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The presence or absence of phenolglycosides in *Salix* (Salicaceae) leaves and the level of dietary specialisation of some of their herbivorous insects

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Summary. European *Salix* species fall into at least 2 groups on the nature of the secondary compounds in their leaves. Some species such as *S. nigricans*, *S. purpurea* and *S. fragilis* contain phenolglycosides in their leaves. Additionally, there are species such as *S. alba, S. caprea* and *S. cinerea* with leaves containing no phenolglycosides.

Using published food plant list for the weevils, the sawflies and the caterpillars of the British moth, I tried to see if the presence or absence of phenolglycosides in the willow leaves is related to the degree of dietary specialisation of the insects feeding on these leaves.

The results show that the *Salix* with phenolglycosides tend to be the the food of the specialised herbivores and to be avoided by generalist. Conversely, *Salix* without phenolglycosides tend to be eaten by more generalist insects and are avoided by the more specialised.

Moreover, the faunas of the different *Salix* species with phenolglycosides in their leaves are more similar to each other than to the faunas of the *Salix* species having no phenolglycosides in their leaves.

Introduction

European *Salix* fall into at least two groups on the nature of the secondary compounds in their leaves. Some species, such as *S. babylonica, S. fragilis, S. incana, S. nigricans, S. pentadra, S. purpurea, S. repens* and *S. triandra* contain in their leaves phenolglycosides, e.g. salicin, but no proanthocyanidins, a form of condensed tannins (Hegnauer 1973). The surfaces of these leaves are usually glabrous. Additionally, there are species such as *S. alba, S. aurita, S. caprea, S. cinerea* and *S. viminalis* with leaves containing no phenolglycosides but rich in proanthocyanidins (Hegnauer 1973). At least the lower surfaces of these leaves are usually covered with trichomes.

The larvae of some species of willow-feeding Chrysomelinae, such as *Phratora vitellinae, Chrysomela populi, C. tremulae* and *C. 20-punctata* use salicin, one of the phenolglycosides present in the leaves on which they feed, as a precursor of the salicylaldehyde they secrete for their own defense (Rowell-Rahier and Pasteels 1982; Pasteels et al. 1983). The primitive defensive secretion of the larvae of this group of beetles consists of methylcyclopentanoid monoterpenes, which are probably autogenously synthesized (review in Pastells et al. 1982). The secretion of aromatic compounds such as salicylaldehyde is thought to be a secondary adaptation subsequent to a shift of host plant from herbs to trees and shrubs (Pasteels, in press). The first group of *Salix* species may have provided appropriate hosts for the initial switch from herbs to trees. These trees, without proanthocyanidins in their leaves, may be more accessible and easier to digest for herbivores adapted to feeding on herbs.

Salicin does not influence the food plant preference of *Ph. vitellinae;* rather, the trichomes on the leaves of the willow species not containing phenolglycosides act as a mechanical deterrent and determine food plant choice (Rowell-Rahier and Pasteels 1982). *Ph. vitellinae* is however a specialised herbivore. Although there is some geographic variation, each local population can show a clear food preference for one of the willows with salicin-rich leaves (Rowell-Rahier, in preparation).

Salicin is known to be toxic if taken internally, the salicylate produced when it is hydrolysed and oxidided uncouples oxidatives phosphorylation in mitochondrial preparations (Marks et al. 1961). Phenolic compounds e.g. salicylic acid may protect higher plants against invasion by fungi or viruses through inhibition of their growth (Vickery and Vickery 1981). Salicin, and the other phenolglycosides present in the leaves of some willow species, may act as wide range feeding deterrents against non-adapted herbivores and thus protect the willow leaves from herbivore damage. Salicin has been show to have a deterrent effect against the mammalian herbivore Opossum (*Trichoserus vulpecula*). The palatability of 50 different *Populus* clones to caged Opossum was inversely correlated with the level of salicin in the leaves (Edwards 1978). Salicin is also a deterrent for *Ph. vulgatissima*, a willow-feeding Chrysomelinae which retains the monoterpene defensive secretion and does not normally feed on those *Salix* species with leaves rich in phenolglycosides (Rowell-Rahier, personal observations). If phenolglycosides play a part in repelling herbivores, their presence or absence in the leaves should be related to the level of dietary specialisation in insects feeding on *Salix*. To test this hypothesis is the primary aim of this study.

With a cluster analysis method, Berenbaum (1981) showed that the faunas of Umbelliferous plant species with similar secondary chemistry are more similar to each other than the faunas of the species having different chemistry. If the presence or absence of phenolglycosides on the one hand or of proanthocyanidins and hairs on the other is one of the factors influencing food plant choice by willow-feeders, willow species with similar secondary chemistry, for these two groups of compounds, should have more similar faunas than those with different chemistry. I have tested this hypothesis by cluster analysis.

Material and methods

Information on the food plants of weevils (Hoffman 1958), sawflies (Lorenz and Krauss 1957) and the caterpillars of the British moths (South 1948) was collected from published data.

In this paper, insects feeding only on Salicaceae are defined as oligophags and insects feeding on Salicaceae as well as on at least one other genus of host plant not belonging to the Salicaceae are defined as polyphags. Data on the food plants of both categories are considered in this analysis. Insects feeding on *Populus* only are, however, not taken into account because available data on the secondary chemistry of *Populus* leaves and on host plant species are incomplete.

I divided the willow feeding insects according to the chemistry of the leaves they eat and the number of *Salix* species or the number of families of host plants from which they are recorded. The probability of chance occurrence of the resulting contingency table was calculated by the G statistic or the Fisher exact test (Sokal & Rohlf 1969). To ensure the independence of the two classifications used, the insects feeding on both types of *Salix* leaves are excluded from the analysis when the number of *Salix* species eaten are considered.

The secondary chemistry of willow leaves is summarized in Hegnauer (1973). More detailled results can be found in the work of Thieme (1965, 1970). To confirm these data for some common European willows (*S. alba, S. caprea, S. cinerea, S. fragilis, S. purpurea* and *S. nigricans*) I made qualitative and quantitative phenolglycosides analysis of methanolic extracts of their dried leaves using the method described by Sticher et al. (1981). No deviation from the results summarized in Hegnauer was found.

Hierarchical cluster analysis (Barr et al. 1975) was performed to help identify clusters of willow species that have similar fauna of weevil and sawflies. All the sawflies and weevils for which adequate data were found were pooled for this analysis. Two different coefficients were used; first a binary coefficient reflecting the presence (1) or the absence (0) of each insect species on each species of willow and, secondly a weighting coefficient equal to the inverse of the number of genera of host plants (Berenbaum 1981). The latter coefficient gives more weight to the choice of specialised herbivores (oligophags). Further analyses were carried out after separating the oligophagous and the polyphagous insects. Additionally, a weighting coefficient equal to the number of genera of host plant was used for the polyphags, thus stressing the importance of the food plant choice of the more polyphagous insects.

Results

1 Partition of the different willow species by weevils and sawflies according to the chemistry of the Salix leaves they eat

The distribution of the sawflies and the weevils according to the chemistry of the leaves they eat and their degree of food specialisation is homogeneous for the two taxonomic groups ($P > 5\%$ by G statistic or Fisher exact test) and both sets of data can therefore be pooled. The sawflies and weevils feeding on *Salix* are rather specialised, 95 (75%) of the 127 species for which adequate data were found feed exclusively on plants belonging to the Salicaceae (oligophags) (Table 1).

Table 1. Distribution of the weevils and sawflies feeding on *Salix*

Secondary chemistry of the *Salix* leaves eaten	Number of families of host plants		
	1: Oligophags	2 or more: Polyphags	Total
Phenolglycosides absent	54	19	73
Phenolglycosides present	41	13	54
Total	95	32	$N = 127$ $G_{adj.} = 0$

Table 2. Distribution of sawflies and weevils feeding on *Salix*

Secondary chemistry of *Salix* leaves eaten	Number of *Salix* species eaten		
	1 or 2	3 or more	Total
Phenolglycosides absent	61	12	73
Phenolglycosides present	17	0	17
Total	78	12	$N = 90$ $P_{Fisher} = 0.07$

The subdivision of the weevils and the sawflies into oligophags (feeding only on the Salicaceae) and polyphags (feeding on at least one other family of host plant) is independent of the presence or absence of phenolglycosides in the leaves they eat (Table 1, $P > 5\%$). It is however worth noticing that the polyphagous species feeding on *Salix* leaves rich in phenolglycosides are not extremely polyphagous. They feed on only one or two of the following other genera of food plants (*Alnus, Betula, Quercus, Ulmus, Rosa* and *Rumex*). The other food plants of the polyphags feeding on the *Salix* leaves devoid of phenolglycosides are more numerous and taxonomically diverse. For the sawflies alone, they include *Atropa, Betula, Carex, Coryllus, Crataegus, Fraxinus, Galeopsis, Laurus, Mentha, Plantago, Prunus, Quercus, Rosa, Rubus, Senecio, Solanum, Solidago, Sorbus, Stachys, Tussilago* and *Veronica*.

The number of weevils and sawflies feeding on each of the two categories of willow leaves and the number of *Salix* species they are recorded from is given in Table 2. We notice that a large proportion (81%) of the species feed on *Salix* without phenolglycosides in their leaves and that all the species feeding exclusively on *Salix* with leaves rich in phenolglycosides are extremely specialised, feeding on just one or two species of *Salix*, whereas those feeding on leaves devoid of phenolglycosides are less specialised. This distribution yields by the Fisher exact test a probability of chance occurrence of 7%. The secondary chemistry of the leaves thus shows some correlation with the degree of specialisation among the insect feeding upon them.

2 Partition of the different willow species by the caterpillars of the british moths according to the chemistry of the leaves they eat

This group of herbivores is globally much more polyphagous than the weevils and the sawflies; 83% of the 133

Table 3. Distribution of the caterpillars of the British moths feeding on *Salix*

Secondary chemistry of *Salix* leaves eaten	Number of families of host plants		
	1: Oligophags	2: or more Polyphags	Total
Phenolglycosides absent	4	98	102
Phenolglycosides present	20	11	31
Total	24	109	$N = 133$ $G_{adj.} = 47.81$

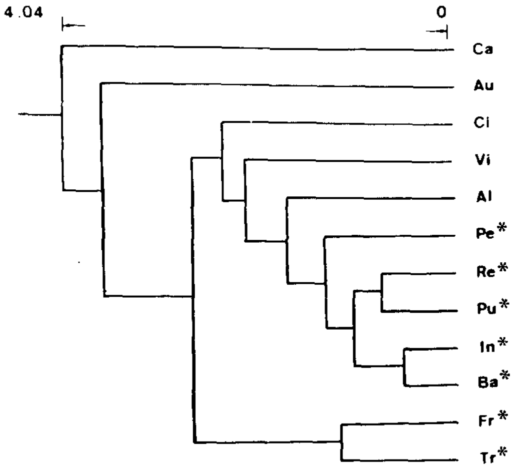

Fig. 1. Cluster analysis showing similarity of *Salix* species using the presence/absence data of all their sawflies and weevils herbivores. The units of the abscissa give the maximum diameter of a cluster in Euclidean metric (see Barr et al. 1979). *Abbreviations: Ca = S. caprea; Au = S. aurita; Ci = S. cinerea; Vi = S. viminalis; Al = S. alba; Pe = S. pentandra; Re = S. repens; Pu = S. purpurea; In = S. incana; Ba = S. babylonica; Fr = S. fragilis; Tr = S. triandra;* * = species containing phenolglycosides in their leaves

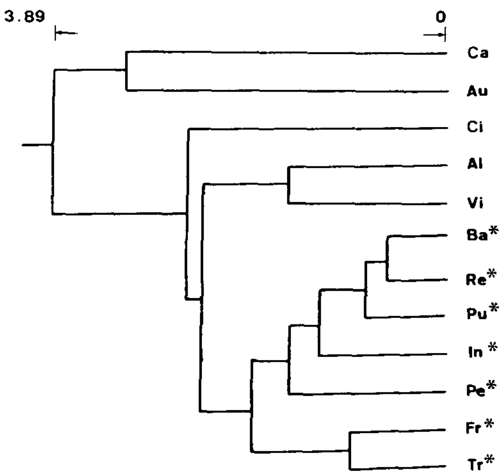

Fig. 2. Cluster analysis showing similarity of *Salix* species using the presence/absence data of only the oligophagous sawflies and weevils. Abbreviations and units see Fig. 1

species feeding on *Salix* are polyphagous, that is, they feed on at least two families of host plants.

Table 3 shows that for this sample of caterpillars dietary specialisation is not independent ($P < 0.001$) of the secondary chemistry of the leaves, when dietary specialisation is measured as the number of families of host plants. The majority (61%) of the caterpillars tolerating phenolglycosides feed only on the Salicaceae whereas a large proportion (91%) of the caterpillars not normally feeding on phenolglycoside-rich leaves are polyphagous.

Analysis could not be carried to the level of the number of *Salix* species eaten because the caterpillars are usually recorded from only one or two species of *Salix,* mostly *S. caprea.*

3 The number of herbivores species recorded on the two chemical categories of willow species

In both the predominantly oligophagous groups (weevils and sawflies) and the largely polyphagous (caterpillars of the British moths) group of phytophagous insects studied, species richness is higher on *Salix* species without phenolglycosides in their leaves but rich in proanthocyanidins (see last columns of Tables 1 and 3).

4 Faunal similarities between the different Salix species

4.1 Oligophags and polyphags. When a binary coefficient reflecting the presence or absence of each weevil and sawfly species on the different willow species is used for the calculations, the species with phenolglycosides form two distinct clusters and are therefore not as clearly separated from those without phenolglycosides as in the following cluster analyses. Indeed, *S. alba, S. viminalis* and *S. cinerea* which do not have phenolglycosides in their leaves are grouped with the *Salix* species rich in phenolglycosides in their leaves (Fig. 1).

4.2 Oligophags. When the cluster analysis is carried out on the basis of the presence or absence of only the oligophagous weevils and sawflies on the different willows, the *Salix* species with leaves rich in phenolglycosides are grouped in a cluster. The *Salix* species without phenolglycosides in their leaves but rich in proanthocyanidins show no particular faunal similarity (Fig. 2).

4.3 Polyphags. When the analysis is made only on the basis of the presence or absence of polyphagous weevils and sawflies on the different willows, the resulting similarities between willow species do not reflect in any way the presence or absence of phenolglycosides and proanthocyanidins in the leaves (Fig. 3).

4.4 Weighting coefficient. When for each insect species (oligophags and polyphags) a weighting coefficient equal to the inverse of the number of genera of host plants is used, the resulting pattern (Fig. 4) is identical to the one obtained on the basis of the presence or absence of the oligophagous species on the different willows (Fig. 2).

When however for each polyphagous species a weighting coefficient equal to the number of genera of host plants is used, the willows with phenolglycosides in their leaves appear extremely similar in their faunas and those with proanthocyanidins very different (Fig. 5).

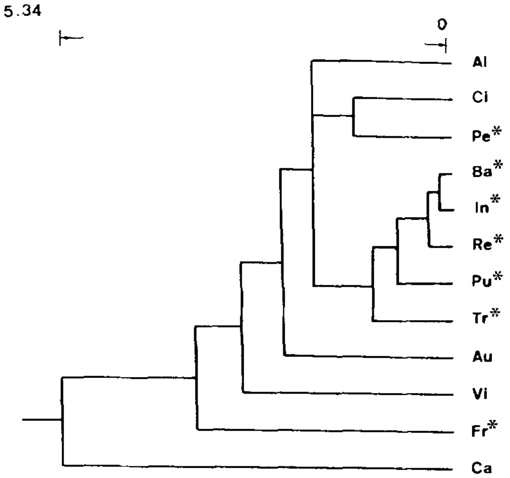

Fig. 3. Cluster analysis showing similarity of *Salix* species using the presence/absence data of only the polyphagous sawflies and weevils. Abbreviations and units see Fig. 1

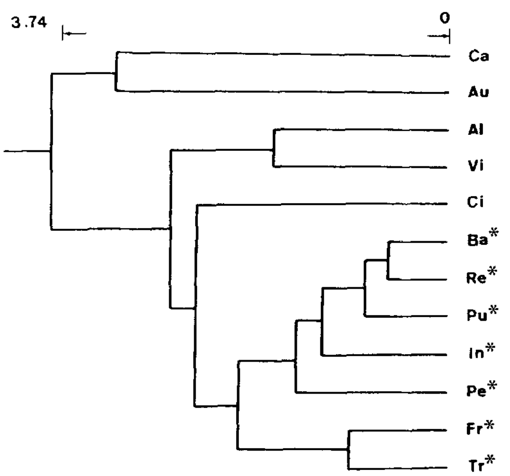

Fig. 4. Cluster analysis showing similarity of *Salix* species using weighted data for all their weevils and sawflies herbivores. Abbreviations and units see Fig. 1

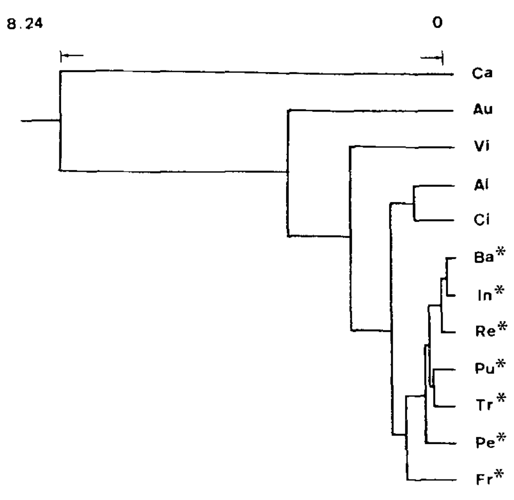

Fig. 5. Cluster analysis showing similarity of *Salix* species using weighted data for the polyphagous weevils and sawflies alone. Abbreviation and units see Fig. 1

Discussion and conclusions

1 The use of faunal lists

I have utilised published faunal lists to give the number of insect herbivore species feeding on willow leaves with different secondary chemistry. Southwood et al. (1982a and b) showed that knockdown sampling and use of faunal lists lead to comparable estimates of species richness.

The faunal list may not give the complete host plant range of the different herbivore species. I think, however, that it is reasonable to assume that it does not bias the data: indeed all the willow species taken into account in this study are common and have similar geographic distributions. There is no reason to believe that the host plant range of the faunas of the willow species belonging to one chemical category might be systematically under or overestimated. Rather I consider the host plant range established from the faunal list as resulting from random sampling.

2 The dietary specialisation of the willow-feeding insects

Within each taxonomic group, the insect species tolerating phenolglycosides in their food tend to have a narrower diet breadth than those not recorded on *Salix* species rich in phenolglycosides. This difference in the degree of food plant specialisation is observed at different host plant taxonomic levels which seems to vary with the more global feeding habits of the insect group.

The weevils and the sawflies feeding on willows are mostly oligophagous. There is then a correlation between food plant secondary chemistry and dietary specialisation when this is estimated as the number of *Salix* species eaten (Table 2). If however it is estimated by the number of families of host plants eaten such a correlation is not found (Table 1). Presumably other factors, such as host plant forms, size, habitat or secondary chemistry other than that of phenolglycosides and proanthocyanidins, play a more important role in determining the distribution of these mostly oligophagous insects between families of host plants, whereas the exact composition of the different phenolglycosides mixtures in the leaves of each *Salix* might be instrumental in determining preference or avoidance of the different *Salix* species.

On the other hand, the caterpillars of the British moths are largely polyphagous and in this case a correlation between food plant chemistry and the degree of dietary specialisation is found when the latter is measured as the number of families of host plant eaten. The presence or absence of phenolglycosides might play a role in the preference or avoidance of the Salicaceae by these mostly polyphagous caterpillars.

Both the lower number of herbivores found on the willows with leaves rich in phenolglycosides and the higher degree of dietary specialisation of these insects is consistent with the idea that the phenolglycosides have a defensive function in willow leaves.

3 The faunal similarities between the different willow species

A corroboration of the protective function of phenolglycosides in willow leaves is afforded by the faunal similarities found between the different *Salix* species. If the degree of dietary specialisation of *Salix* herbivores is linked to the presence or absence of phenolglycosides or proanthocyanidins in the leaves then these chemicals probably influence food plant choice proximately or ultimately. In this case, the faunas of the different willow species with similar chemistry, as far as the presence or absence of phenolglycosides and proanthocyanidins are concerned, should be more similar to each other than the faunas of *Salix* species having different secondary chemistry. The results of cluster analyses show that this is the case for the *Salix* species with leaves rich in phenolglycosides.

On the contrary *Salix* with leaves rich in proanthocyanidins show virtually no similarity in their faunas. The presences of proanthocyanidins (and trichomes) in the leaves does not seems to require special adaptation from the insects feeding on these *Salix* species.

4 The coefficient used in cluster analyses

When the cluster analysis is made simply on the basis of the presence or absence of each insect species (oligophags and polyphags) on the different willow species, the similarity pattern between the willows with phenolglycosides in their leaves does not show clearly. It is probably masked by the effect of other factors likely to play a role in food plant choice, particularly that of the non-oligophags.

For the mostly oligophagous sawflies and weevils, the pattern of similarity is however clear when only the oligophagous species are used in a binary cluster analysis. Thus, as proposed by Berenbaum (1981), the use in the cluster analysis of a weighting coefficient stressing the importance of oligophags, seems, at least in the case of a rather specialised group of herbivores such as the weevils and the sawflies, to reflect the chemical ressemblance between the different willow species better than a binary coefficient.

On the other hand, when only the polyphags are used in the cluster analysis, a pattern of faunal similarities matching the presence of phenolglycosides in the leaves is only obtained when the food plant choice of the more generalised feeder among the polyphags is given more weight (with a coefficient equal to the number of genera of host plant). This probably reflects the avoidance of the leaves rich in phenolglycosides by the extremely polyphagous insects.

As pointed out by Lawton (1978), the effect of secondary chemistry on food plant choice by herbivores is difficult to demonstrate unambiguouly. I would like to suggest that the herbivore species with particularly narrow or wide food plant ranges might show these effects more clearly and that the importance of those species should be stressed when looking for effects of secondary chemistry of the leaves on the food plant choice of a group of herbivores.

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