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## Sequestration as defense mechanism in insects: A review

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### Abstract

Herbivorous insects commonly use sequestration of chemical defences from host plants to avoid predation. The ability to create and handle bioactive chemicals is a critical component in the co-evolution of plants and insects. Plants create bioactive natural chemicals to defend themselves; however some insects detoxify and/or sequester the substances, allowing new niches with fewer competitors to emerge. The cyanogenic glucosides, which are found in over 2500 plant species and are thought to play a significant role in plant defence against herbivores due to their bitter taste and release of lethal hydrogen cyanide upon tissue damage, are one of the most important bioactive components.

**Keywords:** Detoxify, predation, sequestration, cyanogenic glucosides and defence

### 1. Introduction

More than 250 insect species, including beetles (*Termonia et al.*, 2001)<sup>[91]</sup>, true bugs (Duffey and Scudder, 1972; Scudder and Duffey, 2011)<sup>[26, 82]</sup>, and sawflies (Björkman and Larsson 1991)<sup>[7]</sup>, have been found to sequester plant compounds into animal tissues for defence (Duffey, 1977; Opitz and Müller, 2009)<sup>[27, 70]</sup>. Sequestration specific, selective uptake and accumulation of plant toxins. Selective uptake, transport, modification, storage and deployment of plant secondary chemicals in insects for own defence. Toxins typically connects the first tropic level (plants) via second tropic level (insect herbivore) to the third tropic levels (predators and parasitoids). Many sequestering species have evolved to deal with plant defences (Opitz and Müller, 2009)<sup>[70]</sup>, but they aren't always immune to these toxins (Adler *et al.*, 1995; Björkman and Larsson, 1991; Dimarco and Fordyce, 2017; Mason and Bowers, 2017; Smilanich *et al.*, 2009; Zalucki *et al.*, 2001; Zvereva and Kozlov, 2016; Zvereva *et al.*, 2017)<sup>[3, 7, 25, 61, 86, 98, 99, 100]</sup>. Coleoptera and Lepidoptera are the most common hosts, but Heteroptera, Hymenoptera, Orthoptera, and Sternorrhyncha are also frequent hosts (Opitz and Müller, 2009)<sup>[70]</sup>. Averting detection, avoiding attack, and tricking predators are just a few of the many defensive techniques (Ruxton *et al.*, 2004)<sup>[81]</sup>. Selectivity of uptake can be realised by one (or) by a combination of many mechanisms. Plant poisons can be taken up and stored by a variety of insect herbivores as a form of self-defence against their natural enemies. These compounds are efficient against a wide range of predators, including invertebrates such as spiders and ants, as well as vertebrates such as birds and mammals (Trigo, 2000; Nishida, 2002; Opitz and Müller, 2009; Trigo, 2011)<sup>[93, 67, 70, 92]</sup>. Tolerance methods that maintain plant poisons functioning are closely tied to toxin sequestration. The herbivore is thought to be able to manage the spatiotemporal dynamics of toxin buildup by using specific transporters. This pattern has also been observed in the adults of the nymphalid butterfly *Euphydryas phaeton*, which acquired defensive iridoid glycosides as larvae from two different host plants (Bowers, 1980; Bowers, 1988)<sup>[9, 8]</sup>, and the adults of the heliconiine butterfly *Heliconius erato*, whose larvae feed on four different *Passiflora* species (Hay-Roe and Nation, 2007)<sup>[43]</sup>. Specific enzymes are also present in some herbivores, which help to increase the bioactivity of the toxins that have been sequestered. Plants in various groups (e.g., Asteraceae, Boraginaceae, and Leguminosae) produce these chemicals, which are sequestered by specialised grasshoppers, hemipterans, beetles, moths, and butterflies (Trigo, 2011; Macel, 2011; Eisner and Meinwald, 1995)<sup>[92, 56, 28]</sup>, where they have defensive and sexual communication roles. PAs are always present in N-oxide form in specialist insects (Hartman and Ober, 2008)<sup>[38]</sup>. Plant toxin sequestration has been investigated by ecologists for decades. The recently discovered molecular pathways, when combined with transient, non-transgenic

approaches to regulate insect gene expression, will aid in understanding the role of toxin sequestration in natural food web dynamics. *Utetheisa ornatrix*, an arctiine rattlebox moth, is one of the most studied species in terms of its ecological dependence on PAs (Trigo, 2011; Eisner and Meinwald, 1995; Conner and Jordan, 2009; Eisner *et al.*, 2002; Conner and Iyengar, 2015) [92, 28, 19, 29, 18]. *U. ornatrix* can be found in the Neotropics and warm Nearctic regions (Da Costa, 2010) [21], where it eats a variety of *Crotalaria* legume species (Sourakov, 2015) [87]. These alkaloids are sequestered by *U. ornatrix* larvae and passed on to pupae and adults. Males pass PAs to females during mating, who then pass them on to the eggs. As a result, PAs defend all life stages of *U. ornatrix* from predators (Eisner and Meinwald, 1995) [28]. Sequestration of cardenolides has been investigated in terms of host plant chemistry (Brower and Moffitt, 1974; Brower *et al.*, 1982; Martin *et al.*, 1992) [11, 12, 59], compound polarity (Malcolm *et al.*, 1989; Nelson 1993; Roeske *et al.*, 1976) [57, 65, 79], toxicity to vertebrate predators (de Roode *et al.*, 2008, Gowler *et al.*, 2015; Sternberg *et al.*, 2012) [23, 34, 88]. Different cardenolide compounds have been proven to be poisonous to

monarch butterflies as well as vertebrates (Rasmann *et al.*, 2009; Petschenka *et al.*, 2018; Seiber *et al.*, 1983) [76, 74, 83], and possibly parasites (Sternberg *et al.*, 2012) [88].

### 1.1. Sequestered compounds include various

- Aromatic compounds
- Nitrogen-containing metabolites
  - Alkaloids, 1.1.2.2. Cyanogenic glycosides and
  - Glucosinolates

### 1.2 Isoprenoids

- Cardiac glycosides, 1.1.3.2. Cucurbitacins and
- Iridoid glycosides.

### 1.3 Plant families which are sequestering of toxic compounds in has been investigated

Aristolochiaceae, Asteraceae, Fabaceae, Apocynaceae and Plantaginaceae. Sequestration Coleoptera and Lepidoptera (predominant). Heteroptera, Hymenoptera, Orthoptera and Sternorrhyncha (frequently) (Nishida, 2002) [67].

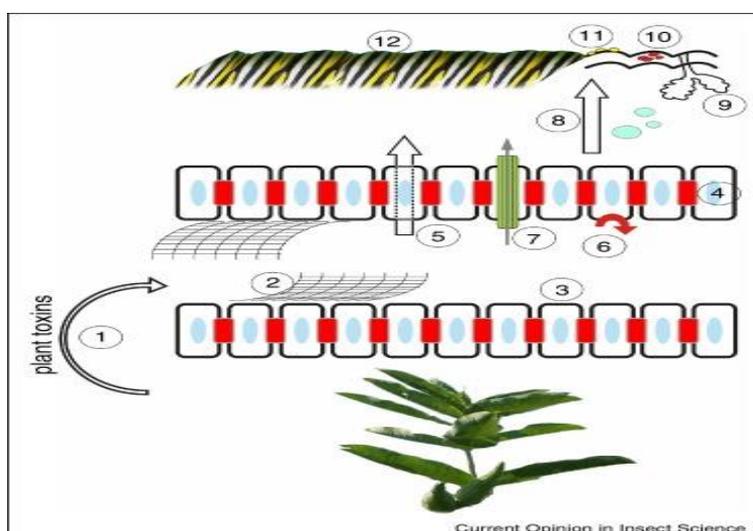
**Table 1.1:** Plant allelochemicals sequestered by Lepidoptera/others, and their examples

Sl. No.	Chemical Class of Sequestrates	Representative Sequestereres	Examples
<b>A. Terpenic Metabolites</b>			
1.	Cardenolides (CGS)	Danainae, Arctiidae, Ctenuchidae	Milkweed butterflies, Tiger moths, <i>Ctenucha virginica</i>
2.	Grayanoides (GTS)	Geometridae	Loopers
3.	Iridoide Glycosides (IGS)	Nymphalidae, Geometridae, Sphingidae	Brush-footed butterflies, Loopers, Hawk moth
<b>B. Nitrogen Containing Metabolites</b>			
1.	Pyrrolizidine alkaloids (PAs)	Danainae, Arctiidae	Milkweed butterflies, Tiger moths
2.	Quinolizidine alkaloids (QAs)	Pyralidae	Snout moth
3.	Cyanogenic glycosides (CNS)	Zygaenidae	Burnet moth

**Table 1.2:** Compound investigated in insects

Compound	Insects	References
Benzoxazinoid glucosides	Western corn rootworm, <i>Diabrotica virgifera virgifera</i>	Robert <i>et al.</i> , 2017 [77]
Cardenolides (CGS)	Milkweed butterflies, Tiger moths, <i>Ctenucha virginica</i>	Nishida, 2002 [67] Matthew <i>et al.</i> , 2012 [62]
Pyrrolizidine alkaloids (PAs)	Milkweed butterflies, Tiger moths	Nishida, 2002 [67]
Cyclopentenyl cyanogens	Lepidopterans	Hagg <i>et al.</i> , 2014 [37]; Lechtenberg, 2011 [54]
$\beta$ -cyanoalanine	<i>Clossiana euphrosyne</i>	Kebeish <i>et al.</i> , 2015 [51]
Cyclopentenyl glycine derived CNGs	Euptopia hegesia	Szewczyk and Zidorn, 2014 [90]

## 2. Sequestration mechanism in insects (Physiological mechanism involved in sequestration) (Petschenka and Agrawal, 2016) [73].



**Fig 2.1:** Physiological mechanism involved in sequestration

1. Plant toxins are injected.
2. Contact the peritropic membrane. The peritropic envelope may be involved in the process of sequestration as plant toxins could bind to the envelope and toxins could be retained in the gut.
3. Sequestration may be modulated by active and passive barrier functions as well as metabolism by degrading enzymes.
4. Polar toxins cannot be sequestered passively as paracellular diffusion across the mid gut epithelium and sequestration may be prohibited.
5. Non-polar (lipophilic) compounds can be sequestered passively as they can cross cell membrane.
6. Proteins like efflux carriers can mediate an active barrier to prevent toxins from reaching the body cavity.
7. One possibility for how polar toxins can be sequestered via specific carrier proteins. Within the haemolymph, Sequestered plant metabolites can be metabolized.
8. Potential binding to haemolymph proteins. Some toxins are transported across the haemolymph.
9. In to glands
10. Reservoirs (or) in to the integument were they are stored.
11. Exposed to predators in the form of droplets.
12. Protection mediated by sequestered plant toxins is often displayed by aposematic colouration.

### 3. Sequestration of secondary metabolites by insects and effects on higher trophic levels

#### 3.1 Cardenolides

Many of the plant (Apocynaceae: Milk weeds) produce toxic chemicals Cardiac glycosides (or) Cardenolides that help to deter herbivore. These compounds specifically bind and inhibit the sodium and potassium pumps found in heart muscle cells. In susceptible insects Cardenolides bind to the  $\alpha$  subunits of Na<sup>+</sup>, K<sup>+</sup> ATPase inhibiting the function ultimately affecting many important physiological process, including muscle contraction, neural function and ion transport. Without working pumps sodium levels in the cell rise, setting of a chain of events that ultimately disrupts the muscle contraction in the cardiac tissue. At high enough dose, can be lethal to insects. Feeding upon milkweed plants (Asclepiadaceae) as larvae, the butterflies sequester a series of cardenolides (found predominantly as cardiac glycosides, CGs) that render the adult unpalatable to predators. These toxins are degraded by inducible CYPs (P 450s) that are expressed in the midgut and also in the fat body. CGs are cardio-active steroids causing toxic effects in various vertebrates. Predatory birds avoid the butterflies primarily because they experience an obnoxious bitter taste and/or emesis after ingestion (Matthew *et al.*, 2012). While the *D. plexippus*-NaC/KC-ATPase is consistently more resistant than the non-adapted insect NaC/KC-ATPases of *E. core* and *S. gregaria*, 11 of the 16 cardenolides examined were more effective than ouabain against its NaC/KC-ATPase. Calactin and calotropin, which are preferentially sequestered by monarchs from *Asclepias* spp. (Cheung *et al.*, 1988; Groeneveld *et al.*, 1990) <sup>[16, 35]</sup>, were the most poisonous cardenolides, inhibiting *D. plexippus*-NaC/KC-ATPase up to 60 times more effectively than ouabain (Cheung *et al.*, 1988; Groeneveld *et al.*, 1990) <sup>[16, 35]</sup>. Monarch caterpillars sequester large amounts of calactin and calotropin, as well as obtaining these substances enzymatically from other milkweed cardenolides like uscharidin (Seiber *et al.*, 1980) <sup>[84]</sup>. Furthermore, monarchs appear to only sequester cardenolides

with a specific polarity range, and chemicals like calactin and calotropin may be easier to retain in bodily tissues than more lipophilic cardenolides (Roeske *et al.*, 1976) <sup>[79]</sup>. While non-polar cardenolides are more easily absorbed via the gut, polar cardenolides appear to elicit emesis in vertebrates at lower doses, according to Duffey, (1977) <sup>[27]</sup>. Cardenolide glycosides have been demonstrated to be more hazardous than equivalent genins in whole organism vertebrate experiments in the past (Hoch, 1961) <sup>[44]</sup>. Sugar residues in cardiac glycosides have been shown to stabilise the NaC/KC-ATPase inhibitor complex by interactions of the sugar molecule's hydroxyl groups with proton-donating as well as proton-accepting groups on the NaC/KC-ATPase (Yoda, 1973) <sup>[96]</sup>, and sugars can prevent the enzyme from reactivating after cardiac glycoside inhibition (Yoda, 1973; Cornelius *et al.*, 2013) <sup>[96, 20]</sup>. Furthermore, the sugar moiety is required for cardiac glycosides to be fixed to the heart muscle (Hoch, 1961) <sup>[44]</sup>. Sugars' influence on any given NaC/KC-ATPase appears to be dependent on the number and chemical identity of the sugar molecules in general (Farr *et al.*, 2002) <sup>[30]</sup>. Responses to sugar removal in the vertebrate NaC/KC-ATPase have been reported to diminish or increase inhibition strength (O'Brien *et al.*, 1993; Farr *et al.*, 2002; Paula *et al.*, 2005; Cornelius *et al.*, 2013) <sup>[69, 30, 71, 20]</sup>.

The peculiar properties of sugars found in cardiac glycosides suggest that they have an adaptive value for plants, but the processes of natural selection have yet to be discovered. Apart from absorption, these sugars may contribute to heart glycoside hydrolytic stability, which could be an anti-predator tactic (e.g., prevention of hydrolysis in the animal gut). Cardenolides from the Asclepiadoideae (milkweed) family, which feature cyclic bridges, are particularly resistant to acid hydrolysis (Seiber *et al.*, 1983) <sup>[83]</sup> and should thus be given special attention in the future. Given the increased inhibition of NaC/KC-ATPase in glycosides compared to genins, we hypothesise that glycosylation of cardiac glycoside genins may result in metabolic activation rather than detoxification. Milkweed cardenolides is the trans-bent junction of rings A and B in the steroid, which is cis-bent in Digitalis and other medicinally significant cardenolides (Seiber *et al.*, 1983; Malcolm, 1991) <sup>[83, 58]</sup>. Based on their toxicity (Brown and Thomas, 1984) <sup>[13]</sup>, these trans-bent 5 $\alpha$ -cardiac glycosides are thought to have a poor affinity for mammalian NaC/KC-ATPase and have a low biological efficacy (Paula *et al.*, 2005; Katz *et al.*, 2010) <sup>[71, 49]</sup>. The fact that uzarigenin has a significantly lower water solubility than digitoxigenin highlights the importance of the A/B ring junction arrangement for basic physicochemical features, and hence might readily alter milkweed cardenolide absorption and sequestration in herbivorous insects.

Dog bane beetle (*Chrysochus* spp) which are resistant to ouabain, a toxic cardenolide found in milk weed host plant (Despres *et al.*, 2007) <sup>[24]</sup>. Ouabain had a stronger inhibitory potency than ouabagenin on lamb-, shark-, and human NaC/KC-ATPase (Farr *et al.*, 2002; Paula *et al.*, 2005; Katz *et al.*, 2010; Cornelius *et al.*, 2013) <sup>[30, 71, 49, 20]</sup>, which was consistent with our data on insect NaC/KC-ATPase.

#### 3.1.1 Cardenolides sequestration in large milk weed bug: *Onchopeltus fasciatus*

Chemical defence in the large milk weed bug *Onchopeltus fasciatus* derives from maternal but not from paternal. Newcombe *et al.*, (2013) <sup>[66]</sup> investigated that weather transmission of Cardenolides from the host diet to the eggs in maternal,

paternal (or) biparental in the large milk weed bug *Oncopeltus fasciatus*. They reared individual bugs on either milkweed seeds [MW; *Asclepias syriaca* L. (Apocynaceae)] that contain Cardenolides, or on sunflower seeds [SF; *Helianthus annuus* L. (Asteraceae)] that do not contain cardenolides. They mated females and males so that all four maternal/paternal diet combinations were represented: MW/MW, MW/SF, SF/MW, and SF/SF. They conducted two-choice predation trials with green lace wing to assess whether maternal, paternal, or biparental transmission of cardenolides into the eggs of *O. fasciatus* increased protection against predation. They concluded that predation trials that a maternal diet of milkweed makes eggs more distasteful than a paternal diet of milkweed (Newcombe *et al.*, 2013) [66]

### 3.2 Pyrrolizidine alkaloids: (PAs)

Crotalaria (Leguminosae: Papilionoideae: Crotalariaeae) is a Pantropical weed genus that includes about 702 species (Polhill, 1982; le Roux *et al.*, 2013) [75, 53]. There are 31 native and 11 non-native species in the Neotropics, notably in Brazil (Flores, 2004) [32]. PAs are abundant in Crotalaria species (Roque-Albelo *et al.*, 2009) [80], with larger quantities in seeds than in leaves (Ferro *et al.*, 2006) [31]. Extrafloral nectaries (EFNs) are also present, which attract predatory ants and wasps (Ferro *et al.*, 2006; Guimarães *et al.*, 2006; Franco and Cogni, 2013; Pereira and Trigo, 2013) [31, 36, 33, 72]. PAs are toxic secondary metabolites found in wide variety of plant taxa, including *Senecio jacobae*, Eupatorium (Asteraceae), Crotalaria (Fabaceae), Parsonsia (Apocynaceae). Danaine butterfly, *Idea leuconoe*, specializing on *Parsonsia laevigata* (Apocynaceae). The larvae of this primitive Danaine acquire PAs and store them as N-oxides throughout all stages of their life. Males use volatile metabolites derived from one PA as a sex pheromone. Females use macrocyclic host PAs (*e.g.*, parsonsine) as specific oviposition cues (Nishida, 2002) [67]. The predatory neotropical orb-weaving spider *Nephila clavipes* (Nephilidae) weaves its web in forest clearings and corridors, which serve as insect flight lanes (Robinson and Mirick, 1971) [78]. PAs are responsible for *U. ornatix* and other arctiine moths' chemical defence (Trigo, 2011; Eisner and Meinwald, 1995; Conner and Jordan, 2009) [92, 28, 19], although a dose-dependent strategy has been less well described. As with other arctiines, the sequestration of unaltered PAs or the translation of plant PAs into insect PAs is varied (Hartmann *et al.*, 2004; Hartmann *et al.*, 2005; Hartmann *et al.*, 2004; Hartmann *et al.*, 2005) [39, 41, 40, 42].

### 3.3 Glucosinolates

The cabbage aphid is a damaging agricultural insect that feeds on plants in the Brassicales order, which includes crops like cabbages and mustards. They are found mainly in the family "Brassicaceae". The enzyme myrosinase, which is stored in specialised tissues. It converts the glucosinolates to the toxic isothiocyanates. Glucosinolates affects higher tropic levels, via reduced host (or) prey quality. Because specialist herbivores may sequester the glucosinolates for their own defence (Despres *et al.*, 2007) [24]. *Athalia rosea* (Turnip saw fly) sequesters ingested glucosinolates mainly in the hemolymph. *A. rosea* larvae easily bleed hemolymph containing the sequestered glucosinolate. A defensive response that offers them protection against the Ant (*Myrmica rubra*) (Hymenoptera: Formicidae) and the wasp (*Vespa vulgaris*) (Muller *et al.*, 2001) [63]. Harlequin bug, *Murgantia histrionica* (Heteroptera:

Pentatomidae) - also contains sequesters- bugs- rejected - two species of bird predators. Aphids are attacked by the predatory two-spotted lady beetle, *Adalia bipunctata*, sequestered glucosinolates and aphid myrosinase mix and volatile isothiocyanates and nitriles are released hydrolytically, conferring a toxic effect on the predator (Aliabadi *et al.*, 2002) [5]. Aphids *Brevicoryne brassicae* and *Lipaphis erysimi* special cases of sequestration of in that the concentration different types of glucosinolates 15- 20 times higher in their haemolymph (relative to the concentrations found in total leaf tissues) (Hopkins *et al.*, 2009) [45]. Although the mechanism governing the selective sequestration of specific glucosinolates is unknown, it is thought to include specific transporters such as the ABC transporters, which allow plant-derived molecules to traverse cellular membranes (Petschenka and Agrawal, 2016; Strauss *et al.*, 2013) [73, 89]. Specialist herbivores are more likely than generalist herbivores to sequester plant defence chemicals (Beran *et al.*, 2014; Bramer *et al.*, 2017; Kazana *et al.*, 2007; Müller *et al.*, 2001) [6, 10, 50, 63]. It's one of a number of adaptations made by Brassicales-feeding herbivores to help them colonise glucosinolate-rich host plants (Cao *et al.*, 2018; Cole, 1997; Louis *et al.*, 2012) [12, 17, 55]. These same glucosinolates, when activated by the insect, can function as defences against predators and parasitoids (Chaplin-Kramer *et al.*, 2011; Kos *et al.*, 2011) [15, 52]. The endogenous cabbage aphid myrosinase (BMY) allows the cabbage aphid to manufacture defensive ITCs from sequestered glucosinolates as long as the enzyme can take the sequestered glucosinolates as substrates (Jones *et al.*, 2002; Kazana *et al.*, 2007) [48, 50]. Because of their better interactions with enzyme binding sites, aliphatic glucosinolates may be favoured as substrates (Husebye *et al.*, 2005) [49].

### 3.4 Cyanogenic glycosides

Six spot Burnet moth *Zygaena filipendulae* are toxic, when they are injured, they released cyanide, highly poisonous chemical. Cyanide compound are found at some levels at each stage of the life cycle from egg to adult. The larvae sequester and store some cyanogenic compound from their food plants – Birds foot trefoil – *Lotus corniculatus*. Cyanogenic glucosides are one of plants' most common defensive compounds (Jensen *et al.*, 2011; Simon *et al.*, 2010) [47, 89]. These glucosides are degraded to a reactive hydroxynitrile, which releases lethal hydrogen cyanide when plant tissue is disrupted (Agerbirk and Olsen, 2012) [4]. Herbivorous arthropods duplicated an old horizontal transfer of a gene originally involved in Sulphur amino acid production in bacteria to detoxify plant-produced cyanide (Wybouw *et al.*, 2014) [95]. The occurrence of CNGs in animals appears to be restricted to a single phylum out of the currently known 31 Arthropod species, in contrast to the taxonomically extensive distribution of CNGs within the plant kingdom (Nahrstedt, 1996) [64].

#### 3.4.1 *Eutoptia hegesia* (Papilionoidea)

The Cyclopentenyl glycine derived (CNGs) from its host plant *Turnera ulmifolia* were hypothesized and sequestered by *Eutoptia hegesia* (Heliconiinae: Papilionoidea). The sequestering was proposed because of significantly higher CNG levels in larvae reared on cyanogenic plants compared to siblings reared on a cyanogenic plants (Zagrobelyny *et al.*, 2004) [97].

### 3.4.2 Cyanogenic glucosides in *Zygaenidae*

The poisonous amino acid -cyano-L-alanine has been discovered in 21 *Zygaenidae* species that synthesise and store the cyanoglucosides linamarin and lotaustralin. -cyanoalanine is also reported for the first time from other cyanogenic lepidoptera, such as the Acraeinae and Heliconiinae of the nymphalid family, as well as other Nymphalidae species that were previously unknown to be cyanogenic. For the first time, cyanogenesis is demonstrated in Heterogynidae, with the existence of -cyano-alanine and the cyanoglucosides linamarin and lotaustralin (Witthohn and Naumann, 2014) <sup>[94]</sup>. *Zygaena* species have long been known to be resistant to HCN, and they may, for example, survive for an hour in a concentrated HCN atmosphere and then swiftly recover when exposed to clean air (Noriega, 2014) <sup>[68]</sup>.

### 4. Conclusions

Among the enormous number of secondary metabolites with known toxic properties only few classes of chemicals have been selected as sequesterable elements by insects, ultimately selected by natural enemies through a food chain. The density of sequestered plant secondary metabolites may progress as follows: a) Produced by a plant, b) Ingested by insects without deterrent, c) Absorbed through the gut without elimination, d) Stored in the particular tissues without disturbing the sensitive tissues, e) Retained and Redistributed to the adults without major loss. Picked up by a bird and taste rejected (or) delivered to the stomach and then vomited (or) and causing other pharmacological affects. Male insects uses sequestered compound for production of sex pheromone. Female insects used for oviposition cues. Gaining unpalatability for an insect may not be a simple process, in that the process deals with consecutive selective factors, each of which may involve physiological and ecological costs.

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