



Review Article

Heavy Metals and Glucosinolates Based Defence Mechanisms in Metal Accumulating Plants and Counter Adaptations by Insect Herbivores: A Review

Saeed Ahmad Asad^{1*}, Rafiq Ahmad² and Muhammad Shahzad²

¹Centre for Climate Research and Development, COMSATS University, Park Road Chak Shahzad Islamabad-45550, Pakistan

²Department of Environmental Sciences, University Road, Tobe Camp Abbottabad-22060, Pakistan

*For correspondence: saeed.asad@comsats.edu.pk

Abstract

Heavy metals (HM) and glucosinolates (GS) have been studied extensively over the last three decades, because of their active involvement in plant defences against a variety of insect herbivores. These inorganic (HM) and organic (GS) chemicals defend the plants through various mechanisms. Heavy metals defend the plants either by deterring the herbivores from feeding on the heavy metal accumulator plant or through toxicity of the accumulated metal after ingestion. Glucosinolates, naturally occurring secondary metabolites in brassica plants, after herbivory are hydrolysed by myrosinase enzyme in to different toxic compounds such as; isothiocyanates, thiocyanates and nitriles, which prevent further herbivory. These compounds deter the insect attack either through direct toxicity or by hindering digestion of the tissue after ingestion. In response to these defence mechanisms, insect herbivores have co-evolved/counter adapted numerous strategies to avoid the toxicity caused by heavy metals and glucosinolates. As a counter adaptation strategy of herbivores, heavy metal toxicity is avoided by switching host or detoxifying through dietary dilution. Toxicity of GS and its hydrolysis products is countered by employing various tactics including leaf snipping, speedy absorption of GS and diverting the conversion of GS to harmless compounds. This review addresses the mechanisms of heavy metals and GS based plant defences. Moreover, counter adaptation mechanisms evolved by feeding herbivores to detoxify these plant defence compounds are encompassed. © 2018 Friends Science Publishers

Keywords: Glucosinolates; Heavy metals accumulators; Plant defences; Herbivores; Counter adaptation mechanisms

Introduction

Plants in the natural environment are exposed to myriads of external threats including abiotic and biotic stresses and thus have evolved numerous strategies and mechanisms to confront the attackers. Amongst abiotic stresses, heavy metals or transition elements are of great concern both for plants and animals. Some of these heavy metals (e.g., Hg, Se, Cd, As and Pb) are not involved in any known physiological function and hence their minute concentrations cause toxicity in plants (Rascio and Navari-Izzo, 2011). For example, cadmium (Cd) and lead (Pb), render extreme toxicity to plants even in trace amounts resulting in reduced germination and growth, disturbed nutritional equilibrium and reduced productivity (Daghan *et al.*, 2010). Contrary to this, some heavy metals are essential (e.g., Cu, Mo, Fe, Co, Mn, Zn and Ni) for normal growth and metabolism of plants and don't exhibit any toxicity symptoms towards plants, but their supra-optimal concentration render them poisonous (Rascio and Navari-Izzo, 2011; Clemens *et al.*, 2013). For instance, Zn being an essential element is involved in key physiological functions such as; photosynthesis, nucleic acid formations

(Hajiboland and Amirazad, 2010; Gurmani *et al.*, 2012; Gupta *et al.*, 2016). On the other hand, its exceeded limits ($> 300 \text{ mg Zn kg}^{-1}$) in plant tissue may result in stunted growth, chlorophyll reductions, Fe-deficiency induced chlorosis and may even hamper the uptake of other essential nutrients such as; P, Mg and Mn (Chaney, 1993). Similar repercussions have been noted with all other heavy metals.

Interestingly, a group of plants called "hyperaccumulators" contain many folds of these heavy metals in their aerial parts without showing any toxicity symptoms (Boyd, 2010; Rascio and Navari-Izzo, 2011). Amongst many possible reasons behind this hyperaccumulation phenomenon, elemental defence hypothesis (Boyd and Martens, 1992; Boyd, 2007; Rascio and Navari-Izzo, 2011) has proven to be the best explanation so far, which states that accumulated heavy metals protect the plants against insect herbivores; a major biotic stress for plants. Heavy metals including; Zn, (Behmer *et al.*, 2005; Stolpe *et al.*, 2017), Se (Galeas *et al.*, 2008), Cd (Jiang *et al.*, 2005; Stolpe *et al.*, 2017), As (Rathinasabapathi *et al.*, 2007), and Ni (Jhee *et al.*, 2006) all have been reported to be involved in plant defence against herbivores from various feeding guilds. Recently many researches have elaborated

that heavy metal content in plant tissue defines the plant-herbivore interactions (Stolpe *et al.*, 2017), where plants may resist the herbivores from feeding on metal containing foliage or reduce the damage after herbivore starts feeding the tissue (Mithöfer and Boland, 2012).

Accumulation of heavy metals by plants could be one type of defence deterring specific enemies but for others there could be another chemical to keep the herbivore away from feeding. For instance, brassica plants possess a wide range of organic compounds notably, glucosinolates which actively defend the host plants against a variety of generalists and specialists insect herbivores (Kessler and Baldwin, 2002; Asad *et al.*, 2015, Asad *et al.*, 2015a). Glucosinolates (GS) are nitrogen, sulphur and carbon containing natural secondary metabolites which are constitutively present in brassica plants but are also induced in response to herbivory. GS by themselves pose rare danger to feeding herbivores because they are distantly located from the enzyme 'myrosinase'. Once the plant tissues are ruptured by herbivores, GS and myrosinase molecule come into contact and various hydrolysis products such as; isothiocyanates, epithionitriles and nitriles are produced which are toxic and deter herbivores from feeding (Fahey *et al.*, 2001; Textor and Gershenzon, 2009). The contents of GS oscillate depending on plant growth stage (Strauss *et al.*, 2004; Stolpe *et al.*, 2017), availability of resources (Bryant *et al.*, 1987; Fine *et al.*, 2006) and type of damage caused by herbivore (Mithöfer and Boland, 2012; Kos *et al.*, 2012). The same glucosinolates which act as deterrents for one type of herbivores might behave as attractants for other species. This phenomenon was further explained by Traw and Dawson (2002), who observed no change in foliar glucosinolates in black mustard, 12 h after feeding by brassica plant specialist, *Pieris rapae*, with chewing mouth parts. Opposite to this behaviour, Travers-Martin and Muller (2007) recorded enhanced indole and aromatic glucosinolates, 24 h after feeding by the generalist folivore, *Spodoptera exigua* on the same brassica plant.

Heavy metals accumulation and GS production for self defense are well known characteristics of brassica plants. Difference amongst these defences are that; GS are synthesized and maintained at the metabolic cost (Mauricio and Rauscher, 1997), but heavy metals are naturally present in the soil environment to be taken up by plants and hence do not incur metabolic cost for synthesis. Therefore, metal based defences are thought to be better option for plant defense (Boyd and Martens, 1992), meaning that glucosinolates may not be required in these plants. This was further elaborated by Pollard and Baker (1997) in choice experiments, where herbivores preferred to feed on low-Zn plants compared with high-Zn containing counterparts. Similar responses have been noted with herbivores feeding on plants containing Ni and Cd (Jhee *et al.*, 2006; Stolpe and Müller, 2016).

Intriguingly, in response to defence strategies developed by plants, herbivores have evolved various

mechanisms to counter these defences. For instance, to avoid the Ni toxicity, green peach aphid inserts mouthparts directly in to cell sap bypassing the metals stored in the epidermal cell walls (Davis *et al.*, 2001). Similarly, herbivores feeding on GS containing plants have developed several evading/aversion strategies to cope with toxicity caused by GS and/or its hydrolysis products. To cope with isothiocyanates (GS), desert locust (*Schistocerca gregaria*) via sulfatase enzyme in its gut rapidly desulfate the GS molecule, thereby inhibiting its hydrolysis (Falk and Gershenzon, 2007).

A growing body of literature exists on investigations regarding plant chemical defences, however detailed overview of plant defence mechanisms against broad range of feeding herbivores have attracted the least attention particularly from ecological point of view. Understanding of these mechanisms may broaden our knowledge of insect-plant interactions in different environments. Parallel with plant defence strategies, herbivores have co-evolved counter measures to evade defences, but these counter adaptations have been studied mostly in generalist herbivores with chewing mouthparts thereby ignoring the specialist feeders, which have evolved advanced mechanisms to cope with the toxicities caused by plant chemicals. Current review is aimed to get insights on the mechanisms of heavy metals and GS-based plant defences in response to herbivore attack. Moreover, different mechanisms employed by insect herbivores from different feeding guilds to detoxify/evade the heavy metals and GS based plant defences are also discussed in this article.

Mechanisms of Action of Plant Chemical Defences

Plants are always exposed to an array of insect herbivores threatening their survival. To safeguard themselves, plants have evolved different defence mechanisms including; mechanical, visual, and chemical defences (Mello and Silva-Filho, 2002; Boyd, 2010; Robertson *et al.*, 2015). Chemical defences amongst these are the most studied and vary from constitutive to induced, production of volatiles, proteins synthesis and trichome (Pare and Tumlinson, 1999; Dicke and Hilker, 2003; Ahuja *et al.*, 2010; Boyd, 2010). Heavy metals and glucosinolates based chemical defences are highly researched in brassica plants. These defences have been known to work effectively both in isolation as well as in consortium (Boyd, 2010; Boyd, 2012; Kazemi-Dinan *et al.*, 2014). Detailed mechanisms by which heavy metals and glucosinolates protect plants from insect herbivory are described in the following sections.

Mechanisms of Action of Heavy Metals

Heavy metals protect plants from herbivores either by deterring the herbivores from feeding on the heavy metal accumulator plant or through toxicity caused by accumulated metal after ingestion imparting lethal/sub-lethal effects

(Boyd and Jhee, 2005; Zhang *et al.*, 2014; Yuan *et al.*, 2016). Many researches have revealed that toxicity only occurs at higher concentrations of elements through consuming plant material or feeding on artificial medium under no-choice experiments (Behmer *et al.*, 2005; Boyd and Jhee, 2005; Coleman *et al.*, 2005; Scheirs *et al.*, 2006; Boyd, 2007; Gonçalves *et al.*, 2007; Boas *et al.*, 2014). Huitson and Macnair (2003) observed that *Arabidopsis halleri* seedlings containing Zn concentrations of 100 μM could not resist the herbivores from feeding. The most obvious reason behind this no-defence was observed as the lower foliar concentrations in the seedlings, not enough to hinder the feeding herbivores. This reason was supported in another investigation, where high Ni in the leaves of Ni hyperaccumulator, *Streptanthus polygaloides* caused 90% mortality to apolyphagous herbivore *Spodoptera exigua* (Boyd and Moar, 1999). In a pollination ecology experiment, same plant species exposed to elevated levels of Ni, attracted significantly lower percentage of pollinating bees and flies as compared with control plants or those counterparts receiving lower metal concentrations (Meindl and Ashman, 2014). Exhibiting the acute metal toxicity by accumulator plants towards herbivores is of paramount importance to defend the metal accumulators at early growth stages. In fact, herbivore damages the plant until it has ingested a lethal dose which limits the effectiveness of the defence (Macnair, 2003). Herbivorous insects can do significant damage before metal toxicity starts functioning and has a benefit to the plant, so plant defence at early growth stage is of immense value for plant survival.

Another way by which accumulated heavy metal defends plants from herbivores is through avoidance of feeding on metal accumulator, with herbivores preferring plants containing lower concentrations of metals. Complete deterrence is very much rare and has so far been reported only by Pollard and Baker (1997) and Jhee *et al.* (2006). These researchers reported complete rejection of high Zn and Ni leaves by caterpillars; cabbage-maggot and cross-striped cabbage-worm respectively. According to Boyd (1998), such deterrence is very important formulating the most effective defence strategy preventing initial herbivory damage. Other experiments (Jhee *et al.*, 1999; Hanson *et al.*, 2004; Behmer *et al.*, 2005; Boyd and Jhee, 2005; Jhee *et al.*, 2006; Gonçalves *et al.*, 2007) have also shown aversion responses in many different invertebrates once animals were given the choice to feed on low or high metal containing plant/artificial medium. Overall, it appears that accumulated elements play significant role to defend the host plant from herbivores. Perhaps, efficacy of accumulated elements within a plant species will magnify the evolutionary pressure on increasing the elemental concentrations within the plant (Boyd *et al.*, 1994).

The mechanisms of herbivore deterrence by heavy metals have been studied by many researchers (Pollard and Baker, 1997; Jhee *et al.*, 1999; de Brito Sanchez *et al.*, 2007; de Brito Sanchez and Giurfa, 2011 and the references

therein) and the negative consequences of herbivore feeding on metal enriched biomass are well documented. For example, Behmer *et al.* (2005) while studying the mechanisms of heavy metal accumulator plant defence against herbivores noted that after post ingestion of metal contaminated food, insects develop aversion through associative learning rather than after first contact with metal contaminated food or metal itself. Such mechanisms have been noted in grasshopper and operate either as a feedback from the taste receptors in mouth parts or via learned association with some food characteristics. Nutrient regulation in migratory locust, *Locusta migratoria* is regulated via direct nutrient feedback (Simpson and Raubenheimer, 1993) but gustatory responsiveness to the target metal is compulsory for such mechanisms to operate. Despite considerable research on the deterrence mechanisms, it is still unclear whether herbivores are deterred after tasting the first meal or other mechanisms. According to Behmer *et al.* (2005), herbivores are deterred after consuming the metal containing meal, but de Brito Sanchez and Giurfa (2011) noted that honeybees like insect have limited gustatory receptors but still can detect various compounds in nectar (de Brito Sanchez *et al.*, 2007). For Zn, post-ingestive feedback seems to be working as reported by Lee and Bernays (1990) in *Schistocerca americana*. They observed that insects rejected the food which was initially acceptable, as *S. americana* nymphs learnt to associate the gustatory cues of previously accepted food, because nymphs were injected with toxic plant secondary metabolites in the haemolymph after feeding. Similarly, grasshoppers modified their feeding behaviour once they learnt to associate their food taste when mixed with unstable sterols and flavours in synthetic food.

Extended hours of feeding on heavy metal containing food, elevate the metal concentration in the food thereby affecting the post ingestion processes and having serious repercussions on the development time and mass gain in the herbivore. With the passage of time, heavy metal decreases the conversion ability of ingested food in to body mass but surprisingly without affecting the digestion process. Limited studies exist describing the influence of metals on insect physiology, one of them (Martoja *et al.*, 1983) observed that Hg and Cd injected in to the haemolymph of grasshoppers (locusts) altered biochemical processes in the fat body which scaled down energy storages and stopped the weight gain process. Moreover, Cd toxicity reduced the accumulation of glycogen and increased synthesis of 'phosphorylase a' (Samarawickrama, 1979). Another effect of heavy metals on insect physiology could be a direct relationship between the amount of metal and herbivore's ability to utilize ingested and digested food. Behmer *et al.* (2005) while studying the effects of Zn on *Schistocerca gregaria* observed that higher Zn concentrations in the plant material or synthetic foods were associated with lower mass gained per unit of meal intake or digested. One thing lacking the most in nearly all studies investigating the herbivores metal accumulator plants

interactions was the conversion of ingested food in to body nitrogen or lipids and to provide information on growth, metabolism, and storage in the insect body.

Empirical evidences indicate that hyperaccumulation of heavy metals effectively deter polyphagous herbivores, because in most of the heavy metals deterrence starts after first meal. Possibly due to this reason, metals based plant defences are not effective against large vertebrate herbivores (Martens and Boyd, 2002). Conclusively, metal based plant defence could strongly depend on the test metal, amount accumulated in the plant body and feeding mode of the herbivore (Konopka *et al.*, 2013).

Mechanisms of Action of Glucosinolates

Glucosinolates (GS) are naturally present in brassica plants but are also induced in response to abiotic and biotic stresses. Inducible defences play key role in devising the defense strategy of the plant (Agrawal *et al.*, 1999; Perkins *et al.*, 2013), depending on the insect species involved, which also determine the occurrence of future attack. Although intact glucosinolates in the plant deter insect attack to some extent (Kim and Jander, 2007), their defence capabilities are enhanced once the plant tissue is eaten/damaged by herbivores, followed by activation of myrosinase enzyme (b-thioglucoside glucohydrolase) to hydrolyze GS core structure (Halkier and Gershenzon, 2006).

Distant locations of substrate and enzymes in plant cells prevent auto-toxicity (Koroleva and Cramer, 2011). During hydrolysis process, myrosinase enzyme dissociates the glucose molecule from the main GS structure to make an unstable aglycones, which undergoes instant loosen rearrangement (Andreasson and Jørgensen, 2003). These structures further reorganize to yield nitriles, epithionitriles, thiocyanates and isothiocyanates like products (Fig. 1) depending on the nature of side chain amino acids, pH, and ferrous ion concentrations (Bones and Rossiter, 2006; Burow *et al.*, 2006; Wittstock and Burow, 2010). Amongst these hydrolysis products, isothiocyanates have been reported to be the most toxic which negatively influence the growth and development and sometimes cause lethal effects in the wide range of insect herbivores (Lambrix *et al.*, 2001; Beekwilder *et al.*, 2008; Müller *et al.*, 2010). After ingestion, isothiocyanates diffuse in to the cellular membranes and move intracellularly in the gut epithelium, finally reaching in to the haemolymph of the insect. The reactive groups of nitrogen, carbon, and sulphur in the isothiocyanate molecules cause biological damage because of their reactivity towards nucleophiles, working either as an acceptor for thiol or side chains (amines) of glutathione and proteins (Kawakishi and Kaneko, 1987). However, isothiocyanates don't damage or react with nucleic acids directly (Xiao *et al.*, 2012) but indirectly reacting with protein thiols and amines of glutathione (GSH) transforming the secondary and tertiary structures of proteins and hence modifying their functions to create toxicity (Mi *et al.*, 2011).

Contrary to isothiocyanates, nitriles are less toxic and are generally excreted with feces (Wittstock *et al.*, 2003). An empirical study by de Vos *et al.* (2008) investigating the oviposition behavior of *Pieris rapae* revealed that nitrile production via epithiospecifier protein (ESP) renders them less attractive for oviposition for specialist herbivores even though some indole glucosinolates are oviposition stimulants. ESP proteins also divert the nitriles to less toxic ones as observed by de Vos *et al.* (2008) who noted that ESP lead to increased production of Indole-3-acetonitrile (IAN), a less toxic nitrile in *Arabidopsis thaliana*. Moreover, *Arabidopsis* plants treated with IAN were less preferred by crucifer specialist insect for oviposition compared with those counterparts treated with indole-3-carbinol, a toxic form of nitrile. Strikingly, this strategy of plant defense works better against second attack on the same plant. This was particularly true in case of *Pieris rapae* because during first attack, larvae of this insect excreted Indole-3-acetonitrile (IAN), a less appealing form of nitrile for adults of *P. rapae* to oviposit (de Vos *et al.*, 2006). Jasmonate signaling is known to be involved in such kind of defense mechanisms (Reymond *et al.*, 2000), *Pieris rapae* feeding induced jasmonates, which up-regulated plant defense mechanism rendering it unfavorable oviposition cues. Exogenous application of Jasmonate on *Brassica oleraceae* (cabbage) plants also reduced oviposition by two crucifer specialists, *Pieris rapae* and *P. brassicae* (Bruinsma *et al.*, 2007) which further confers the involvement of jasmonates in these chemical based defenses.

Sulphur is an integral part of all the approximately 150 discovered glucosinolates (few examples are shown in Table 1) and all sulphur containing chemicals protect the plants against pathogens and insect herbivores (Dubuis, 2004; Cooper and Williams, 2004). Recently, an interesting study by Aziz *et al.* (2016) noticed that plant growth promoting rhizobacteria (PGPRs) enhanced sulphur accumulation by the model plant *Arabidopsis thaliana*. Accumulated sulphur concentration increased the production of aliphatic and indolic types of GS which rendered greater protection against a generalist herbivore, *Spodoptera exigua*. It was further demonstrated that microbes triggered sulphur uptake could possibly be regarded as integration of microbial signaling in plant defense. Sulphur containing compounds, thionin and defensin exhibiting antimicrobial properties are well documented and present in many plant species (Broekaert *et al.*, 1995). Along with being anti herbivorous, antimicrobial activity of sulphur has been observed in *Theobroma cacao* and brassica plants (Cooper *et al.*, 1996; Rohloff and Bones, 2005).

After tissue damage, GS are induced and reach the climax within a certain time period and in fact, these induced defences are crucial when the attack is unpredictable (Haukioja, 1999). As reported earlier that hydrolysis products are toxic to many insect species attacking the plants, but not all compounds are equally

Table 1: Chemical and common names of glucosinolates identified in higher plants

Chemical names	Common names
3-Butenyl	Glucanapin
4-Hydroxybenzyl*	[Gluco] sinalbin
2(R)-2-Hydroxy-3-butenyl	Progoitrin
2(S)-2-Hydroxy-3-butenyl	Epiprogoitrin
2-Hydroxy-4-pentenyl	[Gluco] napoleiferin
Indol-3-ylmethyl	Glucobrassicin
1-Methoxyindol-3-ylmethyl	Neoglucobrassicin
1-Methylethyl	Isopropyl
1-Methylpropyl	Glucocochlearin
4-Methylsulfinyl-3-butenyl	Glucoraphenin
4-(Methylsulfinyl) butyl	Glucoraphanin
5-(Methylsulfinyl) pentyl	Glucoalyssin
3-(Methylsulfinyl) propyl	Glucoiberin
4-(Methylthio) butyl	Glucoerucin
5-(Methylthio) pentyl	Glucoberteroin
3-(Methylthio) propyl	Glucoberverin
1-Pentenyl	Glucobrassicinapin
4-Pentenyl	Glucobrassicinapin
2-Phenylethyl	Phenethyl
2-Propenyl*	Allyl Sinigrin

*The glucosinolates in *Sinapis alba* (white mustard) and *Brassica nigra* (black mustard) (Marsh and Waser, 1970), Sinigrin and Sinalbin are amongst the first identified GS. Source: Fahey *et al.* (2001)

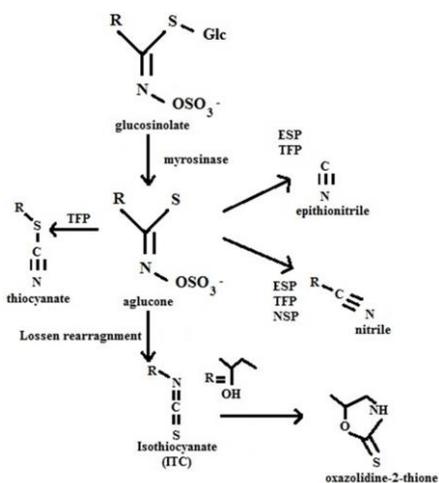


Fig. 1: Hydrolysis products of glucosinolate and myrosinase reaction. Glucosinolates are hydrolyzed by plant myrosinase whereby they remove glucose moiety resulting in the formation of unstable aglucone which rearranges in to different products such as nitrile, isothiocyanates (modified from Jeschke *et al.*, 2016)

effective. Herbivore performance is not only affected by different major classes of GS concentrations, but by different compounds from within those classes, where specific GS molecules regulate the herbivore performance more strongly than total concentrations of GS (Kos *et al.*, 2011). This characteristic of GS enables the plant to have more diverse chemistry, effective against a wide range of insect herbivores (Newton *et al.*, 2009; Kos *et al.*, 2011). Different feeding guilds are affected differently from same

compound or hydrolysis product of GS. In most of the cases, specialist herbivores are less affected by plant chemical defences than generalists, which is perhaps evolutionary adaptation to plant chemicals where mechanisms for detoxification, sequestration or excretion are developing (Opitz and Müller, 2009; Winder and Wittstock, 2011). This can be best explained by an example of brassica specialist lepidopteran, *Pieris rapae* and brassica plants naturally containing glucosinolate based defence. In this case, a protein in the insect gut directs the hydrolysis reaction to produce nitrile instead of isothiocyanates (Wittstock *et al.*, 2004). Similarly, diamond back moth, *Plutella xylostella* detoxify GS via sulfatase enzyme in its gut, which outcompetes the thioglucosidase enzyme and generate desulfoglucosinolates rather than toxic nitriles and isothiocyanates (Ratzka *et al.*, 2002).

Glucosinolate induction triggered by herbivores can be site specific, systemic, or may occur throughout the plant body. According to Chew (1988), in response to herbivore attack, the quantity of at least one class of glucosinolates increases which reduces the further attack by herbivore and formulates the defense strategy of the plant. Herbivore induced production of glucosinolates can be spontaneous occurring within days, or long lasting (Hopkins *et al.*, 2009) and because of this, significant differences between herbivore damaged and undamaged plants can be observed from the start of damage to eight weeks later. This long-lasting induction of glucosinolates put some credence to the hypothesis that early season insect herbivores may deter the late season herbivory thereby altering the food quality (Bartlett *et al.*, 1999; Poelman *et al.*, 2008). Moreover, due to the systemic induction of defense metabolites, root herbivores may modify the quantity of glucosinolates in the above ground biomass. For example, Bezemer and van Dam (2005) observed reduced performance of specialist *P. rapae* due to enhanced glucosinolate level because of root herbivory by the endoparasitic nematode, *Pratylenchus penetrans* in a variety of plant species.

Counter Adaptation of Herbivores against Chemical Defenses

Counter adaptations against heavy metal-based defenses: Heavy metals have since long been known to deter/repel insect herbivores from feeding on metal contaminated food. Ingestion of metal containing plants results in weight loss and acute toxicity leading to death of feeding attackers. Most of these insects can cope with the heavy metals based defenses but with great challenge, because of non-biodegradable nature of heavy metals. One possible adaptive mechanism by herbivores is the avoidance of metal rich tissues and preference to feed on low metal containing plant tissue (Boyd, 1998). For example, aphids feeding on the phloem sap, easily bypass Zn and Ni which are primarily concentrated in the leaf epidermis (Vázquez *et al.*, 1994; Mesjasz-Przybyłowicz *et al.*, 1997; Boyd and

Martens, 2000). Active and mobile herbivores limit the heavy metal toxicity by feeding on different plant species thereby diluting the metal concentration in the ingested diet. In case of lethargic herbivores, avoidance of toxins before feeding is necessary, so adults select low metal hosts for oviposition and after attaining certain growth level, instars move to less favorable plant species (Davis *et al.*, 2001).

Host switching strategy by herbivores to avoid the metal toxicity varies from species to species. Many lepidopteran species (e.g., *Lymantria dispar*) keep moving from one host to another during larval development (Mauffette and Lechowicz, 1984) depending on the host quality and phenology (Liebhold *et al.*, 1986). Interestingly, this host switching sometime becomes lifeline for the insects where one time switching the host significantly improved the growth rate of *Lymantria dispar* (gypsy moth) under field conditions (Stoyenhoff *et al.*, 1994). This improved growth on favorable host dictate the larval performance during later development stages on less favorable food. Many polyphagous herbivores use host switching strategy to combat the heavy metal toxicity. Different strategies evolved by insect herbivores to counter the heavy metal toxicity are enlisted in Table 2.

Counter adaptation against glucosinolate-based defense:

Counter adaptation strategies of herbivorous insects against plant defences could be behavioural, biochemical, and/or physiological. Amongst organic chemical defences, GS-MS is one of the most studied in brassica plants. Both, the substrate-GS and enzyme-MS are compartmentalized separately; GS are located in the vacuoles while enzymes are found somewhere at the periphery of cells. This defence system only becomes active after plant tissues are eaten/damaged by feeding insects and enzyme gets access to substrate resulting in the toxic by-products such as isothiocyanates and nitriles etc. Isothiocyanates the most lethal one. Therefore, counter adaptation by most of the brassica specialized herbivores is to avoid the conversion of GS into isothiocyanates. This may be achieved either by less damage, conversion of GS to less toxic compounds (e.g., nitriles), rapid passage of intact glucosinolates and secreting through honey dew. All major forms of nitriles (e.g., 5-methylsulfinyl, 4-hydroxyphenylacetone nitrile) were excreted in the feces of a brassica specialist herbivore, *Pieris rapae* while feeding on *A. thaliana* plants. Different mechanisms adapted by different herbivores to detoxify GS-myrosinase defence system of plants are listed in Table 3. Chewing insects, predominantly feed on Brassicales and have evolved countermeasures to defend themselves from GS and myrosinase defence system. These herbivores direct the conversion of GS to nitrile rather than isothiocyanates through the mediation of a nitrile specifier protein (Wittstock *et al.*, 2004) or by dissecting off the sulfate from GS molecule with the help of sulfatase enzyme located in the gut of herbivore and hence produce desulfoglucosinolates which myrosinase is unable to activate (Falk and Gershenzon, 2007; Opitz *et al.*, 2011). An interesting study by Malka *et al.* (2016), while studying the GS detoxification by whitefly (*Bemisia tabaci*) demonstrated that only mechanism of GS detoxification in whitefly is desulfation. This can be further ascertained that most of the 7000 gut-specific genes in various species of whitefly have been reported to be involved in nutrient absorption and sucrose hydrolysis (Ye *et al.*, 2014). Moreover, other detoxification related genes (glucuronosyltransferase (GST) and cytochrome P450) have also been reported specifically expressed in the gut of whitefly.

Another counter adaptation strategy employed by herbivores to detoxify isothiocyanates involves conjugation of these compounds with glutathione which renders them less toxic (Schramm *et al.*, 2012). Conjugation of isothiocyanates was demonstrated by *Pieris rapae* larvae through conversion of benzylglucosinolate into phenylacetyl-glycine releasing through faeces afterwards (Vergara *et al.*, 2006). Phenyl acetic acid and phenyl acetonitrile were reported as the intermediates during this isothiocyanate conversion process. Surprisingly, *P. rapae* faeces contained additional conjugates of glycine and isoserine with benzoate and indole-3-carboxylate.

Contrary to chewers, phloem feeders (Aphididae) circumvent the GS-MS defense system in a very different way. These insects penetrate their piercing-sucking mouthparts (stylets) between the epidermal and parenchymal cells to reach the sap thus causing minimal damage (Walling, 2008). Penetration of stylets through the apoplast keeps the substrate and remotely located hydrolysis enzyme in their own compartments (Pentzold *et al.*, 2014; Pentzold *et al.*, 2015). A generalist herbivore, green peach aphid (*Myzus persicae*), a typical phloem feeder passes intact glucosinolates through the gut (Barth and Jander, 2006). However, some contradictory results have revealed that most of the indolic GS are degraded and conjugated with glutathione and amino acids while aliphatic GS pass through the gut while intact (Kim *et al.*, 2008). Other GS detoxification mechanisms employed by *M. persicae* include; repression by secreted effectors, metabolism, and rapid excretion (Kim *et al.*, 2008; Elzinga *et al.*, 2014). Avoiding the large-scale tissue damage is not restricted to specialists only, but also amongst lepidopteran generalists (Pentzold *et al.*, 2014). Lepidopteran herbivores apply leaf-snipping and speedy feeding technique to avoid the tissue disruption and collision of β -glucosidases and cyanogenic glucoside to avoid hydrolysis. Pentzold *et al.* (2015) while investigating the feeding mode and hydrolysis aversion strategy of lepidopteran, observed that *Zygaena filipendulae*, a specialist lepidopteran fed very quickly on cyanogenic glucoside-rich *Lotus corniculatus* plants thereby snipping the leaves in a way that damage was not enough to bring the β -glucosidases enzyme and its cyanogenic glucoside substrate together. This is perhaps the smartest way of circumventing the toxicity of ingested food, which these specialists might have learnt through evolutionary adaptation.

Table 2: Published material supportive of adaptation strategies of insect herbivores to counter the heavy metal based defenses and toxicity

Herbivore	Heavy metal	Detoxification strategy	Reference
Gypsy moth (<i>Lymantria dispar</i> L.)		Host switching during early larval instars development. Such instars exhibit better ability to feed on less favorable food.	Stoyenhoff <i>et al.</i> (1994)
Centipedes (<i>Lithobius forficatus</i>)	Cd, Pb	Permanent storage of metals in the granules of midgut epithelial cells. Midgut epithelium also acts as barrier to stop excessive storage of few metals.	Vandenbulcke <i>et al.</i> (1998)
Green peach aphid (<i>Myzus persicae</i>)	Ni	Insert stylets, directly into cell sap or phloem, bypassing metals accumulated in epidermal cell walls. Rapid metabolism and excretion is also their counter adaptive strategy against Ni toxicity.	Davis <i>et al.</i> (2001)
Cabbage butterfly (<i>Pieris brassicae</i>)	Zn	Complete rejection of feeding on high Zn concentration containing <i>N. caerulescens</i> foliage	Pollard and Baker (1997)
White butterfly (<i>Pieris rapae</i>)	Ni	Limit Ni toxicity by consuming a mixed diet of high- and Low-Ni foliage	Boyd <i>et al.</i> (1994)

Table 3: Counter adaptation mechanisms of insect herbivores against glucosinolate- myrosinase system of plant defense. These adaptations are inducible in generalist herbivores whereas they are often constitutive in specialist feeders (Pentzold *et al.*, 2014)

Insect herbivore	Feeding Mode	Detoxification strategy	Reference
Aphid (<i>Myzus persicae</i>)	Piercing/Sucking	Avoids cell disruption/Rapid excretion/ repression by secreted effectors.	Kim <i>et al.</i> (2008); Elzinga <i>et al.</i> (2014)
Turnip Sawfly (<i>Athalia rosae</i>)	Chewing	Sequestration of intact GS and accumulation in hemolymph.	Opitz <i>et al.</i> (2011).
Cabbage white butterfly (<i>Pieris rapae</i>)	Chewing	Redirection; Nitrile specifier protein mediates the conversion of GS to less toxic compounds (nitrile) instead of isothiocyanates.	Wittstock <i>et al.</i> (2004).
Silver leaf whitefly (<i>Bemisia tabaci</i>)	Stylets/sucking	Desulfation; Cleaves the sulfate from GS molecule before GS hydrolysis.	Malka <i>et al.</i> (2016).
Diamondback moth (<i>Plutella xylostella</i>)	Chewing/lower surface of leaf	Rapid metabolization of GS; Conversion of GS to desulfoglucosinolates and excretion with feces.	Ratzka <i>et al.</i> (2002).
Fall armyworm (<i>Spodoptera frugiperda</i>)	Chewing	Conjugation: detoxify by conjugating isothiocyanates with glutathione.	Yu (1987).
Cabbage looper (<i>Trichoplusia ni</i>)	Chewing	Conjugation: detoxification of isothiocyanates by conjugating with glutathione.	Wadleigh and Yu (1988).
Foxglove aphid (<i>Aulacorthum solani</i>)	Sap sucking	Detoxify by conjugating with glutathione (artificial diet experiment).	Francis <i>et al.</i> (2005).
Cabbage bug (<i>Murgantia histrionica</i>)	Sucking	Sequestration of intact GS.	Aliabadi <i>et al.</i> (2002).
Cabbage aphid (<i>Brevicoryne brassicae</i>).	Sucking	Ingestion of intact GS and active sequestration of them from the phloem sap through the gut wall.	Winde and Wittstock (2011).
Turnip or mustard aphid (<i>Lipaphis erysimi</i>)	Sucking	Sequestration of intact GS and contains own myrosinase in the gut, uses sequestered GS and own myrosinase for self defence.	Bridges <i>et al.</i> (2002).
Desert locust (<i>Schistocerca gregaria</i>).	Chewing	Desulfation; An inducible gut sulfatase activity on glucosinolates to cleave the sulfate from GS.	Falk and Gershenzon (2007).
1. <i>Spodoptera exigua</i> , 2. <i>Spodoptera littoralis</i> , 3. <i>Helicoverpa armigera</i>	Chewing	Glutathione S-transferase activity in the gut.	Schramm <i>et al.</i> (2012).
<i>Zyaena filipendulae</i>	Leaf-snipping and speedy feeding	Minimal tissue damage to avoid substrate-enzyme interaction or shortening the time of possible interaction between enzyme and substrate.	Pentzold <i>et al.</i> (2015).
White butterfly (<i>Pieris rapae</i>)	Chewing	Intermediated products of GS conversion process form conjugates with glycine and released through feces.	Vergara <i>et al.</i> (2006)

Conclusions and Perspectives

Over the centuries, plants have developed various mechanisms to defend themselves from insect herbivores. Among those, heavy metals and glucosinolates based defences are widely studied and debated. These compounds defend the plants either deterring the herbivore from feeding or exhibit toxicity after ingestion has taken place. In heavy metal accumulator plants of *Brassicaceae* family, efficacies of these defences grows many folds, especially when heavy metals and GS work in consortia and do pose a serious threat for insect survival and sometimes become lethal for feeding herbivore. Because of sharing the common niche with plants, herbivores have co-developed mechanisms to circumvent these defences. These counter adaptations include; avoidance of feeding on food containing these compounds and/or diluting the ingested chemicals by consuming less contaminated meal. After ingestion of such

compounds, conversion of more toxic compounds to less toxic ones (e.g., isothiocyanates to nitrile) formulate the counter adaptation strategy of herbivores. Limited literature exists on the influence of soil microbial populations on such kind of defences which needs to be incorporated in future studies. For example, some sulphur oxidizing microbes have been investigated which accelerate the heavy metal uptake by plants and enhance their defence capacity against some specialist insects. Moreover, interactions between plant defences and feeding herbivores also need to be investigated from climate change perspective. This is because, variations in climate have been recognized as an additional stressor to influence plant metabolites on one hand and introduction of alien insect species and disappearance of old populations on the other. Studies on possible role of climate change in insect-plant interactions would be quite helpful to understand the broader picture of these complex relations between insects and their food plants. Multidisciplinary

research involving ecology, physiology, microbiology, and climatology may provide insights in to the co-evolution of insect herbivores and plants.

References

- Agrawal, A.A., C. Laforsch and R. Tollrian, 1999. Transgenerational induction of defences in animals and plants. *Nature*, 401: 60–63
- Ahuja, I., J. Rohloff and A.M. Bones, 2010. Defence mechanisms of *Brassicaceae*: implications for plant-insect interactions and potential for integrated pest management. *A review. Agron. Sustain. Dev.*, 30: 311–348
- Aliabadi, A., J.A.A. Renwick and D.W. Whitman, 2002. Sequestration of glucosinolates by harlequin bug *Murgantia histrionica*. *J. Chem. Ecol.*, 28: 1749–1762
- Andreasson, E. and L.B. Jørgensen, 2003. Localization of plant myrosinases and glucosinolates. *Recent Adv. Phytochem.*, 37: 79–99
- Asad, S.A., S. Muhammad, M. Farooq, A. Afzal, M. Broadley, S. Young and H. West, 2015. Anthocyanin production in the hyperaccumulator plant *Noccaea caerulea* in response to herbivory and zinc stress. *Acta Physiol. Plant.*, 37: 1715
- Asad, S.A., S. Young and H. West, 2015a. Effect of zinc and glucosinolates on nutritional quality of *Noccaea caerulea* and infestation by *Aleyrodes proletella*. *Sci. Total Environ.*, 511: 21–27
- Aziz, M., R.K. Nadipalli, X. Xie, Y. Sun, K. Surowiec, J.L. Zhang and P.W. Paré, 2016. Augmenting sulfur metabolism and herbivore defense in *Arabidopsis* by bacterial volatile signaling. *Front. Plant Sci.*, 7: 458
- Barth, C. and G. Jander, 2006. *Arabidopsis* myrosinases TGG1 and TGG2 have redundant function in glucosinolate breakdown and insect defense. *Plant J.*, 46: 549–562
- Bartlett, E., G. Kiddle, I. Williams and R. Wallsgrove, 1999. Wound-induced increases in the glucosinolate content of oilseed rape and their effects on subsequent herbivory by a crucifer specialist. *Entomol. Exp. Appl.*, 91: 163–167
- Beekwilder, J., W. van Leeuwen, N.M. van Dam, M. Bertossi, V. Grandi, L. Mizzi, M. Soloviev, L. Szabados, J.W. Molthoff, B. Schipper, H. Verbocht, de Vos, M. Piero, G.M.A. Mark and B. Arnaud, 2008. The impact of the absence of aliphatic glucosinolates on insect herbivory in *Arabidopsis*. *PLoS One*, 3: 2068
- Behmer, S.T., C.M. Lloyd, D. Raubenheimer, J. Stewart-clark, J. Knight, R.S. Leighton, F.A. Harper and J.A.C. Smith, 2005. Metal hyperaccumulation in plants: mechanisms of defense against insect herbivores. *Funct. Ecol.*, 19: 55–66
- Bezemer, T.M. and N.M. van Dam, 2005. Linking aboveground and belowground interactions via induced plant defences. *Trends Ecol. Evol.*, 20: 617–624
- Boas, L.V., S.C. Gonçalves, P. António, F. Helena and M.T. Gonçalves, 2014. A Ni hyperaccumulator and a congeneric non-accumulator reveal equally effective defenses against herbivory. *Sci. Total Environ.*, 466–467: 11–15
- Bones, A.M. and J.T. Rossiter, 2006. The enzymic and chemically induced decomposition of glucosinolates. *Phytochemistry*, 67: 1053–1067
- Boyd, R.S., 2012. Plant defense using toxic inorganic ions: conceptual models of the defensive enhancement and joint effects hypotheses. *Plant Sci.*, 195: 88–95
- Boyd, R.S., 2010. Elemental defenses of plants by metals. *Nat. Edu. Knowledge*, 1: 6–9
- Boyd, R.S., 2007. Defense hypothesis of elemental hyperaccumulation: status, challenges, and new directions. *Plant Soil*, 293: 153–176
- Boyd, R.S., 1998. Hyperaccumulation as a plant defensive strategy. In: *Plants that Hyperaccumulate Heavy Metals*, pp: 181–201. Brooks, R.R. (eds.). CAB International, Oxford, UK
- Boyd, R.S. and E.M. Jhee, 2005. A test of elemental defence against slugs by Ni in hyperaccumulator and non-hyperaccumulator *Streptanthus* species. *Chemoecology*, 15: 179–185
- Boyd, R.S. and S.N. Martens, 2000. Aphids are unaffected by the elemental defense of the nickel hyperaccumulator, *Streptanthus polygaloides* (Brassicaceae). *Chemoecology*, 9: 1–7
- Boyd, R.S. and S.N. Martens, 1992. The raison d'être for metal hyperaccumulation by plants. In: The vegetation of ultramafic (Serpentine) soils. *Proceedings of the First International Conference on Serpentine Ecology*, pp: 279–289. Baker, A.J.M., J. Procter and R.D. Reeves (eds.). Andover, Intercept, UK
- Boyd, R.S. and W.J. Moar, 1999. The defensive function of Ni in plants: response of the polyphagous herbivore *Spodoptera exigua* (Lepidoptera: Noctuidae) to hyperaccumulator and accumulator species of *Streptanthus* (Brassicaceae). *Oecologia*, 118: 218–224
- Boyd, R.S., J.J. Shaw and S.N. Marten, 1994. Nickel hyperaccumulation defends *Streptanthus polygaloides* (Brassicaceae) against pathogens. *Amer. J. Bot.*, 81: 294–300
- Bridges, M., A.M.E. Jones, A.M. Bones, C. Hodgson, R. Cole, E. Bartlett, R. Wallsgrove, V.K. Karapapa, N. Watts and J.T. Rossiter, 2002. Spatial organization of the glucosinolate-myrosinase system in brassica specialist aphids is similar to that of the host plant. *Proc. Royal Soc. London*, 269: 187–191
- Broeckaert, W.F., F.R.G. Terras, B.P.A. Cammue and R.W. Osborn, 1995. Plant defensins: novel antimicrobial peptides as components of the host defense system. *Plant Physiol.*, 108: 1353–1358
- Bruinsma, M., N.M. van Dam, J.J.A. Van Loon and M. Dicke, 2007. Jasmonic acid induced changes in *Brassica oleracea* affect oviposition preference of two specialist herbivores. *J. Chem. Ecol.*, 33: 655–668
- Bryant, J.P., T.P. Clausen, P.B. Reichardt, M.C. McCarthy and R.A. Werner, 1987. Effect of nitrogen fertilization upon the secondary chemistry and nutritional value of quaking aspen (*Populus tremuloides* Michx.) leaves for the large Aspen tortrix (*Choristoneura conflictana* (Walker)). *Oecologia*, 73: 513–517
- Burow, M., J. Markert, J. Gershenzon and U. Wittstock, 2006. Comparative biochemical characterization of nitrile-forming proteins from plants and insects that alter myrosinase-catalysed hydrolysis of glucosinolates. *FEBS J.*, 273: 2432–2446
- Chaney, R.L., 1993. Zinc phytotoxicity. In: *Zinc in Soil and Plants*, pp: 135–150. Robson, A.D. (ed.). Kluwer Academic Publishers, Dordrecht, the Netherlands
- Chew, F.S., 1988. Biological effects of glucosinolates. In: *Biologically Active Natural Products for Potential Use in Agriculture*, pp: 155–181. Cutler, H.G. (ed.). American Chemical Society, Washington
- Clemens, S., M.G. Aarts, S. Thomine and N. Verbruggen, 2013. Plant science: the key to preventing slow cadmium poisoning. *Trends Plant Sci.*, 18: 92–99
- Coleman, C.M., R.S. Boyd and M.D. Eubanks, 2005. Extending the elemental defense hypothesis: dietary metal concentrations below hyperaccumulator levels could harm herbivores. *J. Chem. Ecol.*, 31: 1669–1681
- Cooper, R.M. and J.S. Williams, 2004. Elemental sulphur as an induced antifungal substance in plant defence. *J. Exp. Bot.* 55: 1947–1953
- Cooper, R.M., L.V.R. Mario, J. Flood and M.G. Rowan, 1996. Detection and cellular localization of elemental sulphur in disease-resistant genotypes of *Theobroma cacao*. *Nature*, 379: 159–162
- Daghan, H., M. Arslan, V. Uygur, N. Koleli and A. Eren, 2010. The cadmium phytoextraction efficiency of SCMTII gene bearing transgenic tobacco plant. *Biotechnol. Equip.*, 24: 1974–1978
- Davis, M.A., R.S. Boyd and J.H. Cane, 2001. Host-switching does not circumvent the Ni-based defence of the Ni hyperaccumulator *Streptanthus polygaloides* (Brassicaceae). *S. Afr. J. Sci.*, 97: 554–557
- de Brito Sanchez, G. and M. Giurfa, 2011. A comparative analysis of neural taste processing in animals. *Philos. Trans. Royal Soc. London B: Biol. Sci.*, 366: 2171–2180
- de Brito Sanchez, G., J. Ortigão-Farias, M. Gauthier, F. Liu and M. Giurfa, 2007. Taste perception in honeybees: just a taste of honey? *Arthropod Plant Interact.*, 1: 69–76
- de Vos, M., K.L. Kriksunov and G. Jander, 2008. Indole-3-Acetonitrile production from indole glucosinolates deters oviposition by *Pieris rapae*. *Plant Physiol.*, 46: 916–926
- de Vos, M., W. Van Zaanen, A. Koornneef, J.P. Korzelijs, M. Dicke, L.C. Van Loon and C.M.J. Pieterse, 2006. Herbivore-induced resistance against microbial pathogens in *Arabidopsis*. *Plant Physiol.*, 142: 352–363

- Dicke, M. and M. Hilker, 2003. Induced plant defences; from molecular biology to evolutionary ecology. *Basic Appl. Ecol.*, 4: 3–14
- Dubuis, P.H., 2004. *Effect of Sulfur Deficiency on the Resistance of Oilseed Rape to Fungal Pathogens and Expression Profiling of the Glutathione S transferase Family of Arabidopsis thaliana*. Dissertation, University of Fribourg, Switzerland
- Elzinga, D.A., M. DeVos and G. Jander, 2014. Suppression of plant defenses by a *Myzus persicae* (green peach aphid) salivary effector protein. *Mol. Plant Microbe Int.*, 27: 747–756
- Fahey, J.W., A.T. Zalcmann and P. Talalay, 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, 56: 5–51
- Falk, K.L. and J. Gershenzon, 2007. The desert locust, *Schistocerca gregaria*, detoxifies the glucosinolates of *Schouwia purpurea* by desulfation. *J. Chem. Ecol.*, 33: 1542–1555
- Fine, P.V.A., Z.J. Miller, I. Mesones, S. Irazuzta, H.M. Appel, M.H.H. Stevens, I. Sääksjärvi, J.C. Schultz and P.D. Coley, 2006. The growth-defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology*, 87: 150–162
- Francis, F., N. Vanhaelen and E. Haubruge, 2005. Glutathione S-transferases in the adaptation to plant secondary metabolites in the *Myzus persicae* aphid. *Arch. Insect Biochem. Physiol.*, 58: 166–174
- Galeas, M.L., E.M. Klamper, L.E. Bennett, J.L. Freeman, B.C. Kondratieff, C.F. Quinn and E.A. Pilon-Smits, 2008. Selenium hyperaccumulation reduces plant arthropod loads in the field. *New Phytol.*, 177: 715–724
- Gonçalves, M., S. Gonçalves, A. Portugal, S. Silva, J. Sousa and H. Freitas, 2007. Effects of nickel hyperaccumulation in *Alyssum pintodasilvae* on model arthropods representatives of two trophic levels. *Plant Soil*, 293: 177–188
- Gupta, N., H. Ram and B. Kumar, 2016. Mechanism of Zinc absorption in plants: uptake, transport, translocation and accumulation. *Rev. Environ. Sci. Biotechnol.*, 15: 89–109
- Gurmani, A.R., S.U. Khan, R. Andaleep, K. Waseem and A. Khan, 2012. Soil application of zinc improves growth and yield of tomato. *Int. J. Agric. Biol.*, 14: 91–96
- Hajiboland, R. and F. Amirazad, 2010. Growth, photosynthesis, and antioxidant defense system in Zn-deficient red cabbage plants. *Plant Soil Environ.*, 56: 209–217
- Halkier, B.A. and J. Gershenzon, 2006. Biology and biochemistry of glucosinolates. *Annu. Rev. Plant Biol.*, 57: 303–333
- Hanson, B., S.D. Lindblom, M.L. Loeffler and E.A. Pilon-Smits, 2004. Selenium protects plants from phloem-feeding aphids due to both deterrence and toxicity. *New Phytol.*, 162: 655–662
- Haukioja, E., 1999. Ecology: Bite the mother, fight the daughter. *Nature*, 401: 22–23
- Hopkins, R.J., N.M. van Dam and J.J. Van Loon, 2009. Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annu. Rev. Entomol.*, 54: 57–83
- Huitson, S.B. and M.R. Macnair, 2003. Does zinc protect the zinc hyperaccumulator *Arabidopsis halleri* from herbivory by snails? *New Phytol.*, 159: 453–459
- Jeschke, V., J. Gershenzon and D.G. Vassão, 2016. A mode of action of glucosinolate-derived isothiocyanates: Detoxification depletes glutathione and cysteine levels with ramifications on protein metabolism in *Spodoptera littoralis*. *Insect Biochem. Mol. Biol.*, 71: 37–48
- Jhee, E.M., R.S. Boyd, M.D. Eubanks and M.A. Davis, 2006. Nickel hyperaccumulation by *Streptanthus polygaloides* protects against the folivore *Plutella xylostella* (Lepidoptera: Plutellidae). *Plant Ecol.*, 183: 91–104
- Jhee, E.M., K.L. Dandridge, A.M. Christy and A.J. Pollard, 1999. Selective herbivory on low-zinc phenotypes of the hyperaccumulator *Thlaspi caerulescens* (Brassicaceae). *Chemoecology*, 9: 93–95
- Jiang, R.F., D.Y. Ma, F.J. Zhao and S.P. McGrath, 2005. Cadmium hyperaccumulation protects *Thlaspi caerulescens* from leaf feeding damage by thrips (*Frankliniella occidentalis*). *New Phytol.*, 167: 805–914
- Kawakishi, S. and T. Kaneko, 1987. Interaction of proteins with allyl isothiocyanate. *J. Agric. Food Chem.* 35: 85–88
- Kazemi-Dinan, A., S. Thomaschky, R.J. Stein, U. Krämer and C. Müller, 2014. Zinc and cadmium hyperaccumulation act as deterrents towards specialist herbivores and impede the performance of a generalist herbivore. *New Phytol.*, 202: 628–639
- Kessler, A. and I.T. Baldwin, 2002. Plant responses to insect herbivory: The emerging molecular analysis. *Annu. Rev. Plant Biol.*, 53: 299–328
- Kim, J.H. and G. Jander, 2007. *Myzus persicae* (green peach aphid) feeding on *Arabidopsis* induces the formation of a deterrent indole glucosinolate. *Plant J.*, 49: 1008–1019
- Kim, J.H., B.W. Lee, F.C. Schroeder and G. Jander, 2008. Identification of indole glucosinolate breakdown products with antifeedant effects on *Myzus persicae* (green peach aphid). *Plant J.*, 54: 1015–1026
- Konopka, J.K., K. Hanyu, S.M. Macfie and J.N. McNeil, 2013. Does the response of insect herbivores to cadmium depend on their feeding strategy? *J. Chem. Ecol.*, 39: 546–554
- Koroleva, O.A. and R. Cramer, 2011. Single-cell proteomic analysis of glucosinolate-rich S-cells in *Arabidopsis thaliana*. *Methods*, 54: 413–423
- Kos, M., B. Houshyani, R. Wietsma, P. Kabouw, L.E.M. Vet, J.J.A. van Loon and M. Dicke, 2012. Effects of glucosinolates on a generalist and specialist leaf-chewing herbivore and an associated parasitoid. *Phytochemistry*, 77: 162–170
- Kos, M., C. Broekgaarden, P. Kabouw, L.K. Oude, E.H. Poelman, L.E.M. Vet, M. Dicke and J.J.A. van Loon, 2011. Relative importance of plant-mediated bottom-up and top-down forces on herbivore abundance on *Brassica oleracea*. *Funct. Ecol.*, 25: 1113–1124
- Lambrix, V., M. Reichelt, T. Mitchell-Olds, D.J. Kliebenstein and J. Gershenzon, 2001. The *Arabidopsis* epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. *Plant Cell*, 13: 2793–2807
- Lee, J.C. and E.A. Bernays, 1990. Food tastes and toxic effects: Associative learning by the polyphagous grasshopper *Schistocerca americana* (Drury) (Orthoptera: Acrididae). *Anim. Behav.*, 39: 163–173
- Liebholt, A.M., J.S. Elkinton and W.E. Wallner, 1986. Effect of burlap bands on between-tree movement of late-instar gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae). *Environ. Entomol.*, 15: 373–379
- Macnair, M.R., 2003. The hyperaccumulation of metals by plants. *Adv. Bot. Res.*, 40: 63–107
- Malka, O., A. Shekhov, M. Reichelt, J. Gershenzon, D.G. Vassão and S. Morin, 2016. Glucosinolate desulfation by the phloem-feeding insect, *Bemisia tabaci*. *J. Chem. Ecol.*, 42: 230–235
- Marsh, R.E. and J. Waser, 1970. Refinement of the crystal structure of sinigrin. *Acta Crystallographica*, 26: 1030–1037
- Martens, S.N. and R.S. Boyd, 2002. The defensive role of Ni hyperaccumulation by plants: a field experiment. *Amer. J. Bot.*, 89: 998–1003
- Martoja, R., J.M. Bouquegneau and C. Verthe, 1983. Toxicological effects and storage of cadmium and mercury in an insect *Locusta migratoria* (Orthoptera). *J. Invert. Pathol.*, 42: 17–32
- Mauffette, Y. and M.J. Lechowicz, 1984. Differences in the utilization of tree species as larval hosts and pupation sites by the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae). *Can. Entomol.*, 116: 685–690
- Mauricio, R. and M.D. Rauscher, 1997. Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution*, 51: 1435–1444
- Meindl, G.A. and T.L. Ashman, 2014. Nickel accumulation by *Streptanthus polygaloides* (Brassicaceae) reduces floral visitation rate. *J. Chem. Ecol.*, 40: 128–135
- Mello, M.O. and M.C. Silva-Filho, 2002. Plant-insect interactions: an evolutionary arms race between two distinct defense mechanisms. *Braz. J. Plant Physiol.*, 14: 71–81
- Mesjasz-Przybyłowicz, J., W.J. Przybyłowicz, V.M. Prozesky and C.A. Pineda, 1997. Quantitative micro-PIXE comparison of elemental distribution in Ni-hyperaccumulating and non-accumulating genotypes of *Senecio coronatus*. *Nucl. Instr. Meth. Phys. Res. Section B: Beam Interact. Mat. Atoms*, 130: 369–373
- Mi, L., A.J. Di Pasqua and F.L. Chung, 2011. Proteins as binding targets of isothiocyanates in cancer prevention. *Carcinogenesis*, 32: 1405–1413

- Mithöfer, A. and W. Boland, 2012. Plant defense against herbivores: chemical aspects. *Annu. Rev. Plant Biol.*, 63: 431–450
- Müller, R., M. de Vos, J.Y. Sun, I.E. Sønderby, B.A. Halkier, U. Wittstock and G. Jander, 2010. Differential effects of indole and aliphatic glucosinolates on lepidopteran herbivores. *J. Chem. Ecol.*, 36: 905–913
- Newton, E.L., J.M. Bullock and D.J. Hodgson, 2009. Glucosinolate polymorphism in wild cabbage (*Brassica oleracea*) influences the structure of herbivore communities. *Oecologia*, 160: 63–76
- Opitz, S.E.W. and C. Müller, 2009. Plant chemistry and insect sequestration. *Chemoecology*, 19: 117–154
- Opitz, S.E.W., A. Mix, I.B. Winde and C. Müller, 2011. Desulfation followed by sulfation: metabolism of benzyl glucosinolate in *Athalia rosae* (Hymenoptera: Tenthredinidae). *Chembiochem*, 12: 1252–1257
- Pare, P.W. and J.H. Tumlinson, 1999. Plant volatiles as a defense against insect herbivores. *Plant Physiol.*, 121: 325–332
- Pentzold, S., M. Zagrobelny, N. Bjarnholt, J. Kroymann, H. Vogel, C.E. Olsen, B.L. Møller and S. Bak, 2015. Metabolism, excretion, and avoidance of cyanogenic glucosides in insects with different feeding specialisations. *Insect Biochem. Mol. Biol.*, 66: 119–128
- Pentzold, S., M. Zagrobelny, F. Rook and S. Bak, 2014. How insects overcome two-component plant chemical defence: plant β -glucosidases as the main target for herbivore adaptation. *Biol. Rev.*, 89: 531–551
- Perkins, L.E., B.W. Cribb, P.B. Brewer, J. Hanan, M. Grant, M. de Torres and M.P. Zalucki, 2013. Generalist insects behave in a jasmonate-dependent manner on their host plants, leaving induced areas quickly and staying longer on distant parts. *Proc. Royal Soc. London B: Biol. Sci.*, 280: 20122646
- Poelman, E., C. Broekgaarden, J. Van Loon and M. Dicke, 2008. Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. *Mol. Ecol.*, 17: 3352–3365
- Pollard, A.J. and A.J.M. Baker, 1997. Deterrence of herbivory by zinc hyperaccumulation in *Thlaspi caerulescens* (Brassicaceae). *New Phytol.*, 135: 655–658
- Rascio, N. and F. Navari-Izzo, 2011. Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? *Plant Sci.*, 180: 169–181
- Rathinasabapathi, B., M. Rangasamy, J. Froeba, R.H. Cherry, H.J. Mcauslane, J.L. Capinera, M. Srivastava and L.Q. Ma, 2007. Arsenic hyperaccumulation in the Chinese brake fern (*Pteris vittata*) deters grasshopper (*Schistocerca americana*) herbivory. *New Phytol.*, 175: 363–369
- Ratzka, A., H. Vogel, D.J. Kliebenstein, T. Mitchell-Olds and J. Kroymann, 2002. Disarming the mustard oil bomb. *Proc. Natl. Acad. Sci. USA*, 99: 11223–11228
- Reymond, P., H. Weber, M. Damond and E.E. Farmer, 2000. Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. *Plant Cell*, 12: 707–719
- Robertson, G.F., M.P. Zalucki and T.D. Paine, 2015. Larval host choice of the monarch butterfly (*Danaus plexippus* L.) on four native California desert milkweed species. *J. Insect Behav.*, 28: 582–592
- Rohloff, J. and A.M. Bones, 2005. Volatile profiling of *Arabidopsis thaliana* putative olfactory compounds in plant communication. *Phytochemistry*, 66: 1941–1955
- Samarawickrama, G.P., 1979. Biological effects of cadmium in mammals. In: *The Chemistry, Biochemistry, and Biology of Cadmium*, pp: 341–421. Webb, M. (ed.) Elsevier, Amsterdam, The Netherlands
- Scheirs, J., I. Vandevyvere, K. Wollaert, R. Blust and L. De Bruyn, 2006. Plant-mediated effects of heavy metal pollution on host choice of a grass miner. *Environ. Pollut.*, 143: 138–145
- Schramm, K., D.G. Vassão, M. Reichelt, J. Gershenzon and U. Wittstock, 2012. Metabolism of glucosinolate-derived isothiocyanates to glutathione conjugates in generalist lepidopteran herbivores. *Insect Biochem. Mol. Biol.*, 42: 174–182
- Simpson, S.J. and D. Raubenheimer, 1993. The central role of the hemolymph in the regulation of nutrient intake in insects. *Physiol. Entomol.*, 18: 395–403
- Stolpe, C. and C. Müller, 2016. Effects of single and combined heavy metals and their chelators on aphid performance and preferences. *Environ. Toxicol. Chem.*, 35: 3023–3030
- Stolpe, C., K. Ute and M. Caroline, 2017. Heavy metal (hyper) accumulation in leaves of *Arabidopsis halleri* is accompanied by a reduced performance of herbivores and shifts in leaf glucosinolate and element concentrations. *Environ. Exp. Bot.*, 133: 78–86
- Stoyenhoff, J.L., J.A. Witter and M.E. Montgomery, 1994. Effects of host switching on gypsy moth (*Lymantria dispar* L.) under field conditions. *Oecologia*, 97: 143–157
- Strauss, S.Y., R.E. Irwin and V.M. Lambrix, 2004. Optimal defence theory and flower petal colour predict variation in the secondary chemistry of wild radish. *J. Ecol.*, 92: 132–141
- Textor, S. and J. Gershenzon, 2009. Herbivore induction of the glucosinolate-myrosinase defense system: major trends, biochemical bases and ecological significance. *Phytochem. Rev.*, 8: 149–170
- Travers-Martin, N. and C. Muller, 2007. Specificity of induction responses in *Sinapis alba* L. and their effects on a specialist herbivore. *J. Chem. Ecol.*, 33: 1582–1597
- Traw, B.M. and T.E. Dawson, 2002. Reduced performance of two specialist herbivores (Lepidoptera: Pieridae, Coleoptera: Chrysomelidae) on new leaves of damaged black mustard plants. *Environ. Entomol.*, 31: 714–722
- Vandenbulcke, F., C. Grelle, M.C. Fabre and M. Descamps, 1998. Implication of the midgut of the centipede *Lithobius forficatus* in the heavy metal detoxification process. *Ecotoxicol. Environ. Saf.*, 41: 258–268
- Vázquez, M.D., C. Poschenrieder, J. Barceló, A.J.M. Baker, P. Hatton and G.H. Cope, 1994. Compartmentation of zinc in roots and leaves of the zinc hyperaccumulator, *Thlaspi caerulescens* J & C Presl. *Bot. Acta*, 107: 243–250
- Vergara, F., A. Svatoš, B. Schneider, M. Reichelt, J. Gershenzon and U. Wittstock, 2006. Glycine conjugates in a lepidopteran insect herbivore—the metabolism of benzylglucosinolate in the cabbage white butterfly, *Pieris rapae*. *Chembiochem*, 7: 1982–1989
- Wadleigh, R.W. and S.J. Yu, 1988. Detoxification of isothiocyanate allelochemicals by glutathione transferase in three lepidopterous species. *J. Chem. Ecol.*, 14: 1279–1288
- Walling, L.L., 2008. Avoiding effective defences: strategies employed by phloem-feeding insects. *Plant Physiol.*, 146: 859–866
- Winde, I. and U. Wittstock, 2011. Insect herbivore counter adaptations to the plant glucosinolate–myrosinase system. *Phytochemistry*, 72: 1566–1575
- Wittstock, U. and M. Burow, 2010. *Glucosinolate Breakdown in Arabidopsis: Mechanism, Regulation and Biological Significance*, p: 134. The Arabidopsis Book 8
- Wittstock, U., N. Agerbirk, E.J. Stauber, C.E. Olsen, M. Hippler, T. Mitchell-Olds, J. Gershenzon and H. Vogel, 2004. Successful herbivore attack due to metabolic diversion of a plant chemical defense. *Proc. Natl. Acad. Sci. USA*, 101: 4859–4864
- Wittstock, U., N. Agerbirk, E.J. Stauber, C.E. Olsen, M. Hippler, T. Mitchell-Olds, J. Gershenzon and H. Vogel, 2003. Successful herbivore attack due to metabolic diversion of a plant chemical defense. *Acad. Sci. USA*, 101: 4859–4864
- Xiao, Z., L.X. Mi, F.L. Chung and T.D. Veenstra, 2012. Proteomic analysis of covalent modifications of tubulins by Iothiocyanates. *J. Nutr.*, 142: 1377–1381
- Ye, X.D., Y.L. Su, Q.Y. Zhao, W.Q. Xia, S.S. Liu and X.W. Wang, 2014. Transcriptomic analyses reveal the adaptive features and biological differences of guts from two invasive whitefly species. *BMC Genom.*, 15: 370
- Yu, S.J., 1987. Microsomal oxidation of allelochemicals in generalist (*Spodoptera frugiperda*) and semi specialist (*Anticarsia gemmatilis*) insect. *J. Chem. Ecol.*, 13: 423–436
- Yuan, H., F. Qin, W. Guo, H. Gu and A. Shao, 2016. Oxidative stress and spermatogenesis suppression in the testis of cadmium-treated *Bombyx mori* larvae. *Environ. Sci. Pollut. Res.*, 23: 5763–5770
- Zhang, Y.P., D.N. Song, H.H.W. Yang HM, J.Z. Zhang, L.J. Li, E.B. Ma and Y.P. Guo, 2014. Effect of dietary cadmium on the activity of glutathione S-transferase and carboxylesterase in different developmental stages of the *Oxya chinensis* (Orthoptera: Acridoidea). *Environ. Entomol.*, 43: 171–177

(Received 15 August 2017; Accepted 29 December 2017)