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The food plant preferences of *Phratora vitellinae* (Coleoptera: Chrysomelinae)

B. A laboratory comparison of geographically isolated populations and experiments on conditioning

M. Rowell-Rahier
Zoologisches Institut der Universität, Rheinsprung 9, Basel 4051, Switzerland

Abstract. The specialisation of the beetles from the Petite Camargue on *Salix nigricans* was confirmed by laboratory food-plant trials. When *S. nigricans* is not present in the choice offered, other *Salix* species are accepted and the following ranking, in decreasing order of preference, can be established: *S. nigricans, S. purpurea, Populus nigra, P. tremula, S. alba, S. caprea* and *S. cinerea*. *S. nigricans* leaves are the richest in salicin of the 4 most acceptable plant species. The 3 least acceptable species have the undersurface of their leaves covered with trichomes.

The food-plant preferences of 2 populations from central Europe and 2 from Belgium differ both in the field and in the laboratory. The level of dietary specialisation also differs from population to population. The populations originating from localities (Oignie in Belgium and the Petite Camargue in central Europe) where their favorite food-plants in the field and in the laboratory (respectively *P. tremula* and *S. nigricans*) are abundant, show clearer preferences than the populations from localities (Grammont in Belgium and Herrliberg in central Europe) where these plants are scarcer or even absent, due to human influence.

The Salicaceae tested, as seen by the more specialised beetles of Oignie and the Petite Camargue, include both a strongly preferred plant (*P. tremula* or *S. nigricans*) and two or three strongly disliked species (*S. alba, S. caprea* and *S. cinerea*). For these beetles, laboratory preferences and field observations of host plant species are in agreement. It is interesting that *S. nigricans*, although not present in Belgium, is the second most preferred food of the Belgian beetles in laboratory trials. Similarly, *P. tremula*, the preferred food of the Belgian beetles, is well accepted by the central European beetles.

For the less specialised beetles of Grammont and Herrliberg, the same Salicaceae include strongly disliked species but no single strongly preferred species. In the Herrliberg population, with rather poorly defined preferences, preference for the favorite species of the more specific population from the same geographical area (Petite Camargue) can readily be produced in the laboratory by conditioning.

Adults of *Phratora vitellinae* are found in the field on different species of Salicaceae over the range of the species (Rowell-Rahier 1984a). Studies of an Upper Rhine population in the field and in the laboratory indicate however that this beetle is rather specialised in its food plant preference (Rowell-Rahier and Pasteels 1982).

The aim of this paper is to compare on the basis of laboratory studies the food plant preferences of 4 different beetle populations found on four different species of Salicaceae, giving particular attention to their level of dietary specialisation and to the possibility of conditioning their food plant preferences.

The four populations studied comprised two groups; two from central Europe and two from Belgium. In each of these two areas one population was chosen from a natural habitat (swamp) and one from a habitat disturbed by human activities (a garden and a poplar plantation). In its central European swamp habitat *Ph. vitellinae* was found on *Salix nigricans*, a frequently recorded host plant (Rowell-Rahier, 1984a). The geographic range of *S. nigricans* is however smaller than that of *Ph. vitellinae*. In Belgium, *S. nigricans* is absent and *Ph. vitellinae* is found on *Populus tremula* in one natural habitat. With the exception of *S. nigricans*, all the other willows and poplars tested in this work are present and relatively common in both countries.

In the other 2 habitats studied the diversity of the natural resource had been either modified (garden) or increased by the introduction of susceptible new host plants (poplar plantation).

For all these willows, the content of water, total nitrogen and salicin of the leaves and their variation with time of the year were determined. The presence or absence in the leaves of phenylglycosides other than salicin was also ascertained. As the trichomes of *S. caprea* act as a mechanical deterrent for the larvae of *Ph. vitellinae* (Rowell-Rahier and Pasteels 1982), the morphology of the undersurface of *S. nigricans* and *S. caprea* leaves was observed by scanning electron microscopy.

This study was designed to provide answers to the following questions:

– Is there geographic variation in the food plant preferences of *Ph. vitellinae* tested in the laboratory?
– Are these preferences in agreement with the field observations?
– Is the level of dietary specialisation of the different populations comparable?
– Can food plant preference be induced?

Material and methods

The four populations studied

The first population was found on *S. nigricans* in an undisturbed swamp in Alsace (Petite Camargue, Saint Louis, France). Details of the food plant preferences of this population in the field and in the laboratory have already been reported (Rowell-Rahier and Pasteels 1982; Rowell-Rahier 1984).

The second central European population was found on *S. purpurea* in a suburban garden (Herrliberg, Zürich, Switzerland). *S. purpurea* was the only willow species present in this garden. The original vegetation of this area was probably very similar to that of the Petite Camargue swamp but has been disturbed for at least 25 years (approximatively 50 generations of beetles).

The third population was found on *P. tremula* in a swamp in Southern Belgium (Oignie). *S. purpurea*, *S. alba* and *S. caprea* are also present but *S. nigricans* does not occur in Belgium.

The fourth and last population was found on *P. trichocarpa* × *deltoïdes* in a poplar selection station (Grammont, Belgium) where exotic poplars and hybrids have been introduced. Finet (1982) showed that many of these are more susceptible to *Ph. vitellinae* attacks than the indigenous *P. nigra*.

The plants tested

The food plant trials were all made with willows and poplars collected daily in the Petite Camargue in early June 1982. *S. nigricans*, *S. purpurea* and *P. tremula* were chosen for the trials because these comprised the natural food plants of three of the studied populations. *P. nigra* was used as it is present in three of the four collecting sites and because in the poplar plantation (population 4) *Ph. vitellinae* is at least occasionally found on it. Leaves of all these four Salicaceae contain phenolglycosides (Hegnauer 1973) and have no hairs on their undersurfaces. Three other less acceptable willow species (*S. alba*, *S. caprea* and *S. cinerea*) (Rowell-Rahier and Pasteels 1982) were also utilised in the trials.

Leaf characteristics

Leaves of different *Salix* and *Populus* species were collected in the Petite Camargue at different times during the summer of 1981 and 1982. The pilosity of the fresh leaves was noted. In August, trichomes were removed from the leaves as described by Rowell-Rahier and Pasteels (1982). The dry weight of the trichomes was determined by weighing "unshaved" and "shaved" discs of equal area.

The water content of the leaves was determined by weighing them before and after drying at 40° C. Dried leaf samples were stored in air-tight containers.

The total nitrogen content of the leaves was determined by the Kjeldahl method (McKenzie and Wallace 1954).

The salicin content of the willow leaves was determined by quantitative HPLC analysis of a methanolic extract of their dried leaves, using the method described by Sticher et al. (1982). Qualitative HPLC was used to detect the presence or absence of other phenolglycosides in the leaves, but no attempt was made to identify them. The extract of *P. nigra* could not be analysed for technical reasons.

The trichome complement of young and mature *S. caprea* and *nigricans* leaves was examined by scanning electron microscopy of fresh leaves fixed in glutaraldehyde.

The feeding trials

The feeding trials were conducted as described by Rowell et al. (1984). Each beetle population was tested with all possible binary combination (28) of the 7 different plant species, each combination being repeated 11–20 times.

For each trial a relative score was allotted to each of the two plant tested. These scores corresponded to the following categories: much more eaten, slightly more eaten, equally eaten, slightly less eaten and much less eaten. The categories were allotted arbitrary numerical scores of $+2$, $+1$, 0, -1, -2, respectively. For each combination a mean score was obtained by averaging the scores of the different repetitions. For each plant, the 6 scores so obtained were then summed and the total used to determine a rank order in palatability. To assess the significance of the difference between any two plants, a binomial test was used. The rank order of preferences obtained for the four populations were compared using the Spearman test of rank correlation.

The use of relative scores, as outlined above, compensates for differences between individual insects, e.g. in hunger, and the large number of insects used (250 for each population) minimises the effects of individual idiosyncracies. It is assumed that male and female have the same qualitative preferences.

Conditioning of food plant preference

Adults from both the Petite Camargue and the Herrliberg populations were fed exclusively on *S. nigricans* or on *S. purpurea* in the laboratory for 4 weeks. Their choice between *S. nigricans* and *S. purpurea* was ascertained before and after this period. To test further the possible conditioning of adult choice by the larval food plant, larvae, derived from eggs laid on *S. nigricans* or *S. purpurea*, were fed on these two plants during their entire development. The resulting adults were tested upon emergence or after one week of feeding on their larval food plant.

Results

Chemical and physical characteristics of willow leaves

The chemical characteristics of the leaves of the different *Salix* and *Populus* species studied in the Petite Camargue, and their variation with age, are presented in Table 1. As expected, the content of water and total nitrogen is higher in the younger leaves than in the older ones. There are however no marked differences between species. It is interesting to note that *S. nigricans* leaves are richer in salicin than the other willow species with leaves containing phenolglycosides. In *S. nigricans*, the content of salicin is higher in the young leaves than in the older ones. The increase in salicin in *S. purpurea* leaves in June corresponds to the sharp increase in phenolglycoside content shown by Thieme (1965) in the early Spring. The same increase cannot be observed in the *S. nigricans* sample collected at the same date, perhaps because these leaves are more precocious (Rowell-Rahier 1984a). The HPLC chromatograms suggest

Table 1. Content of water, total nitrogen and salicin of the leaves of different Salicaceae collected in the Petite Camargue at different times of the year. (1): young leaves, (2): mature leaves, (3): old leaves; + (or −): other phenylglycosides than salicin present (or absent)

Plant, date			H_2O (% FW)	Total N_2 (% DW)	Salicin (% DW)	Other phenylglycosides
S. nigricans:						
1981	April	(1)	70.3	3.6	5.6	−
	June	(2)	68.1	2.9	2.3	−
	July	(2)	64.8	2.4	1.5	−
	August	(1)	73.4	3.9	4.1	−
	August	(2)	64.0	2.3	1.4	−
	August	(3)	60.5	2.2	1.9	−
	September	(3)	53.2	2.3	1.4	−
1982	May	(1)	66.2	3.5	2.9	
	June	(2)	63.7	2.4	2.5	
	August	(2)	59.2	1.9	1.9	
	August	(3)	57.2	1.8	1.4	
	September	(2)	52.8	2.2	1.2	
	September	(3)	52.7	1.9	0.9	
S. purpurea:						
1981	April	(1)	66.5	3.7	0.2	+
	June	(2)	66.6	3.3	1.6	+
	July	(2)	67.4	3.1	1.3	+
	August	(2)	61.7	2.6		
	September	(3)	53.0	2.5	1.0	+
1982	May	(1)		2.7		
	June	(2)	64.3	2.5		
P. nigra italica:						
1982	June	(2)	64.9	2.5		
P. tremula:						
1982	June	(2)	61.4	1.8	1.3	
S. cinerea:						
1981	April	(1)	66.4	3.9	0	−
	June	(2)	64.1	2.9		
	July	(2)	68.3	2.8		
	August	(1)	65.5	3.6		
	August	(3)	68.3	2.6		
	September	(3)	61.1	2.4	0	−
1982	June	(2)	61.7	2.1		
S. caprea:						
1981	April	(1)	70.1	4.2	0.05	−
	June	(2)	69.8	3.5		
	July	(2)	72.9	3.1	0.1	−
	August	(2)	62.8	2.7		
	September	(3)	64.7	2.5	0.05	−
1982	June	(2)	58.8	2.4		
S. alba:						
1981	April	(1)	69.8	3.5	0	−
	June	(2)	69.6	3.1		
	July	(2)	68.8	2.6	0	−
	August	(2)	62.1	2.3		

that the complexity of the mixtures of phenolglycosides in the leaves is greater in *S. purpurea* than in *S. nigricans*. No phenolglycosides were detected in leaves of *S. caprea*, *S. cinerea* and *S. alba*.

The physical characteristics of *S. caprea* and *S. nigricans* leaves are illustrated in Fig. 1. The trichomes on the lower surfaces of mature *S. caprea* leaves in August represent up to 16% of their dry weight. Figure 1 shows that, in July, the trichomes are denser on the young *S. caprea* leaves than on the mature ones. All the trichomes are probably already present on young leaves and their density decreases as the leaf surface increases with age. Although the trichomes of *S. caprea* are unbranched, they are often hooked. This contrasts with the trichomes of *S. nigricans* which are always straight. Moreover, on *S. nigricans*, trichomes are found only on the veins and never cover the entire lower surface of the leaves.

Rank order of palatability of different salicaceous trees

Figure 2 shows the the order of preference in which the willows and poplars were eaten by the four populations of *Ph. vitellinae*. The rank orders derived from the four populations are fairly similar (see Table 2), especially between the pairs of populations originating from the same geographical area (Oignie-Grammont, Petite Camargue-Herrliberg).

However, when populations from Belgium and central Europe are compared (Oignie-Petite Camargue; Oignie-Herrliberg; Grammont-Petite Camargue; Grammont-Herrliberg) the most preferred food plant is different, *P. tremula* being preferred in Belgium and *S. nigricans* in central Europe. These laboratory preferences correspond to the observed food plant of the populations originating from the Petite Camargue or Oignie in the field. It is interesting to note that *S. nigricans*, although not present in Belgium, is the second most preferred food of the Belgian beetles in the laboratory trials. Similarly, *P. tremula*, the preferred food of the Belgian beetles, is also well accepted by the central European beetles and does not differ significantly from *S. nigricans* in the laboratory trials (see Fig. 3).

The least acceptable food plants are the same for all four populations studied. Expressed otherwise, this means that the different populations have some geographic variation in their criteria for acceptance but are broadly similar in their criteria for rejection of their food plant species.

Variation in the degree of specialisation of the four populations

The distribution of significant differences in palatability, calculated as described in the methods, is indicated in Fig. 3. It should be noted that, as discussed in Rowell et al. (1984), the criteria of significance derive ultimately from the magnitude of the individual differential score.

The Salicaceae tested, as seen by the Oignie and the Petite Camargue beetles, include both a strongly preferred and two or three strongly disliked species. The same plants as seen by the Grammont and Herrliberg beetles include the same strongly disliked species but no single strongly preferred species.

Trials with the four populations produced more significant difference in palatability in many of the combinations involving the three least preferred plants *S. caprea*, *S. alba*

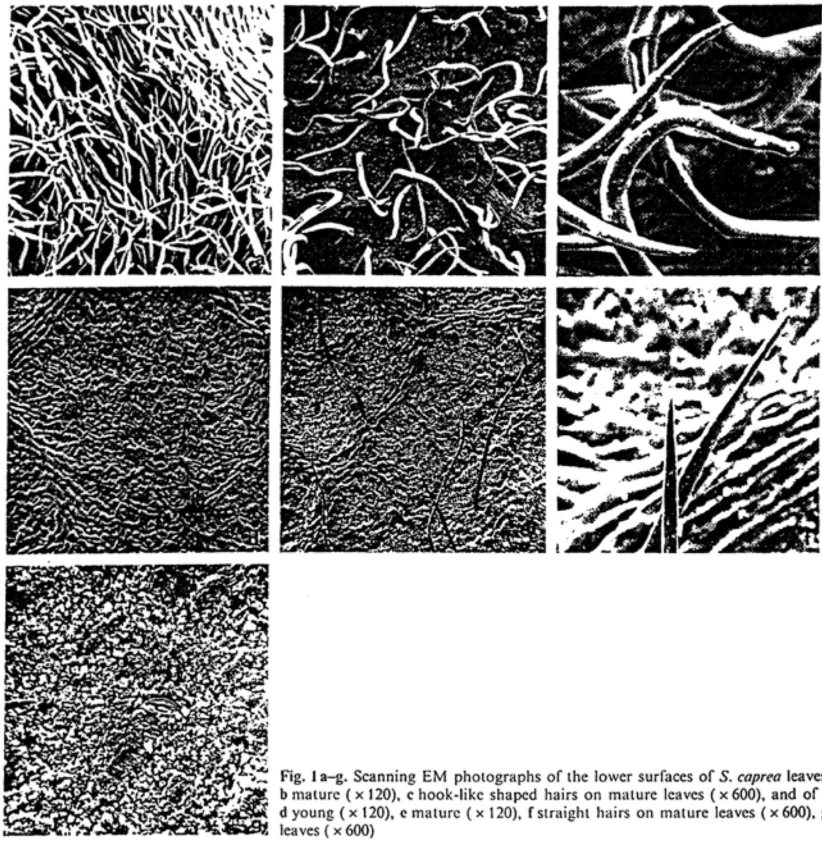

Fig. 1a–g. Scanning EM photographs of the lower surfaces of *S. caprea* leaves, **a** young (×120), **b** mature (×120), **c** hook-like shaped hairs on mature leaves (×600), and of *S. nigricans* leaves, **d** young (×120), **e** mature (×120), **f** straight hairs on mature leaves (×600), **g** waxes on mature leaves (×600)

Table 2. Spearman coefficient of rank correlation (r_s) between the order of preferences of the Salicaceae tested obtained for each of the four populations studied

Populations compared	r_s	p
Oignie – Grammont	0.96	<0.01
Petite Camargue – Herrliberg	0.89	<0.01
Oignie – Petite Camargue	0.71	$=0.05$
Oignie – Herrliberg	0.81	$0.05>p>0.01$
Grammont – Petite Camargue	0.75	$0.05>p>0.01$
Grammont – Herrliberg	0.93	<0.01

and *S. cinerea* (see lower 3 lines of the matrixes shown in Fig. 3) than in those involving the four most preferred plants *S. nigricans*, *S. purpurea*, *P. tremula* and *P. nigra*.

Only the two populations coming from little altered natural habitats (Oignie and Petite Camargue) produced significant differences between their most preferred host plant species (*P. tremula* and *S. nigricans* respectively) and the other acceptable plant species (see first column of Fig. 3b and d). In contrast, there is no large difference in palatability between the most acceptable host and those ranking second and third in preference in the two populations from highly altered habitats (Grammont and Herrliberg).

In all four populations, the differences between the plant species ranked in second, third and fourth positions are usually not significant.

Conditioning of foot plant preference

Table 3 summarizes the results of the choice between *S. nigricans* and *S. purpurea* ascertained for beetles originating from the Petite Camargue or Herrliberg and reared exclusively on *S. nigricans* or *S. purpurea* in the laboratory.

Even after rearing on *S. purpurea*, the preference of the older experienced Petite Camargue beetles for *S. nigricans* remains clear. On the contrary, the adult beetles collected in Herrliberg on *S. purpurea* and reared on it do not normally show a significant preference for either *S. purpurea*

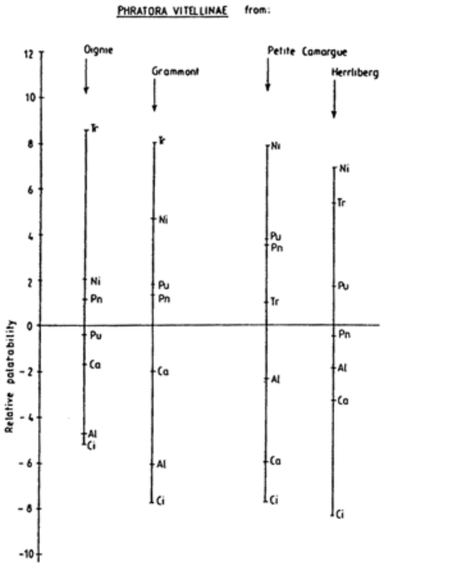

Fig. 2. The relative palatability of the different Salicaceae, plotted on a vertical scale for each of the four populations of *Phratora vitellinae* studied. See text for explanation of how this rank order was derived. *Abbreviations:* Tr, *P. tremula*; Ni, *S. nigricans*; Pn, *P. nigra italica*; Pu, *S. purpurea*; Ca, *S. caprea*; Al, *S. alba*; Ci, *S. cinerea*

Table 3. Results of the choice between *S. nigricans* and *S. purpurea* ascertained for beetles originating from the Petite Camargue and Herrliberg, and reared exclusively on *S. nigricans* or *S. purpurea* in the laboratory

Preference of:	older adults	newly emerged adults	1 week old adults
Rearing of:	adults	larvae	larvae + adults
Petite camargue population			
S. nigricans	+	−	
S. purpurea	+		
Herrliberg population			
S. nigricans	+	−	+
S. purpurea	−		−

+ *S. nigricans* significantly ($p<0.05$) preferred to *S. purpurea*; − *S. nigricans* not significantly ($p<0.05$) preferred to *S. purpurea*

or *S. nigricans*. After feeding on *S. nigricans* in captivity, a preference for *S. nigricans* is, however, induced. This can also be observed with one week old adults derived from larvae reared on *S. nigricans*. The acquired preference does not disapear if the beetles are transferred back to *S. purpurea* and fed on it for up to two weeks ($\beta=0.001$, $n=11$).

Newly emerged adults derived from either Petite Camargue or Herrliberg larvae reared on *S. nigricans* show no strong preference.

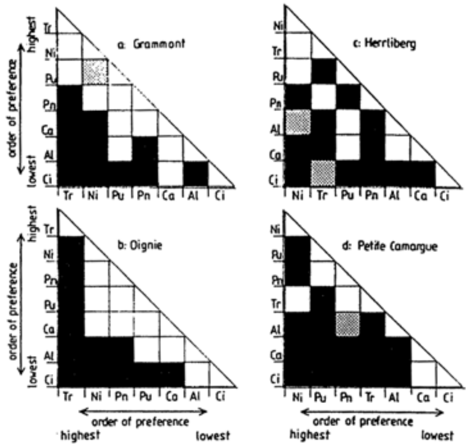

Fig. 3a–d. Matrix showing which combinations of Salicaceae resulted in a significant difference in the feeding behaviour of the beetles. Solid squares: $P<0.05$; dotted squares: $0.05<P<0.07$; open squares: $P>0.07$. A statistically significant difference in this context is the equivalent of a large difference (see text). **a** *Phratora vitellinae* originating from Grammont. **b** *Phratora vitellinae* originating from Oignie. **c** *Phratora vitellinae* originating from Herrliberg. **d** *Phratora vitellinae* originating from Petite Camargue. See Fig. 2 for abbreviations of food plants

Discussion and conclusions

Laboratory feeding trials tend to produce more positive responses than is representative of the wild animal. This is because the beetle is tested in conditions outside the context of its normal searching behavior. For example, the experimenter may confront the animal with a plant which it normally would never encounter. Furthermore the beetle has a restricted choice: if confined with two unpalatable plants, hunger may compel it to eat something which it would normally reject. One can best derive relevant information from this sort of data by considering the differences in apparent palatability between the plants tested. On the one hand, a specialist feeder would be expected to discriminate strongly between the plants it prefers and the majority of other plants. Those, of course, will still vary in palatability and some very distasteful plants may be discriminated against more strongly. On the other hand, a more generalist feeder should discriminate less strongly in favor of any particular species, but would still be expected to discriminate strongly against unpalatable species (Rowell et al. 1984).

The two schemata described above seem to be representative of the results obtained from the two populations from little altered natural habitats (Oignie and Petite Camargue) and from the two populations from the altered habitats (Grammont and Herrliberg), respectively. The populations from the little altered habitats are more specific in their host preferences than those from the the altered habitats.

At least in laboratory trials both specific and less specific populations from a particular geographic area prefer the same species of food plant. The identity of the most preferred plant species is however different in the two geographical areas studied (*P. tremula* in Belgium and *S. nigricans* in central Europe). In the laboratory, *P. tremula* and *S. nigricans* are accepted and well liked by both Belgian and central European beetles, even though *S. nigricans* is not present on Belgium.

Among the leaf characteristics of the less acceptable food plant species, the hairiness of the undersurfaces plays an important deterrent role (Rowell-Rahier and Pasteels 1982). The large proportion of the dry weight invested in trichomes by *S. caprea* supports this idea.

In Grammont, the introduction of exotic poplar hybrids has decreased the relative density of the favored host plant *P. tremula*. Selection for reduced cost and risk (e.g. increased predation) incurred during the search for a specific food plant should favor more opportunistic preference and a greater polyphagy. Similarly, in the suburban habitat of Herrliberg, the host plant favored in the laboratory trials, *S. nigricans*, is probably more difficult to find (if present at all) than in the Petite Camargue.

In the Herrliberg population, which has rather poorly defined preferences, preference for *S. nigricans*, the favorite species of the more specific population (Petite Camargue) from the same geographical area, can readily be produced by conditioning; the reverse (conditioning of the Petite Camargue beetles on *S. purpurea*), however, is not true. This suggests that the Herrliberg population is derived from a more food-plant specific population, such as that of the Petite Camargue, and that dietary specificity needs to be reinforced by feeding on the original food plant. The extent to which the Herrliberg population can be conditioned to prefer willow species is unknown; further experiments would be necessary to determine whether *S. nigricans* is unique in this respect.

It is interesting that in the laboratory, the beetles originating from Grammont prefer *P. tremula*, the favored food plant of the more specific beetles from Oignie. A situation parallel to the one just described in central Europe could thus be present in Belgium, but with *P. tremula* as the natural host in the absence of *S. nigricans*, suggesting a genetic shift in food plant preference to the available host.

S. nigricans leaves are richer in nitrogen and salicin than those of *P. tremula*. Salicin is advantageous to the larvae for at least two reasons. First, it provides them with the precursor for their chemical defense; secondly, it provides a source of glucose otherwise not exploitable (Pasteels et al. 1983). These factors, as well as possible differences in leaf phenology, might make *S. nigricans* a more suitable host plant than *P. tremula* when they are both present, as in the Petite Camargue.

Ph. vitellinae have been found in quantity at least twice on *S. purpurea* in the field (Herrliberg and Lötschental, see Rowell-Rahier 1983a). In the Petite Camargue, a few beetles fed on *S. purpurea*. *S. purpurea* leaves, like those of *P. tremula*, are poorer in salicin than those of *S. nigricans*. Preliminary data suggest that in the laboratory there is no physiological difference (in mortality and speed of development) between the larvae reared on *S. purpurea* and on *S. nigricans*. Some behavioural differences (e.g. in oviposition and mating) between the beetles feeding on the two plants seem however to be present. Microevolutionary differentiation of isolated populations such as that of Herrliberg might lead in time to local specialisation of *Ph. vitellinae* on *S. purpurea*.

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