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Influence of phenolglucosides and trichome density on the distribution of insects herbivores on willows

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Abstract

The effects of both trichome density and phenolglucoside content of leaves of 76 willow hybrids (*Salix alba* × *fragilis*) were measured to estimate their influence on the distribution of *Phratora vitellinae* (L.), *Plagiodera versicolora* Baly (Coleoptera: Chrysomelidae) and *Pontania proxima* (Lepeletier 1823) (Hymenoptera: Tenthredinidae) in a nursery at Gramont, Belgium.

The willows showed differences in their phenolglucoside content and pilosity of leaves and are classified on these basis into four groups by a clustering method. Correlations and multiple regressions showed that these chemical and physical characteristics are good predictors of the abundance of insects. First, the abundance of larvae of *Ph. vitellinae*, adults of *Pl. versicolora* and galls of *P. proxima* is correlated positively with a high phenolglucoside content and a low pilosity of the leaves. Secondly, the distribution of adults of *Ph. vitellinae* and of larvae of *Pl. versicolora* is influenced by neither the chemical nor the physical leaves characteristics studied.

Introduction

Salicaceous plants host a large diversity of insect herbivores (Southwood, 1961) and cultures of willows and poplars may be heavily damaged by them (Morris, 1986). Insect distribution on salicaceous shrubs and trees is influenced by phenolic secondary metabolites (Rowell-Rahier, 1984; Smiley *et al.*, 1985; Tahvanainen *et al.*, 1985; Lindroth & Peterson, 1988), which are the only group of secondary metabolites present in relatively high amounts in the bark and leaves of these plants (Thieme, 1965; Hegnauer, 1973; Palo, 1984).

Two groups of willow species can be distinguished according to the secondary compounds present in their leaves. Some species (e.g. *S. fragilis*, *S. nigricans*, *S. purpurea*) contain high amounts of phenolglucosides but no significant amounts of proanthocyanidins (condensed tannins). The upper and lower surfaces of their leaves are usually glabrous. Other species (e.g. *Salix alba*, *S. caprea*, *S. viminalis*, *S. cinerea*) have leaves containing proanthocyanidins but few phenolglucosides. The undersurface of these leaves is usually densely covered with trichomes (Rowell-Rahier, 1984).

Thus, the leaves of *Salix* can be characterized

by at least 2 variables: a chemical one, i.e. their phenolglucoside content, and a physical one, their trichome density. These 2 variables are negatively associated. Herein, we investigated the relationship between these 2 variables and the abundance in the field of 3 herbivores: *Phratora vitellinae* and *Plagiodera versicolora* (Coleoptera, Chrysomelidae) and *Pontania proxima* (Hymenoptera, Tenthredinidae). These insects were among the most abundant herbivores in the field site selected for the study. They differ in the way they metabolize plant phenolglucoside and in their defensive strategies.

The adults of the leaf beetles *Ph. vitellinae* and *Pl. versicolora* biosynthesize their own defense (isoxazolinone derivatives, Pasteels *et al.*, 1982) and incorporate these products into their eggs (Pasteels *et al.*, 1986). However, the larvae of the first species derive salicylaldehyde for defense from the host-plant phenolglucosides whereas those of *Pl. versicolora* synthesize de novo iridoid monoterpenes (Pasteels *et al.*, 1984; Rowell-Rahier & Pasteels, 1986). Additionally, the females of *Ph. vitellinae* sequester salicin in their eggs, which is used to defend the eggs, but also the neonate larvae before they are able to feed (Pasteels *et al.*, 1986). The larvae of the sawfly (*P. proxima*) live in a characteristic red gall and secrete benzaldehyde of unknown biosynthetic origin (Boevé *et al.*, 1984).

To study the possible influence of phenolglucoside content and trichome density on insect distribution, we selected 2 species of *Salix*, *S. fragilis* and *S. alba*, one belonging to each of the two groups mentioned above, and their numerous hybrids. We quantified the trichome density and the phenolglucoside content of the plants. Although a negative relationship between trichome density and phenolglucoside content has already been reported in *Salix* spp. (see above), it has not previously been studied quantitatively. Trichomes inhibit the feeding of adult *Ph. vitellinae* on *S. caprea*, and phenolglucosides are not necessary to initiate feeding in the laboratory (Rowell-Rahier & Pasteels, 1982). The effect of trichomes on *Pl. versicolora* and *P. proxima* is unknown. Moreover, we do not know how the combination of trichomes and phenolglucosides influence the insect in the field. In this article we clarify these aspects, and we correlate these plant characteristics with insect abundance.

Material and methods

The willow culture. The experimental plot, situated in Gramont (Belgium) in the fields of the 'Rijkstation voor Populierenteelt', contains 329 shrubs, all belonging to the two *Salix* species *S. fragilis* and *S. alba* and their numerous hybrids. Each shrub was placed into one of 5 categories roughly defined by morphological criteria: *S. alba*, *S. 'near alba'*, *S. 'alba* × *fragilis'*, *S. 'near fragilis'* and *S. fragilis* (Steenackers, pers. comm.).

Seventy-six shrubs were selected initially on the basis of their morphological characteristics to include all intermediates between *S. fragilis* and *S. alba* and distributed over the 5 categories as follow: *S. alba*-7 shrubs, *S. 'near alba'*-21 shrubs, *S. 'alba* × *fragilis*-23 shrubs, *S. 'near fragilis'*-15 shrubs and *S. fragilis*-10 shrubs.

All plants were derived from cuttings originating in different geographic areas of Belgium and were planted randomly in the plot in 1981. The shrubs were cut back close to the ground every year, so that at the end of the summer they never exceed 2.5 m in high. It therefore was possible to record all the insects on each plant.

Insect abundance. Larval and adult stages of both *Ph. vitellinae* and *Pl. versicolora* were counted separately. For *P. proxima*, we recorded the number of galls per shrub. In August 1985, all the insects were counted once on each of the 76 trees. In spring and summer 1986, 7 counts were made (on June 1st, 13th and 28th, July 14th and 27th, August 5th and 20th) on a reduced sample of 15 shrubs. These had been selected to include representatives of each of the morphological categories listed above.

The leaf samples. In August 1985, leaf samples were collected from each of the 76 shrubs. One or

two leaves were randomly collected between the 6th and 12th leaf down from the apex, for about twenty branches of each shrub. The samples were air dried at 28 °C and then ground in a mill for phenolglucoside analysis. In 1986, samples of the 15 studied shrubs were collected as above during each of the insect counts, air dried and ground. The estimates of trichome density were made on a separate sample of young leaves (the first 5 apical leaves) and mature leaves (between the 6th and 12th leaf down from the apex) collected from each of the 76 shrubs on the 5th August 1986.

Phenolglucoside analysis. To quantify the phenolglucoside content of the leaves we used a modification of the method described by Smiley *et al.*, 1985. Dried powdered leaves (200 mg) were extracted for 24 h in 6 ml of distilled water acidified with HCl to pH 3.5 to minimize degradation (Meier, pers. comm.). After filtration, serial 1 : 2 dilutions of the extract were applied to silica gel TLC plate POLYGRAM SIL G/UV_{254} with calibrated glass microcapillary tubes. Plates were run in a solvent of methylene chloride and methanol, and the phenolglucosides visualized by spraying the plate with phosphomolybdic acid (3% w/v in ethanol) and heating it at 120 °C for two minutes.

For the 1985 samples we used the solvent in proportion CH_2Cl_2/CH_3OH: 80/20. Under these conditions, 4 compounds were detected. Later we discovered that the proportion 90/10 gives a better separation; 7 compounds were then found as well as in the samples collected in 1986. Salicin and salicortin were identified by comparison with reference substances; a third compound was identified as 2′-O-acetylsalicortin (Meier, pers. comm.). The other compounds present are also salicylates but their identities have not been determined. Quantitative estimates of all compounds were obtained by comparing serial dilutions of the extract with salicin standards on TLC. The validity of this semiquantitative method was confirmed by comparison with quantitative HPLC analysis of samples ($r = 0.97$; $P \leq 0.0001$; $n = 8$) following the method described by Meier (1988).

Trichome density. The number of trichomes on a known area of leaf surface was counted by microscopic examination of the young and mature leaves. The areas (between 6 and 18 mm^2 defined by a ocular grid) were chosen in the middle of the leaves (5 samples per shrub). The data are expressed in trichomes/cm^2.

Statistical analysis. After the experiments were completed, we attempted to obtain a more objective classification of the shrubs that made on the basis of morphological characteristics. For this purpose we used a cluster analysis to group the shrubs according to the phenolglucoside content and the trichome coverage of their leaves. In the cluster analysis, the nearest neighbour method was used with the complete linkage and the squared euclidean distance. This allowed the shrubs to be reclassified into 4 groups (13 in group 1, 23 in group 2, 17 in group 3 and 23 in group 4 – see results).

In a first analysis of the relationship between distribution of the insects and trichome and phenolglucoside values of the shrubs, the Pearson correlation coefficients were calculated. Secondly, to quantify the relative importance of the different factors, we performed stepwise multiple regression analysis. For each species of herbivore studied, a second stepwise multiple regression analysis was performed, taking into account not only trichome and phenolglucoside values but also the data on the other 2 herbivore species present on the shrubs. The objective here was to assess the interactions between the 3 herbivorous species studied.

Results

Classification of the shrubs in groups. The result of cluster analysis is shown in Figure 1. There is 36.8% of similarity between the categories of the morphological classification and the groups of the cluster analysis based on chemical content and pilosity of leaves. This low value of correspondence is not surprising since willows hybridize easily, and since morphological characters used

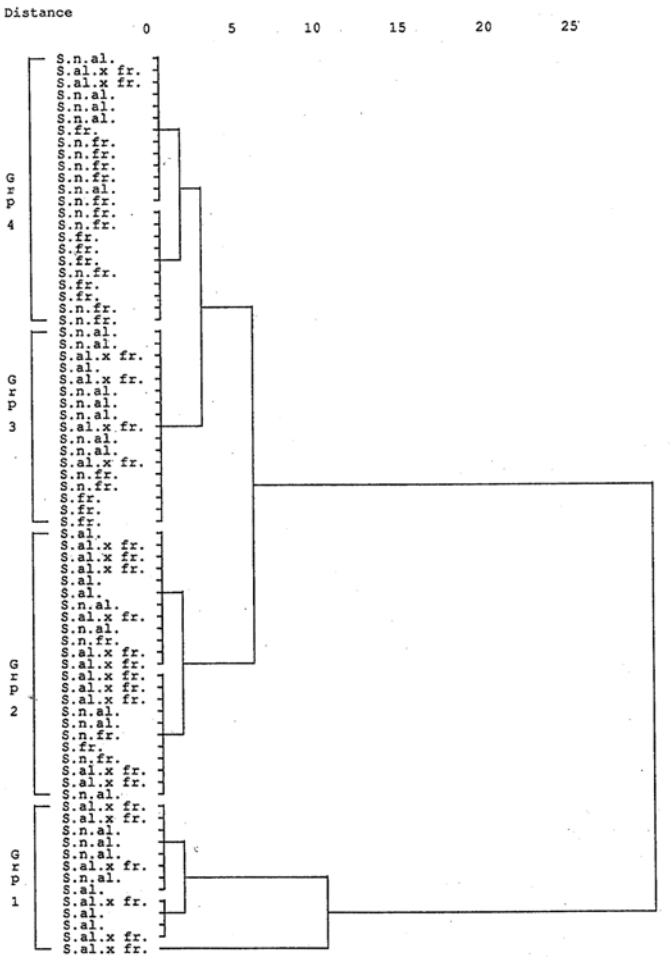

Fig. 1. Cluster analysis (UPGMA) showing the classification of the 76 shrubs into 4 groups on the basis of the chemical and physical characteristics of their leaves (sample of 1985). The abbreviation given for each shrubs represents the morphological category in which each willow was classified initially (S.al. = *S. alba*, S.n.al. = *S. 'near alba'*, S.al. × fr. = *S. 'alba* × *fragilis'*, S.n.fr. = *S. 'near fragilis'* and S.fr. = *S. fragilis*.

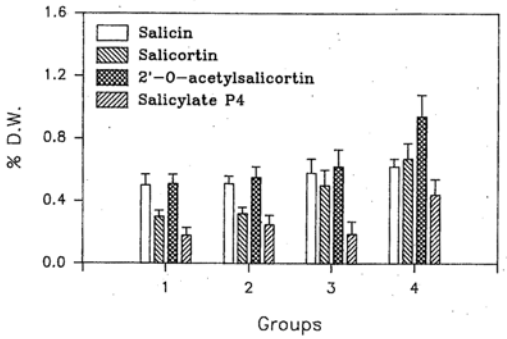

Fig. 2. Average phenolglucoside content of the leaves of the 4 groups of shrubs derived from cluster analysis (sample of 1985). The error bars represent the standard error.

for classical systematics do not necessarily have the same genetic segregation as phenolglucoside content and pilosity of leaves.

In the following discussion, we used the groups defined by the cluster analysis. The 15 shrubs chosen on the basis of their morphology for the study of seasonal variation in 1986 were distributed among these groups as follows: groups 1: 1 shrub, group 2: 3 shrubs, group 3: 5 shrubs and group 4: 6 shrubs.

Phenolglucoside content of the leaves. Four compounds were isolated from a one sample of leaves collected in August 1985. The 3 most abundant and polar ones are salicin (S), salicortin (Sc) and 2′-O-acetylsalicortin (2′-O-acSc). The 4th compound (P4) is a so far unidentified salicylate. The phenolglucoside contents of the leaves of the different groups of shrubs (mean ± standard error) are given in Figure 2. From the first to the fourth group, salicin content slightly increases

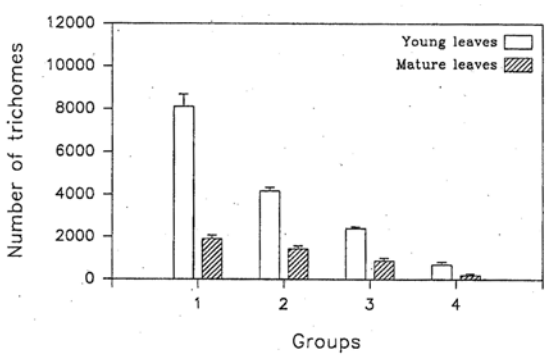

Fig. 3. Average trichome density of the leaves of the 4 groups of shrubs (sample of 1985). The error bars represent the standard error.

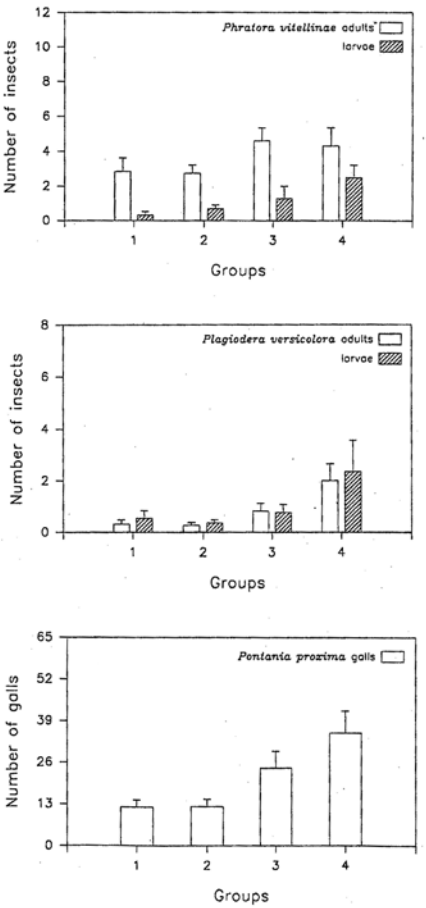

Fig. 4. Average number of insects for the 4 groups of shrubs (sample of 1985). The error bars represent the standard error.

(0.50 ± 0.07 to 0.62 ± 0.05), but is not significantly different between groups. Salicortin and 2′-O-acetylsalicortin increase markedly (respectively 0.30 ± 0.04 to 0.67 ± 0.10 and 0.51 ± 0.06 to 0.94 ± 0.14) and for both compounds, the fourth group is significantly different (respectively $P \leq 0.001$ and $P \leq 0.006$) from the first three groups of shrubs. The last compound P4 slightly increases except in the third group, (0.18 ± 0.05 to 0.44 ± 0.10) but not significantly between groups.

The levels of salicin and salicortin in foliage of the shrubs of group 4 correspond to the values given in the literature for the species *S. fragilis* (Palo, 1984; Hegnauer, 1973); the levels for the first three groups are intermediate between the literature values for *S. alba* and *S. fragilis*. Values for hybrids have not been reported previously. Seven compounds were isolated from the leaves collected in 1986. The first 3 are salicin, salicortin and 2′-O-acetylsalicortin, as before. The other 4 compounds are refered to as P4, P5, P6 and P7. No significant seasonal variation in the total phenolglucoside content was observed in 1986 in the four groups during the period studied.

Trichome density. Figure 3 gives the average number (mean ± standard error) of trichomes on young (TYL) and mature leaves (TML) or each of the 4 groups of shrubs. The results show a gradient from high pilosity in group 1 to low pilosity in group 4. This trend is the reverse of that observed for the phenolglucosides. Moreover, young leaves are always more pubescent than older ones. It is possible that all the trichomes are already present in the young leaves and that their density decreases as a consequence of growth.

The insect counts. The results of the counts of insect abundance in August 1985 are illustrated in Figure 4. The average number of insects per shrub (mean ± standard error) was calculated for the four groups of shrubs. For all the insect stages the numbers increase from the first to the fourth group and are significantly different excepted for the adults of *Ph. vitellinae*.

The counts of 1986 are illustrated in Figure 5 and reflect for each group of shrubs the seasonal variation in the average number of the adults of *Ph. vitellinae* and *Pl. versicolora* and the galls of *P. proxima*. The two chrysomelids are found only in the two last groups; there are two generations

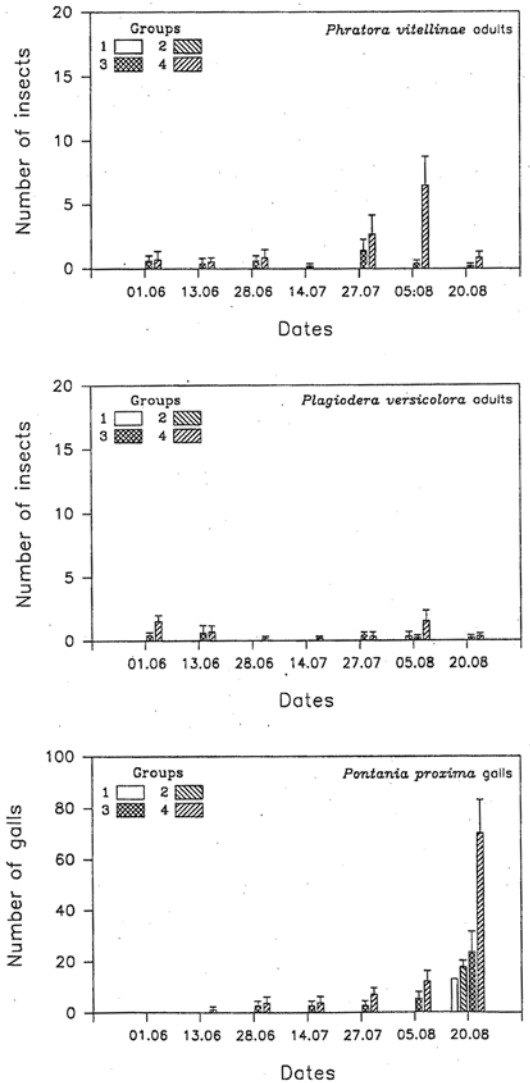

Fig. 5. Seasonal variation of the average number of insects per shrubs (sample of 1986). The error bars represent the standard error.

per year with a high value in the number of adults of *Ph. vitellinae* in the second generation. The number of galls of *P. proxima* increase during the season with a high value in the end of August reflecting a long period of oviposition; Lorenz and Kraus (1957) report one or two generations per year as a function of climatic variations.

Correlation between herbivores, phenolglucosides and trichomes. The results discussed in this section are based on data derived from 2 different samples. For the phenolglucoside analysis, the leaves were collected in August 1985 and for the trichome counts the leaves were collected in August 1986. Comparison of the phenolglucoside values obtained for the same 15 trees in both years shows no major differences between the two years.

The Pearson correlation coefficients between each pair of studied variables are given in Table 1. From these the following facts emerge.

1. Chemical and physical characteristics are not independent. The chemical variables (S, Sc, 2′-O-acSc & P4) are always highly positively correlated with each other ($P \leq 0.001$) and the physical variables (TYL & TML) are also positively correlated ($P \leq 0.001$) with each other. Significant negative correlations ($p \leq 0.001$) are observed between the density of trichomes and all chemical variables except salicin.
   Insect abundance:
2. Only the value for the larvae of *Pl. versicolora* (VEL) are not correlated with those of other insects. All the other values (VIA: adults of *Ph. vitellinae*, VIL: larvae of *Ph. vitellinae*, VEA: adults of *Pl. versicolora* & PP: galls of *P. proxima*) are significantly and positively correlated with each other.
3. The adults of *Ph. vitellinae* (VIA) and the larvae of *Pl. versicolora* (VEL) show no significant correlation with either phenolglucoside content or trichome density.
4. In contrast, the larvae of *Ph. vitellinae* (VIL) and adults of *Pl. versicolora* (VEA) are posi-

Table 1. Correlation coefficient (r) matrix between chemical and physical characteristics of willows and insects' distribution

	S	Sc	2′-O-acSc	P4	VIA	VIL	VEA	VEL	PP	TYL
Sc	0.62									
	+++									
P3	0.60	0.81								
	+++	+++								
P4	0.50	0.53	0.59							
	+++	+++	+++							
VIA	0.04	0.19	0.08	−0.02						
	NS	NS	NS	NS						
VIL	0.33	0.31	0.32	0.15	0.46					
	++	++	++	NS	+++					
VEA	0.33	0.49	0.44	0.25	0.29	0.62				
	++	+++	+++	+	++	+++				
VEL	0.05	−0.08	−0.07	0.01	−0.05	0.02	−0.13			
	NS	NS	NS	NS	NS	NS	NS			
PP	0.35	0.54	0.50	0.36	0.31	0.47	0.58	−0.07		
	++	+++	+++	+++	++	+++	+++	NS		
TYL	−0.17	−0.38	−0.32	−0.25	−0.21	−0.31	−0.35	−0.16	−0.41	
	NS	+++	++	+	NS	++	++	NS	+++	
TML	−0.17	−0.46	−0.41	−0.28	−0.25	−0.38	−0.39	−0.10	−0.54	0.73
	NS	+++	+++	++	+	+++	+++	NS	+++	+++

VIA = *Ph. vitellinae* adult; VIL = *Ph. vitellinae* larvae; VEA = *Pl. versicolora* adult; VEL = *Pl. versicolora* larvae; PP = *P. proxima* gall. S = salicin; Sc = salicortine; 2′-O-acSc = 2′-O-acetylsalicortine; P4 = unidentified compound. TML = trichomes of mature leaves; TYL = trichomes of young leaves. +++: $P \leq 0.001$, ++: $P \leq 0.01$, +: $P \leq 0.05$, NS: $P > 0.05$.

Table 2. Results of multiple regression analysis

Dependent variables (insects)	% of variation explained	Variables in the regression	Level of significance
Phratora vitellinae			
adults	10.35	− TML + Sc − 2′-O-acSc − P4 − TYL − S	NS
	10.33	− TML + Sc − 2′-O-acSc − P4 − TYL	NS
	10.26	− TML + Sc − 2′-O-acSc − P4	NS
	9.12	− TML + Sc − 2′-O-acSc	NS
	6.92	− TML + Sc	NS
	6.24	− TML	+
larvae	23.73	− TML + S-P4 + 2′-O-acSc-Sc − TYL	+ +
	23.56	− TML + S-P4 + 2′-O-acSc-Sc	+ +
	23.34	− TML + S-P4 + 2′-O-acSc	+ + +
	22.92	− TML + S-P4	+ + +
	22.02	− TML + S	+ + +
Plagiodera versicolora			
adults	28.60	Sc − TML + S − TYL + 2′-O-acSc − P4	+ + +
	28.31	Sc − TML + S − TYL + 2′-O-acSc	+ + +
	28.07	Sc − TML + S − TYL	+ + +
	27.74	Sc − TML + S	+ + +
	27.28	Sc − TML	+ + +
larvae	7.47	− TYL − Sc + S − 2′-O-acSc − TML + P4	NS
	7.40	− TYL − Sc + S − 2′-O-acSc − TML	NS
	7.20	− TYL − Sc + S − 2′-O-acSc	NS
	6.94	− TYL − Sc + S	NS
	4.86	− TYL − Sc	NS
	2.56	− TYL	NS
Pontania proxima			
galls	40.62	Sc − TML + 2′-O-acSc + S + P4 − TYL	+ + +
	40.61	Sc − TML + 2′-O-acSc + S + P4	+ + +
	40.49	Sc − TML + 2′-O-acSc + S	+ + +
	40.23	Sc − TML + 2′-O-acSc	+ + +
	39.57	Sc − TML	+ + +

S = salicin; Sc = salicortine; 2′-O-acSc = 2′-O-acetylsalicortine; P4 = unidentified compound. TML = trichomes of mature leaves; TYL = trichomes of young leaves. + + +: $P \leq 0.001$, + +: $P \leq 0.01$, +: $P \leq 0.05$, NS: $P > 0.05$.

tively correlated with phenolglucoside content of the leaves and negatively with trichome density.

5. The number of galls of *P. proxima* (PP) is positively correlated with phenolglucoside content and negatively with the trichome density.

Multiple regression between herbivores, phenolglucosides and trichomes. The results of the multiple regression analysis are presented in Tables 2 and 3. Table 2 suggests that the variation in the number of adults of *Ph. vitellinae* and larvae of *Pl. versicolora* is not explained by the variation in phenolglucoside values and the trichome density. On the other hand, a significant percentage of the variation in the relative abundance of the larvae of *Ph. vitellinae* on different shrubs is explained by the phenolglucoside content of the leaves (positive influence, except P4) and their pilosity (nega-

Table 3. Results of multiple regression analysis

Dependent variables (insects)	% of variation explained	Variables in the regression	Level of significance
Phratora vitellinae			
adults	16.65	PP + VEA – 2′-O-acSc + Sc – P4 – TML – S – TYL	NS
	16.62	PP + VEA – 2′-O-acSc + Sc – P4 – TML – S	NS
	16.52	PP + VEA – 2′-O-acSc + Sc – P4 – TML	NS
	15.64	PP + VEA – 2′-O-acSc + Sc – P4	+
	14.45	PP + VEA – 2′-O-acSc + Sc	+
	13.06	PP + VEA – 2′-O-acSc	+
	11.52	PP + VEA	+
	9.78	PP	+ +
Plagiodera versicolora			
adults	40.50	PP + Sc + VIA – TYL + S – P4 + 2′-O-acSc + TML	+ + +
	40.50	PP + Sc + VIA – TYL + S – P4 + 2′-O-acSc	+ + +
	40.25	PP + Sc + VIA – TYL + S – P4	+ + +
	40.05	PP + Sc + VIA – TYL + S	+ + +
	40.05	PP + Sc + VIA – TYL	+ + +
	39.82	PP + Sc + VIA	+ + +
	39.37	PP + Sc	+ + +
	38.21	PP	+ + +
Pontania proxima			
galls	51.04	VEA – TML + Sc + VIA + P4 + 2′-O-acSc + S + TYL	+ + +
	50.99	VEA – TML + Sc + VIA + P4 + 2′-O-acSc + S	+ + +
	50.94	VEA – TML + Sc + VIA + P4 + 2′-O-acSc	+ + +
	50.65	VEA – TML + Sc + VIA + P4	+ + +
	49.82	VEA – TML + Sc + VIA	+ + +
	48.84	VEA – TML + Sc	+ + +

VIA = *Ph. vitellinae* adult; VEA = *Pl. versicolora* adult; PP = *P. proxima* gall. S = salicin; Sc = salicortine; 2′-O-acSc = 2′-O-acetylsalicortine; P4 = unidentified compound. TML = trichomes of mature leaves; TYL = trichomes of young leaves. + + +: $P \leq 0.001$, + +: $P \leq 0.01$, +: $P \leq 0.05$, NS: $P > 0.05$.

tive influence) (22% by TML & S, $P \leq 0.001$). The abundance of the adults of *P. versicolora* on the different shrubs can be partially explained by the salicortin content (positive influence) and the trichome density of the mature leaves (negative influence) (27%, $P \leq 0.001$). For the galls of *P. proxima*, 39% of the variation in the number of galls per shrub can be explained by the salicortin content and the trichome density of the mature leaves.

Table 3 summarizes the results of the stepwise multiple regression in which the abundances of the other species of herbivore are included in the independent variables. They show that for all the species studied, more variation is explained when the other herbivores are taken into account than when only chemical and physical variables are considered. The contribution of the other herbivores is always positive, suggesting that the same individual shrubs are favored by all 3 species of herbivores.

Discussion and conclusion

The willow plantation studied was an ideal site to study patterns of host plant use by 3 herbivores species common on willows, because the shrubs presented variations in chemical and physical characters while still being part of a taxonomic

continuum between 2 species (*S. alba* and *S. fragilis*). The phenolglucoside content of the leaves did not vary significantly from year to year during the period studied. Seasonal variations have been reported in *Salix pentandra* and *S. purpurea* (Thieme, 1965), in *S. nigricans*, *S. purpurea* and *S. caprea* (Rowell-Rahier, 1984) and *Populus tremuloides* (Lindroth *et al.*, 1987). Several studies have shown the effect of the extraction procedure on the estimation of phenolglucoside content in leaves of Salicaceae (Steele *et al.*, 1969; Lindroth & Pajutee, 1987; Meier *et al.*, 1988). It is likely that the method we selected introduced artefacts due to chemical degradation of the more complex phenolglucosides to more simple one such as salicin. However, we will discuss our results mostly in relation to the total amount of phenolglucosides in the leaves rather than the precise composition of the latter. Two of the 3 species of herbivore studied belong to the same subfamily, although they use very different strategies to deal with the plant toxins. The larvae of one species, *Ph. vitellinae*, uses the phenolglucosides for its own defense against natural enemies; whereas the larvae of *Pl. versicolora* biosynthesize their own defensive compound and excrete the phenolglucosides.

At least for the larvae of *Ph. vitellinae*, the adults of *Pl. versicolora* and the galls of *P. proxima*, our results show clearly that their abundance in the field is not independent of the phenolglucoside content and trichome coverage of the leaves. These 3 species are now discussed separately.

Phratora vitellinae. The correlations between plant characteristics and insect abundance obtained for the larvae of *Ph. vitellinae* are not surprising, since these insects are dependent on salicin for their defense against natural enemies. Additionally, the utilization of salicin allows the larvae to recover some glucose which is linked to an increased growth rate (Rowell-Rahier & Pasteels, 1986). The abundance of the adults of *Ph. vitellinae* in the field is not explained, even in part, by any of the variables studied. Obviously, in the field, the phenolglucosides are not strong attractants or feeding stimulants for the adult of this species. Indeed, it was previously shown that salicin is not a phagostimulant for this insect (Finet, 1982; Soetens, 1986). Phenolglucosides could however influence oviposition, and thus, the distribution of the larvae. Alternatively, the greater abundance of larvae on leaves rich in phenolglucosides and poor in trichomes could simply be due to the better survival and growth of the larvae on these leaves. Further experiments are necessary to determine whether or not phenolglucosides and trichomes influence the oviposition of *Ph. vitellinae*.

Plagiodera versicolora. Since the larvae of *Pl. versicolora* do not use phenolglucosides for defense, or as a nutrient, it is not surprising that the abundance of the larvae is not positively correlated with phenolglucoside content of the leaves. We might have expected a negative correlation, implying that the larvae of this species grow or survive less well on plants rich in phenolglucosides, in view of the general toxicity of these compounds. This however is not the case. *Pl. versicolora* larvae appear to tolerate phenolglucosides, and the adults of *Pl. versicolora* are actually more abundant on shrubs with high phenolglucoside and low trichome coverage. We do not know if this is due to an attractant or phagostimulant effect of the phenolglucoside, or to an avoidance of shrubs with many trichomes, or to yet another plant characteristic positively correlated with high phenolglucoside content and low trichome coverage. Tavhanainen *et al.* (1985) report that the adults of *Pl. versicolora* favors leaves with moderate to low total phenolglucoside concentration in choice experiments with different *Salix* species. Raupp and Denno (1984) report *Pl. versicolora* on willows with potentially high phenolglucoside content. Because the larvae are found equally commonly on all types of leaves, phenolglucosides and pilosity of leaves probably does not affect oviposition by the adults.

Pontania proxima. The distribution of this gall-forming sawfly is closely correlated with high phenolglucoside content of the leaves and low

trichome density. The larvae in the galls secrete benzaldehyde of unknown origin for their defense, but they are also shielded by the (bright red) gall tissue. Preliminary analysis suggest that gall tissue contains the same amount of phenolglucosides as the leaves. Galls of high phenolglucoside content might offer better protection, and thus afford an ultimate explanation for the distribution observed in the field. The response of the ovipositing female to either phenolglucosides or trichomes might provide a proximal explanation.

In summary, we were able to show that for specialised insects feeding on willow, the phenolglucoside content and trichome density of the leaves are good predictor of the abundance of some but not all species and life stages. In the case of the larvae of one species, *Phratora vitellinae*, the phenolglucosides have a defensive function as well as a nutritional one. This positive effect on the larvae is reflected in the distribution of the larvae in the field.

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References

Boevé, J. L., J. C. Braekman, D. Daloze, M. Houart & J. M. Pasteels, 1984. Defensive secretions of Nematinae Larvae (Symphyta-Tenthredinidae). Experientia 40: 546.

Finet, Y., 1982. Préférences alimentaires de *Phratora vitellinae* L. (Coleoptera, Chrysomelidae) vis à vis de différents cultivars de peupliers. Thèse de doctorat. Université Libre de Bruxelles.

Hegnauer, R., 1973. Salicaceae. In: Chemotaxonomie der Pflanzen 6: 240–258.

Lindroth, R. L., M. T. S. Hsia & J. M. Scriber, 1987. Seasonal patterns in the phytochemistry of three *Populus* species. Biochem. Syst. Ecol. 15: 681–686.

Lindroth, R. L. & M. S. Pajutee, 1987. Chemical analysis of phenolic glycosides: art, facts, and artifacts. Oecologia 74: 144–148.

Lindroth, R. L. & S. S. Peterson, 1988. Effects of plant phenols on performance of southern armyworm larvae. Oecologia 75: 185–189.

Lorenz, H. & M. Kraus, 1957. Die Larvalsystematik der Blattwespen. Akademie-Verlag. Berlin.

Meier, B., 1988. Analytik, Chromatographisches Verhalten und Potentielle Wirksamkeit der Inhaltsstoffe Salicylathaltiger Arzneipflanzen Mitteleuropas. Habilitationsschrift. Eidgenössischen Technischen Hochschule. Zürich.

Meier, B., R. Julkunen-Tiitto & O. Sticher, 1988. Comparative high-performance liquid and gas-liquid chromatographic determination of phenolic glycosides in Salicaceae species. J. Chrom. 442: 175–186.

Morris, R. C. 1986. The control of insect pests in energy plantations – An international survey of *Alnus*, *Salix* and *Populus*. Report N° 1. IEA/BA. Biomass Growth and Production Technology.

Palo, T., 1984. Distribution of Birch (*Betula* spp.), Willow (*Salix* spp.), and Poplar (*Populus* spp.) secondary metabolites and their potential role as chemical defense against herbivores. J. Chem. Ecol. 10: 499–520.

Pasteels, J. M., J. C. Braekman, D. Daloze & R. Ottinger, 1982. Chemical defense in Chrysomelid larvae and adults. Tetrahedron 38: 1891–1897.

Pasteels, J. M., M. Rowell-Rahier, J. C. Braekman & D. Daloze, 1984. Chemical defense in leaf beetle and their larvae. The Ecological, Evolutionary and Taxonomic significance. Bioch. Syst. Ecol. 12: 395–406.

Pasteels, J. M., D. Daloze & M. Rowell-Rahier, 1986. Chemical defence in Chrysomelid eggs and neonate larvae. Physiol. Entomol. 11: 29–37.

Raupp, M. J. & R. F. Denno, 1984. The suitability of damaged willow leaves as food for the leaf beetle, *Plagiodera versicolora*. Ecol. Entomol. 9: 443–448.

Rowell-Rahier, M., 1984. The presence or absence of phenolglycosides in *Salix* (Salicaceae) leaves and the level of dietary specialization of some of their herbivorous insects. Oecologia 62: 26–30.

Rowell-Rahier, M. & J. M. Pasteels, 1982. The significance of salicin for a *Salix*-feeder, *Phratora* (= *Phyllodecta*) *vitellinae* Proc. 5th. Int. symp. Insect-Plant Relationships, Wageningen. 73–78.

Rowell-Rahier, M. & J. M. Pasteels, 1986. Economics of chemical defence in Chrysomelinae. J. Chem. Ecol. 12: 1189–1203.

Smiley, J. T., J. M. Horn & N. E. Rank, 1985. Ecological effects of salicin at three trophic levels: new problems from old adaptations. Science 229: 649–651.

Soetens, Ph., 1986. Sensibilité différentielle de *Salix alba*, *S. fragilis* et leurs hybrides aux insectes phyllophages: *Phratora vitellinea*, *Plagiodera versicolora* (Coleoptera: Chrysomelidae) et *Pontania proxima* (Hymenoptera: Tenthredinidae). Travail de fin d'études, Université Libre de Bruxelles.

Southwood, T. R. E., 1961. The number of species of insect associated with various trees. J. Anim. Ecol. 30: 1–8.

Steele, J. W., M. Bolans & R. C. S. Audette, 1969. Phytochemistry of the Salicaceae. II. The effect of extraction procedures on the apparent free phenolic glycoside content of *Salix* species. J. Chrom. 40: 370–376.

Tahvanainen, J., R. Julkunen-Tiitto & J. Kettunen, 1985. Phenolic glycosides govern the food selection pattern of willow feeding leaf beetles. Oecologia 67: 52–56.

Thieme, H., 1965. Die Phenolglykoside der Salicaceen. Pharmazia 20: 688–691.