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Review

Flavonoid–insect interactions: recent advances in our knowledge

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Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

Recent contributions to the role of phenolics, especially flavonoids, in different aspects of insect–plant interactions are reviewed, including data on the effects of rutin on the feeding behaviour of a range of noctuid larvae.

Keywords: Quercetin; Rutin; Sequestered flavonoids; Anthocyanins; Tricin; Isoflavonoids; Feeding behaviour; *Spodoptera*; *Heliothis*; *Helicoverpa*

Contents

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1. Introduction

Our knowledge about the role of phenolics in different aspects of insect–plant interactions continues to expand, but not as fast as our knowledge about the distribution and diversity of phenolics in different plant species. This is in part because the majority of recent work on phenolics, especially flavonoids, relate to either their benefits to human health or their use in phylogenetic studies.

Advances in our knowledge about the health benefits of flavonoids, not only in both crop and medicinal plants, have prompted plant breeders to use traditional and genetic engineering methods to increase the levels of these compounds in crops (Johnson and Felton, 2001; Galili et al., 2002). There has also been an increase in the proportion of land being used to grow medicinal plants that often naturally contain high levels of phenolics. Environmental stress from increased levels of ozone, lack of water, pollution and herbivory are also reported to increase the levels of phenolics in plants (Bi et al., 1997). Thus in theory we could be creating a world of plants richer in flavonoids. Would this influence insect ecology?

There is an intellectually compelling logic to the concept that plant-derived compounds have already played a role in the evolution of insect–plant interactions and that insects would adapt to changes in the levels of a group of compounds. However, Futuyama (2000) argues that there is very little evidence to support this concept. On the other hand, there is evidence suggesting that the fitness of insects can be influenced by the plants they feed on (Schoonhoven et al., 1998). This fitness does not just relate to the fact that a plant can be considered a suitable host because the insect can feed and develop on it, but because it gains some secondary benefits from feeding on a specific species or group of plants. For example, some plants contain compounds, such as flavonoids, that insects can sequester into their body cuticle for protection against pathogens and predators or into their wings to attract mates. Thus insects might benefit from plants having higher levels of flavonoids, making insect pests more difficult to control using biological control agents such as viral pathogens. However, the effect of increasing the levels of specific flavonoids in plants on the host-selection behaviour of insects is unknown.

Phenolics can have negative effects on non-adapted insects. Feeny (1970) showed that ingestion of phenolics by insects reduced the nutritive value of their food. However, some of these early experiments might have overestimated the anti-nutritional effects of phenolics because they were carried out using diets that lacked the enzymes present in leaves that assist insects utilise phenolics. The negative post-ingestive effects of phenolics usually occur in the midgut of insects through oxidative mechanisms, resulting in the formation of superoxide radicals and other reactive oxygen species (Summer and Felton, 1994; Barbehenn, 2002). Ingested phenolics can be tolerated or detoxified depending on the levels of antioxidant, cytochrome P450 monoxygenase and esterase enzymes present in the insects as well as the pH of the gut (Rey et al., 1999, 2000). When the oxidative stress exceeds the antioxidant resources of the tissue in the midgut, then protein oxidation and lipid peroxidation can occur.

Cytochrome P450 enzymes are some of the most numerous group of enzymes involved in the biosynthesis of compounds and occur in all living organisms (Scott et al., 1998). Advances in our knowledge about the diversity and specificity of these enzymes (http://drnelson.utmem.edu/CytochromeP450.html) will most likely result in a better understanding of not only their role in the ability of insects to detoxify and metabolise ingested plant metabolites, but also in the evolution of plant–insect selection behaviour (Berenbaum, 2002; Scott and Wen, 2001).

This review covers some of the recent publications (2000–2002) on different aspects of flavonoid–insect interactions. It also presents data from research on the role of rutin on the feeding behaviour of species of noctuids. These data were previously presented in an early research publication that was not easy to obtain (Blaney and Simmonds, 1983).

2. Phenolics in trees

Salix species are known to contain salicylates that influence herbivory, but the leaves also contain an array of other compounds that could modulate the feeding and fitness of insects that feed on the foliage (Ruuhola et al., 2001). For example, *Salix pentandra* L. contains acetylated salicylates as well as chlorogenic acid and flavonoids and is a favoured host for *Salix*-specialist insects but not polyphagous feeders, whereas another species, *S. phylicifolia* L., contains flavonoids but not salicylates and leaves of this species are eaten by polyphagous species.

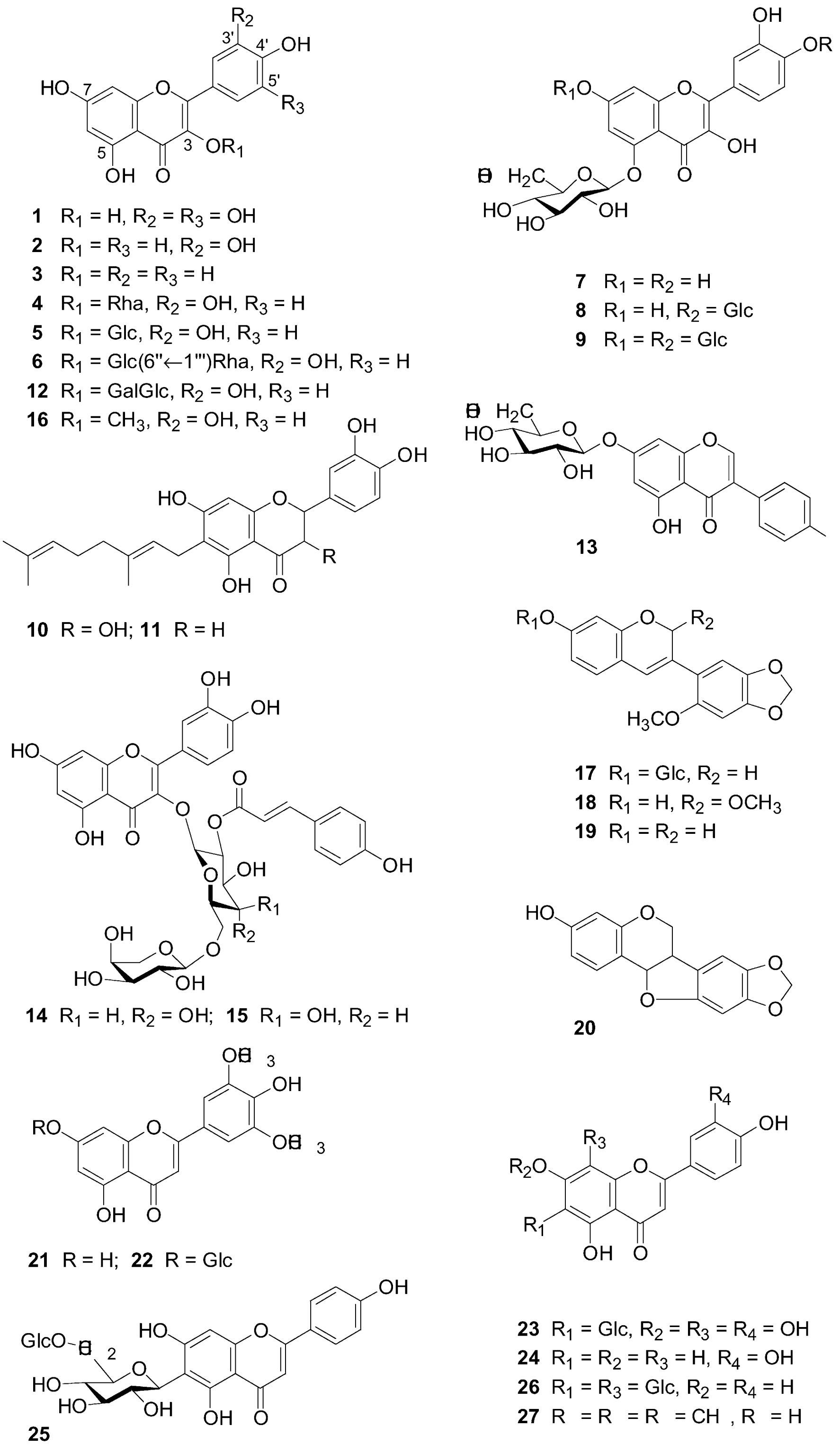
In order to investigate the role of salicylates and flavonoids in species of *Salix* on the feeding and growth of the polyphagous larvae of *Operophtera brumata* L (Geometridae), Ruuhola et al. (2001) studied the chemistry of three *Salix* species (*S. pentandra, S. myrsinifolia* Salisb. and *S. phylicifolia)*. The authors analysed the levels of a range of phenolics in these species of *Salix* and in the frass of larvae exposed to the *Salix* leaves, and from these values calculated the percentage of these compounds degraded by the larvae. Leaves of *S. phylicifolia* contained high levels of flavonoids (173 mg/g dry weight, DW), low levels of chlorogenic acid derivatives (0.97 mg/g DW) and no salicylates. The larvae degraded 66% of both the flavonoids and chlorogenic acids. Leaves of *S. pentandra* contained salicylates (52 mg/g DW), chlorogenic acids (38 mg/g DW) and flavonoids (30 mg/g DW) and larvae degraded between 64 and 73% of these compounds. The highest levels of salicylates (126 mg/g DW) were in leaves of *S. myrsinifolia*, along with chlorogenic acids (49 mg/g DW) and low levels of flavonoids (6 mg/g DW). The larvae degraded 66% of the salicylates, 72% of the flavonoids and 100% of the chlorogenic acids. The growth of larvae was greatest on *S. phylicifolia* and 60% less on the least suitable species, *S. myrsinifolia*. Overall, the authors showed that the levels of the salicylates and chlorogenic acid correlated negatively with growth. Thus larvae exposed to leaves rich in these compounds grew slowly and larvae were shown to consume less material. This suggests that these two groups of compounds could be considered as antifeedants to *O. brumata*. In contrast, Ruuhola et al. (2001) showed that the levels of flavonoids, especially myricetin [Fig. 1 (**1**)], correlated positively with the growth of larvae of *O. brumata*. Ruuhola et al. (2001) proposed that the levels of salicylates in *Salix* are more important than the levels of flavonoids in modulating the feeding of specialists and generalist feeders. It would be interesting to know if the gustatory response of neurons in the taste sensilla of these specialists and generalists to stimulation with salicylates and flavonoids, such as myricetin (**1**), would differ. Until these experiments are undertaken we will not know whether the compounds in *Salix* act directly on the sensory inputs involved in host selection or via a secondary feedback mechanism.

Recent research on the European silver birch (*Betula pendula* Roth) (Saleem et al., 2001) showed that increased production of anti-oxidant phenolics, such as quercetin (**2**) and chlorogenic acid, increased resistance of trees to ozone damage by scavenging free oxygen, hydroxyl radicals and hydrogen peroxide. Similar changes in the concentration of phenolics have been recorded in other species of trees. For example, in needles of *Pinus nigra* Ait. increased production of phenolics is associated with an activation of the senescence process (Giertych et al., 1999). These plants show a shift in the allocation of carbon for the production of chemical defence rather than growth. An increase in phenolics could make the needles more resistant to attack by insects. The activity of these phenolics could be very insect specific. About 40 phenolic compounds have been identified in birch trees and gallotannin occurs in high concentrations in young leaves. The variation in the levels of the phenolic compounds has been shown to modulate the growth of the polyphagous larvae *Epirrita autumnata* Bkh. (Kause et al., 1999). One of the phenolics, 1-*O*-galloyl-D-glucopyranose (glucogallin) was investigated to see if it was detrimental to larvae and was shown not to be an effective defence compound (Alonso et al., 2002).

3. Sequestered flavonoids

Insects are reported to utilise flavonoids to increase their fitness. For example, the blue butterfly, *Polyommatus icarus,* feeds on a range of plants and is known to sequester flavonoids in its wings. Burghardt et al. (2000) studied the flavonoid content in butterflies fed on different species of plants with known flavonoid content. They were able to show that the levels of flavonoids in the insects were positively associated with the amounts of flavonoids in the food that they had consumed. These caterpillars were also able to sequester flavonoids from diets they normally did not feed on and females sequester more flavonoids than males. The amounts sequestered varied depending on the types of flavonoids in the diet. Larvae selectively sequestered and metabolised derivatives of quercetin (**2**) and kaempferol (**3**), the predominant flavonoids in the plants analysed. Other flavonoids such as myricetin derivatives, flavones and isoflavonoids were mostly excreted. Flavonoid rhamnogalactosides and highly polar multipli-glycosylated derivatives are less readily sequestered when compared to glucosides or galactosides. Overall, females sequestered flavonoids in their wings 59% more efficiently than males. This suggests that females are better adapted to sequester these compounds than males. Burghardt et al. (2000, 2001) proposed that the accumulation of the flavonoids in the smaller wings of the female is used in visual communication, as flavonoid-rich females are more attractive to males than flavonoid-free females.

Early research using paper chromatography reported that the silkworm, *Bombyx mori* sequestered flavonoids in their cocoon and that the flavonoids differed from those in leaves of its host the mulberry tree, *Morus alba* L. (Fujimoto et al., 1959). Further experiments were undertaken by Fujimoto and Hayashiya (1972) who reared *B. mori* larvae on an artificial diet containing quercitrin (**4**), isoquercitrin (**5**) or rutin (**6**) and found that when these larvae pupated the cocoons contained flavonoids, but the flavonoids were not identified. Recent research has shown that three of the flavonoid 5-glucosides, quercetin 5-*O*-glucoside (**7**), quercetin 5,4′-di-*O*-glucoside (**8**) and quercetrin 5,7,4′-tri-*O*-glucoside (**9**) found in the cocoon shell were not present in mulberry leaves (Tamura et al., 2002). These flavonoids occurring in *B. mori* are thought to be metabolites formed from the quercetin (**4**) in their diet. However, it is not known how these are metabolised within the larvae. Tamura et al. (2002) reported that although mammals can metabolise quercetin glycosides, there were no reports of a glucosyltransferase in mammals that can



1 $R_1 = H, R_2 = R_3 = OH$
2 $R_1 = R_3 = H, R_2 = OH$
3 $R_1 = R_2 = R_3 = H$
4 R_1 = Rha, $R_2 = OH, R_3 = H$
5 R_1 = Glc, $R_2 = OH, R_3 = H$
6 R_1 = Glc(6"←1"')Rha, $R_2 = OH, R_3 = H$
12 R_1 = GalGlc, $R_2 = OH, R_3 = H$
16 $R_1 = CH_3, R_2 = OH, R_3 = H$

7 $R_1 = R_2 = H$
8 $R_1 = H$, R_2 = Glc
9 $R_1 = R_2$ = Glc

13

10 R = OH; **11** R = H

17 R_1 = Glc, $R_2 = H$
18 $R_1 = H, R_2 = OCH_3$
19 $R_1 = R_2 = H$

14 $R_1 = H, R_2 = OH$; **15** $R_1 = OH, R_2 = H$

20

21 R = H; **22** R = Glc

23 R_1 = Glc, $R_2 = R_3 = R_4 = OH$
24 $R_1 = R_2 = R_3 = H, R_4 = OH$
26 $R_1 = R_3$ = Glc, $R_2 = R_4 = H$
27 R = R = R = CH , R = H

25

Fig. 1. (**1**) myricetin, (**2**) quercetin, (**3**) kaempferol, (**4**) quercitrin (quercetin 3-*O*-rhamnoside), (**5**) isoquercitrin (quercetin 3-*O*-glucoside), (**6**) rutin (quercetin 3-*O*-rhamnosylglucoside), (**7**) quercetin 5-*O*-glucoside, (**8**) quercetin 5,4′-di-*O*-glucoside, (**9**) quercetin 5,7,4′-tri-*O*-glucoside, (**10**) diplacol, (**11**) diplacone, (**12**) quercetin 3-*O*-glucosylgalactoside, (**13**) genistin (genistein 7-*O*-glucoside), (**14**) quercetin 3-*O*-arabinosyl(1‴→6″)[2″-*O*-(*E*)-*p*-coumaroyl]glucoside, (**15**) quercetin 3-*O*-arabinosyl(1‴→6″)[2″-*O*-(*E*)-*p*-coumaroyl]galactoside, (**16**) quercetin 3-methyl ether, (**17**) judaicin 7-*O*-glucoside, (**18**) 2-methoxyjudaicin, (**19**) judaicin, (**20**) maackiain, (**21**) tricin, (**22**) tricin 7-*O*-glucoside, (**23**) isoorientin, (**24**) luteolin, (**25**) isovitexin 6″-*O*-glucoside, (**26**) schaftoside, (**27**) sideroxylin, (**28**) 8-demethylsideroxylin.

transfer a glucose moiety to the C-5 hydroxy of quercetin (**4**). However, such an enzyme may be present in *B. mori*. The three flavonoid 5-glucosides were present in the cocoon shells of a range of strains of *B. mori*, so the ability to sequester these compounds could be a feature of *B. mori* and not just a strain-specific event. The 5-glucosides are more polar than quercetin (**4**) and this might influence any biological function they have in the ecology of *B. mori*. Quercetin glycosides with a sugar at C-5 are not common in plants (Harborne, 1967) and it would be interesting to know whether insects feeding on plants containing these compounds sequester them.

Hamamura et al. (1962) were one of the first groups to show that larvae of the silkworm *Bombyx mori* could differentiate among quercetin glycosides found in the leaves of *M. alba*: quercetin-3-*O*-glucoside (**5**) was a feeding stimulant, quercetin-3-*O*-rhamnoside (**4**) was a deterrent and quercetin-3-*O*-rutinoside (**6**) was inactive. It is not known whether the proportion of these glycosides in the diet of silkworms influence their ability to sequester the flavonoid 5-glucosides found in the cocoon. Recently, Bernays and Chapman (2000) showed that rutin (**6**) ingested by locusts is hydrolysed in the gut and excreted, but some is absorbed and metabolised to β-3-*O*-glucoside and sequestered in the cuticle.

4. Flavonoids on the leaf surface

Insects landing or walking on a leaf would encounter the flavonoid aglycones that can accumulate on the leaf surface before encountering the more polar glycosides that occur in vacuoles within the leaf. The aglycones on the leaf surface are thought to provide protection to the leaf. For example, resin on the surface of leaves of the shrub *Mimulus aurantiacus* Cortis contributes to 30% of the leaves dry weight (Hare, 2000a). This resin contains flavonoids and it reduced the fitness of *Euphydryas chalcedona* (Lepidoptera, Nymphalidae), a primary herbivore of the shrub (Lincoln, 1985; Lincoln and Walla, 1986). It could also contribute to lowering water loss as well as providing leaves with protection against UV light. Lincoln and Walla (1986) hypothesised that the high levels of *ortho*-dihydroxyflavonoids in the resin would be associated with the resistance of the shrub to insects. This hypothesis was based on the research by Elliger et al. (1980). They showed that *ortho*-dihydroxyflavonoids when incorporated into an artificial diet were active against the generalist *Heliothis zea*. Hare (2002a,b) studied the proportion of six flavanones in the resin of *M. aurantiacus* and showed that the relative amounts of some of the flavanones differed among populations of plants and seasons. Hare (2002a,b) found that the two most abundant flavonoids in the resin, the *ortho*-dihydroxyflavonoids, diplacol (**10**) and diplacone (**11**), occurred in both the resistant and susceptible plants and thus are not responsible for the resistance of *M. aurantiacus* to attack by *E. chalcedona*. These compounds might not be active against *E. chalcedona* because the larvae have adapted to the levels of the two compounds in their host, although the compounds may provide some protection against other herbivores. Now that the flavonoids in the resin have been identified, the contribution they make individually or in different combinations to the resistance of *M. aurantiacus* to insects could be investigated.

5. Behavioural responses to quercetin-derivatives

Results of some of the earlier research on the effects of quercetin (**4**) and quercetin glycosides on insects were collated by Harborne and Grayer (1994) and Simmonds (2001). The responses of insects to these compounds vary greatly. For example, rutin (**6**) is a phagostimulant to *Heliothis virescens* (Blaney and Simmonds, 1983) and the locusts *Schistocerca americana* (Bernays et al., 1991), *Schistocerca albolineata* (Thomas) and *Melanoplus differentialis* (Thomas) (Bernays and Chapman, 2000). Its influence on larvae of *Helicoverpa zea*, *H. armigera, Spodoptera littoralis, S. exiqua* and *S. exempta* depends on the concentration tested; at concentrations between 10^{-4} and 10^{-5} M it stimulated feeding but at higher concentration it was a deterrent (Fig. 2) (Blaney and Simmonds, 1983). Thus the role of quercetin-derivatives in insect–plant interactions appears to be complex. This complexity is illustrated by the research on the host selection behaviour of *Yponomenta* larvae that consume plants containing rutin (Van Drongelen, 1979). Caterpillars use neurons in taste sensilla on their mouthparts to detect compounds in food, many of these compounds stimulate neurons that elicit a behavioural response resulting in either food acceptance or rejection (Schoonhoven and van Loon, 2002). However, rutin might not be used by the insects to select food as electrophysiological studies on the neurons in the maxillary taste sensilla of larvae of *Yponomenta* showed that none of the neurons in either the lateral or medial maxillary styloconic sensilla responded to stimulation with rutin (van Drongelen, 1979). Similar research with the diamondback moth, *Plutella xylostella,* showed that no neurone in either the lateral or medial styloconic sensilla of larvae responded to stimulation with flavonoids from its host plants (Van Loon et al., 2002). Bernays and Chapman (2001) showed that the neural response of the medial styloconic sensilla of larvae of the polyphagous *Grammia geneura* to stimulation with quercetin (**2**) did not differ from the response to the electrolyte, KCl, although in one insect the deterrent neurone, called "cell 1″, did

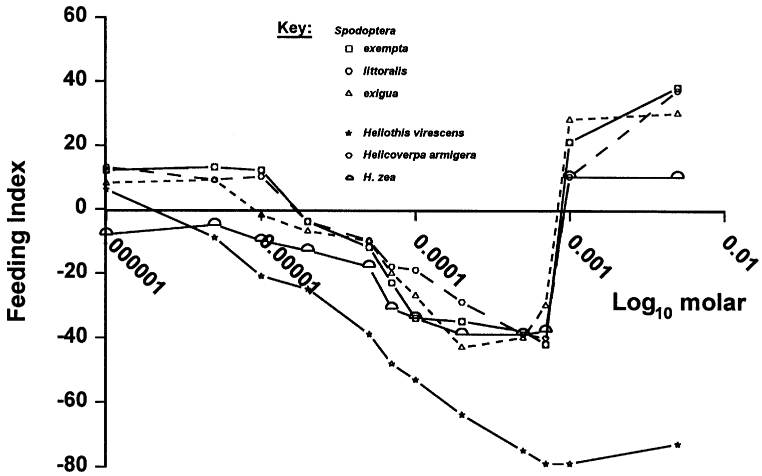


Fig. 2. Effects of rutin **6** on the feeding behaviour of *Spodoptera exempta*, *S. littoralis*, *S. exiqua*, *Heliothis virescens*, *Helicoverpa armigera* and *H. zea* (based on data presented in Blaney and Simmonds, 1983). Feeding Index ((C−T)/(C+T))% presents the mean amount of sucrose treated control (C) and rutin-treated (T) glass-fibre discs eaten in a 18 h bioassay. A negative value indicates a phagostimulant and a positive value an anti-feedant. Each species was tested with 12 concentrations of rutin and 5–10 replicates per concentration. The SEM (standard error of the mean) values have not been presented but varied from 4 to 21%; the values being higher at low concentrations.

respond. This was the same neurone in the medial sensilla that responded to the deterrent caffeine. Two neurones in the medial sensilla responded to chlorogenic acid. However, neither quercetin (**2**) nor chlorogenic acids stimulated a strong response from neurons in the lateral sensilla. Thus although the behaviour of insects can be modulated by quercetin-derived compounds we do not yet know how they perceive these compounds. When insects encounter flavonoids in plants they will also be encountering an array of other compounds. Most electrophysiological experiments have utilised single compound extracts dissolved in an electrolyte so we do not know if the neurons would detect flavonoids if presented in more complex mixtures.

Examples from recent behavioural experiments show that an insects' behavioural responses to a specific compounds can be modulated by other compounds. For example, feeding experiments with the flavonoids rutin (**6**) and quercetin 3-glucosylgalactoside (**12**), that occur in leaves of *Glycine max* Merrill, in combination with the isoflavone genistin (**13**), acted synergistically to disrupt the consumption and accumulation of food by larvae of *Trichoplusia ni* (Hoffmann-Campo, 1995 in Harborne and Williams, 2000). Quercetin 3-glucoside (**5**), that occurs in the pollen of the sunflower *Helianthus annuus* L., contributes to the stimulus that promotes feeding in the western corn root worm *Diabrotica virgifera*. However, other constituents in the pollen, such as the lipids, are thought to be more potent stimulants (Lin and Mullin, 1999). Both the lipids and flavonoids could contribute to the strength of the feeding stimulus in pollen.

A mixture of two recently characterised acylated flavonol glycosides, quercetin 3-*O*-arabinosyl-(1‴→6″)-[2″-*O*-(*E*)-*p*-coumaroyl]-glucoside (**14**) and quercetin 3-*O*-arabinosyl-(1‴→6″)-[2″-*O*-(*E*)-*p*-coumaroyl]-galactoside (**15**) in the narrowleaf vetch, *Vicia angustifolia* L., stimulate the probing behaviour of the bean aphid, *Megoura crassicauda* Mordvilko (Takemura et al., 2002). When tested individually at 1 gle/ml (gle = gram leaf equivalent) the two isomers did not have a significant effect on probing, but when combined at a concentration of 1 gle/ml they elicited a response that was equal to that produced by 60% of the crude aqueous extract of the vetch. The combination effect of the two isomers was additive rather than synergistic.

A recent study by Green et al. (2002) showed that a methanol extract from the surface of pods of a cultivar (ICPL 87) of *Cajanus cajan* Druce stimulated feeding of 5th instar *Helicoverpa armigera*. This extract contained four phenolic compounds, isoquercitrin (**5**), quercetin (**2**), quercetin-3-methyl ether (**16**) and a stilbene, 3-hydroxy-4-prenyl-5-methoxystilbene-2-carboxylic acid (Green et al., in press). The stilbene had been previously shown to occur in leaves of pigeon pea challenged with *Botrytis cinerea* (Cooksey et al., 1982). A binary choice glass-fibre disc bioassay showed that neither isoquercitrin (**5**) nor quercetin (**2**) stimulated larvae to feed, whereas quercetin-3-methyl ether (**16**) stimulated feeding but larvae consumed as much of the water-treated control disc as they did of the quercetin-3-methyl ether-treated disc (Green et al., in press). The stilbene was a potent antifeedant. Thus when the compounds were tested individually against 5th instar *H. armigera* at the concentration extracted from the pod surface, none of the four phenolics stimulated feeding in the same way as the crude methanol extract. This suggests that there must be some interaction among the compounds not only to stimulate feeding but to mask the antifeedant activity of the stilbene. Thus the phenolics were tested in combinations. Quercetin-3-methyl ether (**16**) was shown to stimulate feeding when present on both discs even when one disc contained the stilbene. However, this appetitive response was lost when one disc had the quercetin-3-methyl ether (**16**) in combination with the stilbene and the other disc had just the stilbene.

Phytochemical analysis of extracts from cultivars of *C. cajan* that varied in their susceptibility to *H. armigera* showed that the relative amounts of the four phenolics did vary among cultivars (Green et al., in press). The most susceptible cultivar was the only cultivar to contain quercetin (**2**) and this cultivar had the lowest concentration of the stilbene. Overall, the ratio of the quercetin-3-methyl ether (**16**) to the stilbene related to the susceptibility of the pigeon pea cultivars: as the proportion of the stilbene increased the susceptibility decreased. Thus the relative proportion of phenolic compounds in *C. cajan* influenced the feeding behaviour of *H. armigera* and could explain the differences in susceptibility of pigeon peas to this pest observed in field trials. It would be interesting to know if these compounds also influence the selection behaviour of the gravid female.

6. Anthocyanins

The "signals" that are released by a plant under stress can influence the biosynthesis of flavonoids and thus alter the profile of compounds in that plant. For example, when aphids feed on sorghum leaves the leaves develop red spots. These red spots are associated with the expression of anthocyanins, a response that is also associated with water-stress. The induction of the accumulation of the anthocyanin red-spots is thought to be a consequence of the de-activation of phytoalexins (3-deoxy-anthocyanidins) that occurs after insect or fungal attack. The elicitors being oligosaccharides that are produced by enzymes involved in the degrading of plant cell walls. The resistance of plants to pathogens has been attributed to an increase in anthocyanins (Stonecipher et al., 1993). However, recent research has questioned their role in making a plant more resistant to attack by insects. When sorghum leaves with the aphid-induced reddish colouration are fed to aphids there is a decrease in aphid fecundity; however, when water stressed leaves that have the same red pigmentation are fed to aphids there is no decrease in aphid fecundity (Costa-Arbulu et al., 2001). The red pigmentation in both the aphid-stressed and water-stressed leaves was qualitatively similar. The authors suggest that the red-pigmentation is a symptom that is correlated with induced resistance but is not the factor that causes a decrease in aphid fitness.

As our knowledge about the distribution and diversity of anthocyanins in grasses increases (Fossen et al., 2002) it would be interesting to see if they influence the host selection behaviour of other grass pests such as locusts and caterpillars. For example, all grasses have been found to contain glycosides of cyanidin, peonidin, pelargonidin and delphinidin, but the profiles of these anthocyanins in crops such as rice, bamboo, wheat and sorghum differ (Fossen et al., 2002). We have very little comparative information as to the influence that altering the levels of these compounds in plants has on the fitness of grass-feeding pests or on insects that differentiate among flowers depending on their anthocyanin content. A study of *Penstemon* Mitch. (Scrophulariaceae) by Scogin and Freeman (1987) showed that the type of aglycone anthocyanidin expressed in the flowers influenced the types of pollinators that visited the flowers. A study of anthocyanins in two species of *Mimulus* showed that the red flowers of *M. cardinalis* Dougl. Ex. Benth. contained more anthocyanins than occurred in the pink flowers of *M. lewisii* Pursh (Wilbert et al., 1997). The hummingbird-pollinated flowers of *M. cardinalis* contained a higher proportion of pelargonidin-derivatives than *M. lewisii*, whereas bumblebee-pollinated flowers of *M. lewisii* contained a higher proportion of cyanidin-derivatives. Blue flowers are usually pollinated by bees and Harborne and Williams (2000) have recently collated data on the different pigments in blue flowers which includes many examples of delphinidin-derivatives.

7. Isoflavonoids

Four isoflavonoids [judaicin 7-*O*-glucoside (**17**), 2-methoxy-judaicin (**18**), judaicin (**19**) and maackiain (**20**)] from different wild relatives of *Cicer* were tested against the feeding behaviour and development of *Helicoverpa armigera,* an important chickpea pest, and for activity on the feeding behaviour of four other species of noctuids (Simmonds and Stevenson, 2001). Overall, judaicin (**19**) and maackiain (**20**) were the most active compounds against *H. armigera,* but their activity varied among the bioassays and among the instars. All four isoflavonoids had antifeedant activity against *H. armigera* at 100 ppm and the larvae did not habituate to the compounds during the 8 h bioassay. Maackiain (**20**) was the most potent of the four isoflavonoids and was the only isoflavonoid to be active against *S. frugiperda*. Judaicin (**19**) was the only isoflavonoid to be active against *S. littoralis* and none of the four isoflavonoids influenced the feeding behaviour of *H. virescens* or *S. exiqua*. Thus if isoflavonoids present in wild relatives of the chickpea, *C. arietinum* L., were expressed in higher concentrations in cultivars of *C. arietinum* they might confer resistance to *H. armigera*. As yet there is no information as to whether these compounds would influence other insect pests of chickpea, especially root feeders as these compounds usually occur in roots. Previous research showed maackiain (**20**) contributes to the resistance of wild chickpea to fungal pathogens (Stevenson and Haware, 1999).

8. Tricin derivatives

A range of flavonoids were identified in *Hyparrhenia hirta* Stapf. (Poaceae), a grass that occurs in the Mediterranean region and arid parts of Africa (Bouaziz et al., 2001). A methanol extract of the plant and four of the 10 flavonoids identified in the plant were tested in a glass-fibre bioassay to see if they would influence the feeding behaviour of two locusts, *L. migratoria* and *S. gregaria,* and larvae of *Spodoptera frugiperda* (Bouaziz et al., 2001). The methanol extract stimulated feeding of all three species, and three of the flavonoids, tricin (**21**), tricin 7-*O*-glucoside (**22**) and isoorientin (**23**) also stimulated the feeding of the locusts but not *S. frugiperda*. The fourth flavonoid tested, luteolin, did not influence the feeding of the three species of insects. Tricin (**21**) and tricin-derivatives have been identified in many species in the Poaceae (Harborne and Williams, 1976) and they could play a role in the selection of grasses by insects. However, there is very little research into the role these compounds have on the feeding behaviour of Poaceae feeding insects.

9. Other flavonoids

The butterfly *Pieris napi oleracea* will oviposit on the European crucifer garlic mustard, *Alliaria petiolata* Cavara & Crande, but the larvae do not usually survive. Renwick et al. (2001) identified two compounds, alliarinoside and the flavone glycoside, isovitexin-6″-*O*-glucoside (**25**), from ethanolic extracts of leaves of *A. petiolata*. The responses of larvae to these compounds varied among the instars and were also influenced by the diet the insects were reared on. Larvae reared on cabbage were more responsive to the flavone glycoside than those reared on a wheat germ diet and larval instars 3, 4 and 5 were more responsive than 1st and 2nd instars. Larvae (4th instar) made no attempt to feed or even bite cabbage discs treated with the flavone glycoside. Results show that alliarinoside inhibits feeding by early instars through a post-ingestive mechanism, whereas the flavone glycoside **25** acts as a feeding deterrent. Further research on *A. petiolata* showed that the levels of the active flavone glycoside **25**, and alliarinoside along with a number of other flavonoids that did not influence the feeding of larvae of *P. napi oleracea*, varied among sites and seasons. The qualitative and quantitative variations in these compounds could explain the variation in susceptibility of *A. petiolata* to herbivory (Haribal and Renwick, 2001).

Flavonoid glycosides have been reported to stimulate the probing of rice planthoppers (Adjei-Afriyie et al., 2000a,b), whereas apigenin-*C*-glycosides such as schaftoside (**26**) deterred feeding (Grayer et al., 1994).

Elliger et al. (1980) reported that the unusual *C*-glycosidic flavonoid, 4″-hydroxy-maysin from *Zea mays* inhibited the development of *Heliothis zea*. Thus this compound recently isolated from the touch sensitive legume *Mimosa pudica* L. (Lobstein et al., 2002) could contribute to the resistance of this plant to generalist insect herbivores. However, this hypothesis has not yet been tested.

The *C*-methylated flavones, sideroxylin (**27**) and 8-demethylsideroxylin (**28**), and a phenylpropene derivative coniferaldehyde were isolated from *Eucalyptus saligna* (Sm.) (Sarker et al., 2001). A glass-fibre bioassay was used to evaluate the influence of these compounds on the feeding behaviour of *Spodoptera exiqua*, *Heliothis virescens* and *Locusta migratoria*. Sideroxylin was the only one of the three compounds to influence the feeding behaviour of the insects (Sarker et al., 2001). It showed mild antifeedant activity against *S. exigua* and *L. migratoria*. It is possible that these phenolics could influence the feeding of herbivores feeding on species of *Eucalyptus*.

10. Conclusions

Overall, this review of recent literature on flavonoid-insect interactions has shown that researchers are furthering our understanding of the range of flavonoids that influence host selection behaviour of phytophagous insects. However, the neural mechanisms by which these compounds modulate behaviour, especially feeding behaviour, remain unknown. In humans, we have a similar situation. We know that the taste of food-plants is influenced by the diversity and concentration of flavonoids they contain but there is no clear structure–activity relationship associated with bitterness or sweetness (Bohm, 1998). However, there are some trends in the behavioural data that suggest that the type and position of the saccharides influence the bitterness of flavonoids and many sweet flavonoids are dihydrochalcones. However, the specificity of the binding sites on human taste receptors to flavonoids that elicit sweetness or bitterness is not fully understood.

Plants currently bred for increased levels of phenolics might well influence different aspects of insect–plant interactions but as yet we do not have enough data available to evaluate what these effects might be. Studies into these interactions are needed if we are to have a better understanding of how increasing the expression of a group of compounds like flavonoids in plants could influence insect ecology.

The field of flavonoid chemistry has benefited greatly by the research undertaken by Jeffrey Harborne and his colleagues and collaborators. His team has played a key role in collating information about the ecological function and diversity of flavonoids. The significance of his contributions to this subject is illustrated by the high proportion of publication on flavonoids that make reference to Harborne et al.

Jeffrey Harborne's major contribution to flavonoid research was into the use of flavonoids in chemotaxonomy. Recently he wrote that the golden age of chemotaxonomy is long past (Harborne, 2000). However, his legacy in phytochemistry, along with the legacies of Tony Swain and Edgar Bate-Smith, provide us with fundamental phytochemical knowledge for use in modern disciplines such as evolutionary-development, molecular ecology and agricultural bio-technology.

The role of flavonoids in insect ecology was an area of research close to the heart of Jeffrey Harborne. The identification of a gustatory neural code used to modulate the behavioural response of an insect to flavonoids would be a fitting epitaph for entomologists to dedicate to Jeffrey.

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