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INFLUENCE OF PHENOLGLUCOSIDES ON THE DISTRIBUTION OF HERBIVORES ON WILLOWS

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Variation in the biochemical characteristics of host plants and their importance as key factors in explaining the distribution of herbivores is a widely studied area (review in Rosenthal & Janzen, 1979). First we will review briefly some of the evidence showing how one group of plant secondary compounds, the phenolglucosides, present in the leaves of willows and poplars, could influence herbivore distribution, and more particularly the leaf beetles of the subfamily Chrysomelinae which are important among the herbivores causing leaf damage to willows.

According to Thieme (1965) and Palo (1984) phenols are the only group of secondary metabolites present in significant amounts in the leaves of the Salicaceae. However, they are not present in the leaves of all species of willow and poplar. The toxicity of these phenolglucosides is established (Vickery & Vickery, 1981).

In a recent study, Tahvanainen et al. (1985) showed that the concentration and the composition of the different phenolglucoside blends are species specific in willows. For 4 common leaf beetle species (*Phratroa vitellinae*, *Plagioderma versicolora*, *Lochmaea caprea*, *Galerucella lineola*), The patterns of food plant selection observed in multiple choice preference experiments are closely related to the phenolglucoside spectra of the willows tested. Indeed, the second choice of the beetles was always the willow species which was chemically the most similar to the preferred host plant. The phenolglucoside blends can have both stimulatory and inhibitory influences on the leaf beetles.

The phenolglucosides of the leaves can also be used as precursors of the glandular defensive secretion of the larvae. Indeed, the secretion of salicylaldehyde by the larvae of several chrysomeline species feeding on salicaceous trees offers an excellent example of the use of plant secondary compounds in insect defense (review in Rowell-Rahier & Pasteels, 1986). Pasteels et al. (1983) have previously demonstrated the utilisation of the phenolglucoside salicin as the substrate for the production of salicylaldehyde. Since then, using the methods described by Rowell-Rahier & Pasteels (1982), we have investigated the role of salicortin, a further phenolglucoside found in the leaves of many willows, and have demonstrated that this compound, an acetylated salicin derivative, can also be used by the larvae of the beetle *Ph. vitellinae* as a precursor of salicylaldehyde.

Smiley et al. (1985) recently studied the relationship between a Californian Sierra Nevada beetle (*Chrysomela aenicollis*) and its salicaceous host plants. The willow leaves they encounter in the field have

highly variable concentrations of salicin, salicortin and tremulacin. No other phenolglucosides could be detected in significant amounts. In leaves of *S. lasiolepis* the salicin concentration varied between neighbouring plants by one hundred fold (from 0.08 to 8% DW). Damage due to herbivory is significantly higher on plant specimens rich in salicin and beetle larvae placed on high salicin plants have a higher survival rate.

The importance of phenolglucosides as defensive precursors for some chrysomelinae thus established, we decided to compare the influence of phenolglucosides (mainly salicin and salicortin) on the distribution in the field of herbivores using and not using these compounds for their own benefit. This is the subject of this paper.

An ideal site for this study was offered by a plantation (Gramont, Belgium) of small willow trees which included both *S. alba* and *S. fragilis* together with numerous hybrids between these species. *S. alba* contains very little or no phenolglucosides in its leaves whereas *S. fragilis* is rich in phenolglucosides. 89 individual trees were randomly selected. A sample of the leaves of each of them was collected and analysed for phenolglucoside content. All herbivores present on these trees were counted; this was possible only because of the small size of the young trees. Variation in phenolglucosides and in insect distribution within individual trees was not considered in this study. Detailed data are presented in Soetens (1986). We will discuss only the results which concern the 3 main herbivore species encountered. These are:

Phratora vitellinae, a Chrysomelinae, the larval of which use phenolglucosides for their defense;

Plagioderia versicolora, a Chrysomelinae, the larval defense of which is independent of host plant secondary chemistry, and

Pontania proxima, a gall forming sawfly, which was the most numerous herbivore encountered.

The adults of both leaf beetle species have a low motility.

Before discussing the results, we would like to make what we believe to be a very important methodological comment. To analyse the phenolglucoside content of the leaves we first used a time-consuming methanol extraction (3 consecutive extractions in a large volume of solvent) followed by reverse phase HPLC (methods in Meier et al., 1985). To spare time, we then tried to replace this precise method by a quicker and simpler water extraction (overnight in a small volume of water) followed by semiquantitative TLC (methods in Smiley et al., 1985). We of course ran controls to compare all 4 aspects of the 2 procedures. That is, we compared water and methanol extracts of the same material, and we also compared TLC and HPLC analysis of the same extracts. It became clear that the analysis methods (TLC and HPLC) gave comparable results, but that the extraction methods (large volume of methanol or small volume of water) gave very different results. Moreover, when we analysed the data, there was nearly no correlation to be found between herbivore distribution and the salicin and salicortin content of methanolic leaf extracts. Relatively clear correlations could however be observed between herbivore distribution and glucoside content determined phenol from aqueous leaf extracts. This, in fact, is perhaps not surprising, because after all the digestive systems of insects are not full of methanol.

In Table 1, the results of a multiple regression analysis are summarised. The dependent variables are the abundance of each of the herbivores studied and the independent variables are the content in 4 different phenolglucosides of aqueous leaf extracts. Because the phenolglucosides might influence the defense of larvae but not that of adults, the adults and the larvae of *Ph. vitellinae* and *Pl. versicolora* were recorded separately.

The variation in the content of phenolglucosides has no clear impact on the distribution of *Ph. vitellinae* adults. We have shown in previous studies that at least salicin is not a phagostimulant for this species at the concentration present in the leaves, but rather that physical factors, such as leaf trichomes, are important in determining food plant choice. Trichomes are commoner on leaves of species poor in phenolglucosides.

The larvae of *Ph. vitellinae* show a weak correlation with the phenolglucoside content of the leaves. We expected this result because the plant phenolglucosides are important as larval defensive precursors for this species. The most important component of the multiple correlation is the content in P3, a still unidentified compound.

Table 1. Multiple regression analysis.

Independent variables: salicin, salicortin (-SC), P3, P4.

Dependent variable	% VARIATION EXPLAINED	OVERALL SIGNIFICANCE	MAIN® COMPONENT
<i>Ph. vitellinae</i>			
Adults	4	ns	SC
Larvae	8	*	P3
<i>Pl. versicolor</i>			
Adults	24	***	SC
Larvae	1	ns	-
<i>P. proxima</i>			
Galls	30	***	P3 SC

ns, not significant; *, $p < .10$; ***, $p < .005$.

®, single independent variable with the largest influence.

The pattern for *Pl. versicolora* is somewhat different. The distribution of the adults of this species is highly positively correlated with the phenolglucosides content of the leaves. A stepwise multiple regression showed that only one of them, salicortin, was responsible for this: 22% of the variation in abundance of *Pl. versicolora* adults can be explained by the variation in salicortin content of the leaves.

The distribution of the larvae, on the other hand, does not seem to be influenced at all by the variation in phenolglucosides, and indeed this species does not use them as precursors for its larval defense (Rowell-Rahier & Pasteels, 1986).

The most numerous herbivore species in the studies willow field was the gall-building sawfly *Pontania proxima*. These are also highly positively correlated with the phenolglucoside content of the leaves. 30% of the variation in gall number can be explained by variation in

phenolglucoside content. Among the 4 compounds analysed, P3 and salicortin have the largest influence. It is possible that the sawflies select plants which are rich in phenolglucosides so that their larvae benefit from the extra protection brought by the larger quantity of phenolglucosides in the gall tissue. This hypothesis remains to be examined.

Thus, the distribution of the three most frequent herbivores on the studied willows can in part be predicted by the phenolglucoside content of the leaves. A further point remains to be examined: are there any interactions between the three species?

To answer this we did some further multiple regression analyses looking at the relationship between the distribution of each species, for example *Plagiodera versicolora* adults (see Table 2), and both the phenolglucosides and the abundance of the other herbivores on the tree. It is clear from this analysis that the abundance of *Plagiodera* is positively correlated with the phenolglucosides (among them salicortin plays the largest role) and the abundance of *Ph. vitellinae* larvae and the galls of *Pontania proxima*. 47% of the variation in the number of *Plagiodera* adults can be explained by this set of independent variables. This means that at least on the level of the individual tree the 3 species do not exclude each other. On the contrary, all 3 of them favour the same individual trees rich in salicortin. The phenolglucosides alone explained only 24% of the variation. This observed distribution must be explained by other factors, for example, some plant characteristics which have a positive influence on the 3 species and are positively correlated with the salicortin content of the leaves. Also, we can not exclude the possibility that the glucosides could be an induced defense response of the tree to herbivory.

Table 2. Multiple regression analysis.

Dependent variable: *Pl. versicolora* adults
 Independent variables: salicin, salicortin, P3, P4, *Ph. vitellinae* adults, *Ph. vitellinae* larvae, *P. proxima* galls.

Multiple correlation coefficient: .68
 variation explained: 47%
 Overall significance: $p < .001$

PARTIAL COEFF. FOR:	BETA	P	
Salicin	.013	.87	ns
Salicortin	.221	.12	ns
P3	.024	.84	ns
P4	-.035	.73	ns
<i>Ph. vit.</i> adults	.030	.73	ns
" " larvae	.301	<.001	***
<i>Pont. prox.</i> galls	.332	<.001	***

Our results suggested to us that the effects of the glucosides on the distribution of the beetles were different for the 2 species observed. To explore the cause of these difference, we are planning to test directly the phagostimulant or deterrent effect of salicin and salicortin in the laboratory by giving the beetles the choice between extracts of a control plant (without phenolglucosides) to which increasing quantities of salicin

and salicortin are added. To explain the larval distribution, we are planning to test the impact of salicin and salicortin on oviposition. Preliminary results of John Smiley (pers. comm.) suggest that for *Ch. aenicollis*, which use glucosides as larval defensive precursor, salicin stimulates oviposition.

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