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# Phenolglucosides and Interactions at Three Trophic Levels: Salicaceae — Herbivores — Predators

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## I. INTRODUCTION

The significance of plant secondary metabolites for animal-plant interactions is a widely studied area.[47] Indeed, biochemical and behavioral adaptations of herbivores are often key factors in determining their distribution patterns and level of dietary specialization.

Among plant secondary metabolites, phenolics are the most common.[64] More particularly, the role of flavonoids and especially tannins has been discussed at length in the context of both the "apparency plant defense theory"[8,46] and the "resource availability hypothesis".[4] We will not discuss plant defensive strategies further here.[2,7]

In this review we will summarize our knowledge of the role of one group of simple phenols — the phenolglucosides, in a set of interactions between three trophic levels. For this purpose, we will use the example of the host-plant family Salicaceae, the herbivores feeding on them, and their natural enemies. This system is particularly interesting, since it involves antagonistic and agonistic interactions such as toxic, deterrent, or phagostimulant effects, and more complex effects such as the sequestration of plant toxins for defense of the beetles against their natural enemies.

Price[38,39] and Bernays and Graham[1] have stressed the potential importance of natural enemies for the speciation and dietary specialization of herbivores. They suggest that the use of specific plant toxins by herbivores to reduce predation could be one of the strategies leading to their dietary specialization.

## II. PHENOLGLUCOSIDES

### A. CHEMICAL STRUCTURE AND METHODS OF ANALYSIS

The Salicaceae include (within the genera *Salix* and *Populus*) many of the shrub and tree species of the boreal and montane regions of the northern hemisphere. Because of their relatively rapid growth rate, they are often planted for wood production.

Their chemistry is rather well known thanks to work of Thieme and Benecke,[62] Pearl and Darling,[37] and Steele et al.[57] and has been reviewed by Hegnauer,[11] Palo,[29] and Reichardt et al.[45] The Salicaceae as a family is characterized by the presence of phenolic glucosides in the bark of all species and the leaves of some species; no secondary metabolites other than phenolics have been identified so far in the leaves of Salicaceous plants. In northern temperate European species of *Salix* there is a separation between those with phenolglucosides in their leaves and those with condensed tannins (see below).

The structures of several of the more common phenolglucosides found in the Salicaceae are given in Figure 1. Salicin and salicortin are the simplest and most frequently identified ones. Salicin is well known in pharmacology for its analgesic and antipyretic effects.[26] The active metabolite is salicylic acid obtained after removal of the glucose moiety and oxidation of the remaining aglycone saligenin. Salicin is also toxic to some insects (such as ants,[30] and some Lepidoptera).[22,23] Phenolglucosides inhibit the feeding of some mammals also such as the Finnish mountain hare *(Lepus timidus)*[58] and the opossum *Trichosurus vulpecula* in New Zealand.[6]

The instability of many phenolglucosides has been reported by Thieme[60] and Steele et al.[56] Lindroth and Pajutee[21] summarize various forms of hydrolytic degradation and interconversion of phenolglucosides (Figure 2). These seriously affect the results of quantitative and qualitative chemical analysis and are dependent on the sample preparation technique (fresh, freeze-dried, or oven-dried leaves) and the extraction method utilized (water or methanol as a solvent, variation in pH, variation of relative volume of solvent, variation in extraction time).

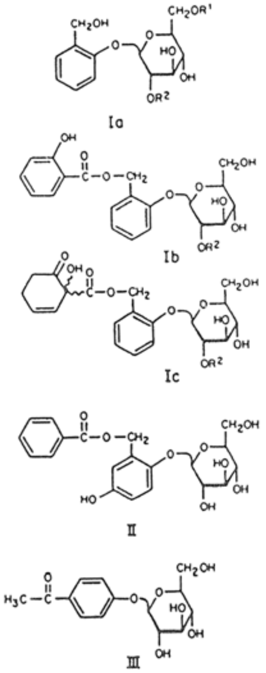

FIGURE 1. Structures of different phenolglucosides.[28] Ia: Salicin ($R^1 = H$, $R^2 = H$), Fragilin ($R^1 = CH_3CO$, $R^2 = H$); Ib: Salicoylsalicin ($R^2 = H$); Ic: Salicortin ($R^2 = H$), 2-O-Acetylsalicortin ($R^2 = CH_3CO$), Tremulacin ($R^2 = C_6H_5CO$); II. Salireprosid; III: Picein. (From Meier, B., Sticher, O., and Bettschart, A., *Dtsch. Apoth.*, 7, 341, 1985. With permission.)

In light of these chemical artefacts, great caution should be exercised when comparing the results from different studies where the chemical analyses are performed with different methods. Moreover, it is important to consider which one of the many possible sample preparation and extraction techniques is the more appropriate for the question being asked. For example, what a herbivorous insect gets from a plant might well differ from what is found in a methanol extraction.[52]

## B. DISTRIBUTION

### 1. Interspecific Variation

The barks of all Salicaceae species are rich in phenolglucosides. This is not the case for the leaves; there is usually a larger quantity and a greater diversity of phenolglucosides in the bark than in the leaves.[29] In fact, the leaves of some *Salix* species

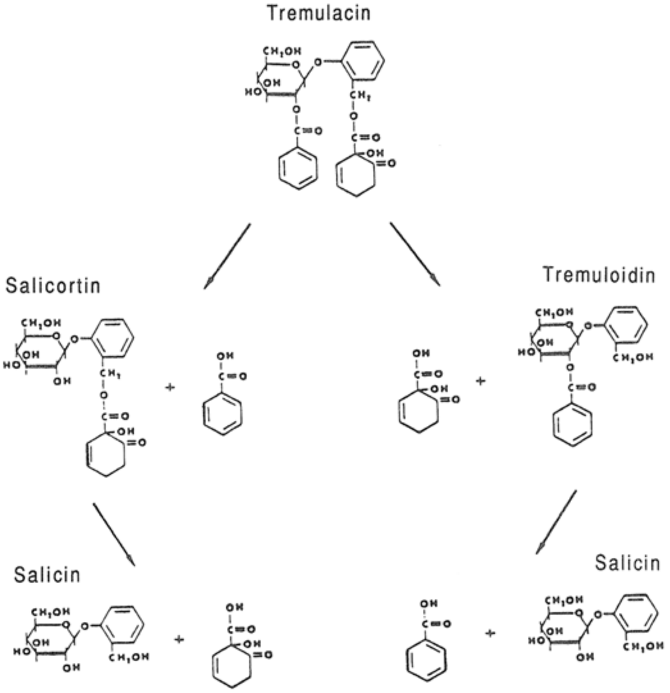

FIGURE 2. Pathways of hydrolytic degradation and interconversion of phenolic glycosides. (From Lindroth, R. L. and Pajutee, R. S., *Oecologia*, 74, 144, 1987. With permission.)

do not contain any phenolglucoside at all, although the level of total phenolics in *Salix* and *Populus* leaves is generally high. The occurrence of phenolglucosides in the leaves of different *Salix* and *Populus* species is reviewed by Palo.[29] Additional information is found in Julkunen-Tiito,[16] who gives a chemotaxonomic survey of phenolics in the leaves of Northern species of Salicaceae. Meier et al.,[28] Rowell-Rahier,[49] Horn,[13] and Lindroth et al.[24] also analyzed the leaves of different *Salix* and *Populus* species. The profile of the different glucosides in *Salix* and *Populus* leaves appears to be relatively species specific despite intraspecific variations described in the next section.

### 2. Intraspecific Variations and Abiotic Factors

There are many other sources of variation in the phenolglucoside content of leaves, other than species differences. Content also varies with sex of the plant (the Salicaceae are dioecious); there is usually a higher level of glucosides in the leaves of female plants than in those of males.[60] Further, the phenolglucoside content of leaves tends to exhibit temporal variation. In general, it decreases later in the season (Figure 3)[13,37,49,61] but this is not always the case (e.g., *P. tremuloides* and *P. deltoides*).[20] Denno et al.[5] showed that mature leaves of *S. viminalis, S. dasyclados,* and *S. fragilis* are richer in

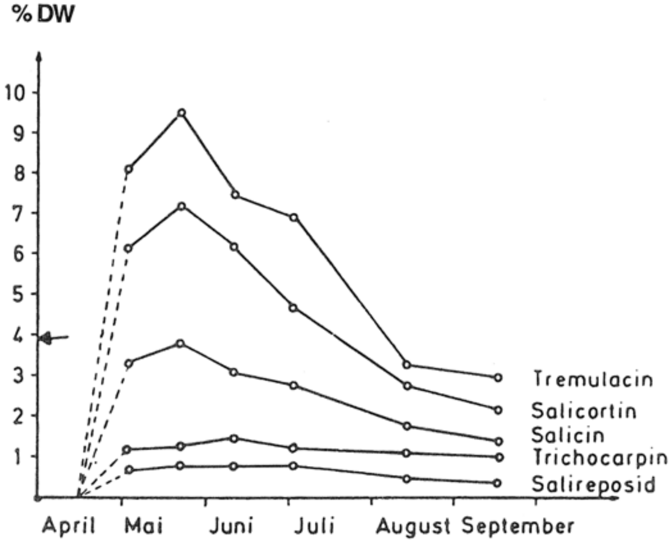

FIGURE 3. Seasonal variation in phenolglucoside concentration in the leaves of *P. trichocarpa* Hopk.[61] (From Thieme, H. and Benecke, R., *Pharmazie*, 25, 228, 1970a. With permission)

phenolglucosides than the young ones closer to the apex of the shoot. The reverse was observed in *P. tremuloides*, but this observation needs to be confirmed by additional measurements.[20] Since the Salicaceae produce new leaves during most of the growing season, two factors have to be differentiated when comparing leaves of different "ages": first, developmental variations (i.e., position on the shoot at a given moment in time),[5] and secondly, seasonal variations (i.e., same developmental stage at different time).[20] Diurnal variations have also been observed, showing decreasing quantity from a maximum in the early morning to a minimum in the evening.[60]

Environmental stress (water, nutrients, light, air pollution) are known to lead to mobilization of nitrogen and should reduce the production of carbon based secondary metabolites.[14] Fast growers should show much plasticity in their production of secondary metabolites, and give priority to growth. A reduction in phenolglucoside is observed in *P. deltoides* when the leaves are submitted to ozone stress (Jones, personal communication) while Larsson et al.[18] produced threefold variation in the phenolics of sapling *S. dasyclados* by varying the light intensity and nutrient supply. Relative carbon availability is linked to phenolic synthesis. Additionally, Bryant et al.[3] demonstrate that nitrogen fertilization results in decreased concentration of condensed tannins and phenolglucosides in the leaves of *P. tremuloides*. Thus if the C/N ratio is decreased, the content of the leaves in carbon-based secondary metabolite also decreases.

Elevation is another possible source of variation; salicin and salicortin are positively correlated with elevation in *S. lasiolepis, S. orestera*, and *S. planifolia*.[13]

Lastly there have been several reports of individual variation between leaves of one branch or of different plants and clones.[20,53-55] Summarizing, the causes of intraspecific variation in the phenolglucoside content of the leaves are numerous and each plant or

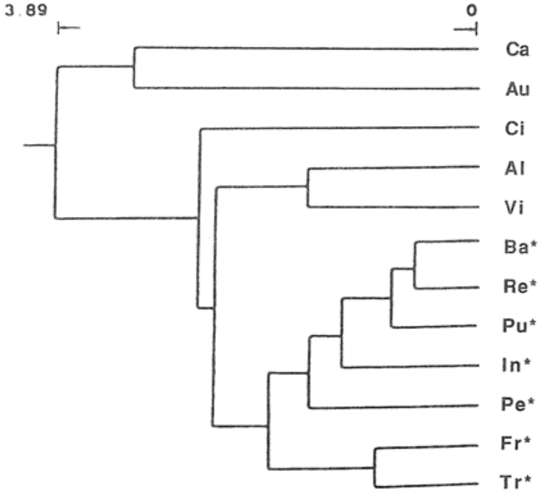

FIGURE 4. Cluster analysis showing similarity of *Salix* species using presence/absence data of oligophagous sawflies and weevils.[48] The units of the abscissa give the maximum diameter of a cluster in Euclidean metric. CA = *S. caprea;* Au = *S. aurita;* Ci = *S. cinerea;* Al = *S. alba;* Vi = *S. viminalis;* Ba = *S. babylonica;* Re = *S. repens;* Pu = *S. purpurea;* In = *S. incana;* Pe = *S. pentandra;* Fr = *S. fragillis;* Tr = *S. triandra.* * = species of Salix containing phenolglucosides in their leaves.

set of leaves probably presents at any given point in time a particular mixture of phenolglucosides to a potential herbivore. Intraspecific variation in the phenolglucosides content of the leaves suggests that the Salicaceae should be considered as an heterogenous habitat sensu Whitham.[66]

## III. PHENOLGLUCOSIDES IN HOST PLANT LEAVES AND DISTRIBUTION PATTERNS OF HERBIVORES

Several recent studies (bibliographical and experimental) suggest that herbivore distribution is affected by the phenolglucoside content of the leaves of different *Salix* species, of which there are more than 30 in Europe alone.

In a bibliographical survey, Rowell-Rahier[48] shows that for British moths, French weevils, and German sawflies, the presence or absence of phenolglucosides (or alternatively the absence or presence of condensed tannins) in *Salix* leaves is related to the degree of dietary specialization of the insects feeding on these leaves. The results show that *Salix* species with phenolglucosides tend to be eaten by specialized herbivores and avoided by generalists. Conversely, *Salix* species without phenolglucosides tend to be the food of more polyphagous insects and tend to be avoided by more specialized ones. Moreover, the faunas of the different *Salix* species with phenolglucosides in their leaves are more similar to each other than are the faunas of the *Salix* species having no phenolglucosides in their leaves; this is particularly true for oligophages (Figure 4).

This affords a corroboration of the protective function of phenolglucosides in willow leaves. Indeed, if the dietary specialization of *Salix* herbivores is linked (proximately or ultimately) to the presence or absence of phenolglucosides, the faunas of the willow species with similar chemistry should be more similar to each other than the faunas of *Salix* species with different secondary chemistry.

In a study of the aphid *Pemphigus betae* on *Populus angustifolia* Zucker[68] shows that the leaves with lower level of total phenolic are more infested than those with higher level. It is also clear from that study that, as suggested by Whitham,[66] the leaves should be considered as a heterogenous habitat; the level of phenol between and within leaves is extremely variable, showing for example a gradient of concentration between the tip and the apex of a single leaf.

Among the herbivores on willows, the leaf beetles (Chrysomelidae) have been intensively investigated by several research groups. In northern and central Europe more than 70 such species are described.[9,17] The leaf beetles are particularly interesting because they offer a model to study not only the impact of plant secondary metabolites on herbivory but also the significance of these compounds for the interaction between herbivores and their enemies. We will now briefly summarize what is known about the relationship between phenolglucosides and (1) laboratory experiments on food plant choice by beetles, (2) abundance of beetles, and (3) herbivory level in the field.

### A. LABORATORY STUDIES

Tahvanainen and co-workers[59] showed that the concentration and composition of the different phenolglucoside blends are species specific in willows. For four common leaf beetle species *(Phratora vitellinae, Plagiodera versicolora, Lochmea 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 in its phenolglucoside content to the preferred host plant (Table 1). Rowell-Rahier[49-50] showed that *Ph. vitellinae* in laboratory food plant choice prefers *Salix* species rich in phenolglucosides *(S. nigricans, S. purpurea, S. fragilis)* to those whose leaves do not contain phenolglucosides *(S. caprea, S. cinerea, S. alba)*. However, geographically isolated populations can be local specialists and have markedly different preferences within those *Salicaceae* species which have phenolglucosides in their leaves. Especially in disturbed habitats (a small number of indigenous species and/or a large number of introduced species), the preferred host in the field might not be the same as the one established in laboratory tests. Denno et al.[5] studied *Ph. vitellinae* and *G. lineola* on *Salix* species (*S. viminalis* — low phenolglucoside content and native; *S. fragilis* — high phenolglucoside content and native; *S. dasyclados* — high phenolglucoside content but introduced). *Ph. vitellinae* oviposits preferentially on *S. fragilis* whereas *G. lineola* prefers to oviposit on *S. viminalis* followed closely by *S. fragilis.* For both beetle species *S. dasyclados* is the least preferred oviposition host. The performances (larval survival, pupal size, developmental time) of the two beetles on *S. fragilis* and *S. viminalis* were quite similar. We will discuss this apparent discrepancy between performance and oviposition in the next section.

Matsuda and Matsuo[27] indicate that for *Chrysomela vingitipunctata costella* and *Pl. versicolora distinctata,* salicin and populin in combination with sucrose have a phagostimulant effect, whereas for *Ch. v. costella* populin alone is active. These compounds have no effect on *L. caprea scribata.*

However, the feeding preference of *Ph. vitellinae* for *Salix* spp. rich in phenolglucosides is not directly caused by a phagostimulatory effect of salicin but is rather an indirect consequence of the pilosity of the leaves of many of the species which do not

**Table 1**
**FOR FOUR SPECIES OF LEAF BEETLES, THERE IS A GOOD CORRESPONDENCE BETWEEN THE RANKS OF PREFERENCE OBTAINED WITH DIFFERENT *SALIX* SPECIES IN MULTIPLE CHOICE FEEDING EXPERIMENTS (RF) AND THE RANKS OF CHEMICAL SIMILARITY (RCS) OF THOSE *SALIX* SPECIES COMPARED TO THE MOST PREFERRED SPECIES (1)**

| *Salix* sp. | *Ph. vitellinae* | | *Pl. versicolora* | | *G. lineola* | | *L. caprea* | |
|---|---|---|---|---|---|---|---|---|
| | RCS | RF | RCS | RF | RCS | RF | RCS | RF |
| **Wild** | | | | | | | | |
| S. ni | 1 | 1 | 4 | 4 | 4 | 4 | 4 | 4 |
| S. ph | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| S. ca | 3 | 4 | 1 | 1 | 1 | 1 | 1 | 1 |
| S. pe | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Cultivated** | | | | | | | | |
| S. aq | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 |
| S. da | 2 | 2 | 2 | 2 | 4 | 4 | 4 | 4 |
| S. vi | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2 |
| S. tr | 4 | 4 | 4 | 4 | 1 | 1 | 1 | 1 |

S. ni = *S. nigricans;* S. ph = *S. phylicifolia;* S. ca = *S. caprea;* S. pe = *S. pentandra;* S. aq = *S. aquatica;* S. da = *S. dasyclados;* S. vi = *S. viminalis;* S. tr = *S. triandra.*

Adapted from Tahvanainen, J., Julkenen-Tiito, R., and Kettunen, J., *Oecologia*, 67, 52, 1985.

contain phenolglucosides. For example, shaved *S. caprea* leaves are preferred to unshaved ones and, *Ph. vitellinae* does not show any preference between shaved *S. caprea* leaves with or without salicin painted on the surface.[50] *Ph. vitellinae* is not the only leaf beetles species for which the presence or absence of trichomes on the leaf surface plays an important role in feeding choice of the adults. *Ph. laticollis* feeds on different species of *Populus* and prefers *P. nigra italica. P. alba* has leaves covered with trichomes and is rejected by *Ph. laticollis.* However, in laboratory test, silica discs coated with methanol and dichloromethane extracts of *P. alba* are preferred to those coated with the extract of *P. nigra.* This suggests that factors other than those present in the extracts are important for the food plant choice of *Ph. laticollis*,[63] In preliminary studies of another beetle belonging to the same genus, *Ph. vulgatissima*, Rowell-Rahier (unpublished) was able to show that this species prefers *Salix* with leaves poor in phenolglucosides (e.g., *S. caprea* and *S. cinerea*) and that salicin is deterrent for the adults of this species.

## B. FIELD STUDIES

In a field study, involving two of the same beetle species as in the previous examples (*Ph. vitellinae* and *Pl. versicolora*), as well as a common sawfly *(Pontania proxima)*, the correlation between herbivore abundance and amount of salicin and sailcortin in the leaves of individual *S. alba* (poor in phenolglucosides), *S. fragilis* (rich in phenolglucosides), and several of their hybrids containing intermediate levels of phenolglucosides

### Table 2
### RESULTS OF A MULTIPLE REGRESSION BETWEEN THE ABUNDANCE OF EACH OF THREE HERBIVORE SPECIES IN THE FIELD (DEPENDENT VARIABLE) AND THE PHENOLGLUCOSIDE CONTENT OF THE *SALIX* LEAVES ON WHICH THEY WERE RECORDED

| Herbivore species | % Variation explained | Overall significance |
|---|---|---|
| *Ph. vitellinae* | | |
| Adults | 4 | ns |
| Larvae | 8 | $p<.10$ |
| *Pl. versicolora* | | |
| Adults | 24 | $p<.005$ |
| Larvae | 1 | ns |
| *P. proxima* | | |
| Galls | 30 | $p<.005$ |

Adapted from Rowell-Rahier, M., Soetens, Ph., and Pasteels, J. M., *Insects-Plants,* Labeyrie, V., Fabres, G., and Lachaise, D., Eds., W. Junk, Dordrecht, 1987, 91.

was tested.[52] Because the phenolglucosides influence larvae and adult beetles differently (see below), the abundances of both life stadia were recorded separately. The results show that phenolglucosides have no significant effect on the distribution of *Ph. vitellinae* adults and *Pl. versicolora* larvae. However, the phenolglucosides may partly explain the observed variation in the distribution of *Ph. vitellinae* larvae, *Pl. versicolora* adults, and *P. proxima* galls (Table 2).

The function and importance of the phenolglucosides for *Ph. vitellinae* larvae will be discussed below. The pattern of distribution of *Pl. versicolora* adults is rather well predicted by phenolglucoside alone (24% of variation explained), but the prediction is even better (multiple correlation coefficient 0.68, 47% of variation explained) when the combination of both phenolglucosides and abundance of other herbivores on the same plant is taken into account. The different herbivores studied prefer the same individual plants, and the observed distribution of *Pl. versicolora* adults can probably be explained by some plant characteristic positively correlated with phenolglucosides and influencing positively the different herbivores. For *Pl. versicolora*, the results obtained in the field and in the laboratory are different. In the laboratory study of Tahvanainen et al.,[59] *Pl. versicolora* preferred willow leaves with moderate to low total phenolglucoside content whereas Soetens[55] found the abundance of this beetle in the field is positively correlated with level of phenolglucosides, mostly salicortin. Obviously, factors other than food plant preferences influence the abundance of the beetles in the field (e.g., natural enemies), but we also have to keep in mind the possibility of local specialization (as demonstrated in *Ph. vitellinae*[49]).

The relationship between intraspecific variation in phenolglucosides in individual trees of *S. lasiolepis* and the damage due to herbivory by a leaf beetle, *C. aenicollis*, was established by Smiley et al.[54] in California. The level of herbivory is also positively correlated with level of phenolglucosides in the attacked leaves (Figure 5). Additionally, the relative success of *C. aenicollis* on *S. lasiolepis* and *S. orestera* is positively correlated with salicin content.

It is possible that the gall-forming sawfly, *P. proxima*, selects plants rich in phenolglucosides so that the larvae are better protected by the surrounding plant tissue.

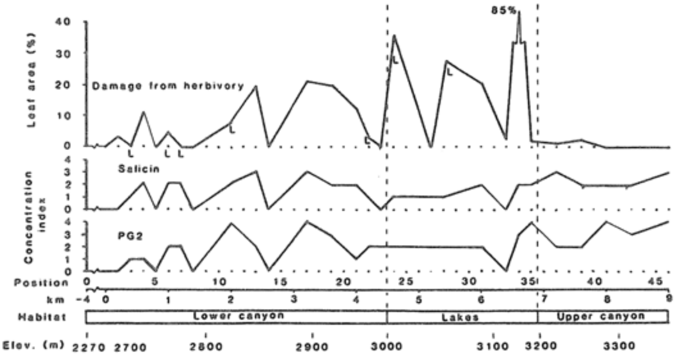

FIGURE 5. Leaf damage and chemical content of willows growing along an elevation gradient in the east-central Sierra Nevada mountains. Sampling points were at 200 m intervals numbered 0 to 46; if no foliage was present none was collected. Data points indicated by L represent *S. lasiolepis*; otherwise they represent *S. orestera*. (From Smiley, J. T., Horn, J. M., and Rank, N. E., Ecological effects of salicin at three trophic levels: new problems from old adaptations, *Science*, 229, 649, 1985. © 1985 by the AAAS. With permission.)

Fritz et al.[10] showed that for four sawfly species (*Euura* spp.; *Pontania* sp.; *Phyllocolpa* sp.) found on *S. lasiolepis* there is a considerable variation among clone phenotypes in their susceptibility to sawfly oviposition; possible influences of phenolglucosides have not been examined.

### C. EFFECT OF ALTITUDE

In Section II we have seen that several factors can influence the intraspecific distribution of phenolglucosides. Altitude was one of them. Indeed, the 100:1-fold variation in phenolglucosides observed by Smiley and Rank[53] within *S. orestera* and *S. lasiolepis* was observed along an elevation gradient (from 2270 m to 3300 m) in the Californian Sierra Nevada. It is difficult to assess the importance of altitude on performance of the beetle, because it affects not only host-plant secondary metabolites, but may also provide an important selection pressure which is experienced by the beetles, namely the direct effect of temperature. Larvae from *C. aenicollis* collected from high elevation site grow approximately 10% faster than individuals from a lower altitude population under controlled temperature and phenolglucoside conditions in the laboratory. Rapid growth is indeed to be expected from the high elevation population, whereas better defense of the beetles against more abundant predators is expected from the lower elevation populations. This seems to contradict the observation of lower concentration of salicin (which is used for insect defense; see following section) at lower elevation. Smiley and Rank[53] suggested that lower salicin content at low elevation could be an adaptation of the plants to escape heavy herbivory by specialists such as *C. aenicollis*, which are dependent on salicin for their own chemical defense.

### D. EFFECT OF LEAF AGE

Leaf age is another factor known to affect the phenolglucoside content of leaves of Salicaceae (see Section II), Raupp and Denno[41] studied leaf age as a predictor of herbivore distribution and abundance using *Pl. versicolora* on *S. babylonica*. Raupp and

Denno did not mention phenolglucosides as a variable in their study, but they established that adult *Pl. versicolora* are mostly found on the young leaves and larvae and eggs on the older leaves of *S. babylonica*. Adults fed young leaves reproduce sooner and have bigger clutches of eggs than those fed old leaves. The larvae fed young leaves have a greater adult weight, have shorter developmental times and lower mortality than those on older leaves. In another study Raupp[40] shows that older leaves erode the cutting surface of the beetles mandibles more than tender leaves, and that this results in a slower consumption rate of older leaves, correlated with reduced fecundity. This mechanical factor is certainly not the only one explaining the better performance of *Pl. versicolora* on *S. babylonica* young leaves. These young leaves probably also have higher nutritive value and a qualitatively and quantitatively different phenolglucoside content than the older ones.

The larvae of two other beetle species (*Ph. vitellinae* and *G. lineola*) perform better (higher survival on *S. viminalis* and *S. dasyclados*, larger pupal size on *S. fragilis,* and faster development on *S. dasyclados*) on mature leaves than on younger ones.[5] In the case of these three willow species mature leaves contain larger amounts of phenolglucosides and other simple phenolics (see Section II). These mature leaves are also poorer in primary nutrients (such as proteinaceous amino acids) and are tougher than the terminal young ones.

### E. EFFECT OF ENVIRONMENTAL STRESS

Adult *G. lineola*[18] have a reduced feeding rate on leaves with high phenolic content, obtained by increasing relative carbon availability (increased the light intensity under low nutrient condition).

Adult and larval *Pl. versicolora* prefer to feed on, and have a higher consumption rate of, ozone-stressed *P. deltoides* (possibly due to reduced levels of phenolglucosides, see Section II).[15] However, the females prefer to oviposit on nonstressed plants.

Adult *Pl. versicolora* avoid previously injured leaves of *S. babylonica.*[44] On damaged *S. babylonica* and *S. alba,* fecundity is reduced, the development of the larvae is slower, and they have a lower weight as adults.[42]

These results may seem contradictory; ozone stress seems to increase consumption by *Pl. versicolora* whereas stress originating from previous injury decreases it. However, the two types of stress probably have very different effects on the phenolglucoside content of the leaves; ozone stress probably decreases it, and injury of the leaves could have the opposite effect as observed by Reichardt et al.[45] in *P. tremuloides.*

### F. SUMMARY

In summary, when looking at the pattern of distribution of herbivores and their relationships to phenolglucosides (Table 3), larvae and adults often have different responses. Studies of feeding and oviposition preference also give different answers. In the next section we will examine how this might be explained.

Most of the results summarized above were obtained by correlative rather than causal studies. Additionally, it is important to remember that in the relationships summarized above, the variation in phenolglucosides is linked to different origins (interspecific differences, age differences, environmental stress, etc.). These factors do not exclusively affect the level of phenolglucosides in the plants, but rather numerous other plant characteristics too, all of which might be important for the herbivore. In other words, the experiments are inadequately controlled in the purity of the prime variable.

### Table 3
### FEEDING PREFERENCES AND GROWTH PERFORMANCE OF TWO LEAF BEETLES: *PH. VITELLINAE* (LARVAE USE PHENOLGLUCOSIDES FOR DEFENSE) AND *PL. VERSICOLORA* (LARVAE DO NOT USE PHENOLGLUCOSIDES FOR DEFENSE)

| Studies | Ph. vitellinae | Pl. versicolora |
|---|---|---|
| *In laboratory:* | | |
| 49, 50 | Feed on spp. rich in phenolglucosides | |
| 59 | Feed on spp. rich in phenolglucosides | Feed on spp. low to poor in phenolglucosides |
| 5 | Oviposit on spp. rich in phenolglucosides | |
| *In the field:* | | |
| 55 | Abundance of adults not correlated with phenolglucoside content of the leaves; abundance of larvae positively correlated with phenolglucoside content of the leaves | Abundance of adults positively correlated phenolglucoside content of the leaves; abundance of larvae not correlated with phenolglucoside content of the leaves |
| *Other studies:* | | |
| 41 | | Prefer young leaves |
| 5 | Larvae perform better on mature leaves | |
| 15 | | Prefer ozone-stressed leaves but oviposit on nonstressed plants |
| 44 | | Avoid injured leaves, larvae perform less well on injured leaves |

## IV. SPECIALIZATION ON LEAVES RICH IN PHENOLGLUCOSIDES

### A. ANTAGONISTIC EFFECTS OF PHENOLGLUCOSIDES ON HERBIVORES: DETOXIFICATION MECHANISMS IN THE TIGER SWALLOWTAIL BUTTERFLY

Although the evidence reported in the previous section strongly suggests that phenolglucosides mediate the distribution of phytophagous insects on salicaceous plants, there are surprisingly very few data on the precise action of those phenols on behavior and physiology of herbivores. There are few direct demonstrations of the negative impact of the condensed tannins on the growth of herbivores, but Bryant et al.[3] have demonstrated that larval growth of large aspen tortrix *(Choristoneura conflictana)* is decreased by increasing concentration of condensed tannins and phenolglucosides in artificial diets. The toxicity of the phenolglucosides salicortin and tremulacin is due to their degradation product 6-hydroxycyclohexenane.[45]

There is no information on the detoxification of phenolglucosides in the guts of leaf beetles not dependent on these compounds for their own defense. However, recent studies by Lindroth et al.[19,25] provide insight into the way another insect, the tiger swallowtail (*Papilio glaucus canadensis* and *P. g. glaucus*) feeding on salicaceae (*P. tremuloides*) deals with the phenolic glycosides. *P. g. canadensis* is adapted to these toxins and grows well on *P. tremuloides. P. g. glaucus* does not grow so well on the same plants. Lindroth et al.[23,25] showed that the toxic phenolics are salicortin and tremulacin and not salicin. They cause gut lesions.[23] The active toxic compound is a cyclohexane saligenin ester.[25] The hypothetical degradation pathways of salicortin and tremulacin are summarized in Figure 6. The two species of tiger swallowtail differ at least in two ways in their enzymatic degradation potential. First, the midgut-β-glucosidase activity of the adapted *P. g. canadensis* is one third to one half that of the less adapted *P. g. glaucus.* The specificity of this β-glucosidase is still unknown. Ingestion

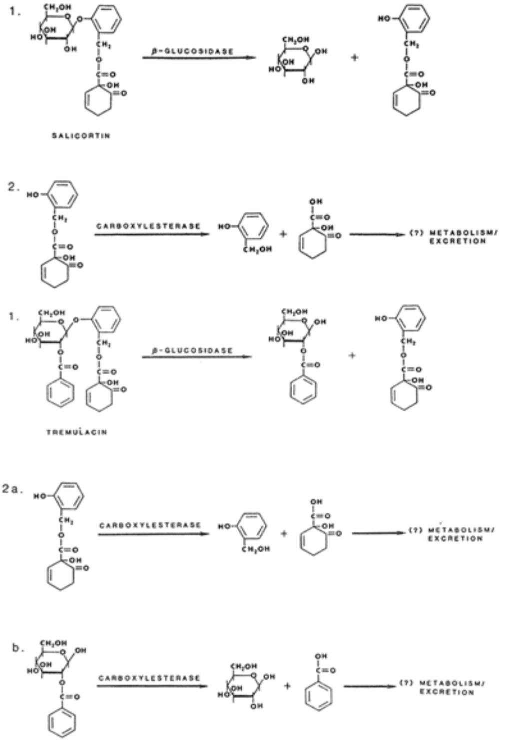

FIGURE 6. Hypothetical pathway of metabolism of salicortin and tremulacin by *Papilio*. Step 1 is cleavage of the glycosidic bond by β-glucosidases; Step 2a involves detoxification of the cyclohexenone saligenin ester via hydrolysis by carboxylesterases. In Step 2b the benzoyl ester of glucose provides an alternative substrate for carboxylesterase activity thus competitively inhibiting the ability of the enzymes to hydrolyze the biologically active cyclohexenone saligenin ester (Step 2a). (From Lindroth, R. L., Scriber, M., and Hsia, M. T. S., *Ecology*, 69, 814, 1988. With permission.)

of phenolglucosides suppresses β-glucosidase activity in *P. g. canadensis* but induces it in *P. g. glaucus.*[19] Secondly, the actively toxic cyclohexane saligenin ester (liberated by the β-glucosidase) is further degraded (hydrolyzed) to harmless products by a carboxylesterase. The activity of the carboxylesterase is thus a determinant: this enzyme should act as fast as possible to avoid damage. Lindroth suggests that the evolution of an optimal carboxylesterase is a critical part of the adaptation to phenolglucosides. The biological activity of tremulacin is twice that of salicortin, since the benzoyl glucose moiety released by the β-glucosidase may provide an alternative substrate for the carboxylesterase.[25]

### Table 4
### DISTRIBUTION OF DEFENSE COMPOUNDS IN CHRYSOMELINAE LARVAE

| Beetles | Host plants | Secretions |
|---|---|---|
| **Subtribe Chrysomelina** | | |
| 6 genera, 11 spp. | Salicacae<br>Betulaceae<br>Ranuncalaceae<br>Apiaceae<br>Polygonacae<br>Scrophulariaceae<br>Cruciferae | Iridoid monoterpenes |
| *Chrysomela interrupta* | Betulaceae | Phenylethyl esters |
| 6 spp. of *Chrysomela* | Salicaceae | Salicylaldehyde<br>Benzaldehyde |
| *Gastrolina depressa* | *Juglandaceae* | Juglone |
| **Subtribe Phyllodectina** | | |
| 3 spp. of *Phratora* | Salicaceae | Iridoid monoterpenes |
| *Phratora vitellinae* | Salicaceae | Salicylaldehyde |

Adapted from Pasteels, J. M., Duffey, S., and Rowell-Rahier, M., *J. Chem. Ecol.*, 16, 1990.

## B. AGONISTIC EFFECTS OF PHENOLGLUCOSIDES ON LEAF BEETLES: CHEMICAL DEFENSE

As shown in the data reviewed above, the distribution of leaf beetles on the Salicaceae seems to be linked, positively or negatively, to the phenolglucoside content of the leaves. Leaf beetles are known for their extremely diverse defensive mechanisms, which include chemical defense. Both adults and larvae can be so defended. Chemical defense of adult leaf beetles has been recently reviewed.[33] Chemical defense of adult leaf beetles feeding on the Salicaceae bears no direct relationship to the phenolglucosides of the leaves. The phenolglucosides are not directly sequestered by adults for defensive purpose. On the other hand, the phenolglucosides are known to influence the nature of the defensive secretion of the larvae of several species of the subtribe Chrysomelina and *Phratora* in the subtribe Phyllodectina.[35,51] Defensive compounds are identical in larval and adult stages of the subtribe Chrysolinina and the subfamily Galerucinae, which do not possess glands at all in the larval stage. This contrasts sharply with the Chrysomelina and Phyllodectina, which possess nine pairs of segmental exertile larval defensive glands. The compounds generally liberated by the larval defensive glands are listed in Table 4. The larval secretions from these glands are volatile and highly reactive compounds detectable at a distance.

In Table 4, we can see that the larvae of some species of *Chrysomela* and *Phratora* secrete not monoterpenes, as expected from their taxonomic position, but rather aromatic compounds such as salicylaldehyde and benzaldehyde. It has long been suggested that some chrysomelid larvae utilize salicin and possibly other phenolglucosides as precursors of salicylaldehyde produced in specialized defensive glands[12,36,65] and this utilization is now well documented. These species feed exclusively on trees belonging to the Salicaceae. The secretion of salicylaldehyde is an adaptation to the secondary chemistry of the Salicaceae. To confirm the role of salicin and salicortin as precursors of the salicylaldehyde secreted by the larvae of *Ph. vitellinae*, the consequence of a salicin-free diet on the defensive secretion of the larvae was examined. In nature, adult *Ph. vitellinae* are never seen on *S. caprea* and the larvae do not normally accept these hairy leaves which are salicin free. After being denuded of trichomes, however, the leaves of *S. caprea* were readily accepted by the larvae. The larvae developed normally on shaved leaves but did not produce any secretion. Addition of salicin or salicortin (from Dr. B. Meier) to the shaved leaves of *S. caprea* restored the secretion of salicylaldehyde. Salicin and salicortin contained in the food plant are thus the precursors of the larval secretion of salicylaldehyde.[50] Experimental feeding of *Ch. tremulae* larvae with labeled salicin confirmed the role of salicin as a precursor of salicylaldehyde. The transformation of salicin into salicylaldehyde occurs in the defensive glands; the β-glucosidase activity was found to be four times higher in the glands than in the guts.[34] Both β-glucosidase and oxidase needed to transform salicin in salicylaldehyde occur in the secretion itself, and the derivation of salicylaldehyde from salicin occurs in the gland reservoirs[31] (Duffey and Pasteels, in preparation). The concentration of glucose and aldehyde in the secretion are far from equimolar and this indicates that the glucose formed by the salicin hydrolysis is in great part recovered by the larvae and transferred back into the blood.[34] The recovered glucose should benefit the beetles. Thus the cost of defense is expected to be maximal in those species synthesizing defensive secretion *de novo* and minimal or negative in those which profit from the use of the plant phenolglucosides. Rowell-Rahier and Pasteels[51] showed experimentally that the autochthonously produced secretion of some beetle species entails an appreciable cost, expressed as a loss of weight. These costs can be avoided by the use of an appropriate plant precursor such as salicin, in which case the recovery of the glucose moiety of the salicin contributes significantly to the larval energy budget.

At hatching the larvae are clustered and immobile on the leaves. An early defense would therefore seem to be critical. However, only those species secreting salicylaldehyde have functional glands on hatching. Species producing *de novo* secretion are not able to secrete at birth. Paradoxically, then, only those larvae which depend on salicin normally found in their food seem to be able to produce a secretion before feeding. The obvious explanation is that the salicin is sequestered in the eggs and used as a precursor by the neonate larvae. This hypothesis has been confirmed experimentally; the eggs of some of the Salicaceae feeders contain salicin, and those species are also those whose larvae secrete salicylaldehyde. The adults do not sequester salicin for their own defense (they produce *de novo* isoxazolinone glycosides[33]), but females are able to sequester salicin in their eggs for the benefit of both eggs and neonate larvae.[30]

Based on evidence from *Plagiodera versicolora*, Pasteels et al.[31] suggested that the biochemical determinant of the chemical nature of the larval defensive secretion is the oxidase, rather than the β-glycosidase necessary for the transformation of salicin into salicylaldehyde. In other words, a change in the specificity of the oxidase, say the acquisition of the ability to oxidize saligenin, would permit an insect to develop a switch from *de novo* biosynthesis of iridoid monoterpenes to sequestered defense via the utilization of the plant glucoside salicin. The larval gland reservoirs of *Pl. versicolora* contain besides the iridoid aldehyde and lactone, glucose and a nonspecific β-glucosidase able to hydrolyze salicin. It was thus suggested that species producing monoterpenes also contain in the glandular reservoirs a β-glucosidase and possibly an oxidase, yet to be demonstrated, needed in the final steps of the biosynthesis of the oxidized iridoid monoterpenes from a reduced iridoid glucoside.[31]

Thus, the significance and function of phenolglucosides such as salicin and salicortin varies between species of beetles. *Ph. vitellinae* and several *Chrysomela* spp. are dependent on the glucosides for the defense of their larvae and eggs against natural enemies. Other species *(Pl. versicolora. G. lineola)* do not directly use salicin and salicortin. Accordingly, we expect the first set of species to be extremely well adapted to handling phenolglucosides compared with the second group of species. Beetle larvae using salicin and salicortin for defensive purpose should be particularly good at transporting these toxins into the glands, whereas the latter set should be adapted to detoxify the phenolglucosides. This remains to be documented. Additionally, we should expect that the beetles producing salicylaldehyde in the larval stage will select Salicaceae species rich in salicin and other phenolglucosides to lay their eggs. This was confirmed (see previous section).

## V. PHENOLGLUCOSIDES AND NATURAL ENEMIES OF LEAF BEETLES

In view of the data viewed in the second section of this paper it is clear that the phenolglucosides from host plants influence, directly or otherwise, the distribution and performance of leaf beetles feeding on the Salicaceae. It is also clear (see Section IV) that the phenolglucosides are utilized by some beetle species for their own defense, whereas in other species detoxification mechanisms for these same compounds are present.

Although several studies compare the performance of different beetle species on *Salix* with different levels of phenolglucosides in their leaves, few, to our knowledge, try to explain the observed patterns by the interaction of the herbivores with their predators.[5] Larvae of *Ph. vitellinae* fed on leaves rich in salicylaldehyde precursors are immediately regurgitated alive by predaceous coccinellid larvae (a predator present in the field). On the other hand, the larvae of *Ph. vitellinae* fed on leaves lacking the precursor of their defensive secretion do not survive attack by the same predaceous coccinellid. This kind of interaction might explain why *Ph. vitellinae* avoid species of Salicaceae poor in salicin as oviposition hosts although the larvae develop well on these plants in the absence of predation! *G. lineola*, on the other hand, is not chemically defended by exocrine secretion and does not rely on phenolglucosides for its defense. In this species the oviposition preference of the adult matches the larval performance.

Smiley et al.[54] measured the effect of host derived defense on larval survival in the field, and showed a differential survival of *C. aenicollis* on willow clones containing different levels of salicin. Larvae feeding on salicin-rich clones were able to produce salicylaldehyde and had significantly greater survivorship than those larvae reared on salicin-poor clones, not producing salicylaldehyde.

There is, to our knowledge, no evidence demonstrating whether the beetle larvae defended by secretion synthesized *de novo* or the larvae of species which use plant phenolglucosides as a precursor of their defensive secretion are better protected from natural enemies. But it is clear that the efficiency of one type of compound is not a fixed characteristic. For example, Pasteels and Grégoire[32] showed that, when given a choice between larvae of *Pl. versicolora* and *Ph. vitellinae*, predaceous sawflies select the type of prey they have previously eaten. Clearly, chemical defense of larvae is not foolproof: predation on *Pl. versicolora* larvae by ladybird larvae has been observed[67] and predation on *Ph. vitellinae* larvae by spiders (*Xysticus* sp.) and neuropteran larvae *(Anisochrysa prasina)* has also been observed.[35] Some reports, still anecdoal, suggest that predatory wasps are "temporally" specialized on larvae producing salicylaldehyde.[35]

Raupp and Denno[41] showed that the generalist ladybird *Hippodamia convergens* has an enhanced search behavior on leaves of willows *(S. babylonica)* covered with feces of adult *Plagiodera versicolora*. The plant phenolglucosides could influence this behavior indirectly, since the distribution of the *Plagiodera* adults seems to be correlated with the phenolglucoside content of the plants.[55] Additionally, it is unknown what the cue for the change in the predator behavior is, but one cannot exclude a chemical factor, such as, for example, the presence of active compounds resulting from the degradation of phenolglucosides in the feces.

Defense is not the only reported function of the larval exocrine secretion of the leaf-beetles. At least in some cases, it has been demonstrated that the secretions have an impact on the distribution of other herbivores. Raupp et al.[43] showed that the iridoid monoterpenes secreted by the larvae of *Pl. versicolora* negatively influence the distribution of conspecific adults as well as the leaf consumption by another herbivore, *Nymphalis antiopa* larvae. *Prenelopis* ants tending aphids are repelled by salicylaldehyde[35] and stop tending the aphids.

## VI. CONCLUSIONS

Phenolglucosides clearly influence the distribution and food seleciton of herbivores in a complex way. Indeed the interactions between plant and herbivores are part of a dynamic process of which we are only able to observe a frozen sequence in evolutionary time. On the basis of this single observation, we try to deduce the logical course of evolution designating it as coevolution, diffuse or not. It is outside the scope of this review to discuss the terminology which should be utilized to describe the dynamic changes in the interaction between two trophic levels (the host plants and their herbivores). Rather we have stressed that this process involves not only plants and herbivores but also the third trophic level which consists of the herbivores' natural enemies.

Our present understanding of the impact of phenolglucosides on herbivores is mostly speculative since many of the data are descriptive or correlative and very few studies include causal analysis. With these restrictions in mind we would like to divide the interaction between phenolglucosides and herbivores in four rough evolutionary "steps".

- First: The phenolglucosides in the leaves of the Salicaceae have a defensive function against nonadapted herbivores.
- Secondly: Some herbivores have developed adaptation to escape the negative impact of the phenolglucosides, for example, the detoxification mechanism present in *Papilio* spp.
- Thirdly: Some herbivores are able to use the phenolglucosides for their own benefit, for example, the sequestration and utilization which has evolved several times in larvae of leaf beetles.
- Fourthly: At the third trophic level, some natural enemies of the herbivores have adapted to handle the defense which the insects derive from the host-plant phenolglucosides. It is not excluded that some predators can use these plant-derived defensive volatiles as cues to find their prey.

Why are leaves of some northern temperate *Salix* defended by phenolglucosides against herbivory, whereas those of others produce tannins and trichomes? Although it has been suggested[54] that some *Salix* species could escape specialized herbivores which search for plants rich in phenolglucosides by reducing the phenolglucoside content of their leaves, it would be highly hazardous to speculate that it is only the selective pressure of particular herbivores that has prompted such a shift in plant defense. Phenolglucosides could be classified as qualitative defense (sensu Feeny[8]). However, the two types of leaf defense present in *Salix* are not obviously correlated to the apparency of the *Salix* species. Whether or not these two defensive mechanisms fit the resource allocation theory of Coley et al.[4] remains an open question. Additionally, it is important to remember that caution is necessary when dividing the *Salix* species in two categories.

There are contradictory data in the literature on the presence of phenolglucosides in some species which might be explained by geographic variation or by the ease with which hybridization occurs in this group.

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