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# FEEDING DETERRENCY OF FLAVONOIDS AND RELATED PHENOLICS TOWARDS *SCHIZAPHIS GRAMINUM* AND *MYZUS PERSICAE*: APHID FEEDING DETERRENTS IN WHEAT

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**Key Word Index**—*Triticum aestivum*; Gramineae; aphids; *Schizaphis graminum*; *Myzus persicae*; Aphididae; Homoptera; insect feeding deterrents; flavonoids; tricin; dihydrochalcones; host plant resistance.

**Abstract**—A number of naturally occurring flavonoids have been tested for their feeding deterrent activity against two aphid species, *Schizaphis graminum* and *Myzus persicae*. Most flavonoids, including a number of dihydrochalcones related to phloretin, showed strong deterrency at concentrations well within the range often found in plants. Flavanone and flavone glycosides showed weak feeding deterrency relative to their corresponding aglycones. *S. graminum* and *M. persicae* responded similarly towards the compounds tested. The feeding deterrency of wheat extracts towards *S. graminum* was confined to the phenolic fraction, which included the flavone tricin. The more polar phenolic fraction showed the strongest feeding deterrency towards *S. graminum*.

## INTRODUCTION

There has been much work on the essential components of synthetic diets for maintaining aphids [1]. On the other hand, there is only a limited amount of data available on how secondary plant constituents affect aphid growth. Such secondary plant compounds may play a major role in determining the host range of some aphid species. Four cases have been reported in which natural products act as feeding stimuli towards aphids. These are the effect of sinigrin on *Brevicoryne brassicae* [2], the alkaloid sparteine on *Acyrthosiphon spartii* [3] and various simple phenolic substances towards *Aphis fabae* [4]. The waxy *n*-alkane ($C_{32}H_{66}$) surface coating of bean leaves was attractive to *Acyrthosiphon pisum* and might play a role in the first phases of host plant selection by this aphid [5].

The adverse effects of natural products on aphids can take the form of feeding deterrence and/or cause a decrease in the growth rate or rate of reproduction. Klingauf [6] reported that the dihydrochalcone, phlorizin is a probing stimulant to *Aphis pomi* (de Geer) and *Rhapalosiphum insertum* (Walk.) both of which are hosts on apples. On the other hand, phlorizin is a deterrent to a non-host aphid, *Acyrthosiphon pisum* (Harris). In another study, phlorizin has been reported [7] to be a feeding deterrent towards *Myzus persicae* (Sulz.) and *Amphorophora agathonica*, and an ingestion deterrent towards these two species as well as *Aphis pomi*. Schoonhoven and Derksen-Koppers [8] have measured the feeding deterrency and effect on reproduction of 24 natural products towards *M. persicae*. Among the diverse substances tested were naringin which was inactive as a feeding deterrent and phlorizin which showed intense feeding deterrency. Todd *et al.* [9] tested the antibiosis of a variety of readily available phenolic materials towards the greenbug, *Schizaphis graminum* (Rondani) which can be a severe pest on sorghum, winter wheat and other small grains in the U.S.A. Among the compounds tested were the flavonoids quercetin and naringenin. The former was expecially toxic towards *S. graminum*. In a more detailed study [10], a variety of phenolic sorghum constituents were tested as feeding deterrents towards *S. graminum*. All of the feeding deterrent activity in the polar extracts of sorghum was associated with the phenolic fraction. In studies on resistance of barley to greenbugs, benzyl alcohol has been shown to influence their rate of reproduction [11, 12]. Zanthophylline, a 2-quinolone alkaloid, has been tested for feeding deterrent activity against *S. graminum* [13]. However, in this case, the results appear to be flawed since the bioassay method used was inappropriate for a sucking insect. 2-Tridecanone isolated from a wild tomato species, *Lycopersicon hirsutum* f. *glabratum* was toxic towards three aphid species including *M. persicae* [14, 15]. A correlation has been found between the concentration of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and resistance to the corn leaf aphid *Rhopalosiphum maidis* (Fitch) as well as *Metopolophium dirhodum* (Walker) in corn [16] and other Gramineae [17]. Finally, a number of diterpene acids have been surveyed as feeding deterrents towards *S. graminum* and, although the structure–activity relationships are not clear, the substances tested showed a wide range of deterrent activity [18].

## RESULTS AND DISCUSSION

### *Aphid feeding deterrency of flavonoids*

In view of the widespread occurrence of flavonoids in grasses, the fact that many members of this family are hosts to aphids and the apparent differences in the aphid feeding deterrent activity among flavonoids (see Introduction), the feeding deterrency of a number of more

polar naturally occurring flavonoids has been surveyed to see if some indication of the structure–activity relationships can be obtained.

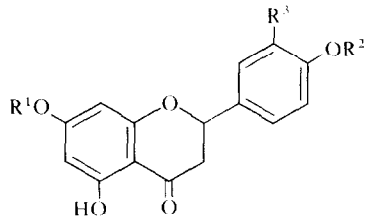
The previously described bioassay [10] was used and consisted of a synthetic diet to which compounds to be tested could be added. The percentage of aphids feeding, when compared with the appropriate controls, was a measure of the feeding deterrency. Pure compounds were tested at a series of concentrations so that a dose–response curve could be constructed. From this curve the $ED_{50}$ could be calculated. The $ED_{50}$ is the concentration of material in the diet on which half of the aphids will feed relative to the controls. The data obtained are displayed in Table 1. Because of the reported [6–8] effect of phlorizin on other aphid species, it and a series of related dihydrochalcones were included in the compounds tested on *S. graminum*. The naturally occurring dihydrochalcone (DHC), phlorizin showed the highest activity but its aglycone, phloretin, was also highly active. A closely related series of semi-synthetic dihydrochalcones used as synthetic sweeteners [19, 20] were less active by a factor of 10 (Table 1).

The flavanone glycosides were inactive at all concentrations tested whereas the corresponding flavanone aglycones showed good feeding deterrent activity. The flavonol and flavonol glycosides tested were uniformly active as feeding deterrents. Phlorizin is one of the few substances which has been tested against more than one aphid species. Phlorizin showed the same feeding deterrent activity towards both *M. persicae* and *Amphorophora agathonica* [7]. In order to gain some indication that feeding deterrents might have different

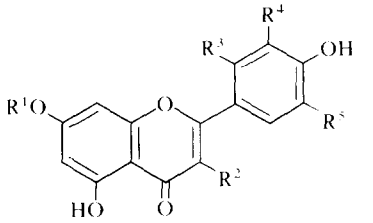
Table 1. Aphid feeding deterrency of flavonoids

| Compound | *S. graminum* $ED_{50}$ (%) | *M. persicae* $ED_{50}$ (%) |
|---|---|---|
| Dihydrochalcones: | | |
| Phloretin | 0.04 | 0.14 |
| Phlorizin | 0.02 | 0.14 |
| Neohesperidin DHC | 0.2 | 0.2 |
| Naringin DHC | 0.17 | 0.16 |
| 4′-*O*-Carboxymethylhesperetin DHC | 0.07 | |
| 4′-*O*-Sulfopropylhesperetin DHC | 0.13 | |
| 4′-*O*-β-Glucosylhesperetin DHC | 0.1 | |
| Hesperetin DHC | 0.1 | |
| Flavanones: | | |
| Naringenin | 0.15 | 0.25 |
| Naringin | No activity at 1.0% | Weak activity at <1% |
| Eriodictyol | 0.02 | 0.02 |
| Homoeriodictyol | 0.04 | 0.07 |
| Hesperetin | * | * |
| Hesperidin | No activity at 0.25% | No activity at 1% |
| Neohesperidin | No activity at 0.25% | No activity at 1% |
| Dihydroquercetin | 0.06 | 0.06 |
| Flavones: | | |
| Apigenin | † | † |
| Vitexin | 0.25 | 0.1 |
| Luteolin | 0.03 | |
| Luteolin 7-*O*-glucoside | No activity at 1.0%‡ | Weak activity at <1% |
| Tricin | † | * |
| Tricin 4′-*O*-methyl ether | | * |
| Flavonols: | | |
| Quercetin | 0.08 | 0.03 |
| Quercitrin | 0.06 | 0.06 |
| Rutin | 0.02 | Weak activity at <1% |
| Rhamnetin | † | † |
| Morin | 0.04 | 0.04 |
| Myricitrin | 0.07 | |
| Misc. phenolics: | | |
| Chlorogenic acid | 0.2 | 0.35 |
| *p*-Hydroxybenzaldehyde | 0.13‡ | 0.4 |
| *p*-Hydroxybenzoic acid | 0.36‡ | 0.5 |

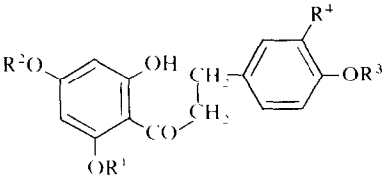
*Active but $ED_{50}$ uncertain due to low solubility of substrate in diet.
†Apparently inactive but substrate has low solubility in diet.
‡Data from ref. [10].



| | |
|---|---|
| Naringin | $R^1$ = Neohesperidosyl; $R^2 = R^3 = H$ |
| Naringenin | $R^1 = R^2 = R^3 = H$ |
| Hesperidin | $R^1$ = Rutinosyl; $R^2$ = Me; $R^3$ = OH |
| Hesperetin | $R^1$ = H; $R^2$ = Me; $R^3$ = OH |
| Eriodictyol | $R^1 = R^2$ = H; $R^3$ = OH |
| Homoeriodictyol | $R^1 = R^2$ = H; $R^3$ = OMe |
| Neohesperidin | $R^1$ = Neohesperidosyl; $R^2$ = Me; $R^3$ = OH |
| Eriocitrin | $R^1$ = Rutinosyl; $R^2$ = H; $R^3$ = OH |



| | |
|---|---|
| Apigenin | $R^1 = R^2 = R^3 = R^4 = R^5 = H$ |
| Kaempferol | $R^1 = R^3 = R^4 = R^5$ = H; $R^2$ = OH |
| Luteolin | $R^1 = R^2 = R^3 = R^5$ = H; $R^4$ = OH |
| Quercetin | $R^1 = R^3 = R^5$ = H; $R^2 = R^4$ = OH |
| Quercitrin | $R^1 = R^3 = R^5$ = H; $R^2$ = rhamnosyloxy; $R^4$ = OH |
| Rutin | $R^1 = R^3 = R^5$ = H; $R^2$ = rutinosyloxy; $R^4$ = OH |
| Rhamnetin | $R^1$ = Me; $R^2 = R^4$ = OH; $R^3 = R^5$ = H |
| Morin | $R^1 = R^4 = R^5$ = H; $R^2 = R^3$ = OH |
| Myricitrin | $R^1 = R^3$ = H; $R^2$ = rhamnosyloxy; $R^4 = R^5$ = OH |
| Vitexin | 8-*C*-Glucosylapigenin |
| Tricin | $R^1 = R^2 = R^3$ = H; $R^4 = R^5$ = MeO |



| | |
|---|---|
| Phlorizin | $R^1$ = Glc; $R^2 = R^3 = R^4$ = H |
| Phloretin | $R^1 = R^2 = R^3 = R^4$ = H |
| Neohesperidin DHC | $R^1$ = H; $R^2$ = neohesperidosyl; $R^3$ = Me; $R^4$ = OH |
| Naringin DHC | $R^1 = R^3 = R^4$ = H; $R^2$ = neohesperidosyl |
| 4′-*O*-Carbomethylhesperetin DHC | $R^1$ = H; $R^2$ = $-CH_2COOH$; $R^3$ = Me; $R^4$ = OH |
| 4′-*O*Sulfopropylhesperetin DHC | $R^1$ = H; $R^2$ = $-CH_2CH_2CH_2SO_3H$; $R^3$ = Me; $R^4$ = OH |
| 4′-*O*-β-Glucosylhesperetin DHC | $R^1$ = H; $R^2$ = Glc; $R^3$ = Me; $R^4$ = OH |

degrees of activity towards different aphid species some of the flavonoids were also tested against *M. persicae* (Table 1).

The feeding deterrent activity of the phenolics tested against *M. persicae* paralleled their activity against *S. graminum*. With the exceptions of vitexin and quercetin, *M. persicae* was slightly more tolerant of the flavonoids than *S. graminum*. This may be a reflection of the wider host range of *M. persicae* compared to *S. graminum*. The latter is largely confined to a limited number of the Gramineae as hosts. As a result of the wider host range, *M. persicae* must cope with a greater variety of phenolic substances and may well have evolved the necessary mechanism for dealing with a variety of phenolic materials.

Although the $ED_{50}$ is a convenient way to express the biological results, the actual situation may be more complex. This is illustrated in Fig. 1 which shows the dose–response curve for quercitrin and dihydroquercetin.

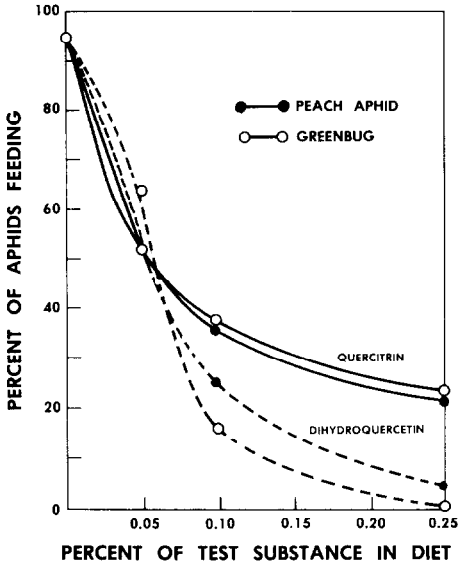



Fig. 1. Dose–response curve for quercitrin and dihydroquercetin. For all data points s.e. $\leqq \pm 5\%$.

The plot shows that even though the two compounds have about the same $ED_{50}$ (0.06%), at higher concentrations (0.25%) quercitrin is much less active than dihydroquercetin.

As the data in Table 1 indicate, the concentrations of flavonoids which have an affect on aphid feeding are well below the level at which such substances are often found in plants. On this basis it might be expected that flavonoids would exert a profound effect on aphid feeding in the plant. That this is not often the case is probably due to the selective feeding of aphids on tissues which are relatively low in deterrent flavonoids. In plants, flavonoids are often restricted to specific storage tissues. While they may be translocated to the phloem [21] the steady-state level is probably low relative to their concentration in storage tissues [22]. As a result, since aphids are often phloem feeders or feed on specific tissues they may not normally encounter high flavonoid concentrations. Moreover, the flavonoids involved in phloem transport are very likely in the form of their glycosides, and as shown in this study, the flavanone and flavone glycosides are largely inactive as feeding deterrents.

The widely different response of greenbug to different flavonoids raises questions regarding the mode of action of these substances. The inactivity of the flavanone and flavone glycosides compared with their aglycones is the most striking difference. The feeding deterrent results, to a large degree, parallel the antibacterial action of flavonoids against three different test organisms [23]. Thus flavonoid glycosides were inactive, flavonols (morin most active) were more active than the flavones, and dihydroquercetin was more active than quercetin as antibacterial agents. These results suggest that the major influence of feeding deterrent phenolics may be on the microflora rather than on the aphid itself. The microbiological degradation of flavonoids to smaller fragments has been well studied [24] and the classic case in higher animals is the action of bovine rumen microflora on flavonoids [25, 26]. The pattern of activity against aphids found for flavonoids in this study is very different from that reported by Elliger *et al.* [27] against the chewing insect *Heliothis zea*.

If the dietary flavonoid passed through the aphid without modification, one might expect it to accumulate, unchanged, in the honeydew along with the excess sugar which is not utilized by the insect. On the other hand, hydrolysis of flavonoid glycosides by the aphid might lead to the accumulation of aglycones in the honeydew. To test these possibilities greenbugs were allowed to feed on diets with high (1%) naringin and luteolin-7-glucoside concentrations. Neither of these compounds showed feeding deterrent activity, so high feeding rates were obtained. The honeydew was collected and examined for phenolic compounds by paper chromatography and UV spectroscopy but there was no indication of any flavonoids. These results suggest that the aphid, or perhaps more especially its symbiotic flora, has degraded the substrates to non-flavonoid fragments.

*Aphid feeding deterrents in wheat*

A search for aphid feeding deterrent compounds in a *S. graminum* (biotype C) [28] resistant line of wheat was also undertaken. This biotype is a severe pest on wheat in the Pacific Northwest of the United States. The bioassay was used as a guide so that activity could be followed in the fractionation procedures in the same way as previously described [10] for monitoring feeding-deterrent activity of sorghum fractions and constituents towards *S. graminum*.

Two lines of wheat were studied for their greenbug feeding deterrent activity. One was Amigo, a greenbug-resistant line and the other was Triumph 64, a greenbug-susceptible line [29, 30]. The methanol extracts of each, after removal of non-polar waxes, chlorophyll and solvent showed modest aphid feeding deterrent activity. When the methanol extracts were passed through a column of XAD-2, all the feeding deterrent activity was confined to the fraction which was adsorbed on the XAD. The XAD non-retained material, which contained large amounts of carbohydrate, was devoid of feeding deterrent activity. The bulk of the XAD-retained material was phenolic as judged by TLC and viewing under UV light with $AlCl_3$ or spraying with $FeCl_3$. Most of the XAD-retained material was very polar and was separated into ethyl-acetate-soluble and -insoluble fractions. The small amount of

ethyl-acetate-soluble material was chromatographed on silicic acid to give tricin, a known component of wheat and other grasses [31, 32].

The more polar ethyl-acetate-insoluble phenolic fraction was chromatographed on Sephadex LH-20 with methanol. Feeding deterrent activity was distributed through all of the collected fractions. These fractions appeared largely to contain the mixtures of glycoflavones previously reported [33] from wheat (see Experimental). Both lines of wheat gave essentially the same results. At this juncture, information became available on the mode of feeding for *S. graminum*, biotype C (B. C. Campbell, private communication). In view of these results, and the results detailed in the preceding section, it appeared more profitable to abandon work utilizing whole-plant extracts in favor of an approach in which chemical composition work could be confined to just those tissues on which the insect feeds.

## EXPERIMENTAL

*Bioassay.* The same bioassay technique and diet was used for determining feeding deterrency of compounds to *M. parsicae* as that previously described [10] for *S. graminum*, except that the percentage of feeding was determined after 8 hr instead of 24 hr. This shorter period was necessary for those cases where strong feeding deterrents were being tested since many of the peach aphids which did not feed on the diet died within 24 hr. On the other hand, no fatalities occurred within 8 hr and the controls ran at 92–96% feeding in 8 hr.

*Isolation.* The isolation procedure used was the same for both Amigo and Triumph 64 varieties. Stems and leaves of wheat were dried, ground and extracted with MeOH. The MeOH extracts were treated with charcoal to remove non-polar waxes and fats. Solvent was removed from the extracts and the residue warmed with repeated batches of EtOAc. The EtOAc-soluble fraction was chromatographed on silicic acid. Those fractions showing a $FeCl_3$ positive spot were combined, and solvent removed to give a residue which was crystallized from MeOH to give tricin. The UV and IR spectra were identical with those of a sample isolated from alfalfa.

The EtOAc-insoluble material was taken up in water, filtered to remove any water-insoluble material and filtered through a column of XAD-2. The column was washed with a large amount of water to remove non-aromatic materials. This fraction was freeze-dried and the residue tested for feeding deterrency. The XAD column was then washed with a large vol. of MeOH until the eluants gave a negative $FeCl_3$ test. Solvent was removed from the MeOH eluants. The residue was tested for feeding deterrency. This residue was chromatographed on Sephadex LH-20 with MeOH. The feeding deterrency was distributed through all of the collected fractions. These fractions were monitored by TLC and components detected with $AlCl_3$/UV light or $FeCl_3$.

*Metabolism of naringin and luteolin 7-glucoside.* Diet capsules were made up with either 1% naringin or luteolin 7-glucoside, as previously described [10]. The capsules were mounted in a plastic vial cap which fitted snugly on a 35-ml glass vial. For each substrate, 10 such vials were loaded with about 100 greenbugs each. After the aphids had fed for 3 days the caps were removed and the honeydew taken up in MeOH. The MeOH-soluble honeydew from each vial was pooled for each substrate and concd to about 1 ml. The MeOH extracts were paper chromatographed (15% HOAc) along with appropriate standards and controls, and separate papers sprayed with $FeCl_3$, diazotized sulfanilic acid and $AlCl_3$ with examination under UV light. The UV spectra of the MeOH extracts were also examined with added $AlCl_3$ and NaOH shift reagents.

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