Arabidopsis leaves and flower stalks undergo programmed cell death at early stages of differentiation. *Plant Journal*., **64**, 456–469

Koroleva, O. a.; Cramer, R., 2011: Single-cell proteomic analysis of glucosinolate-rich S-cells in *Arabidopsis thaliana*. *Methods*., **54**, 413–423

Krieger, R. I.; Feeny, P. P.; Wilkinson, C. F., 1971: Detoxication enzymes in the guts of caterpillars: an evolutionary answer to plant defenses? *Science (New York, N.Y.)*., **172**, 579–581

Kuhn, J.; Pettersson, E. M.; Feld, B. K.; Nie, L.; Tolzin-Banasch, K.; M'Rabet, S. M. H.; Pasteels, J.; Boland, W., 2007: Sequestration of plant-derived phenolglucosides by larvae of the leaf beetle *Chrysomela lapponica*: Thioglucosides as mechanistic probes. *Journal of Chemical Ecology*., **33**, 5–24

Lambrix, V.; Reichelt, M.; Mitchell-Olds, T.; Kliebenstein, D. J.; Gershenzon, J., 2001: The Arabidopsis epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. *The Plant cell*., **13**, 2793–2807

Lankau, R. A., 2007: Specialist and generalist herbivores exert opposing selection on a chemical defense. *New Phytologist*., **175**, 176–184

Law, C. W.; Chen, Y.; Shi, W.; Smyth, G. K., 2014: Voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biology*., **15**, R29

Leisinger, A. K.; Janzen, D. H.; Hallwachs, W.; Igloi, G. L., 2013: Amino acid discrimination by the nuclear encoded mitochondrial arginyl-tRNA synthetase of the larva of a bruchid beetle (*Caryedes brasiliensis*) from northwestern Costa Rica. *Insect Biochemistry and Molecular Biology*., **43**, 1172–1180

Li, Q.; Eigenbrode, S. D.; Stringam, G. R.; Thiagarajah, M. R., 2000: Feeding and growth of *Plutella xylostella* and *Spodoptera eridania* on *Brassica juncea* with varying glucosinolate concentrations and myrosinase activities. *Journal of Chemical Ecology*., **26**, 2401–2419

Li, X.; Baudry, J.; Berenbaum, M. R.; Schuler, M. A., 2004: Structural and functional divergence of insect CYP6B proteins: From specialist to generalist cytochrome P450. *Proceedings of the National Academy of Sciences*., **101**, 2939–2944

Li, X.; Schuler, M. A.; Berenbaum, M. R., 2007: Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Annual review of entomology*., **52**, 231–253

Lichtenstein, E. P.; Strong, F. M.; Morgan, D. G., 1962: Naturally occurring insecticides, identification of 2-Phenylethylisothiocyanate as an insecticide occurring naturally in the edible part of turnips. *Journal of Agricultural and Food Chemistry*., **10**, 30–33

Liebminger, E.; Grass, J.; Jez, J.; Neumann, L.; Altmann, F.; Strasser, R., 2012: Myrosinases TGG1 and TGG2 from *Arabidopsis thaliana* contain exclusively oligomannosidic N-glycans. *Phytochemistry*., **84**, 24–30

Lindroth, R. L., 1989: Host plant alteration of detoxication activity in *Papilio glaucus glaucus*. *Entomologia Experimentalis et Applicata*., **50**, 29–35

Lindroth, R. L.; Weisbrod, A. V., 1991: Genetic variation in response of the gypsy moth to aspen phenolic glycosides. *Biochemical Systematics and Ecology*., **19**, 97–103

Liu, R.; Nyoike, T. W.; Liburd, O. E., 2016: Evaluation of site-specific tactics using bifenazate and *Neoseiulus californicus* for management of *Tetranychus urticae* (Acari: Tetranychidae) in strawberries. *Experimental and Applied Acarology*., **70**, 189–204

Mainguet, A. M.; Louveaux, A.; El Sayed, G.; Rollin, P., 2000: Ability of a generalist insect, *Schistocerca gregaria*, to overcome thioglucoside defense in desert plants: Tolerance or adaptation? *Entomologia Experimentalis et Applicata*., **94**, 309–317

Malcolm, S. B.; Brower, L. P., 1989: Evolutionary and ecological implications of cardenolide sequestration in the monarch butterfly. *Experientia*., **3**, 284-295

Malka, O.; Shekhov, A.; Reichelt, M.; Gershenzon, J.; Vassão, D. G.; Morin, S., 2016: Glucosinolate desulfation by the phloem-feeding insect *Bemisia tabaci*. *Journal of Chemical Ecology*., **42**, 230–235

Mao, Y. B.; Cai, W. J.; Wang, J. W.; Hong, G. J.; Tao, X. Y.; Wang, L. J.; Huang, Y. P.; Chen, X. Y., 2007: Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nature Biotechnology*., **25**, 1307–1313

Mao, Y. B.; Tao, X. Y.; Xue, X. Y.; Wang, L. J.; Chen, X. Y., 2011: Cotton plants expressing CYP6AE14 double-stranded RNA show enhanced resistance to bollworms. *Transgenic Research*., **20**, 665–673

Marazzi, C.; Städler, E., 2004: *Arabidopsis thaliana* leaf-surface extracts are detected by the cabbage root fly (*Delia radicum*) and stimulate oviposition. *Physiological Entomology*., **29**, 192–198

Matile, P., 1980: The mustard oil bomb compartmentation of the myrosinase system. *Biochemie und Physiologie der Pflanzen*., **175**, 722–731

Meena, N. K.; Rampal; Barman, D.; Medhi, R. P., 2013: Biology and seasonal abundance of the two-spotted spider mite, *Tetranychus urticae*, on orchids and rose. *Phytoparasitica*., **41**, 597–609

Migeon, A.; Nouguier, E.; Dorkeld, F., 2010: Spider Mites Web: A comprehensive database for the Tetranychidae. *Trends in Acarology SE - 96*., pp. 557–560

Mikkelsen, M. D.; Hansen, C. H.; Wittstock, U.; Halkier, B. A., 2000: Cytochrome P450 CYP79B2 from Arabidopsis catalyzes the conversion of tryptophan to indole-3-acetaldoxime, a precursor of indole glucosinolates and indole-3-acetic acid. *Journal of Biological Chemistry*., **275**, 33712–33717

Mikkelsen, M. D.; Petersen, B. L.; Glawischnig, E.; Jensen, A. B.; Andreasson, E.; Halkier, B. A., 2003: Modulation of *CYP79* genes and glucosinolate profiles in *Arabidopsis* by defense signaling pathways. *Plant Physiology*., **131**, 298 LP-308

Mitchell, R., 1973: Growth and population dynamics of a spider mite (*Tetranychus urticae* K ., Acarina: Tetranychidae). *Ecology*., **54**, 1349–1355

Mithöfer, A.; Wanner, G.; Boland, W., 2005: Effects of feeding *Spodoptera littoralis* on lima bean leaves. II. Continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. *Plant Physiology*., **137**, 1160–1168

Modarres Najafabadi, S. S., 2012: Comparative biology and fertility life tables of *Tetranychus urticae* Koch (Acari: Tetranychidae) on different common bean cultivars. *International Journal of Acarology*., 1–9

Müller, C.; Agerbirk, N.; Olsen, C. E.; Boevé, J. L.; Schaffner, U.; Brakefield, P. M., 2001: Sequestration of host plant glucosinolates in the defensive hemolymph of the sawfly *Athalia rosae*. *Journal of Chemical Ecology*., **27**, 2505–2516

Müller, C.; Wittstock, U., 2005a: Uptake and turn-over of glucosinolates sequestered in the sawfly *Athalia rosae*. *Insect Biochemistry and Molecular Biology*., **35**, 1189–1198

Müller, C.; Wittstock, U., 2005b: Uptake and turn-over of glucosinolates sequestered in the sawfly *Athalia rosae*. *Insect Biochemistry and Molecular Biology*., **35**, 1189–1198

Müller, C.; Sieling, N., 2006: Effects of glucosinolate and myrosinase levels in *Brassica juncea* on a glucosinolate-sequestering herbivore and vice versa. *Chemoecology*., **16**, 191–201

Müller, C., 2009: Interactions between glucosinolate- and myrosinase-containing plants and the sawfly *Athalia rosae*. *Phytochemistry Reviews*.

Müller, R.; de Vos, M.; Sun, J. Y.; Sønderby, I. E.; Halkier, B. A.; Wittstock, U.; Jander, G., 2010: Differential effects of indole and aliphatic glucosinolates on lepidopteran herbivores. *Journal of Chemical Ecology*., **36**, 905–913

Nahrstedt, A.; Davis, R. H., 1986: Uptake of linamarin and lotaustralin from their foodplant by larvae of *Zygaena trifolii*. *Phytochemistry*., **25**, 2299–2302

Nishida, R., 2002: Sequestration of defensive substances from plants by Lepidoptera. *Annual Review of Entomology*., **47**, 57–92

Ode, P. J., 2006: Plant chemistry and natural enemy fitness: Effects on herbivore and natural enemy interactions. *Annu. Rev. Entomol*., **51**, 163–185

Oerke, E. C. C., 2006: Crop losses to pests. *Journal of Agricultural Science*., **144**, 31

Opitz, S. E. W.; Müller, C., 2009: Plant chemistry and insect sequestration. *Chemoecology*.

Opitz, S. E. W.; Jensen, S. R.; Müller, C., 2010: Sequestration of glucosinolates and iridoid glucosides in sawfly species of the genus *Athalia* and their role in defense against ants. *Journal of Chemical Ecology*., **36**, 148–157

Oppenoorth, F. J.; Van Asperen, K., 1960: Allelic genes in the housefly producing modified enzymes that cause organophosphate resistance. *Science*., **132**, 298–299

Oppenoorth, F. J.; van der Pas, L. J. T.; Houx, N. W. H., 1979: Glutathione S-transferase and hydrolytic activity in a tetrachlorvinphos-resistant strain of housefly and their influence on resistance. *Pesticide Biochemistry and Physiology*., **11**, 176–188

Ortelli, F.; Rossiter, L. C.; Vontas, J.; Ranson, H.; Hemingway, J., 2003: Heterologous expression of four glutathione transferase genes genetically linked to a major insecticide-resistance locus from the malaria vector *Anopheles gambiae*. *The Biochemical journal*., **373**, 957–963

Overmeer, W. P. J.; Harrison, R. A., 1969: Notes on the control of the sex ratio in populations of the two-spotted spider mite, *Tetranychus urticae* Koch (Acarina: Tetranychidae). *New Zealand Journal of Science*., **12**, 920–928

Pan, L.; Wen, Z.; Baudry, J.; Berenbaum, M. R.; Schuler, M. A., 2004: Identification of variable amino acids in the SRS1 region of CYP6B1 modulating furanocoumarin metabolism. *Archives of Biochemistry and Biophysics*., **422**, 31–41

Park, Y. L.; Lee, J. H., 2002: Leaf cell and tissue damage of cucumber caused by two-spotted spider mite (Acari: Tetranychidae). *Journal of Economic Entomology*., **95**, 952–957

Park, Y. L.; Lee, J. H., 2005: Impact of twospotted spider mite (Acari: Tetranychidae) on growth and productivity of glasshouse cucumbers. *Journal of economic entomology*., **98**, 457–463

Pedras, M. S. C.; Nycholat, C. M.; Montaut, S.; Xu, Y.; Khan, A. Q., 2002: Chemical defenses of crucifers: Elicitation and metabolism of phytoalexins and indole-3-acetonitrile in brown mustard and turnip. *Phytochemistry*., **59**, 611–625

Pentzold, S.; Zagrobelny, M.; Rook, F.; Bak, S., 2014: How insects overcome two-component plant chemical defence: Plant β-glucosidases as the main target for herbivore adaptation. *Biological Reviews*., **89**, 531–551

Peterson, C. J.; Tsao, R.; Coats, J. R., 1998: Glucosinolate aglucones and analogues: Insecticidal properties and a QSAR. *Pesticide Science*., **54**, 35–42

Petschenka, G.; Pick, C.; Wagschal, V.; Dobler, S., 2013: Functional evidence for physiological mechanisms to circumvent neurotoxicity of cardenolides in an adapted and a non-adapted hawk-moth species. *Proceedings of the Royal Society B: Biological Sciences*., **280**, 20123089–20123089

Pinto, F. M.; Armesto, C. P.; Magraner, J.; Trujillo, M.; Martín, J. D.; Candenas, M. L., 1999: Tachykinin receptor and neutral endopeptidase gene expression in the rat uterus: characterization and regulation in response to ovarian steroid treatment. *Endocrinology*., **140**, 2526–2532

Pontoppidan, B.; Ekbom, B.; Eriksson, S.; Meijer, J., 2001: Purification and characterization of myrosinase from the cabbage aphid *Brevicoryne brassicae*, a brassica herbivore. *European*

Journal of Biochemistry., **268**, 1041–1048

Popp, J.; Peto, K.; Nagy, J., 2013: Pesticide productivity and food security. A review. *Agronomy for Sustainable Development*.

Prapanthadara, L. A.; Hemingway, J.; Ketterman, A. J., 1993: Partial purification and characterization of glutathione S-transferases involved in DDT resistance from the mosquito *Anopheles gambiae*. *Pesticide Biochemistry and Physiology*., **47**, 119–133

R Core Team; R Foundation For Statistical Computing, 2014: R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing*.

Ramsey, J. S.; Rider, D. S.; Walsh, T. K.; De Vos, M.; Gordon, K. H. J.; Ponnala, L.; MacMil, S. L.; Roe, B. A.; Jander, G., 2010: Comparative analysis of detoxification enzymes in *Acyrthosiphon pisum* and *Myzus persicae*. *Insect Molecular Biology*., **19**, 155–164

Ranson, H.; Rossiter, L.; Ortelli, F.; Jensen, B.; Wang, X.; Roth, C. W.; Collins, F. H.; Hemingway, J., 2001: Identification of a novel class of insect glutathione S-transferases involved in resistance to DDT in the malaria vector *Anopheles gambiae*. *The Biochemical journal*., **359**, 295–304

Ratlamwala, H., 2014a: *Two spotted spider mite (* Tetranychus urticae*) selection to Arabidopsis thaliana*. University of Western Ontario

Ratzka, A.; Vogel, H.; Kliebenstein, D. J.; Mitchell-Olds, T.; Kroymann, J., 2002: Disarming the mustard oil bomb. *Proceedings of the National Academy of Sciences of the United States of America*., **99**, 11223–11228

Raymond, M., 1987: Inheritance of chlorpyrifos resistance in *culex pipiens* L. (diptera: culicidae) andestimation of the number of genes involved. *Heredity*.

Redovniković, I. R.; Glivetić, T.; Delonga, K.; Vorkapić-Furać, J., 2008: Glucosinolates and their potential role in plant. *Periodicum Biologorum*., **110**, 297–309

Rehrig, E. M.; Appel, H. M.; Jones, A. D.; Schultz, J. C., 2014: Roles for jasmonate- and ethylene-induced transcription factors in the ability of Arabidopsis to respond differentially to damage caused by two insect herbivores. *Frontiers in Plant Science*., **5**

Reintanz, B.; Lehnen, M.; Reichelt, M.; Gershenzon, J.; Kowalczyk, M.; Sandberg, G.; Godde, M.; Uhl, R.; Palme, K., 2001: Bus, a bushy Arabidopsis CYP79F1 knockout mutant with abolished synthesis of short-chain aliphatic glucosinolates. *The Plant Cell*., **13**, 351

Rieu, I.; Powers, S. J., 2009: Real-Time Quantitative RT-PCR: Design, Calculations, and Statistics. *THE PLANT CELL ONLINE*., **21**, 1031–1033

Rioja, C.; Zhurov, V.; Bruinsma, K.; Grbic, M.; Grbic, V., 2017: Plant-herbivore interactions: a case of an extreme generalist, the two-spotted spider mite, *Tetranychus urticae*. *Molecular Plant-Microbe Interactions*., **X**, MPMI-07-17-0168-CR

Roush, R. T.; Hoy, M. A., 1978: Relative toxicity of permethrin to a predator, *Metaseilus occidentalis*, and its prey, *Tetranychus urticae*. *Environmental Entomology*., **7**, 287–288

Rupasinghe, S. G.; Wen, Z.; Chiu, T. L.; Schuler, M. A., 2007: *Helicoverpa zea* CYP6B8 and CYP321A1: Different molecular solutions to the problem of metabolizing plant toxins and insecticides. *Protein Engineering, Design and Selection*., **20**, 615–624

Sances, F. V; Wyman, J. A.; Ting, I. P., 1979: Morphological responses of strawberry leaves to infestations of twospotted spider mite. *Journal of Economic Entomology*., **72**, 710–713

Sarmento, R. A.; Lemos, F.; Bleeker, P. M.; Schuurink, R. C.; Pallini, A.; Oliveira, M. G. A.; Lima,

E. R.; Kant, M.; Sabelis, M. W.; Janssen, A., 2011: A herbivore that manipulates plant defence. *Ecology Letters*., **14**, 229–236

Sarosh, B. R.; Wittstock, U.; Halkier, B. A.; Ekbom, B., 2010: The influence of metabolically engineered glucosinolates profiles in *Arabidopsis thaliana* on *Plutella xylostella* preference and performance. *Chemoecology*., **20**, 1–9

Sasaki-Sekimoto, Y.; Taki, N.; Obayashi, T.; Aono, M.; Matsumoto, F.; Sakurai, N.; Suzuki, H.; Hirai, M. Y.; Noji, M.; Saito, K.; Masuda, T.; Takamiya, K. I.; Shibata, D.; Ohta, H., 2005: Coordinated activation of metabolic pathways for antioxidants and defence compounds by jasmonates and their roles in stress tolerance in Arabidopsis. *Plant Journal*., **44**, 653–668

Sato, M. E.; Silva, M. Z. da; Raga, A.; Souza Filho, M. F. de; da Silva, M. Z.; Raga, A.; de Souza Filho, M. F., 2005: Abamectin resistance in *Tetranychus urticae* Koch (Acari: Tetranychidae): selection, cross-resistance and stability of resistance. *Neotropical Entomology*., **34**, 991–998

Schlaeppi, K.; Bodenhausen, N.; Buchala, A.; Mauch, F.; Reymond, P., 2008: The glutathione-deficient mutant pad2-1 accumulates lower amounts of glucosinolates and is more susceptible to the insect herbivore *Spodoptera littoralis*. *Plant Journal*., **55**, 774–786

Schramm, K.; Vassão, D. G.; Reichelt, M.; Gershenzon, J.; Wittstock, U., 2012: Metabolism of glucosinolate-derived isothiocyanates to glutathione conjugates in generalist lepidopteran herbivores. *Insect Biochemistry and Molecular Biology*., **42**, 174–182

Schuler, M. A., 2012: Insect P450s: Mounted for battle in their war against toxins. *Molecular Ecology*.

Schweizer, F.; Heidel-Fischer, H.; Vogel, H.; Reymond, P., 2017: Arabidopsis glucosinolates trigger a contrasting transcriptomic response in a generalist and a specialist herbivore. *Insect Biochemistry and Molecular Biology*., **85**, 21–31

Scott, J. G.; Zhang, L., 2003: The house fly aliesterase gene (MdαE7) is not associated with insecticide resistance or P450 expression in three strains of house fly. *Insect Biochemistry and Molecular Biology*., **33**, 139–144

Scott, W. S.; Catchot, A.; Gore, J.; Musser, F.; Cook, A. D., 2013: Impact of two-spotted spider mite (Acari: Tetranychidae) duration of infestation on cotton seedlings. *J. Econ. Entomol*., **106**, 862–865

Seifert, J.; Scott, J. G., 2002: The CYP6D1v1 allele is associated with pyrethroid resistance in the house fly, *Musca domestica*. *Pesticide Biochemistry and Physiology*., **72**, 40–44

Seo, S. T.; Tang, C. S., 1982: Hawaiian Fruit Flies (Diptera: Tephritidae): Toxicity of benzyl isothiocyanate against eggs or 1st instars of three species. *Journal of Economic Entomology*., **75**, 1132–1135

Shikita, M.; Fahey, J. W.; Golden, T. R.; Holtzclaw, W. D.; Talalay, P., 1999: An unusual case of ‘uncompetitive activation’ by ascorbic acid: purification and kinetic properties of a myrosinase from *Raphanus sativus* seedlings. *The Biochemical journal*., **341 (Pt 3**, 725–732

Shroff, R.; Vergara, F.; Muck, A.; Svatos, 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*., **105**, 6196–6201

Siemens, D. H.; Mitchell-Olds, T., 1996: Glucosinolates and herbivory by specialists (Coleoptera: Chrysomelidae, Lepidoptera: Plutellidae): consequences of concentration and induced resistance. *Environmental Entomology*., **25**, 1344–1353

Simon, J. C.; D'alençon, E.; Guy, E.; Jacquin-Joly, E.; Jaquiéry, J.; Nouhaud, P.; Peccoud, J.; Sugio, A.; Streiff, R., 2015: Genomics of adaptation to host-plants in herbivorous insects. *Briefings in Functional Genomics*.

Sitnik, J. L.; Francis, C.; Hens, K.; Huybrechts, R.; Wolfner, M. F.; Callaerts, P., 2014: Neprilysins: An evolutionarily conserved family of metalloproteases that play important roles in reproduction in Drosophila. *Genetics*., **196**, 781–797

Soler, R.; Bezemer, T. M.; Van Der Putten, W. H.; Vet, L. E. M.; Harvey, J. A., 2005: Root herbivore effects on above-ground herbivore, parasitoid and hyperparasitoid performance via changes in plant quality. *Journal of Animal Ecology*., **74**, 1121–1130

Sønderby, I. E.; Geu-Flores, F.; Halkier, B. a., 2010a: Biosynthesis of glucosinolates - gene discovery and beyond. *Trends in Plant Science*., **15**, 283–290

Srigiriraju, L.; Semtner, P. J.; Anderson, T. D.; Bloomquist, J. R., 2009: Esterase-based resistance in the tobacco-adapted form of the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) in the Eastern United States. *Archives of Insect Biochemistry and Physiology*., **72**, 105–123

Stahl, E.; Hilfiker, O.; Reymond, P., 2018: Plant–arthropod interactions: who is the winner? *Plant Journal*., **93**, 703–728

Staub, R. E.; Feng, C.; Onisko, B.; Bailey, G. S.; Firestone, G. L.; Bjeldanes, L. F., 2002: Fate of Indole-3-carbinol in cultured human breast tumor cells. *Chemical Research in Toxicology*., **15**, 101–109

Stauber, E. J.; Kuczka, P.; van Ohlen, M.; Vogt, B.; Janowitz, T.; Piotrowski, M.; Beuerle, T.; Wittstock, U., 2012: Turning the 'mustard oil bomb' into a 'cyanide bomb': Aromatic glucosinolate metabolism in a specialist insect herbivore. *PLoS ONE*., **7**

Strauss, S. Y.; Rudgers, J. A.; Lau, J. A.; Irwin, R. E., 2002: Direct and ecological costs of resistance to herbivory. *Trends in Ecology and Evolution*.

Stumpf, N.; Nauen, R., 2002: Biochemical markers linked to abamectin resistance in *Tetranychus urticae* (Acari: Tetranychidae). *Pesticide Biochemistry and Physiology*., **72**, 111–121

Su, Q.; Oliver, K. M.; Xie, W.; Wu, Q.; Wang, S.; Zhang, Y., 2015: The whitefly-associated facultative symbiont *Hamiltonella defensa* suppresses induced plant defences in tomato. *Functional Ecology*., **29**, 1007–1018

Sugio, A.; Kingdom, H. N.; MacLean, A. M.; Grieve, V. M.; Hogenhout, S. A., 2011: Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis. *Proceedings of the National Academy of Sciences*., **108**, E1254–E1263

Suzuki, T.; España, M.U.; Nunes, M.A.; Zhurov, V.; Dermauw, W.; Osakabe, M.; Van Leeuwen, T.; Grbic, M.; Grbic, V., 2017: Protocols for the delivery of small molecules to the two-spotted spider mite, *Tetranychus urticae*. *PLoS ONE*, **12**, e0180658

Tehri, K., 2014: A review on reproductive strategies in two spotted spider mite , *Tetranychus Urticae* Koch 1836 (Acari : Tetranychidae). *Journal of Entomology and Zoology Studies JEZS*., **2**, 35–39

Textor, S.; de Kraker, J. W.; Hause, B.; Gershenzon, J.; Tokuhisa, J. G., 2007: MAM3 catalyzes the formation of all aliphatic glucosinolate chain lengths in Arabidopsis. *Plant Physiology*., **144**, 60–71

Textor, S.; Gershenzon, J., 2009: Herbivore induction of the glucosinolate-myrosinase defense

system: Major trends, biochemical bases and ecological significance. *Phytochemistry Reviews*.

Thangstad, O. P.; Iversen, T. H.; Slupphaug, G.; Bones, A., 1990: Immunocytochemical localization of myrosinase in *Brassica napus* L. *Planta*., **180**, 245–248

Tijet, N.; Helvig, C.; Feyereisen, R., 2001: The cytochrome P450 gene superfamily in *Drosophila melanogaster*: Annotation, intron-exon organization and phylogeny. *Gene*., **262**, 189–198

Tjallingii, W. F.; Esch, T. H., 1993: Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. *Physiological Entomology*., **18**, 317–328

Tomczyk, A.; Kropczynska, D., 1985: Effects on the host plant. In: Helle, W. & M. W. Sabelis (eds.), *Spider Mites, Their Biology, Natural Enemies and Control*. Elsevier Press, New York, pp. 312–330

Tsagkarakou, A.; Navajas, M.; Papaioannou-Souliotis, P.; Pasteur, N., 1998: Gene flow among *Tetranychus urticae* (Acari: Tetranychidae) populations in Greece. *Molecular Ecology*., **7**, 71–79

Uda, Y.; Kurata, T.; Arakawa, N., 1986: Effects of ph and ferrous ion on the degradation of glucosinolates by myrosinase. *Agricultural and Biological Chemistry*., **50**, 2735–3740

Van Dam, N. M.; de Jong, T. J.; Iwasa, Y.; Kubo, T., 1996: Optimal distribution of defences: are plants smart investors? *Functional Ecology*., **10**, 128–136

Van Dam, N. M.; Raaijmakers, C. E., 2006: Local and systemic induced responses to cabbage root fly larvae (Delia radicum) in *Brassica nigra* and *B. oleracea*. *Chemoecology*., **16**, 17–24

Van Dam, N. M.; Tytgat, T. O. G.; Kirkegaard, J. A., 2009: Root and shoot glucosinolates: A comparison of their diversity, function and interactions in natural and managed ecosystems. *Phytochemistry Reviews*.

van de Vrie, M.; McMurtry, J. A.; Huffaker, C. B., 1972: Ecology of tetranychid mites and their natural enemies: A review: III. Biology, ecology, and pest status, and host-plant relations of tetranychids. *Hilgardia*., **41**, 343–432

van Geem, M.; Harvey, J. a.; Gols, R., 2014: Development of a generalist predator, *Podisus maculiventris*, on glucosinolate sequestering and nonsequestering prey. *Naturwissenschaften*., **101**, 707–714

Van Leeuwen, T.; Van Pottelberge, S.; Tirry, L., 2005: Comparative acaricide susceptibility and detoxifying enzyme activities in field-collected resistant and susceptible strains of *Tetranychus urticae*. *Pest Management Science*., **61**, 499–507

Van Leeuwen, T.; Vontas, J.; Tsagkarakou, A.; Tirry, L., 2009: Mechanisms of acaricide resistance in the Two Spotted Spider Mite *Tetranychus urticae*. In: Ishaaya, I. & A. R. Horowitz (eds.), *Biorational Control of Arthropod Pests*. Springer Science, pp. 347–393

Van Leeuwen, T.; Vontas, J.; Tsagkarakou, A.; Dermauw, W.; Tirry, L., 2010: 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 Leeuwen, T.; Dermauw, W., 2016: The molecular evolution of xenobiotic metabolism and resistance in chelicerate mites. *Annual Review of Entomology*., **61**, 475–498

Vergara, F.; Svatoš, A.; Schneider, B.; Reichelt, M.; Gershenzon, J.; Wittstock, U., 2006: Glycine conjugates in a lepidopteran insect herbivore - The metabolism of benzylglucosinolate in the cabbage white butterfly, *Pieris rapae*. *ChemBioChem*., **7**, 1982–1989

Voegele, R. T.; Mendgen, K., 2003: Rust haustoria: nutrient uptake and beyond. *New Phytologist*.

Vontas, J. G.; SMALL, G. J.; HEMINGWAY, J., 2001: Glutathione S-transferases as antioxidant defence agents confer pyrethroid resistance in *Nilaparvata lugens*. *Biochemical Journal*., **357**, 65–72

Vorwerk, S.; Biernacki, S.; Hillebrand, H.; Janzik, I.; Müller, A.; Weiler, E. W.; Piotrowski, M., 2001: Enzymatic characterization of the recombinant *Arabidopsis thaliana* nitrilase subfamily encoded by the NIT2/NIT1/NIT3-gene cluster. *Planta*., **212**, 508–516

Wadleigh, R. W.; Yu, S. J., 1988: Detoxification of isothiocyanate allelochemicals by glutathione transferase in three lepidopterous species. *Journal of Chemical Ecology*., **14**, 1279–1288

Walling, L. L., 2000: The myriad plant responses to herbivores. *Journal Of Plant Growth Regulation*., **19**, 195–216

Warabieda, W., 2015: Effect of two-spotted spider mite population (*Tetranychus urticae* Koch) on growth parameters and yield of the summer apple cv. Katja. *Horticultural Science*., **42**, 167–175

Weech, M. H.; Chapleau, M.; Pan, L.; Ide, C.; Bede, J. C., 2008: Caterpillar saliva interferes with induced *Arabidopsis thaliana* defence responses via the systemic acquired resistance pathway. *Journal of Experimental Botany*., **59**, 2437–2448

Wheat, C. W.; Vogel, H.; Wittstock, U.; Braby, M. F.; Underwood, D.; Mitchell-Olds, T., 2007: The genetic basis of a plant insect coevolutionary key innovation. *Proceedings of the National Academy of Sciences*., **104**, 20427–20431

Wheelock, C. E.; Shan, G.; Ottea, J., 2005: Overview of carboxylesterases and their role in the metabolism of insecticides. *Journal of Pesticide Science*., **30**, 75–83

Willinger, G.; Dobler, S., 2001: Selective sequestration of iridoid glycosides from their host plants in *Longitarsus* flea beetles. *Biochemical Systematics and Ecology*., **29**, 335–346

Winde, I.; Wittstock, U., 2011: Insect herbivore counteradaptations to the plant glucosinolate-myrosinase system. *Phytochemistry*., **72**, 1566–1575

Windsor, A. J.; Reichelt, M.; Figuth, A.; Svatoš, A.; Kroymann, J.; Kliebenstein, D. J.; Gershenzon, J.; Mitchell-Olds, T., 2005: Geographic and evolutionary diversification of glucosinolates among near relatives of *Arabidopsis thaliana* (Brassicaceae). *Phytochemistry*., **66**, 1321–1333

Wittstock, 2011: Glucosinolate breakdown in Arabidopsis: mechanism, regulation and biological significance. *The Arabidopsis Book*., 1–14

Wittstock, U.; Agerbirk, N.; Stauber, E. J.; Olsen, C. E.; Hippler, M.; Mitchell-Olds, T.; Gershenzon, J.; Vogel, H., 2004: Successful herbivore attack due to metabolic diversion of a plant chemical defense. *Proceedings of the National Academy of Sciences of the United States of America*., **101**, 4859–4864

Wouters, F. C.; Reichelt, M.; Glauser, G.; Bauer, E.; Erb, M.; Gershenzon, J.; Vassão, D. G., 2014: Reglucosylation of the benzoxazinoid DIMBOA with inversion of stereochemical configuration is a detoxification strategy in Lepidopteran herbivores. *Angewandte Chemie - International Edition*., **53**, 11320–11324

Wu, J.; Baldwin, I. T., 2010: New insights into plant responses to the attack from Insect herbivores. *Annual Review of Genetics*., **44**, 1–24

Wu, S.; Peiffer, M.; Luthe, D. S.; Felton, G. W., 2012: ATP hydrolyzing salivary enzymes of caterpillars suppress plant defenses. *PLoS ONE*., **7**

Wybouw, N.; Zhurov, V.; Martel, C.; Bruinsma, K. A.; Hendrickx, F.; Grbic, 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

Yu, L.; Tang, W.; He, W.; Ma, X.; Vasseur, L.; Baxter, S. W.; Yang, G.; Huang, S.; Song, F.; You, M., 2015: Characterization and expression of the cytochrome P450 gene family in diamondback moth, *Plutella xylostella* (L.). *Scientific Reports*., **5**

Yu, S. J., 1989: Purification and characterization of glutathione transferases from five phytophagous lepidoptera. *Pesticide Biochemistry and Physiology*., **35**, 97–105

Zarate, S. I.; Kempema, L. A.; Walling, L. L., 2006: Silverleaf whitefly Induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiology*., **143**, 866–875

Zhang, P.; Zhu, X.; Huang, F.; Liu, Y.; Zhang, J.; Lu, Y.; Ruan, Y., 2011: Suppression of jasmonic acid-dependent defense in cotton plant by the mealybug *Phenacoccus solenopsis*. *PLoS ONE*., **6**

Zhang, P. J.; Zheng, S. J.; van Loon, J. J. A.; Boland, W.; David, A.; Mumm, R.; Dicke, M., 2009: Whiteflies interfere with indirect plant defense against spider mites in Lima bean. *Proceedings of the National Academy of Sciences*., **106**, 21202–21207

Zhang, Y.; Talalay, P.; Cho, C. G.; Posner, G. H., 1992: A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proceedings of the National Academy of Sciences*., **89**, 2399–2403

Zhen, Y.; Aardema, M. L.; Medina, E. M.; Schumer, M.; Andolfatto, P., 2012: Parallel molecular evolution in an herbivore community. *Science*., **337**, 1634–1637

Zhu-Salzman, K.; Bi, J. L.; Liu, T. X., 2005: Molecular strategies of plant defense and insect counter-defense. *Insect Science*., **12**, 3–15

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 de Peer, Y.; Grbic, M.; Clark, R. M. et al., 2013: Reciprocal responses in the interaction between Arabidopsis and the cell-content-feeding chelicerate herbivore spider mite. *Plant Physiology*., **164**, 384–399

Zou, X.; Xu, Z.; Zou, H.; Liu, J.; Chen, S.; Feng, Q.; Zheng, S., 2016: Glutathione S-transferase SlGSTE1 in *Spodoptera litura* may be associated with feeding adaptation of host plants. *Insect Biochemistry and Molecular Biology*., **70**, 32–43

Curriculum Vitae

Education

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Publications

1- Sinclair, B. J., L. V. Ferguson, **G. Salehipour-shirazi**, and H. A. MacMillan. 2013. Cross-tolerance and Cross-talk in the Cold: Relating Low Temperatures to Desiccation and Immune Stress in Insects. *Integr. Comp. Biol*. 53(4):545-56.

2- Sinclair, B. J., **G. Salehipour-shirazi**, and L. V. Ferguson. 2013. Does cold activate the Drosophila melanogaster immune response? *J. Insect Physiol*. Submitted.

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