

# Interference of host plant morphology and phenology and their correlation with abundance patterns of the leaf galling sawfly *Pontania proxima*

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**Abstract** The host taxon preference of *Pontania proxima* (Serville 1823) (Tenthredinidae, Hymenoptera) was investigated by observing densities of galls on previously genotypically and phenotypically characterized clones representing three taxa of a hybrid complex, i.e., *Salix alba*, *Salix* × *rubens*, and *Salix fragilis*. Gall densities among these three taxa were observed by using two experimental designs: (1) an indoor experiment in a greenhouse flight cage, and (2) an outdoor experiment on a plantation of cuttings. Subsequently, observed gall densities were related with selected, characteristic phenotypical properties of the host plants. In the indoor experiment, *S. fragilis* was clearly preferred by *P. proxima*, while in the outdoor experiment, *S. × rubens* revealed the highest gall densities. The factor “foliation start” was excluded in the indoor experiment, but best explained the preference of *S. × rubens* in the outdoor experiment. The results of a linear regression model affirmed on the one hand that morphological properties should be expected as relevant signals during the host plant selection process of *P. proxima*. On the other hand, it was shown that other phenotypical factors such as foliation start in spring contribute to an interference of factors being relevant for host plant selectivity. The earlier foliation start of the morphological intermediate *S. × rubens* in the outdoor experiment shifts resource availability towards that taxon at an early oviposition phase of *P. proxima* and therefore entails a different gall distribution pattern with a preference for the hybrid under natural conditions.

**Keywords** Foliation phenology · Host plant selection · Nematinae · Plant gall · Plant herbivore interaction · *Salix*

## Introduction

Host plant selectivity in herbivorous insects is based on host plant suitability, i.e., host plant quality and substrate quantity, factors which are expected to enhance reproduction success. Many herbivorous species have been shown to reveal preferences within closely related host plant species or for single taxa (Roininen and Tahvanainen 1989; Rank et al. 1998; Ikonen 2002; Fritz et al. 2003; Ferrier and Price 2004; Niemi et al. 2005). Such preferences are based on the host plant phenotype, which results in visual, olfactory, gustatory or mechanosensory traits attracting herbivores (Schoonhoven et al. 2005). Phenotypic traits can be affected by environmental factors but are mainly defined by the genotype, which leads to an effect of plant genetic diversity on herbivore abundance patterns in plant communities (Fritz et al. 1994, 1998; Fritz 1999; Whitham et al. 2003; Czesak et al. 2004; Hochwender and Fritz 2004).

Plant genetic differences in hybrid complexes have been shown to significantly affect the distribution and abundance of herbivorous species and pathogens (e.g., Fritz et al. 1996; Orians et al. 1997; Hjalten et al. 2000; Hochwender and Fritz 2004; Hochwender et al. 2005; Ito and Ozaki 2005; Tovar-Sanchez and Oyama 2006). However, host plant selection and acceptance by herbivores is driven by a variety of different host plant traits and by phenological synchronization (Schoonhoven et al. 2005), phenotypic diversity and interference of several traits which are of major interest with regard to resulting herbivore patterns.

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Various members of *Salix* (Salicaceae) are well known to hybridize frequently and have been the subject of a considerable number of studies on herbivore abundance patterns. In a study on 19 clones of the *Salix alba*–*Salix fragilis* aggregate (Kehl et al. 2008), the hybrid taxon *Salix* × *rubens* showed, as expected, intermediate morphological, phytochemical and genotypic traits compared to its parent taxa, but a significantly earlier leaf phenology. Since all these phenotypic characteristics may be relevant for host plant selection of herbivores, we chose a leaf galling sawfly species to investigate the interferences of host plant morphology and phenology in this hybrid complex with the distribution and abundance patterns of a specialized herbivore species. The facultatively bivoltine, gall-inducing sawfly species *Pontania proxima* (Serville 1823) (Tenthredinidae, Nematinae) is known to induce leaf blade galls during oviposition on the *S. alba*–*S. fragilis* hybrid complex (Carleton 1939; Kopelke 1985, 1999, 2005). Earlier gall quantifications indicated that *P. proxima* has a clear preference for *S. fragilis*, which leads to an inhomogeneous pattern of gall densities within the host plant aggregate as a whole (Kopelke 1999), and are in accordance with the findings of Soetens et al. (1991), who reported negative correlations of *P. proxima* gall frequencies with leaf trichome densities of cultivated plant individuals of the species complex, meaning a higher rate of gall inductions on *S. fragilis* which exhibits lower leaf pilosity.

Almost nothing is known about the mechanisms of the host plant perception by *P. proxima* from a greater distance, but it can be expected that visual cues and probably volatiles also play a key role, as has been shown for other Symphytean species (Aluja and Prokopy 1993; Barker et al. 2006). In contrast, the behavior of *P. proxima* during host plant surface evaluation prior to gall induction and oviposition has been well documented (Kopelke 1985, 1998). Thus, morphological traits are expected to affect host plant selection of this species as for other herbivorous insects (Myers and Bazely 1991; Zvereva et al. 1998; Bjorkman and Ahrne 2005) as well as presence and composition of specific non-volatile phenolic glucosides, being typical secondary metabolites in *Salix* (Binns et al. 1968; Egloff 1982; Shao 1991). These compounds are also well known as sawfly attractants (Roininen and Tahvanainen 1989; Kolehmainen et al. 1994; Ikonen 2002) and as stimulators for oviposition (Roininen et al. 1999). Host plant foliation phenology, however, has also been shown to affect herbivore populations (Yukawa 2000; Miyamoto and Nakamura 2004; Mopper 2005; Jones and Despland 2006).

The *S. alba*–*S. fragilis* aggregate is represented by a wide variety of intermediate forms, derived from natural hybridization and backcrossing (Triest et al. 1999, 2000; Triest 2001; De Cock et al. 2003) of the two morphologically and

phytochemically clearly distinct parent species, *S. alba* L. and *S. fragilis* L. *Salix alba* is distributed all over Europe, Asia Minor and the Caucasus and occurs mainly in valleys of rivers and streams. *Salix fragilis* is distributed in northern Asia Minor and the Armenian High Plateau, but according to Skvortsov (1999) is also widespread in cultural and semi-cultural landscapes and along river banks in Central Europe, where it is considered to disperse mainly vegetatively (Beismann et al. 1997a, 2000). Here, *S. fragilis* most frequently hybridizes with *S. alba* (Beismann et al. 1997b; Triest et al. 1997; Skvortsov 1999; De Cock et al. 2003). A recent morphological study even states that pure *S. fragilis* is relatively rare in Central Europe, and most plants identified as *S. fragilis* are probably already hybrids (Belyaeva 2009).

The presence of an outdoor plantation with individuals of the *S. alba*–*S. fragilis* complex representing a manageable selection of pheno- and genotypically characterized plant clones (each represented by up to 15 individuals), which largely represent the variation in local populations of the host plant complex, allowed the setting up of an experimental design for studying the host plant preference of the locally ubiquitous species *P. proxima* and the identification of the phenotypic factors relevant for the observed rates of gall induction patterns. The present study is focussed on gall induction frequencies at the level of host plant taxa or clones, but ignores possible linkages between preference patterns and larval fitness and parasitization.

## Materials and methods

To account for the taxon selectivity of *P. proxima* and for the relevance of determining phenotypic traits of the host plants, i.e., leaf morphology and foliation phenology, two parallel experiments were set up: (1) an outdoor experiment (under natural climatic conditions) using 19 pheno- and genotypically characterized plant clones and a natural population of *P. proxima* monitored during the vegetation period of 1 year, and (2) an indoor experiment with bred *P. proxima* females and three clones representing the three host plant taxa in a flight cage under controlled conditions during 24 h, in order to exclude the factor ‘foliation phenology’ which is attended by temporal and climatic conditions.

### Indoor experiment

The proposed preference for *S. fragilis* in its host plant complex was tested in a biotest setup with *P. proxima* females and one genotypically characterized, representative clone of each of the three host taxa, i.e., *S. alba* (clone 18), *S. × rubens* (clone 12), and *S. fragilis* (clone 11) derived from one locality (Table 1). The biotest in a flight

**Table 1** Selected clones for the investigations

Site	Site coordinates	River	Clones according to taxonomic group		
			<i>S. alba</i>	<i>S. × rubens</i>	<i>S. fragilis</i>
1	49°55'14"N, 11°34'59"E	Roter Main			1, 2
2	49°57'06"N, 11°33'47"E	Roter Main		5	3, 4
3	50°07'29"N, 11°17'14"E	Main	6		
4	50°10'53"N, 11°12'38"E	Rodach	9, 10		7, 8
5	50°09'26"N, 11°09'16"E	Rodach	17, 18	12, 14	11, 13, 15, 16
6	50°02'14"N, 10°56'01"E	Main		19	

Affiliation of clones to taxonomic groups is based on genotypic and phenotypic analyses (Kehl et al. 2008)

cage (60 × 60 × 60 cm) was performed in four replications with eight gravid females in total (2 × 1 and 2 × 3 females) and two plants (cuttings) derived from three clones, which were circularly placed at a regular sequence. All cuttings were of similar height (~25 cm) and provided similar numbers of shoot tips, i.e., potential oviposition sites. The females remained in the flight cage for 24 h. The numbers of induced galls were determined after 2 weeks, and relative gall numbers were calculated for every replication.

#### Outdoor experiment

Gall numbers and densities were quantified on 233 cuttings belonging to 19 clones of the *S. alba*/*S. fragilis* aggregate, having been sampled in 2001 at five sites in natural habitats along the rivers Main and Rodach (Upper Franconia, Bavaria, Germany). These plants represent a wide morphological continuum from morphologically typical *S. alba* to *S. fragilis*. Fifteen cuttings of each clone were planted in 2002 in a regular sequence on an experimental plot with homogenous soil quality (the upper 85 cm from the top layered with 50 cm sandy clay, 5 cm sand, 10 cm grit and 20 cm coarse gravel) in an open area of the Ecological Botanical Garden of the University of Bayreuth (Germany). Plants were not cut until data collection in 2005, to avoid the induction of long shoots and to ensure a natural growth habit. The plants revealed a mean height of 142 cm (SD 41.3 cm). For the detailed study design, see Kehl et al. (2008).

Galls were quantified between 7 and 15 June on the 233 vital plants and all galled leaves were labeled. The length of every shoot exhibiting galled leaves was measured during the data acquisition. *Pontania proxima* has two generations per year and, from previous investigations, gall quantification in June refers to the first generation. Gall densities per clone were calculated by the sum of recorded galls per number of available cuttings for each clone.

#### Selection of variables

A previous study (Kehl et al. 2008) on the phenotypic variation of these 19 clones of the *S. alba*–*S. fragilis* aggregate represented by 233 cuttings provided two main factors: (1) morphological leaf traits, with the variables or factors ‘leaf area’, ‘trichome density’, ‘specific leaf weight’ and ‘petiole length’, and (2) growth traits, including phenology (‘foliation start’) and ‘stem diameter growth’. In addition, leaf chemistry was shown to correlate with the first factor (morphological leaf traits). For interpreting observed gall induction differences in the present study, the two factors ‘morphological leaf traits’ (with the variables ‘leaf area’ and ‘leaf trichome density’) and ‘foliation phenology’ were correlated with the gall abundances recorded for the three host taxa.

Leaf area was inferred by use of the ellipse area formula: length (mm) × width (mm) ×  $\pi \times 0.25$  (Clancy et al. 1993). Leaf pubescence was quantified under a stereo lens in five randomly chosen 1-mm segments on the upper leaf surface by counting the hairs along a 1-mm scale. The start of plant foliation (when the first leaf lamina spread at 3–4 positions on the plant) was monitored during April 2005.

#### Data analysis

All data were tested for normality and log-transformed where possible to achieve normality, otherwise non-parametric tests were used. Comparisons of multiple independent samples were conducted by one-way ANOVA if normality (tested with Kolmogorov–Smirnov test) was given or by Kruskal–Wallis ANOVA as the non-parametric approach, using the software package STATISTICA (data analysis software system), version 7 (StatSof 2004; <http://www.statsoft.com>). For the indoor experiment, relative gall numbers per replication were calculated by summarizing gall numbers per clone for each of the four replications and compared using one-way ANOVA ( $n = 4$ ). For the outdoor experiment, gall densities per clone were analyzed using

non-parametric Kruskal–Wallis ANOVA. Mean and SE or median and quartiles are given throughout in graphical form in the figures. To prove the relevance of the selected factors for the observed differences in gall densities in the outdoor experiment, regression analysis using fitted linear models were carried out in R [version 2.9.2, `lm(stats)`, `stepAIC(MASS)`].

## Results

### Host plant preference

The results derived from the indoor biotest document the preference of *P. proxima* for *S. fragilis* (one-way-ANOVA:  $F_{2,9} = 25.10$ ,  $P < 0.001$ ). Altogether, 229 galls were induced by eight females of *P. proxima*. A mean of 70% of the galls were laid on *S. fragilis* cuttings. Gall allocation values for *S. alba* and *S. × rubens* showed the trend as a preference for *S. × rubens* cuttings (25% of galls) compared to *S. alba* cuttings (4% of galls) (Fig. 1a), but were not statistically different.

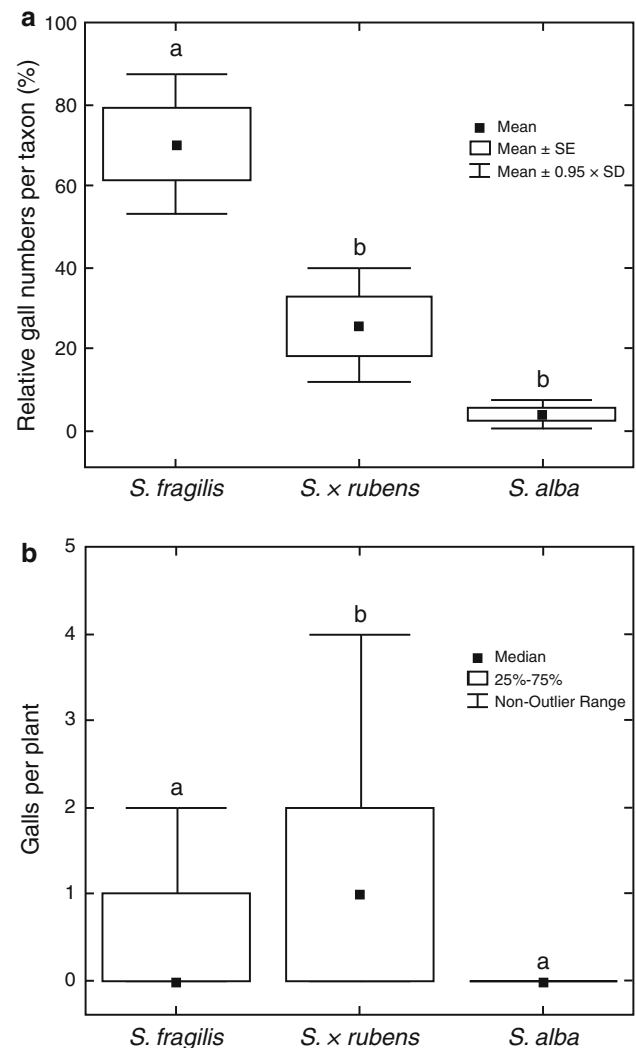
In the outdoor experiment, the observation of the first occurrence of galls on plants on the experimental plot in 2005 took place on 28 April. Counts of the cuttings from the plantation provided 320 galls of *P. proxima* on 76 of the 233 examined individual plants; 10% of the *S. alba* cuttings, 32% of the *S. fragilis* cuttings, and more than 50% of the *S. × rubens* cuttings exhibited galls of *P. proxima*. Again, significant differences in gall numbers among representatives of the three host taxa were observed (Kruskal–Wallis-ANOVA:  $H_{2,233} = 20.16$ ,  $P < 0.001$ ), but the hybrid *S. × rubens* revealed significantly higher gall numbers compared to plants belonging to either *S. fragilis* or *S. alba* (Fig. 1b).

### Coherences with phenotypical factors

The selected phenotypical factors differed significantly between the three investigated taxa (Fig. 2a–c). Both morphological traits recorded on the 233 plants from the outdoor experiment revealed clearly intermediate values for *S. × rubens* concerning leaf area and leaf trichome density (Fig. 2a, b). However, the factor ‘foliation start’ showed lowest values for *S. × rubens*, mean for *S. fragilis* and highest for *S. alba* (Fig. 2c). The median for foliation start in the taxon *S. × rubens* was 5 April, whereas for *S. fragilis* and *S. alba*, it was 12 and 14 April, respectively.

Regression analysis using linear models revealed significant effects of the factors ‘foliation start’ and ‘leaf area’ on the gall numbers of the investigated plants on the experimental plot (Table 2).

The analysis of variances of the lengths of all shoots exhibiting galls of *P. proxima* during this investigation

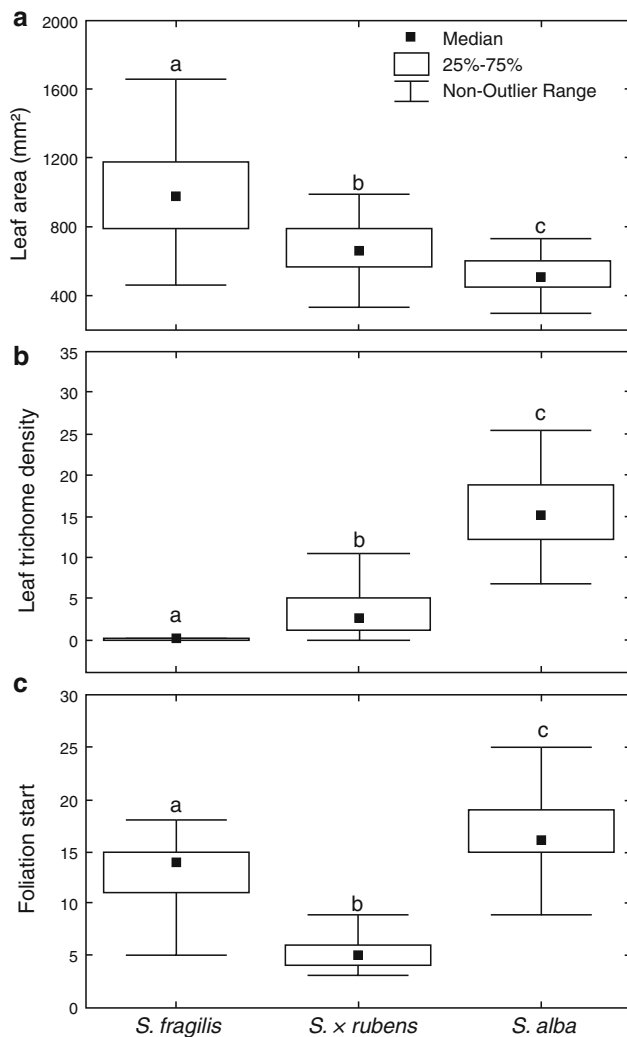


**Fig. 1** Observed gall distributions in the indoor and outdoor experiment. **a** Relative gall numbers per taxon, each represented by eight plant individuals belonging to one clone, in the indoor experiment. **b** Galls per plant in the outdoor experiment. *Salix fragilis* was represented by 138, *S. × rubens* by 54 and *S. alba* by 41 individuals. Significant differences are indicated by different letters (**a** one-way ANOVA, post hoc procedure after Scheffé, **b** Kruskal–Wallis ANOVA, post hoc comparisons of mean ranks of all pairs of groups)

revealed no differences between the three taxa as determined at the end of the oviposition period (one-way ANOVA:  $F_{2,230} = 1.236$ ,  $P = 0.293$ ), and that the length variation of allocated shoots was very high (Fig. 3). These results devalue shoot length as a relevant factor for taxon selectivity in the case of *P. proxima*.

## Discussion

The present analysis of the host plant preference of *P. proxima* is based on phenotypic factors that were previously recognized and referred to as phylogenetically



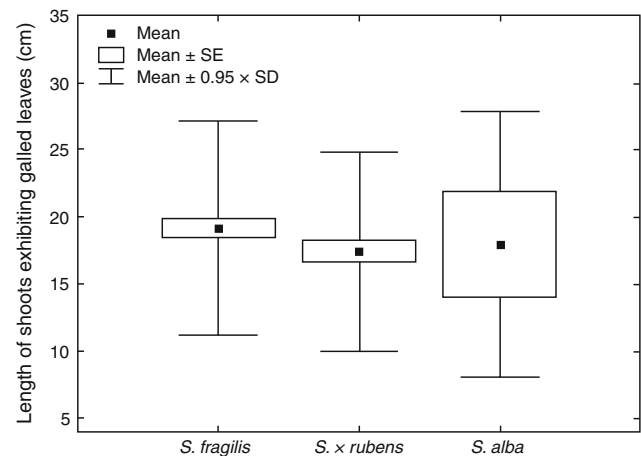
**Fig. 2** Phenotypic properties of the 19 clones (233 plants) representing the three taxa in the outdoor experiment. **a** Leaf area (Kruskal–Wallis ANOVA:  $H_{2,230} = 113.56$ ,  $P < 0.001$ ). **b** Leaf trichome density (Kruskal–Wallis ANOVA:  $H_{2,229} = 167.01$ ,  $P < 0.001$ ). **c** Foliation start (Kruskal–Wallis ANOVA:  $H_{2,233} = 133.30$ ,  $P < 0.001$ ). Significant differences are indicated by different letters (Kruskal–Wallis ANOVA, post hoc comparisons of mean ranks of all pairs of groups)

**Table 2** The fitted linear model for gall densities of *Pontania proxima* (Akaike information criterion) included the mean foliation start, mean leaf area and mean pubescence of the leaf surface as explanatory variables but no interaction terms

	Estimate	SE	<i>t</i>	<i>P</i>
Intercept	−0.460	1.614	−0.285	0.78
Foliation start	−0.137	0.057	−2.400	0.03*
Leaf area	0.427	0.175	2.449	0.03*
Trichome density on leaves	0.676	0.483	1.399	0.18

Adj.  $R^2 = 0.40$ ,  $P = 0.01$ ,  $n = 223$

\* Significant at  $P < 0.05$



**Fig. 3** Mean length (cm) of shoots exhibiting galled leaves until 15 June for the three host plant taxa (one-way ANOVA:  $F_{2,230} = 1.236$ ,  $P = 0.293$ )

significant traits in an analysis of 19 clones of the *S. alba*/*S. fragilis* aggregate, and therefore considered as highly explanatory concerning the morphological differentiation of the three taxa (Kehl et al. 2008). With ‘leaf size’ and ‘leaf trichome density’, two leaf morphological factors were selected that have been shown to correlate with the secondary metabolite composition of the leaves and to autocorrelate. The third factor, ‘foliation start’, referring to a plant phenological trait, was shown not to correlate with leaf morphology. The three selected phenotypic factors applied for the analysis of the investigated clones in both experiments corresponded to those in Kehl et al. (2008). In accordance, clones pertaining to *S. fragilis* had the largest leaves, lowest trichome density and intermediate foliation start, those of *S. x rubens* had intermediate leaf morphology and earliest foliation start, and those of *S. alba* had the smallest leaves, highest trichome density and latest bud break in spring.

Since some degree of evidence had already been given with regard to host plant preference of *P. proxima* (Soetens et al. 1991), data from the recent host plant genotypification (Kehl et al. 2008) allowed a more detailed investigation, even if the small selection numbers of different clones which, in addition, were derived from a restricted area, reduced the value of the results to some degree. We focussed mainly on preference patterns of *P. proxima* within its host plant complex, but not on performance patterns, since previous investigations (A. Kehl and G. Rambold, unpublished data) indicated that no differences exist, at least in the parasitization rates on *S. fragilis* and *S. x rubens* host plants.

The central finding derived from this study is that, after morphological host plant properties, phenological characteristics also correlate with gall densities of *P. proxima*



under natural conditions in the outdoor experiment. But under conditions excluding these phenological effects in the indoor experiment, different taxon selectivity was observed.

Under conditions of exclusion of climatic and temporal effects, like foliation start and eclosion start of the herbivore as well as their phenological synchronization, the expected host plant preference of *S. fragilis* was clearly affirmed by the indoor experiment. This finding is in accordance with those from a similar study, which revealed a certain degree of selectivity for gall-inducing sawflies with regard to closely related willow species, being explained by phylogenetically determined traits of the host species such as leaf morphology (Yamazaki and Ohsaki 2006), aside from plant vigor (Kokkonen 2000) and shoot length (Ferrier and Price 2004).

The results from the indoor experiment also affirmed findings by Soetens et al. (1991), who showed, additionally, that gall numbers were positively correlated with the high phenolic glucoside content of the leaves and low leaf trichome density.

But, in contrast, the results from the outdoor experiment, when the phenotypic synchronization between host plant and herbivore may take effect, revealed a detectable preference of the hybrid *S. × rubens* as host plant. And, indeed, the preference of the hybrid *S. × rubens* as host appeared to be correlated and explained by its relatively early foliation start, i.e., phenology. Thus, *S. fragilis*, being preferred in the indoor experiment, revealed only low frequencies of gall induction rates outdoors, just like *S. alba*. This discrepancy in host plant preference between the two experiments underlines that the additional factor ‘leaf phenology’ of the outdoor experiment must have a determinative effect on host plant selectivity, and therefore leads to a preference of hybrid clones under natural conditions. Foliation start is genetically fixed and triggered by day length, but also influenced by climatic conditions, and the effect can therefore be expected to vary between years. Nevertheless it is one factor which is extremely relevant in natural habitats under natural conditions (Aizen and Patterson 1995), and may remain unrecognized in bioassays.

Foliation start and the phenological synchronization with herbivores searching for feeding or oviposition sites could affect herbivore distribution on host plants, as was shown for gall midges (Yukawa and Akimoto 2006). Therefore, we assume that early bud break of *S. × rubens* clones enlarged the phenological window for oviposition of *P. proxima*. This is also affirmed by the observed phenological data of both host plants and herbivore. Estimating a time span of 10 days (Carleton 1939; Kopelke 1998, 1999) from gall induction to its observation on the experimental plot, the first galls were likely to be induced around 18 April. By this time point, only *S. × rubens* individuals can

be expected to provide enough unfolded leaves for oviposition. This means that the earliest eclosing *P. proxima* females oviposited mainly on *S. × rubens* plants, despite their morphological or phytochemical properties being only suboptimal. In that case, foliation start is interpreted as complying with resource availability, which led to a preference for the morphologically intermediate taxon. Based on these findings, we expect morphological properties to nevertheless provide relevant signals for host plant selection under natural conditions and even in the presence of hybrid individuals, as the constantly low gall frequencies on *S. alba* indicate. That was also clearly affirmed by the regression analysis, which showed that ‘foliation start’ as well as ‘leaf size’ correlated with the gall densities among the three taxa or the 19 clones. The factor ‘trichome density’, even at significantly different intensities between the taxonomic groups, did not, however, contribute to explaining gall density variation among the three taxa represented by 19 clones in the outdoor experiment (Table 2).

Based on our previous investigations on the phenotypic diversity in the host plant complex (Kehl et al. 2008), we admit that leaf size might also be a factor which could be recognized by females and therefore also be a relevant cue for host plant selection. Leaf size is slightly correlated with gall size (Clancy et al. 1993) and may therefore influence the larval performance for *P. proxima*, but this correlation was not investigated in the present study. Kopelke (1998) mentioned that leaf size could actually be recognized by females evaluating the host plant, e.g., of species of *Phyllocolpa*, being inducers of leaf blade folds.

The trichome density on leaves has been shown to affect the host plant selection of leaf beetles (Zvereva et al. 1998; Dalin and Björkman 2003; Björkman and Åhrne 2005), and a similar effect on *P. proxima* females evaluating the host plant leaf surfaces can be presumed. Therefore, we assume that host plant selection, at least in the indoor experiment, was mainly triggered by morphological properties (and/or phytochemical traits), which are expected to act as cues during the process of host plant evaluation.

Additionally, the often observed relevance of shoot length for intra- and interspecific selectivity of gall-inducing sawflies (Price et al. 1987; Craig et al. 1989; Woods et al. 1996; Fritz et al. 2000, 2003; Kokkonen 2000; Ferrier and Price 2004) can be regarded as insignificant for the taxon selectivity of *P. proxima*. This is not surprising since, in contrast to shoot galling species, *P. proxima* is not expected to achieve any direct positive effect by attacking leaves on long shoots. Shoot galling sawflies (genus *Euura*) often overwinter in these shoots, and shorter shoots often die during the winter, so that in this case, a direct advantage of a preference for longer shoots is clear. Furthermore, it is expected that gall-inducing species exhibiting an early

phenology, as shown for *P. proxima* in this study, are not able to distinguish shoots by their length due to low resource heterogeneity early in the year (Price et al. 2004).

This study showed that, in the case of *P. proxima*, a resolvable interference of factors contributing to host plant phenotype correlate with herbivore attack and gall densities. Based on these results, especially the host plant foliation start, can be expected to influence host plant selectivity of *P. proxima* under natural conditions.

The earlier foliation start described for the taxon *S. × rubens* is interpreted as a possible evolutionary advantage due to an elongation of the vegetation period and may have contributed to the widespread occurrence of this hybrid taxon, as described by some authors. On the other hand, the actual findings demonstrate that this phenological property implies stronger infestation by at least one specialized herbivore. Therefore, this relationship may negatively affect the performance of *S. × rubens* and thereby compensate the expected advantages for the hybrid taxon due to its elongated vegetation period.

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## References

- Aizen MA, Patterson WA (1995) Leaf phenology and herbivory along a temperature gradient—a spatial test of the phenological window hypothesis. *J Veg Sci* 6:543–550
- Aluja M, Prokopy RJ (1993) Host odor and visual stimulus interaction during intratree host finding behavior of *Rhagoletis pomonella* flies. *J Chem Ecol* 19:2671–2696
- Barker AM, Molotsane R, Müller C, Schaffner U, Städler E (2006) Chemosensory and behavioural responses of the turnip sawfly *Athalia rosae*, to glucosinolates and isothiocyanates. *Chemoecology* 16:209–218
- Beismann H, Speck T, Bogenrieder A (1997a) The wind in the willows—dispersal mechanisms and distribution of *Salix alba*, *Salix fragilis* and their hybrid *S. × rubens*. In: Geronomidis G, Vincent JFV (eds) Plant biomechanics. Proceedings of the plant biomechanics meeting, Reading, 7–12 September 1997, pp 57–64
- Beismann H, Barker JHA, Karp A, Speck T (1997b) AFLP analysis sheds light on distribution of two *Salix* species and their hybrid along a natural gradient. *Mol Ecol* 6:989–993
- Beismann H, Wilhelm H, Bailleres H, Spatz H-C, Bogenrieder A, Speck T (2000) Brittleness of twig bases in the genus *Salix*: fracture mechanics and ecological relevance. *J Exp Bot* 51:617–633
- Belyaeva I (2009) Nomenclature of *Salix fragilis* L. and a new species, *S. euxina* (Salicaceae). *Taxon* 58:1344–1348
- Binns WW, Blunden G, Woods DL (1968) Distribution of leucoanthocyanidins, phenolic glycosides and imino-acids in leaves of *Salix*-species. *Phytochemistry* 7:1577–1581
- Bjorkman C, Ahrne K (2005) Influence of leaf trichome density on the efficiency of two polyphagous insect predators. *Entomol Exp Appl* 115:179–186
- Carleton M (1939) The biology of *Pontania proxima*, the bean gall sawfly of willows. *J Linn Soc Lond* 40:575–624
- Clancy KM, Price PW, Sacchi CF (1993) Is leaf size important for a leaf-galling sawfly (Hymenoptera: Tenthredinidae)? *Environ Entomol* 22:116–126
- Craig TP, Itami JK, Price PW (1989) A strong relationship between oviposition preference and larval performance in a shoot galling sawfly. *Ecology* 70:1691–1699
- Czesak ME, Knee MJ, Gale RG, Bodach SD, Fritz RS (2004) Genetic architecture of resistance to aphids and mites in a willow hybrid system. *Heredity* 93:619–626
- Dalin P, Björkman C (2003) Adult beetle grazing induces willow trichome defence against subsequent larval feeding. *Oecologia* 134:112–118
- De Cock K, Lybeer B, Van der Mijnsbrugge K, Zwaenepoel A, Van Peteghem P, Quataert P, Breynne P, Goetghebeur P, Van Slycken J (2003) Diversity of the willow complex *Salix alba*–*S. × rubens*–*S. fragilis*. *Silvae Genetica* 52:148–153
- Egloff CP (1982) Phenolglykoside einheimischer *Salix*-Arten. Dissertation, ETH Zürich, Zürich (in German with English abstract)
- Ferrier SM, Price PW (2004) Oviposition preference and larval performance of a rare bud-galling sawfly (Hymenoptera: Tenthredinidae) on willow in northern Arizona. *Environ Entomol* 33:700–708
- Fritz RS (1999) Resistance of hybrid plants to herbivores: genes, environment or both? *Ecology* 80:382–391
- Fritz RS, Nichols-Orians CM, Brunsfeld SJ (1994) Interspecific hybridization of plants and resistance to herbivores: hypotheses, genetics and variable responses in a diverse herbivore community. *Oecologia* 97:106–117
- Fritz RS, Roche BM, Brunsfeld SJ, Orians CM (1996) Interspecific and temporal variation in herbivore responses to hybrid willows. *Oecologia* 108:121–129
- Fritz RS, Roche BM, Brunsfeld SJ (1998) Genetic variation in resistance of hybrid willows to herbivores. *Oikos* 83:117–128
- Fritz RS, Crabb BA, Hochwender CG (2000) Preference and performance of a gall-inducing sawfly: a test of the plant vigor hypothesis. *Oikos* 89:555–563
- Fritz RS, Crabb BA, Hochwender CG (2003) Preference and performance of a gall-inducing sawfly: plant vigor, sex, gall traits and phenology. *Oikos* 102:601–613
- Hjältén J, Ericson L, Roininen H (2000) Resistance of *Salix caprea*, *S. phylicifolia* and their F1 hybrids to herbivores and pathogens. *Ecoscience* 7:51–56
- Hochwender CG, Fritz RS (2004) Plant genetic differences influence herbivore community structure: evidence from a hybrid willow system. *Oecologia* 138:547–557
- Hochwender CG, Janson EM, Cha DH, Fritz RS (2005) Community structure of insect herbivores in a hybrid system: examining the effects of browsing damage and plant genetic variation. *Ecol Entomol* 30:170–175
- Ikonen A (2002) Preferences of six leaf beetle species among qualitatively different leaf age classes of three Salicaceous host species. *Chemoecology* 12:23–28
- Ito M, Ozaki K (2005) Response of a gall wasp community to genetic variation in the host plant *Quercus crispula*: a test using half-sib families. *Acta Oecol* 27:17–24
- Jones BC, Despland E (2006) Effects of synchronization with host plant phenology occur early in the larval development of a spring folivore. *Can J Zool* 84:628–633
- Kehl A, Aas G, Rambold G (2008) Genotypical and multiple phenotypical traits discriminate *Salix × rubens* Schrank clearly from its parent species. *Plant Syst Evol* 275:169–179
- Kokkonen K (2000) Mixed significance of plant vigor: two species of galling *Pontania* in a hybridizing willow complex. *Oikos* 90:97–106

- Kolehmainen J, Roininen H, Julkunen-Tiitto R, Tahvanainen J (1994) Importance of phenolic glucosides in host selection of shoot galling sawfly *Euura amerinae* on *Salix pentandra*. *J Chem Ecol* 20:2455–2466
- Kopelke J-P (1985) Biologie und Parasiten der gallbildenden Blattwespe *Pontania proxima*. *Senckenbergiana Biol* 65:215–239 (in German with English abstract)
- Kopelke J-P (1998) Oviposition strategies of gall-making species of the sawfly genera *Pontania*, *Euura* and *Phyllocolpa* (Hymenoptera: Tenthredinidae: Nematinae). *Entomol Gen* 22:251–275
- Kopelke J-P (1999) Gallenerzeugende Blattwespen Europas—Taxonomische Grundlagen, Biologie und Ökologie (Tenthredinidae: Nematinae: *Euura*, *Phyllocolpa*, *Pontania*). *Senckenbergische Naturforschende Gesellschaft, Frankfurt a. M.* (in German with English abstract)
- Kopelke J-P (2005) The species of the *Pontania proxima* group in Europe. *Senckenbergiana Biol* 85:85–95
- Miyamoto Y, Nakamura M (2004) Plant phenology-mediated indirect effects: the gall midge opens the phenological window wider for a leaf beetle. *Entomol Sci* 7:315–322
- Mopper S (2005) Phenology—how time creates spatial structure in endophagous insect populations. *Ann Zool Fenn* 42:327–333
- Myers JH, Bazely D (1991) Thorns, spines, prickles, and hairs: are they stimulated by herbivory and do they deter herbivores? In: Tallamy DW, Raupp MJ (eds) *Phytochemical induction by herbivores*. Wiley, New York, pp 325–344
- Niemi L, Wennstrom A, Ericson L (2005) Insect feeding preferences and plant phenolic glucosides in the system *Gonioctena linnaeana*–*Salix triandra*. *Entomol Exp Appl* 115:61–66
- Orians CM, Huang CH, Wild A, Dorfman KA, Zee P, Dao MTT, Fritz RS (1997) Willow hybridization differentially affects preference and performance of herbivorous beetles. *Entomol Exp Appl* 83:285–294
- Price PW, Roininen H, Tahvanainen J (1987) Why does the bud-galling sawfly, *Euura mucronata*, attack long shoots? *Oecologia* 74:1–6
- Price PW, Ohgushi T, Roininen H, Ishihara M, Craig TP, Tahvanainen J, Ferrier SM (2004) Release of phylogenetic constraints through low resource heterogeneity: the case of gall-inducing sawflies. *Ecol Entomol* 29:467–481
- Rank NE, Köpf A, Julkunen-Tiitto R, Tahvanainen J (1998) Host preference and larval performance of the salicylate-using leaf beetle *Phratora vitellinae*. *Ecology* 79:618–631
- Roininen H, Tahvanainen J (1989) Host selection and larval performance of two willow feeding sawflies. *Ecology* 70:129–136
- Roininen H, Price PW, Julkunen-Tiitto R, Tahvanainen J, Ikonen A (1999) Oviposition stimulant for a gall-inducing sawfly, *Euura lasiolepis*, on willow is a phenolic glycosid. *J Chem Ecol* 24:943–953
- Schoonhoven LM, van Loon JJA, Dicke M (2005) *Insect–plant biology*. Oxford University Press, Oxford
- Shao Y (1991) *Phytochemischer Atlas der Schweizer Weiden*. Dissertation, ETH Zürich, Zürich (in German with English abstract)
- Skvortsov AK (1999) *Willows of Russia and adjacent countries*. University of Joensuu, Joensuu
- Soetens P, Rowell-Rahier M, Pasteels JM (1991) Influence of phenolglucosides and trichome density on the distribution of insect herbivores on willows. *Entomol Exp Appl* 59:175–187
- Tovar-Sanchez E, Oyama K (2006) Effect of hybridization of the *Quercus crassifolia* × *Quercus crassipes* complex on the community structure of endophagous insects. *Oecologia* 147:702–713
- Triest L (2001) Hybridization in staminate and pistillate *Salix alba* and *S. fragilis* (Salicaceae): morphology versus RAPDs. *Plant Syst Evol* 226:143–154
- Triest L, De Greef B, De Bondt R, Van Bossche D, D’Haeseleer M, Van Slycken J, Coart E (1997) Use of RAPD markers to estimate hybridization in *Salix alba* and *Salix fragilis*. *Belg J Bot* 129:140–148
- Triest L, De Greef B, Vermeersch S, Van Slycken J, Coart E (1999) Genetic variation and putative hybridization in *Salix alba* and *Salix fragilis* (Salicaceae): Evidence from allozyme data. *Plant Syst Evol* 215:169–187
- Triest L, De Greef B, De Bondt R, Van Slycken J (2000) RAPD of controlled crosses and clones from the field suggests that hybrids are rare in the *Salix alba*–*Salix fragilis* complex. *Heredity* 84:555–563
- Whitham TG, Young WP, Martinsen GD, Gehring CA, Schweitzer JA, Shuster SM, Wimp GM, Fischer DG, Bailey JK, Lindroth RL, Woolbright S, Kuske CR (2003) Community and ecosystem genetics: a consequence of the extended phenotype. *Ecology* 84:559–573
- Woods JO, Carr TG, Price PW, Stevens LE, Cobb NS (1996) Growth of the coyote willow and the attack and survival of a mid-rib galling sawfly, *Euura* sp. *Oecologia* 108:714–722
- Yamazaki K, Ohsaki N (2006) Willow leaf traits affecting host use by the leaf-gall-forming sawfly. *Popul Ecol* 48:363–371
- Yukawa J (2000) Synchronization of galls with host plant phenology. *Popul Ecol* 42:105–113
- Yukawa J, Akimoto K (2006) Influence of synchronization between adult emergence and host plant phenology on the population density of *Pseudasphondylia neolitseae* (Diptera: Cecidomyiidae) inducing leaf galls on *Neolitsea sericea* (Lauraceae). *Popul Ecol* 48:13–21
- Zvereva EL, Kozlov MV, Niemela P (1998) Effects of leaf pubescence in *Salix borealis* on host-plant choice and feeding behaviour of the leaf beetle, *Melasoma lapponica*. *Entomol Exp Appl* 89:297–303