For antagonists and mutualists: the paradox of insect toxic secondary metabolites in nectar and pollen

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Abstract

The plant kingdom produces an extraordinary diversity of secondary metabolites and the majority of the literature supports a defensive ecological role for them, particularly against invertebrate herbivores (antagonists). Plants also produce secondary compounds in floral nectar and pollen and these are often similar to those produced for defense against invertebrates elsewhere in the plant. This is largely because the chemical armoury within a single plant species is typically restricted to a few biochemical pathways and limited chemical products but how their occurrence in floral rewards is regulated to mediate both defence and enhanced pollination is not well understood. Several phytochemicals are reviewed here comparing the defensive function alongside their benefit to flower visiting mutualists. These include caffeine, aconitine, nicotine, thymol, linalool, lupanine and grayanotoxins comparing the evidence for their defensive function with their impacts on pollinators, their behaviour and well-being. Drivers of adaptation and the evolution of floral traits are discussed in the context of recent studies. Ultimately more research is required that helps determine the impacts of floral chemicals in free flying bees, and how compounds are metabolized, sequestered or excreted by flower feeding insects to understand how they may then affect the pollinators or their parasites. More work is also required on how plants regulate nectar and pollen chemistry to better understand how secondary metabolites and their defensive and pollinator supporting functions are controlled, evolve and adapt.

Key words

Introduction

Animals and plants show adaptive selection for physical traits that optimize the efficiency of pollen transfer. Typically, adaptations augment greater fidelity or attention from flower visitors to a plant species that optimises pollen transfer between conspecific flowers; typically referred to as specialization (Brosi 2016). One of the most frequently cited examples of pollinator specialization is a physical adaptation; the extraordinarily long nectar spur of the moon orchid, *Angraecum sesquipedale* (Orchidaceae)*.* This flower was predicted by Charles Darwin in 1862 to be pollinated by a moth with an equally long proboscis and 40 years later turned out to be the case with the discovery of Darwin's Sphinx moth *Xanthopan morgani praedicta* (Lepidotpera: Sphingidae) (Arditti et al. 2012). Numerous other examples exist where flower morphology has adapted to restrict nectar access to specialist taxa for example, *Aconitum* spp. (Ranunculaceae) (Thøstesen et al. 1996) and so garner greater pollinator focus and increase pollen transfer.

Phytochemicals also optimise pollination service in many plant species. For example, naïve honeybees show innate attraction to blue colours (Giurfa et al. 1995) which, in flowers, are produced by anthocyanins such as delphinidins (Katsumoto et al. 2007) or more stable metal chelated floral pigments such as commelinin from *Commelina communis* (Commelinacae) (Kondo et al. 1992). In some cases, the capacity to produce blue has driven highly specialised interactions based on mimicry and pseuodocopulation. The blue colour produced at the heart of the flower of *Ophrys speculum* (Orchidaceae) by cyanidin glycosides, predominantly the 3-*O*-(3''-*O*-malonylglucoside), is enhanced by surface structural features giving it a highly

reflective quality that is much closer to the thorax of the mimicked insect (Vignolini et al. 2012).

Flower colours may even change with age or after pollination to dissuade further visitation although this may be dependent upon environmental context and is not common (Ruxton and Schaefer 2016). Pollinators are proposed to be the main selective agent driving the evolution of flower colour (Wang et al. 2013) and in concert with insect pheromone mimicry, illustrate further the extraordinary capacity of plants to mediate the services pollinators and present irresistible attraction for sexually active males. A notable example of floral odour mimicry is the production of a variety of pyrazines by hammer orchids such as *Drakaea thynniphila* (Orchidaceae) which very closely mimic the sex pheromone of the female Thynnid wasps (*Agriomyia* spp.) (Hymenoptera: Thynnidae) and prove irresistible to sexually active males (Bohman et al. 2014). So clearly floral chemistry is understood to play a significant role in optimising pollination service.

Floral phytochemistry has seen increased interest in recent years particularly around the chemistry of nectar and pollen and in part stimulated by Adler (2000) and the contemporary scientific popularity of pollination systems as standard bearers for healthy ecosystems. The most comprehensive phytochemical survey of nectar and pollen to date was published recently, analysing 31 cultivated and wild plant species from multiple sites and of different cultivars and established that pollen typically had the highest concentrations of secondary metabolites (Palmer-Young et al. 2019a, b). Since these plant compounds were typically reported to be associated with defence this finding was consistent with optimal defence theory and the importance of pollen as the male gamete but the occurrence in nectar presents an ecological paradox since this is the reward for flower visitors (Stevenson et al.

2017). And furthermore many allelochemicals occurring in plants are toxic or deterrent to bees (Detzel and Wink 1993).

One of the earliest reviews of secondary metabolites in nectar had postulated a defensive function for these compounds and noted that many of the compound groups occurring in nectar were also deterrent or toxic. Why would a flower secrete a toxin into the nectar since this is the reward for flower visitors that transfer pollen from one flower to a conspecific so provide a service to the plant (Baker 1977)? Subsequent reviews (Adler 2000; Irwin et al. 2014; Stevenson et al. 2017) have synthesised the role of nectar toxins around their various bioactivities – many of which have been convincingly argued to optimise pollen transfer between conspecific flowers.   Palmer-Young et al. (2019a) reported that of over 100 compounds identified in pollen nectar and corolla tissue across the plant taxa, most were unique to a single species and that nectar and pollen chemistry always comprised of compounds that occurred in other plant tissues in the same species. Thus, if defence compounds occur in the foliage to protect against antagonists, they are likely to also occur in the nectar and pollen and be encountered by mutualists. While concentrations of secondary metabolites varied more in nectar than pollen the consistency with which they occur in nectar is nevertheless unexpected particularly since they are often cited as defensive against other invertebrates. Six examples are reviewed here that illustrate how plant secondary metabolites that have a reported defence function may have a dual role that is in conflict with its protective purpose. Existing knowledge gaps are also highlighted relevant to each compound.

Caffeine enhances bee memory and learning.

Caffeine is a purine alkaloid that has established toxicity to invertebrates and is considered a potential natural biopesticide (Hollingsworth 2002). Its toxicity in insects is manifest by paralysing and intoxicating insects through inhibition of phosphodiesterase activity and increasing the intracellular levels of cyclic AMP (Nathanson 1984). It also inhibits feeding in the tobacco armyworm (*Spodoptera litura*) (Lepidoptera: Noctuidae) demonstrated through taste assays using leaf discs from a genetically transformed *Nicotiana tabacum* that biosynthesised caffeine (Uefuji et al. 2005; Kim et al. 2006). Most familiarly caffeine occurs at very high concentrations in coffee beans to protect against insect damage although the coffee berry borer (*Hypothenemus hampei*) (Coleoptera: Curculionidae), the major insect pest of coffee beans, has overcome this mechanism of defence through a symbiosis with a *Pseudomonas* species of gut symbiont that detoxifies caffeine for the host (Ceja-Navarro et al. 2015).

The role of caffeine in plant defence against invertebrate herbivores is therefore apparent so its occurrence in nectar, the food reward for invertebrate pollinators, was unexpected (Kretschmar and Baumann 1999) especially in the knowledge that it is also toxic to honeybees (Detzel and Wink, 1993). Subsequent evaluation of the biological effect of caffeine against bees in learning and taste assays revealed more surprises. Firstly, caffeine is reportedly a feeding deterrent to honey bees in a dose dependent manner and it was detected in the nectar of several commercially important *Citrus* spp (Rutaceae) and *Coffea* spp. (Rubiaceae) (Wright et al. 2013). However, this was at concentrations that were below the bee's taste detection threshold. Secondly, bees fed caffeine at ecologically relevant concentrations during a learning experiment were three times more likely to recall a trait associated with a food reward than bees fed a control diet. This effect lasted for up to 72h and was manifest

through depolarisation of the membrane potential of Kenyon cells in the mushroom body of the bees brain where associative memory formation occurs indicating that this was a pharmacological effect acting on the brain directly (Menzel et al. 1980; Wright et al. 2013). Memory is an important attribute in generalist pollinators facilitating the rapid relocation of previously encountered and reliable sources of food (Menzel and Muller U 1996). Wright et al. (2013) postulated that this enhancement of memory would provide an evolutionary advantage to the plant by manifesting enhanced fidelity to a caffeine containing reward in free flying bees and increased recruitment of nest mates. Shortly after Couvillon et al. (2015) demonstrated this prediction to be the case. How plants manage this dual role in defence and maximising attraction is quantitative as demonstrated by Thomson et al. (2015) who reported that artificial flowers containing caffeinated nectar at 10^{-5} M received more pollen than flowers containing no caffeine or flowers containing artificial nectar with caffeine at 10^{-4} M. The behavioural change that enhances pollination is that caffeine enables bees to recall a trait or cue associated with food and be more likely to locate it, but this has yet to be demonstrated experimentally.

Ultimately it is conceivable that commercial bees utilised to maximise pollination services in horticulture could be trained to provision more efficient pollination services through exposure to the floral odours of target crops flowers while feeding on caffeinated food supplements; effectively training the bees to pollinate a target crop.

Caffeine occurs widely in the plant kingdom in at least 6 families of flowering plants. In floral tissues this includes *Chamelia sinensis* (Theaceae) (P. Stevenson unpubl.), *Coffea* spp. (Wright et al. 2013), *Citrus* spp. (Kretschmar and Baumann 1999), and *Tilia* spp. (Malvaceae) (Naef et al. 2007; Mathon et al. 2014). The flowers of these species are characterised by being small

white and generally indistinct but with a strong aroma so these species may employ odour as the primary tool for attraction. This may suit pollination services by flower visitors susceptible to the effects of caffeine with a similar mechanism across these families. Koch and Stevenson (2017) suggest that this mechanism of increasing floral focus through enhanced memory for the odour associated with the food reward is so effective in *Tilia* spp. it may explain the phenomenon of dead bees under these trees – bees that overvalue the food source and continue to forage long after the tree has stopped producing nectar and ultimately run out of energy and drop to the ground and die since the nectar of *Tilia* spp. is reported to contain caffeine (Naef et al. 2007; Mathon et al. 2014).

More recently caffeine is reported to be a potential antimicrobial that could reduce disease load in honeybees caused by *Nosema bombi* (Dissociodihaplophasida: Nosematidae) (Bernklau et al. 2019) thereby offering an additional benefit to the pollinators that provide this most important service to plants. Although Richardson et al. (2015) reported no effect against another parasite, *Crithidia bombi* (Trypanosomatida)*. The biological activity of nectar and pollen compounds against bee parasites is discussed in more detail below. The biological effects of related purine alkaloids including xanthine, theophylline and theobromine would be worthy of investigation.

Diterpenoid nectar toxins in *Rhododendron* filter specialist pollinators.

Rhododendron (Ericaceae) is a large Genus of >1000 species found across the Northern hemisphere and into the Maleysian Peninsula and islands (Chamberlain et al. 1996). In the British Isles Rhododendrons are popular ornamentals but one species, *R. ponticum* subsp. *baeticum,* introduced from the Iberian Peninsula, has become highly invasive along with

hybrids between *R. ponticum* and congenerics in parts of Britain (Milne and Abbott 2000).

Invasive species may contribute to pollinator decline (Gonzalez-Varo et al. 2013) although

considering the potential impacts only a few studies have investigated this (Stout and

Morales 2009). Grayanoid diterpenes are the compounds responsible for the various

biological activities attributable to mad honey and have been reviewed recently (Hanson

2016). Grayanoid diterpenes are tetracyclic diterpenoids with their biosynthetic origins in

the isoprenyl pathway and the ecological function of these compounds is reportedly in

defence. Rhodojaponin III, grayanotoxin III, and kalmanol were identified as the active

components in flowers of the Chinese insecticidal plant, *R. molle*, with the most abundant

compound rhodojaponin III, showing antifeedancy, growth inhibition and insecticidal

activity against larvae of two Lepidoptera (Klocke et al. 1991). However, only one study by

Scott-Brown et al. (2016) has reported grayanoid diterpenoids as a defense to an herbivore

that targets Rhododendron. Grayanotoxin 1 (GTX I) was recorded in higher concentrations

in young foliage of *R. simsii* and at a concentration that was biologically active as both a

deterrent and as an entomotoxin against the glass house thrips *Heliothrips haemorrhoidalis*

(Thysanoptera: Thripidae) (Scott-Brown et al. 2016). The occurrence of grayanotoxins in

nectar of *Rhododendron* is unexpected but has largely been assumed owing to its

occurrence in honey and was, only very recently, reported directly from the nectar

(Tiedeken et al. 2014). In this study GTX I was identified as the principal diterpene but did

not have a deterrent effect against *Bombus terrestris* (Hymenoptera: Apidae) the buff-tailed

bumble bee which is known to be a pollinator of the plant. However, later studies

investigating the impact of an invasive plant species with toxic nectar in the British Isles on

native fauna established that GTX I was both deterrent to and highly toxic to native honey

bees *Apis mellifera* subsp. *mellifera* (Hymenoptera: Apidae) and a solitary mining species of

bee *Andrena scotica* (Hymenoptera: Andrenidae) but *Bombus terrestris* remained unharmed (Tiedeken et al. 2016). The authors postulated that adaptation by the plant and the pollinator may have arisen to harness specialised foraging on this otherwise entomotoxic nectar and so secure the nectar reward for *Bombus* species which are the primary pollinator of *R. ponticum* in the British Isles. When an introduced species provides nectar containing invertebrate toxins poorly adapted pollinators that did not co-evolve with it could be intolerant of the effects (Callaway & Ridenour 2004). Further, this effect could serve to filter the most efficient pollinators – in this case *Bombus* spp. – and may co-evolve rapidly in the introduced range of *R. ponticum* (Tiedeken et al. 2016). So GTX 1 provides Rhododendron with effective protection against antagonists and enhances pollination service by mutualists in the same plant. How this mechanism adapts in the introduced populations is discussed further below.

Grayanotoxins have been considered as candidates for botanical insecticides since they have activity against a range of pests insect species (Mei-Ying et al. 2015). Commercially produced *B. terrestris* are used increasingly by farmers to supplement pollination services – particularly in polytunnels and greenhouse crops such as tomatoes and strawberries. Since *Bombus* spp. are tolerant of grayanotoxins and these compounds have a wide-ranging activity against invertebrate antagonists it is possible they could provide candidates for bee friendly botanical insecticides.

Diterpene alkaloids in *Aconitum* spp. and nectar robbery.

Species in the Genus *Aconitum* produce potent mammalian toxins with reports of human poisonings associated with the norditerpenoid alkaloids that occur in all plant parts (Kolev et

al. 1996). As with grayanotoxins and caffeine the ecological function to the plant of these compounds is likely to be for defense against herbivory since several compounds of this class from *Aconitum* and related species have deterrent properties against insects (Ulubelen et al. 2001; Gonzalez-Coloma et al. 2004). Flowers of *Aconitum* spp. are notable for having highly adapted nectaries on two long nectar spurs protected by a sepaloid hood or galea restricting nectar access to only long-tongued species of Bumble bee (Thøstesen et al. 1996). Despite these efforts to limit access to a few species of more forage focussed pollinators and increase the likelihood of pollen transfer to conspecific flowers, *Aconitum* flowers suffer from nectar robbery. Nectar robbing occurs when a hole is chewed through the corolla to access nectar that is otherwise unavailable via the route intended by the plants (Inouye, 1980). However, this doesn't appear to come with any fitness cost to *Aconitum* (Utelli and Roy 2000). This can be explained because fewer than 10% of flower visits are by robbing species and only a small fraction of these visits result in a successful robbing event due to the occurrence of insect repellent norditerpenoids in nectar of *Aconitum* flowers (Barlow et al. 2017). These compounds vary in concentrations from one flower to the next and at the highest concentrations are too toxic even for the pollinator but importantly the robbing species of *Bombus* (*B. terrestris* in UK) are poorly adapted to their effects compared to the pollinating species which can tolerate concentrations of the nectar toxins that are 10 times higher than the robbing species.

Bee species may not be particularly sensitive to toxins in food (Wright et al. 2010; de Brito Sanchez et al. 2015) but adaptation to toxins differs among species since the short tongued species *B. terrestris* was more likely to be deterred by artificial food containing aconitine

than a long-tongued specialists like *B. hortorum* and this may be related to short tongued species outcompeting long-tongued species for flowers with generalist pollinator syndrome.

So, again, the limited chemical armoury of *Aconitum* species serves both to protect the plant from antagonists such as folivores and nectar robbers while concurrently conserving the nectar in their morphologically adapted nectaries for long tongued species that can access the nectar trough the intended route, tolerate the toxins and pollinate the plant. It would be interesting to determine how widespread tolerance to these diterpene alkaloid toxins is among long-tongued species including *B. gerstackerii* the Aconitum specialist is and how consistently intolerant short-tongued and potentially robbing species are.

Nectar phytochemistry and pollinator health

Diseases are a major biotic challenge for bees and can contribute to pollinator declines particularly in combination with other stressors including habitat loss, invasive species and pesticide exposure (Vanbergen et al. 2013). Bees transmit and acquire diseases on flowers, but flower phenology including chemistry may influence transmission and disease spread (Mcart et al. 2014; Koch et al. 2017). The role of plant species and variation in these traits is largely unexplored and may inform how disease transmission can be better managed (Adler et al. 2018). Flower chemistry can influence bee disease by killing bee pathogens and reducing transmission, and secondary metabolites in nectar and pollen could be active against gut parasites and reduce levels of infections in foraging adults or larvae (Koch et al. 2017). Indeed recent work has established that nectar phytochemicals are biologically active against *Crithidia bombi* gut parasites of *Bombus impatiens* in artificially inoculated animals

(Richardson et al. 2015). Several commercially available compounds known to occur in nectar and be relevant to foraging by *Bombus* spp. from earlier reports including the alkaloids, nicotine and anabasine, and the phenolic monoterpene thymol were tested in inoculated bees and shown to reduce levels of infection in test bees. This potential benefit to bees is in conflict with the knowledge that some of these compounds have established roles as defence compounds but since honeybees response to nicotine is nuanced when it is encountered it may serve to effect more frequent visits between flowers by pollinators (Köhler et al. 2012) and reduce the amount of nectar required to maintain successful pollen transfer by native pollinators (Kessler and Baldwin 2007; Kessler et al. 2008).

Thymol is a phenolic based essential oil component of many plants; most notably *Thymus vulgaris* (Lamiaceae), and has biological activity against a number of insects with scope for commercialisation as a biopesticide (Isman 2006; Wilson and Isman 2006) and can confer resistance to invertebrate antagonists. Richardson et al. (2015) reported minor activity of this compound against *Crithidia bombi* in *B. impatiens* but tested only at concentrations found in honey. Thymol occurs in nectar in higher levels than in honey and is more active at this ecologically relevant concentration against *C. bombi* (Palmer-Young et al. 2016). So, thymol may have potential benefit for bees in a landscape setting for the provision of nectar but also with the added value of reducing disease incidence. Thymol also occurs in a wide range of species including *T. vulgaris*, a popular and commercially cultivated plant. Furthermore, thymol is less harmful to bees than some of the other compounds tested by Richardson et al. (2015) which can be directly toxic to them (Köhler et al. 2012) or cause malaise behaviours and sublethal effects (Hurst et al. 2014; Oliver et al. 2015). However, thymol did not limit acquisition of parasites and any effects of thymol against *C. bombi* in isolation may reflect

indirect host-mediated, effects of chronic thymol ingestion (Rothchild et al. 2018) so other more consistently effective compounds are required before we can envision medicinal plants for bees in field margins and flower strips. Most studies on the biological activity of nectar compounds against bee pathogens however have not investigated the fate of the metabolites so have not fully explained the chemical processes that might be influencing the effects. Testing compounds in vitro for example may provide some activities but these may not necessarily reflect the chemistry of the insect gut where metabolism of compounds could influence activity – for example glycosylation or methylation having effects on the bioactivity. Phytochemical analysis of the contents of the hindgut where *C. bombi* proliferate would be informative for some target compounds and evaluation of the parasite itself to understand the mechanisms of effects are required. More studies to investigate free flying bees would be welcome can reduce their pathogen load or uptake when foraging on the flowers that produce bioactive compounds in nectar would be informative for understanding their capacity to contribute to bee health or influence transmission.

Pollen chemistry and pollination.

A priori, herbivore defense of pollen makes sense since this is the male gamete; vital plant tissue, so conforms to optimal defence theory as a priority for protection (McCall and Fordyce 2010; Cook et al. 2013). Little surprise then that the concentrations of secondary metabolites in pollen is typically one or two orders of magnitude greater than in nectar (Palmer-Young et al. 2019a, b). Again, the consistency of occurrence of secondary metabolites across tissue reveals that components occurring in pollen are invariably similar to those occurring elsewhere in the plant for defense. For example, the genus *Lupinus* includes several crop species such as *Lupinus mutabilis* (Leguminosae); a food crop cultivated in South America.

Lupinus species produce several quinolizidine alkaloids including sparteine and lupanine which occur throughout the plant but notably in the seeds at very high concentrations (3% by weight) (Hatzold et al. 1983). Evidence suggests their primary function is to protect the plants from insect attack, because D-lupanine is toxic and repellent to insects including beneficial arthropods that feed on the herbivores (Emrich BH 1991; Kordan et al. 2012). More recently D-lupanine along with its 13-*O*-tigloyloxy, 13-*O*-angeloyloxy, 13-hydroxy and 4-*O*-hydroxy-13-*O*-angeloyloxy derivatives have been found in pollen at concentrations in excess of 2mg g^{-1} and although at these concentrations did not increase mortality in *Bombus terrestris*, a pollinating species for Lupins, they did cause experimental micro-colonies to produce fewer and smaller males which could have longer term colony and population impacts (Arnold et al. 2014). The function of toxins in pollen as a defence makes sense but to cause existential impacts on a key mutualist does not. In these experiments, insects were provisioned treated pollen with no choice and it is the choice that may provide a more effective experimental arena to understand how pollen defence chemistry functions. *Bombus* species were deterred from collecting saponin-containing pollen from two *Dipsacus* spp. (Caprifoliaceae) and pollen containing the highest levels of a saponin were less groomed off, meaning the bees carried more pollen for pollination (Wang et al. 2019). Some evidence exists for repellent and attraction to function together to optimise temporal visitation patterns of pollinators to coincide with stigma receptiveness and pollen maturation. *Thrips major* Uzel (Thysanoptera: Thripidae) occurs abundantly on flowers of the common Elder (*Sambucus niger* L.) (Adoxaceae) (Raspudić et al. 2009). *T. major* is widely recognised as a flower damaging pest species (Moritz et al., 2004). However, when *T. major* was excluded from inflorescences of *S. niger* the flowers failed to produce fruit indicating a role in pollination through pollen feeding behaviour. Peak abundance of linalool, the major monoterpenoid in the headspace of the

inflorescence, coincided with the highest numbers of adult thrips on flowers and is an attractive compound to *T. major*. Thrips declined in senescing flowers correlating with higher concentrations of the cyanogenic glycosides prunasin and sambunigrin in reproductive tissue which were deterrent to the thrips. Cyanogenic glycosides are well-established defence compounds for invertebrates (Zagrobelny et al. 2004). This work provides a fresh view of thrips as beneficial insects along with evidence of a mutualistic relationship between *T. major* and *S. nigra* highlighting the possibility that their value in food production maybe overlooked and their systematic control may have unintended negative consequences. So, while plant defence compounds in plants can function simply to protect it the mechanisms of protection may themselves mediate more effective pollination by flower visitors carrying more pollen to conspecific flowers in adapted species. Elsewhere linalool is a deterrent to colonisation of rice by the rice brown planthopper (*Nilaparvata lugens*) (Hemiptera: Delphacideae) indicating its function in some plant species in defense (Xiao et al. 2012). How pest species remain deterred by a single compound while mutualists remain attracted to the same compound remains unclear although Xiao et al. (2012) also report that linalool attracts natural enemies of the brown planthopper so it is possible that the deterrence in the brown planthopper could be through association with greater predation.

Conclusions

For decades scientific evidence has strongly supported the primary adaptive function of secondary metabolites in plants for defense against herbivory – especially against invertebrates. There are countless reports of the biological activity of plant extracts and compounds against insects (Isman and Grieneisen 2014) consolidating this view but surprisingly little on the effects of phytochemicals on pollinators. Understanding how traits

associated with food reward and reward chemistry would help inform the ecology and evolution of plants and pollinators (Parachnowitsch and Manson 2015).

Here, examples have been reviewed that establish ecological functions that benefit the plant through maximizing pollen transfer by repelling less preferred pollinators, supporting heathier pollinators or enhancing their behaviour to deliver more efficient pollination. This compelling evidence for ecological functions based on these biological effects raises the question of what is driving adaptation in plants for secondary metabolites and how does adaptation to herbivory and pollination interact? Ramos and Schiestl (2019) argue that pollination and herbivory are both concurrent drivers of diversity and should be studied together. They showed that plants under selection by pollinators (bees) evolved increased floral attractiveness, but this was compromised by herbivores. Plants under selection from pollinators and herbivores evolved higher degrees of self-compatibility and autonomous selfing, as well as reduced herkogamy.

A similar question is considered across large spatial scales in Egan et al. (2016) who measured levels of the entomotoxic diterpenoids grayanotoxin I (GTX I) and GTX III in leaves and nectar of *Rhododendron ponticum* in its native and introduced ranges. As mentioned above, high concentrations of GTX I in young *Rhododendron* leaves provide defense against the glass house thrips (Scott-Brown et al. 2016) so its primary role appears from this evidence to be defense. But GTX I is also toxic to some pollinating species when encountered in nectar including honeybees and a solitary mining bee species, (*Andrena scotica*) whereas another pollinator, *Bombus terrestris* remains unaffected (Tiedeken et al. 2014, 2016) suggesting adaptation by *Bombus* permitting exclusive access to nectar for the pollinator and specialist pollinator service for the flowers. Occurrence of GTX I, the most active against bees, was significantly lower or absent from nectar in introduced plants whereas GTX III – inactive

against the bees – was similar across two countries. The occurrence of nectar GTX was not affected by environmental variation, and considering the specificity of change to GTX I, and its differential toxicity to some bee species, its occurrence in the plant may have been influenced during invasion by interaction with herbivores or via pollinator-mediated selection owing to local populations of pollinators in the introduced range being poorly adapted to nectar toxins which in the native range serve to filter out preferred pollinators (Egan et al. 2016). A comprehensive survey of the genus and correlation of the presence of toxic nectar and generalist pollinator syndromes or absence of toxins and specialists' flowers may provide stronger evidence that GTX I serves to filter specialists.

Crop domestication can also drive down plant defenses reducing inherent resistance to herbivory and disease so may similarly affect secondary metabolites in nectar and pollen with consequences for pollinators. In *Vaccinium corymbosum* (Ericaceae), domestication altered plant chemistry of nectar and pollen, and reduced pollen chemical diversity. These changes included the antimicrobial caffeic acid ester 4-*O*-caffeoylshikimic acid which could have implications for pollen protection because caffeic acid esters of cyclitols are established herbivore antagonists (Stevenson et al. 1993) but these compounds also protect *Bombus impatiens* against the gut pathogen *Crithidia bombi* at concentrations found in wild but not cultivated plants (Egan et al. 2018). This suggests that domestication changed floral traits with consequences for bee health and investigations of pollinator-dependent crops more generally are required to determine broader implications of domestication on floral chemistry associated with domestication (Egan et al. 2018). Further work to establish how chemical adaptation responds to pollinator needs and herbivore defense including spatially discreet studies of nectar secondary compounds to show how variation affects plant ecology is required along with a broader view of phytochemistry beyond antagonistic interactions, that

integrates the consequences of chemically defended mutualist rewards. Ultimately many of the knowledge gaps would be filled with more work on free flying bees to understand in realistic ecological settings how these effects manifest (e.g., Singaravelan et al. 2005; Manson et al. 2013; Couvillon et al. 2015).

Acknowledgments

References

Adler LS (2000) The ecological significance of toxic nectar. Oikos 91:409–420. doi: 10.1034/j.1600-0706.2000.910301.x

Adler LS, Michaud KM, Ellner SP, et al (2018) Disease where you dine: plant species and floral traits associated with pathogen transmission in bumble bees. Ecology 99:2535–2545. doi: 10.1002/ecy.2503

Arditti J, JOHN E, Kitching IJ, Wasserthal LT (2012) 'Good Heavens what insect can suck it'- Charles Darwin, Angraecum sesquipedale and Xanthopan morganii praedicta. Bot J Linn Soc 169:403–432. doi: 10.1111/j.1095-8339.2012.01250.x

Arnold SEJ, Idrovo MEP, Arias LJL, et al (2014) Herbivore Defence Compounds Occur in

Pollen and Reduce Bumblebee Colony Fitness. J Chem Ecol 40:. doi: 10.1007/s10886-

014-0467-4

Baker HG (1977) Non-Sugar Chemical Constituents of Nectar. Apidologie 8:349–356. doi:

10.1051/apido:19770405

Barlow SE, Wright GA, Ma C, et al (2017) Distasteful Nectar Deters Floral Robbery. Curr Biol

27:. doi: 10.1016/j.cub.2017.07.012

Bernklau E, Bjostad L, Hogeboom A, et al (2019) Dietary phytochemicals, honey bee

longevity and pathogen tolerance. Insects 10:1–12. doi: 10.3390/insects10010014

Bohman B, Phillips RD, Menz MHM, et al (2014) Discovery of pyrazines as pollinator sex

pheromones and orchid semiochemicals: Implications for the evolution of sexual

deception. New Phytol 203:939–952. doi: 10.1111/nph.12800

Brosi BJ (2016) Tansley insight Pollinator specialization : from the individual to the

community. New Phytol 210:1190–1194

Ceja-Navarro JA, Vega FE, Karaoz U, et al (2015) Gut microbiota mediate caffeine

detoxification in the primary insect pest of coffee. Nat Commun 6:1–9. doi:

10.1038/ncomms8618

Chamberlain D, HYAM R, ARGENT G, et al (1996) The genus Rhododendron its classification

and synonymy. Edinburgh: Royal Botanic Garden., Edinburgh

Cook D, Manson JS, Gardner DR, et al (2013) Norditerpene alkaloid concentrations in tissues

and floral rewards of larkspurs and impacts on pollinators. Biochem Syst Ecol 48:123–

131. doi: 10.1016/j.bse.2012.11.015

Couvillon MJ, Toufailia A, Butterfield TM, et al (2015) Caffeinated Forage Tricks Honeybees

into Increasing Foraging and Recruitment Behaviors. Curr Biol 1–4. doi:

10.1016/j.cub.2015.08.052

de Brito Sanchez MG, Serre M, Avargues-Weber A, et al (2015) Learning context modulates aversive taste strength in honey bees. J. Exp. Biol. 218, 949–959. J Exp Biol 218:949–959

Detzel A, Wink M (1993) Attraction, deterrence or intoxication of bees (Apis mellifera) by plant allelochemicals. Chemoecology 4:8–18

Egan PA, Adler LS, Irwin RE, et al (2018) Crop Domestication Alters Floral Reward Chemistry With Potential Consequences for Pollinator Health. Front Plant Sci 9:1–14. doi: 10.3389/fpls.2018.01357

Egan PA, Stevenson PC, Tiedeken EJ, et al (2016) Plant toxin levels in nectar vary spatially across native and introduced populations. J Ecol 104:1106–1115. doi: 10.1111/1365-2745.12573

Emrich BH (1991) Acquired toxicity of the lupin aphid, Macrosiphum albifrons, and its influence on the aphidophagous predators Coccinella septempunctata, Episyrphus balteatus and Chrysoperla carnea. Z Pflanzenk Pflanz 98:398–404

Giurfa M, Nflfiez J, Chittka L, Menzel R (1995) Colour preferences of flower-naive honeybees. J Comp Physiol - A Sensory, Neural, Behav Physiol 177:247–259

González-Coloma A, Reina M, Medinaveitia A, et al (2004) Structural diversity and defensive properties of norditerpenoid alkaloids. J Chem Ecol 30:1393–1408. doi: 10.1023/B:JOEC.0000037747.74665.0a

Gonzalez-Varo JP, Biesmeijer JC, Bommarco R, et al (2013) Combined effects of global change pressures on animal-mediated pollination. Trends Ecol Evol 28:524–530.

Hanson JR (2016) From 'mad honey' to hypotensive agents, the grayanoid diterpenes. Sci Prog 99:327–334. doi: 10.3184/003685016X14720691270831

Hatzold T, Elmadfa I, Gross R, et al (1983) Quinolizidine Alkaloids in Seeds of. J Agric Food
Chem 31:934–938. doi: 10.1021/jf00119a003

Hollingsworth (2002) Caffeine as a repellent for slugs and snails. Nature 417:915–916

Hurst V, Stevenson PC, Wright GA (2014) Toxins induce "malaise" behaviour in the
honeybee (Apis mellifera). J Comp Physiol A. doi: 10.1007/s00359-014-0932-0

Irwin RE, Cook D, Richardson LL, et al (2014) Secondary compounds in floral rewards of toxic
rangeland plants: Impacts on pollinators. J Agric Food Chem 62:7335–7344. doi:
10.1021/jf500521w

Isman MB (2006) Botanical Insecticides, Deterrents, and Repellents in Modern Agriculture
and an Increasingly Regulated World. Annu Rev Entomol 51:45–66. doi:
10.1146/annurev.ento.51.110104.151146

Isman MB, Grieneisen ML (2014) Botanical insecticide research : many publications , limited
useful data. Trends Plant Sci 19:140–145. doi: 10.1016/j.tplants.2013.11.005

Katsumoto Y, Fukuchi-mizutani M, Fukui Y, et al (2007) Engineering of the Rose Flavonoid
Biosynthetic Pathway Successfully Generated Blue-Hued Flowers Accumulating
Delphinidin. Plant Cell Physiol 48:1589–1600. doi: 10.1093/pcp/pcm131

Kessler D, Baldwin IT (2007) Making sense of nectar scents: the effects of nectar secondary
metabolites on floral visitors of Nicotiana attenuata. Plant J 49:840–854. doi:
10.1111/j.1365-313X.2006.02995.x

Kessler D, Gase K, Baldwin IT (2008) Field experiments with transformed plants reveal the
sense of floral scents. Science (80-) 321:1200–1202. doi: 10.1126/science.1160072

Kim YS, Uefuji H, Ogita S, Sano H (2006) Transgenic tobacco plants producing caffeine: A
potential new strategy for insect pest control. Transgenic Res 15:667–672. doi:
10.1007/s11248-006-9006-6

Klocke JA, Hu M, Choo S, Kubo I (1991) Grayanoid diterpene insect antifeedants from. Phytochemistry 30:1797–1800

Koch H, Brown MJ, Stevenson PC (2017) The role of disease in bee foraging ecology. Curr Opin Insect Sci 21:60–67. doi: 10.1016/j.cois.2017.05.008

Koch H, Stevenson PC (2017) Do linden trees kill bees? Reviewing the causes of bee deaths on silver linden (Tilia tomentosa). Biol Lett 13:20170484. doi: 10.1098/rsbl.2017.0484

Köhler A, Pirk CWW, Nicolson SW (2012) Honeybees and nectar nicotine: Deterrence and reduced survival versus potential health benefits. J Insect Physiol 58:286–292. doi: 10.1016/j.jinsphys.2011.12.002

Kolev ST, Leman P, Kite GC, et al (1996) Toxicity following accidental ingestion of Aconitum containing Chinese remedy. Hum Exp Toxicol 15:839–842. doi: 10.1177/096032719601501008

Kondo, T., Yoshida, K., Nakagawa, A., Kawai, T., Tamura, H., Gotoo T (1992) Structural basis of blue-flower colour development in flowerpetals from Commelina communis. Nature 358:515–5185

Kordan B, Dancewicz K, Wróblewska A, Gabryś B (2012) Intraspecific variation in alkaloid profile of four lupine species with implications for the pea aphid probing behaviour. Phytochem Lett 5:71–77. doi: 10.1016/j.phytol.2011.10.003

Kretschmar J a., Baumann TW (1999) Caffeine in Citrus flowers. Phytochemistry 52:19–23. doi: 10.1016/S0031-9422(99)00119-3

Manson JS, Cook D, Gardner DR, Irwin RE (2013) Dose-dependent effects of nectar alkaloids in a montane plant-pollinator community. J Ecol 101:1604–1612. doi: 10.1111/1365-2745.12144

Mathona C, Edder P, Christen P, Bieri S (2014) Unexpected Occurrence of Caffeine in Sleep-

Inducing Herbal Teas. Chim Int J Chem 68:705–709. doi: 10.2533/chimia.2014.705

Mcart SH, Koch H, Irwin RE, Adler LS (2014) Arranging the bouquet of disease: Floral traits and the transmission of plant and animal pathogens. Ecol Lett 17:624–636. doi: 10.1111/ele.12257

McCall AC, Fordyce JA (2010) Can optimal defence theory be used to predict the distribution of plant chemical defences? J Ecol 98:985–992

Mei-Ying H, Klocke JA, Chiu S-F, Kubo I (2015) Response of Five Insect Species to a Botanical Insecticide, Rhodojaponin III. J Econ Entomol 86:706–711. doi: 10.1093/jee/86.3.706

Menzel R. and Muller U (1996) LEARNING AND MEMORY IN HONEYBEES: From Behavior to Neural Substrates. Annu Rev Neurosci 19:379–404. doi: 10.1146/annurev.ne.19.030196.002115

Menzel R, Erber J, MASUHR T (1980) Localization of short-term memory in the brain of the bee, Apis mellifera. Physiol Entomol 5:343–358. doi: 10.1111/j.1365-3032.1980.tb00244.x

Milne RI, Abbott RJ (2000) Origin and evolution of invasive naturalized material of Rhododendron ponticum L. in the British Isles. 9, 541–556. Mol Ecol 9:541–556

Moritz G, Mound LA, Morris DC GA (2004) Pest thrips of the world - an identification and information system using molecular and microscopial methods. In: CBIT, Univ. Queensland,CDROM. http://thripsnet.zoologie.uni-halle.de/key-server-neu/data/0a08090e-0e03-4a0e-8502-070105080e05/media/Html/Thrips_major.htm. Accessed 26 Apr 2019

Naef R, Jaquier A, Velluz A, Bachofen B (2007) From the Linden Flower to Linden Honey-Volatile Constituents of Linden Nectar, the Extract of Bee-Stomach and Ripe Honey. Perspect Flavor Fragr Res 1:31–40. doi: 10.1002/9783906390475.ch3

Nathanson JA (1984) Caffeine and Related Methylxanthines : Possible Naturally Occuring

    Pesticides P. Science (80-) 226:184–187

Oliver CJ, Softley S, Williamson SM, et al (2015) Pyrethroids and nectar toxins have subtle

    effects on the motor function, grooming and wing fanning behaviour of honeybees

    (Apis mellifera). PLoS One 10:. doi: 10.1371/journal.pone.0133733

Palmer-Young EC, Farrell IW, Adler LS, et al (2019a) Chemistry of floral rewards: intra- and

    interspecific variability of nectar and pollen secondary metabolites across taxa. Ecol

    Monogr 89:1–23. doi: 10.1002/ecm.1335

Palmer-Young EC, Farrell IW, Adler LS, et al (2019b) Secondary metabolites from nectar and

    pollen: a resource for ecological and evolutionary studies. Ecology 0:1045540. doi:

    10.1002/ecy.2621

Palmer-Young EC, Sadd BM, Stevenson PC, et al (2016) Bumble bee parasite strains vary in

    resistance to phytochemicals. Sci Rep 6:. doi: 10.1038/srep37087

Parachnowitsch AL, Manson JS (2015) The chemical ecology of plant-pollinator interactions:

    recent advances and future directions. Curr Opin Insect Sci 8:41–46. doi:

    10.1016/j.cois.2015.02.005

Ramos SE, Schiestl FP (2019) Rapid plant evolution driven by the interaction of pollination

    and herbivory. 196:193–196

Raspudić E, M I, M B, S T (2009) Distribution of Thysanoptera species and their host plants in

    Croatia. Acta Agric Slov 93:275–283

Richardson LL, Adler LS, Leonard AS, et al (2015a) Secondary metabolites in floral nectar

    reduce parasite infections in bumblebees. Proc R Soc B Biol Sci 282:20142471–

    20142471. doi: 10.1098/rspb.2014.2471

Richardson LL, Adler LS, Leonard AS, et al (2015b) Secondary metabolites in floral nectar

reduce parasite infections in bumblebees. Proc R Soc B 282:20142471. doi: 10.1098/rspb.2014.2471

Rothchild KW, Adler LS, Irwin RE, et al (2018) Effects of short-term exposure to naturally occurring thymol concentrations on transmission of a bumble bee parasite. Ecol Entomol 43:567–577. doi: 10.1111/een.12631

Ruxton GD, Schaefer HM (2016) Floral colour change as a potential signal to pollinators. Curr Opin Plant Biol 32:96–100. doi: 10.1016/j.pbi.2016.06.021

Scott-Brown AS, Gregory T, Farrell IW, Stevenson PC (2016) Leaf trichomes and foliar chemistry mediate defence against glasshouse thrips; Heliothrips haemorrhoidalis (Bouché) in Rhododendron simsii. Funct Plant Biol 43:1170–1182. doi: 10.1071/FP16045

Singaravelan N, Ne'eman G, Inbar M, Izhaki I (2005) Feeding responses of free-flying honeybees to secondary compounds mimicking floral nectars. J Chem Ecol 31:2791–2804. doi: 10.1007/s10886-005-8394-z

Stevenson PC, Anderson JC, Blaney WM, Simmonds MSJ (1993) Developmental inhibition of Spodoptera litura (Fab.) larvae by a novel caffeoylquinic acid from the wild groundnut, Arachis paraguariensis (Chod et Hassl.). J Chem Ecol 19:2917–2933. doi: 10.1007/BF00980592

Stevenson PC, Nicolson SW, Wright GA (2017) Plant secondary metabolites in nectar: impacts on pollinators and ecological functions. Funct Ecol 31:. doi: 10.1111/1365-2435.12761

Stout JC, Morales CL (2009) Ecological impacts of invasive alien species on bees. Apidologie 40:388–409

Thomson JD, Draguleasa MA, Tan MG (2015) Flowers with caffeinated nectar receive more

pollination. Arthropod Plant Interact 9:1–7. doi: 10.1007/s11829-014-9350-z

Thøstesen AM, Olesen JM, Thostesen AM (1996) Pollen Removal and Deposition by Specialist and Generalist Bumblebees in Aconitum septentrionale. Oikos 77:77. doi: 10.2307/3545587

Tiedeken EJ, Egan PA, Stevenson PC, et al (2016) Nectar chemistry modulates the impact of an invasive plant on native pollinators. Funct Ecol 30:885–893. doi: 10.1111/1365-2435.12588

Tiedeken EJ, Stout JC, Stevenson PC, Wright GA (2014) Bumblebees are not deterred by ecologically relevant concentrations of nectar toxins. J Exp Biol 217:1620–1625. doi: 10.1242/jeb.097543

Uefuji H, Tatsumi Y, Morimoto M, et al (2005) Caffeine production in tobacco plants by simultaneous expression of three coffee N-methyltrasferases and its potential as a pest repellant. Plant Mol Biol 59:221–227. doi: 10.1007/s11103-005-8520-x

Ulubelen A, Meric¸li A, Meric¸li F, et al (2001) Insect repellent activity of diterpenoid alkaloids. Phyther Res 15:170–171. doi: 10.1002/ptr.688

Utelli A-B, Roy BA (2000) Pollinator abundance and behavior on Aconitum lycoctonum (Ranunculaceae): an analysis of the quantity and quality components of pollination. Oikos 89:461–470. doi: 10.1034/j.1600-0706.2000.890305.x

Vanbergen AJ, Garratt MP, Vanbergen AJ, et al (2013) Threats to an ecosystem service: Pressures on pollinators. Front Ecol Environ 11:251–259. doi: 10.1890/120126

Vignolini S, Davey MP, Bateman RM, et al (2012) The mirror crack ' d : both pigment and structure contribute to the glossy blue appearance of the mirror orchid , Ophrys speculum. New Phytol 196:1038–1047

Wang H, Conchou L, Bessière JM, et al (2013) Flower color polymorphism in Iris lutescens

(Iridaceae): Biochemical analyses in light of plant-insect interactions. Phytochemistry 94:123–134. doi: 10.1016/j.phytochem.2013.05.007

Wang X-Y, Tang J, Wu T, et al (2019) Bumblebee Rejection of Toxic Pollen Facilitates Pollen Transfer. Curr Biol 1–6. doi: 10.1016/j.cub.2019.03.023

Wilson JA, Isman MB (2006) Influence of essential oils on toxicity and pharmacokinetics of the plant toxin thymol in the larvae of Trichoplusia ni. Can Entomol 138:578–589. doi: 10.4039/N06-801

Wright GA, Baker DD, Palmer MJ, et al (2013) Caffeine in floral nectar enhances a pollinator's memory of reward. Science (80-) 339:1202–1204. doi: 10.1126/science.1228806

Wright GA, Mustard JA, Simcock NK, et al (2010) Parallel reinforcement pathways for conditioned food aversions in the honeybee. Curr. Biol. 20, 2234–2240. Curr Biol 20:2234–2240

Xiao Y, Wang Q, Erb M, et al (2012) Specific herbivore-induced volatiles defend plants and determine insect community composition in the field. Ecol Lett 15:1130–1139. doi: 10.1111/j.1461-0248.2012.01835.x

Zagrobelny M, Bak S, Vinther A (2004) Cyanogenic glucosides and plant – insect interactions. 65:293–306. doi: 10.1016/j.phytochem.2003.10.016

Caffeine
Nicotine

Aconitine
Grayanotoxin 1
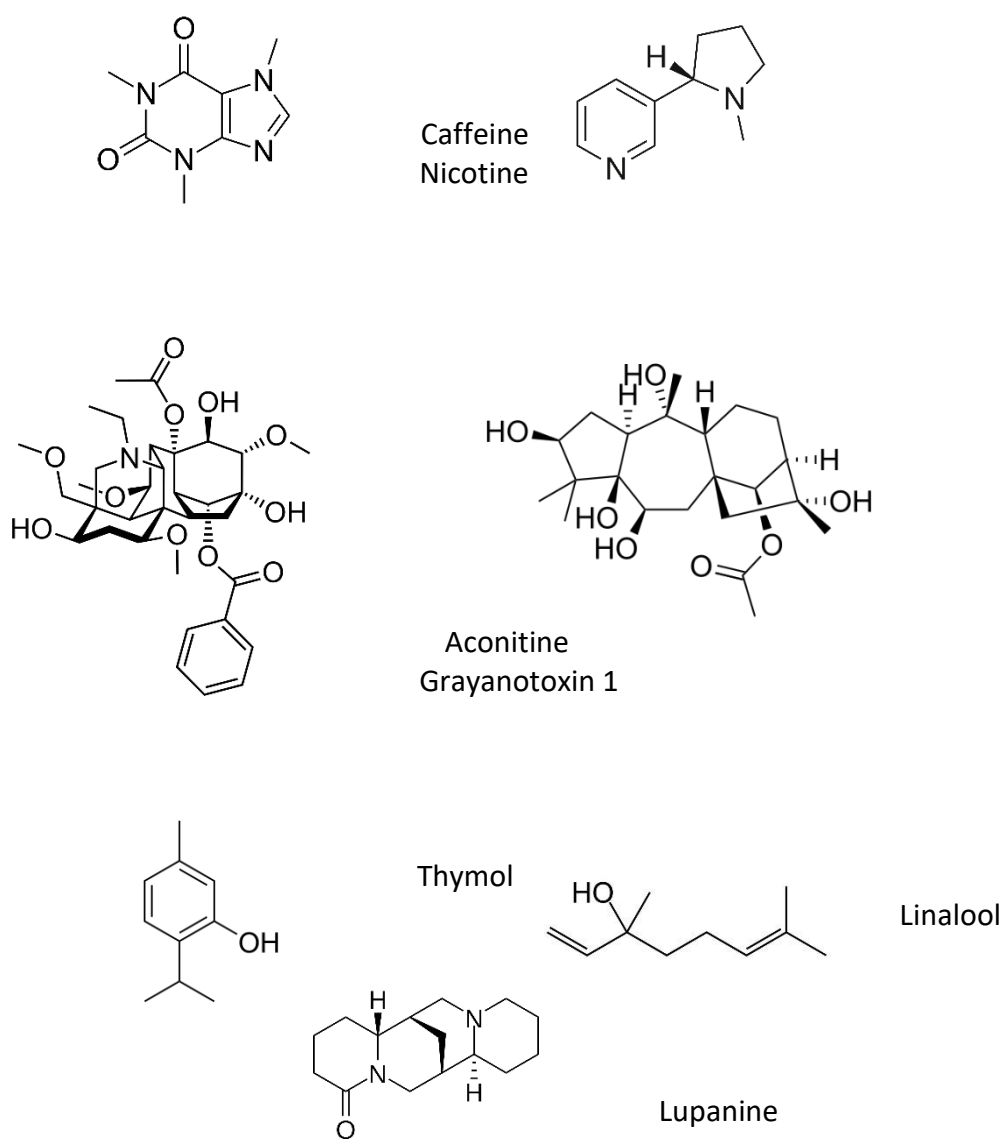
Thymol

Linalool

Lupanine

Fig 1 Chemical structures of phytochemicals having a defensive role against insect antagonists but also various beneficial effects for pollinators.