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

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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 product 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 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.

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Recently many researchers 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 defense 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 Martens, 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, epiphenonitrioles 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 mouthparts. Opposite to this behaviour, Travers and Müller (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 Rausher, 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 oxiridases 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-Fillho, 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 trichrome (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-defense 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. Infact, 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 defend 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) 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 strategies 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*
**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 (Kawahishi 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 for 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 oleracea (cabbage) plants also reduced oviposition by two crucifer specialists, Pieris rapae and P. brassicae (Brunisma 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).
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Table 1: Chemical and common names of glucosinolates identified in higher plants

<table>
<thead>
<tr>
<th>Chemical names</th>
<th>Common names</th>
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<tbody>
<tr>
<td>3-Butenyl</td>
<td>Gluconapin</td>
</tr>
<tr>
<td>4-Hydroxybenzyl</td>
<td>[Gluco] sinalbin</td>
</tr>
<tr>
<td>2(R)-2-Hydroxy-3-butenyl</td>
<td>Progoitrin</td>
</tr>
<tr>
<td>2(Si)-2-Hydroxy-3-butenyl</td>
<td>Epiprogoitrin</td>
</tr>
<tr>
<td>2-Hydroxy-4-pentenyl</td>
<td>[Gluco] nopoliferin</td>
</tr>
<tr>
<td>Indol-3-ylmethyl</td>
<td>Glucobrassicin</td>
</tr>
<tr>
<td>1-Methoxyindol-3-ylmethyl</td>
<td>Neoglucobrassicin</td>
</tr>
<tr>
<td>1-Methylthyl</td>
<td>Isopropyl</td>
</tr>
<tr>
<td>1-Methylpropyl</td>
<td>Glucococharain</td>
</tr>
<tr>
<td>4-Methylsulfanyl-3-butenyl</td>
<td>Glucoraphanin</td>
</tr>
<tr>
<td>4-(Methylsulfanyl) butyl</td>
<td>Glucoraphanin</td>
</tr>
<tr>
<td>5-(Methylsulfanyl) pentyl</td>
<td>Glucosinapin</td>
</tr>
<tr>
<td>3-(Methylsulfanyl) propyl</td>
<td>Glucoiberin</td>
</tr>
<tr>
<td>4-(Methylthio) butyl</td>
<td>Glucosinapin</td>
</tr>
<tr>
<td>5-(Methylthio) pentyl</td>
<td>Glucobrassicinapin</td>
</tr>
<tr>
<td>3-(Methylthio) propyl</td>
<td>Glucobrassicinapin</td>
</tr>
<tr>
<td>1-Pentenyl</td>
<td>Neoglucobrassicinapin</td>
</tr>
<tr>
<td>4-Pentenyl</td>
<td>Neoglucobrassicinapin</td>
</tr>
<tr>
<td>2-Phenethyl</td>
<td>Phenethyl</td>
</tr>
<tr>
<td>2-Propanol*</td>
<td>Allyl Sinigrin</td>
</tr>
</tbody>
</table>

*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 aglucine which rearranges in to different products such as nitrile, isothiocyanates (modified from Jeschke et al., 2016)

After tissue damage, GS are induced and reach the climax within a certain time period and infact, 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 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 defence than generalists, which is perhaps evolutionary adaptation to plant chemicals where mechanisms for detoxification, sequestration or excretion are developing (Opitz and Müller, 2009; Winde 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 xyllostela 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 (Bartlet 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-Przybylowicz 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 vary from species to species. Many lepidopterans 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 in to 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-hydroxyphenylacetonitrile) 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 defense 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 defense 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 sulfatease 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 whitely 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 acetamide 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 chewsers, phloem feeders (Aphididae) circumvent the GS-MS defense system in a very different way. These insects penetrate their piercing-sucking mouthparts (styles) between the epidermal and parenchymal cells to reach the sap thus causing minimal damage (Walling, 2008). Penetration of styli through the apoplast keep 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.
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Table 2: Published material supportive of adaptation strategies of insect herbivores to counter the heavy metal based defenses and toxicity

<table>
<thead>
<tr>
<th>Herbivore</th>
<th>Heavy metal</th>
<th>Detoxification strategy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gypsy moth (Lymnaea dispar L.)</td>
<td>Ni</td>
<td>Host switching during early larval instars development. Such instars exhibit better ability to feed on less favorable food.</td>
<td>Stoyenhoff et al. (1994)</td>
</tr>
<tr>
<td>Centipedes (Lithobius Coll. Pb forficatus)</td>
<td>Ni</td>
<td>Permanent storage of metals in the granules of midgut epithelial cells. Midgut epithelium also acts as a barrier to stop excessive storage of few metal.</td>
<td>Vandenbulcke et al. (1998)</td>
</tr>
<tr>
<td>Green peach aphid Ni (Myzus persicae)</td>
<td>Ni</td>
<td>Insert stylers, 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.</td>
<td>Davis et al. (2001)</td>
</tr>
<tr>
<td>Cabbage butterfly Zn (Pieris brassicae)</td>
<td>Zn</td>
<td>Complete rejection of feeding on high Zn concentration containing N. caerulescens foliage.</td>
<td>Pollard and Baker (1997)</td>
</tr>
<tr>
<td>White butterfly (Pieris Ni rapae)</td>
<td>Ni</td>
<td>Limit Ni toxicity by consuming a mixed diet of high- and Low-Ni foliage.</td>
<td>Boyd et al. (1994)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Insect herbivore</th>
<th>Feeding Mode</th>
<th>Detoxification strategy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphid (Myzus persicae)</td>
<td>Piercing/Sucking</td>
<td>Avoeds cell disruption/Rapid excretion/ repression by secreted effectors.</td>
<td>Kim et al. (2008); Elzinga et al. (2014)</td>
</tr>
<tr>
<td>Turnip Sawfly (Athalia rosae)</td>
<td>Chewing</td>
<td>Sequestration of intact GS and accumulation in hemolymph.</td>
<td>Optitz et al. (2011); Wittstock et al. (2004).</td>
</tr>
<tr>
<td>Cabbage white butterfly (Pieris rapae)</td>
<td>Chewing</td>
<td>Redirection; Nitrile specifier protein mediates the conversion of GS to less toxic compounds (nitrile) instead of isothiocyanates.</td>
<td>Malka et al. (2016); Ratzka et al. (2002).</td>
</tr>
<tr>
<td>Silver leaf whitely (Bermsia tabaci)</td>
<td>Styles/sucking</td>
<td>Desulfation; Cleaves the sulfate from GS molecule before GS hydrolysis.</td>
<td></td>
</tr>
<tr>
<td>Diamondback moth (Plutella xylostella)</td>
<td>Chewing/lower surface of leaf</td>
<td>Rapid metabolism of GS; Conversion of GS to daeulisocinolates and excretion with feces.</td>
<td></td>
</tr>
<tr>
<td>Cabbage looper (Trichoplusia ni)</td>
<td>Chewing</td>
<td>Conjugation: detoxification of isothiocyanates by conjugating with glutathione.</td>
<td></td>
</tr>
<tr>
<td>Foxglove aphid/Audacorhithol solani</td>
<td>Sap sucking</td>
<td>Detoxify by conjugating with pyranate (artificial diet experiment).</td>
<td>Francis et al. (2005).</td>
</tr>
<tr>
<td>Cabbage bug (Margantia histrionica)</td>
<td>Sucking</td>
<td>Sequestration of intact GS.</td>
<td>Aliabadi et al. (2002).</td>
</tr>
<tr>
<td>Cabbage aphid (Brevicoryne brassicae)</td>
<td>Sucking</td>
<td>Ingestion of intact GS and active sequestration of them from the phloem sap through the gut wall.</td>
<td>Wunde and Wittstock (2011).</td>
</tr>
<tr>
<td>Turnip or mustard aphid (Lipaphis Sucking erysiniti)</td>
<td>Chewing</td>
<td>Sequestration of intact GS and contains own myrosinase in the gut, uses Bridges et al. (2002). sequestered GS and own myrosinase for self defence.</td>
<td></td>
</tr>
<tr>
<td>Desert locust (Schistocerca gregaria)</td>
<td>Chewing</td>
<td>Desulfation; An inductive gut sulfatase activity on glucosinolates to cleave Falk and Gershenson the sulfate from GS. (2007).</td>
<td></td>
</tr>
<tr>
<td>Zygaena filipendulae</td>
<td>Leaf-snipping</td>
<td>Leaf-snipping and minimal tissue damage to avoid substrate-enzyme interaction or shortening Pentzold et al. (2015).</td>
<td></td>
</tr>
<tr>
<td>White butterfly (Pieris Ni rapae)</td>
<td>Chewing</td>
<td>Intermediate products of GS conversion process form conjugates with Vergara et al. (2006)</td>
<td></td>
</tr>
</tbody>
</table>

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 snapping 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 adaption.

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, effectiveness of these defenses 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 defenses. These counteradaptations 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 (isothiocyanates to nitrile for example) 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 defense 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 into the co-evolution of insect herbivores and plants.

References


Boyd, R.S. and S.N. Martens, 2000. Aphids are unaffected by the elemental defense of the nickel hyperaccumulator, Streptanthus polygaloides (Brassicaceae). Chemocology, 9: 1-7
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
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