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Field resistance to willow leaf rust *Melampsora epitea* in inter- and intraspecific hybrids of *Salix viminalis* and *S. dasyclados*

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Abstract

Families of interspecific hybrids between *Salix dasyclados* and *S. viminalis*, and intraspecific hybrids of the two species, were monitored for rust infection caused by *Melampsora epitea* during the years 1997–1999. The resistance of interspecific hybrids was compared to intraspecific pure species halfsibs. In addition, uredospores were sampled from the most infected interspecific hybrids to determine whether rust from interspecific hybrids differed from the rust spores that infected the pure species. The results from 1997 and 1999 showed that interspecific hybrids were more resistant than the intraspecific pure species, thus exhibiting a pattern of hybrid resistance. In 1998 the interspecific hybrids showed intermediate resistance compared to intraspecific pure species hybrids, i.e., an additive pattern of resistance. The rust sampled from the interspecific hybrids consisted of the same types of rust that infect the pure species. Comparisons of weather conditions prevailing during the three growing seasons indicated that these conditions might play a role in the infection pattern, in addition to the level of sensitivity to infection shown by the plants.

Introduction

Willows are grown in Sweden as a source of renewable energy. The willow leaf rust caused by *Melampsora epitea* Thüm is the most important disease on cultivated willows. Epidemics caused by this fungus severely affect growth and survival of many common willow species (Parker et al., 1993). Studies carried out on one of the most commonly cultivated species, *Salix viminalis* L., have demonstrated reduced biomass production, disruption of winter dormancy and frost hardiness (Verwijst, 1990), as well as a decline in competitiveness as a result of leaf rust attack (Verwijst, 1993).

Variation in the levels of susceptibility between interspecific hybrids and their parental species of *Salix* has been investigated (Fritz et al., 1994; 1996a; Roche and Fritz, 1998). Under natural conditions, hybridisation is considered common within the genus *Salix* (Lid, 1974, Mossberg et al., 1992). Resistance to pathogens may vary significantly between different willow parental species and/or their interspecific hybrids (Fritz et al., 1996b; Roche and Fritz, 1998). Artificial plant hybridisation is a way of obtaining more rust resistant *Salix* clones.

Plant hybridisation can affect inheritance of resistance in different ways. Hybridisation can lead to: (1) more susceptible plants (hybrid susceptibility); (2) more resistant plants (hybrid resistance); (3) plants having intermediate resistance compared to their parents (additive); or (4) plants in which resistance is equal to one parent (dominance). To what extent hybridisation affects the resistance is dependent on the type of resistance inherited from the parental species (Fritz et al., 1994).

The taxonomic status of *Melampsora* rust and its willow hosts is unclear (Pei et al., 1993; Helfer, 1992). One isolate of *M. epitea* can have a broad host range, whereas another isolate can be so specific that it can infect only one clone of one willow species (Pei et al., 1993). Under natural conditions, mutation, migration and sexual reproduction of the rust on *Larix*, early in spring, could give rise to new rust pathotypes. Natural hybridisation among *Salix* species in combination with the use of selected hybrids for commercial cultivation complicates the situation further. Because hybridisation is known to affect resistances, it is of interest to investigate how resistance in hybrid populations of willows is inherited in order to increase biomass growth and resistance to willow rust (Orians and Floyd, 1997).

In an earlier study carried out under greenhouse conditions, resistance in F_1 hybrids between *S. viminalis* and *S. dasyclados* Wimmer and their pure species halfsibs was compared, after exposure to four different rust pathotypes. The inheritance pattern of resistance was shown to be additive, because the interspecific hybrids showed intermediate resistance compared to their pure species halfsibs (Fritz et al., 1996b). Roche and Fritz (1998) studied hybrids of other willow species under field conditions where plants were exposed to a natural rust population with several pathotypes. They found that the hybrids were more susceptible than their parental species.

An aim of this study was to examine if our earlier results from greenhouse experiments predicting additive resistance are also valid when the hybrids are exposed to a variable population of *Melampsora* rust naturally occurring in the field. The hybrids from the greenhouse experiment were planted in the field and rust infections were monitored regularly throughout the three growing seasons during 1997–1999.

Our earlier studies showed that the interspecific hybrids in general were susceptible to only one of the rust pathotypes, which were derived either from *S. viminalis*, or from *S. dasyclados*. Another aim of the present study was to examine whether the same types of rust infecting the pure species also infect interspecific hybrid plants in the field, or if other types of rust would infect the interspecific hybrids (Pei et al., 1996). To test this, rust spores were sampled from selected interspecific hybrids in the field and used for infection experiments with selected interspecific hybrid clones and their siblings under controlled conditions.

Materials and methods

Plant material

Eight sets of families composed of hybrids between *S. viminalis* females and *S. dasyclados* males and intraspecific crosses of these species were investigated. The parental clones of *S. viminalis* were either of Swedish or Polish origin, whereas the *S. dasyclados* clones were of Swedish origin. The crosses were performed at the Department of Plant Breeding, Swedish University of Agricultural Sciences, Uppsala. The eight fullsib families contained four to sixteen siblings in each hybrid family. Each interspecific hybrid family had two or three associated intraspecific halfsib families with which it shared one parent. Each intraspecific family then consisted of three siblings from two or three different crosses (Figure 1).

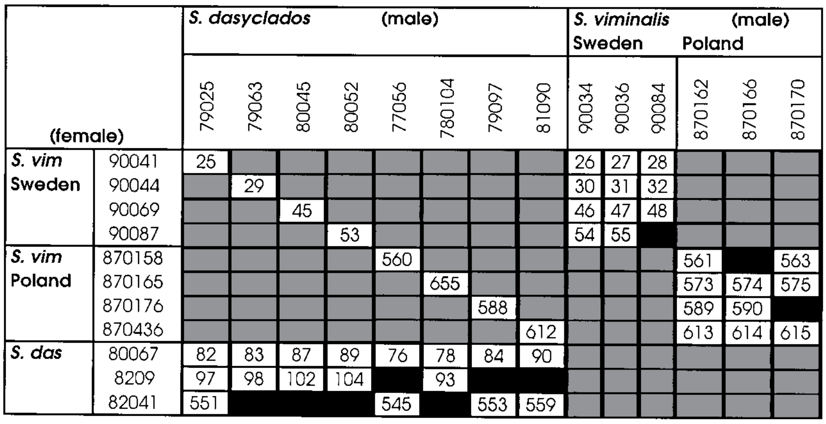
Cuttings were produced and planted in a randomised block design in field plots with 10×12 rows, where each clone was planted in four different blocks. The cuttings were planted in June 1994 and cut down after the 1994 growing season to encourage the development of more shoots during the following season. The entire plantation was fertilised once a year, early in the season with 150 : 15 : 80 kg/ha NPK. The plants were irrigated when needed during the dry periods of the summer.

Measurements of rust infection

The plants were monitored regularly for incidence of *Melampsora* infection once a week during the three summers of 1997–1999. Rust infections normally start during the second half of July in the Uppsala area (Johansson, unpublished). To assure recording of all possible infections, the first observations were started the first week of July, and continued until the middle of September. Rust infections normally decline due to senescence of leaves after the middle of September. Therefore, no recordings were made after September 15.

The average height of the plants varied from 1.30 m the first year to 2.10 m the last year of recording. All shoots in all stools were inspected visually and rust incidence was recorded weekly from the first week of July to September 15th.

Infection intensity was assessed using a modified scoring system suggested by Schreiner (1959) of $S{*}P$. The term S is the severity of infection per leaf, on the majority of the infected leaves according to the



		S. dasyclados (male)								*S. viminalis* (male)					
										Sweden			Poland		
(female)		79025	79063	80045	80052	77056	780104	79097	81090	90034	90036	90084	870162	870166	870170
S. vim **Sweden**	90041	25	▒	▒	▒	▒	▒	▒	▒	26	27	28	▒	▒	▒
	90044	▒	29	▒	▒	▒	▒	▒	▒	30	31	32	▒	▒	▒
	90069	▒	▒	45	▒	▒	▒	▒	▒	46	47	48	▒	▒	▒
	90087	▒	▒	▒	53	▒	▒	▒	▒	54	55	■	▒	▒	▒
S. vim **Poland**	870158	▒	▒	▒	▒	560	▒	▒	▒	▒	▒	▒	561	■	563
	870165	▒	▒	▒	▒	▒	655	▒	▒	▒	▒	▒	573	574	575
	870176	▒	▒	▒	▒	▒	▒	588	▒	▒	▒	▒	589	590	■
	870436	▒	▒	▒	▒	▒	▒	▒	612	▒	▒	▒	613	614	615
S. das	80067	82	83	87	89	76	78	84	90	▒	▒	▒	▒	▒	▒
	8209	97	98	102	104	■	93	■	■	▒	▒	▒	▒	▒	▒
	82041	551	■	■	■	545	■	553	559	▒	▒	▒	▒	▒	▒

Figure 1. Crossing scheme showing parental clones of *Salix dasyclados* and *S. viminalis* and the interspecific hybrid families with their pure species halfsibs. Each family can be identified by number, e.g.'25' or '561'.

following scale: 1 = 1–3 uredinia; 5 = 4–8 uredinia; 25 = 9 or more uredinia. The term P is the percentage of infected leaves on the plant (0 = no infection; 1 = 1–25% infected; 2 = 26–50% infected; 3 = 51–75% infected and 4 = 76–100% infected).

Because of the non-normality of the rust severity data, non-parametric Kruskal–Wallis one-way analyses of variance (ANOVA) were conducted (Kruskal and Wallis, 1952). The maximum Schreiner scores on the different clones were used in the test. Therefore, we used the mean Schreiner score of the maximum scores for each sibling as the dependent variable and compared: (a) hybrids with each pure species; (b) pure species against each other; (c) hybrids against each other; and (d) hybrids against means of pure species. Sequential Bonferroni analyses were used to adjust for multiple comparisons (Rice, 1989).

Inoculation with rust from the field

To find out whether the types of rust infecting interspecific hybrids during field conditions were the same as those infecting the pure species, leaves colonised with uredinia from each of the fifteen most infected interspecific hybrid clones from the 1997 experiment were separately sampled and frozen in liquid nitrogen before storing at −70 °C. This procedure gave fifteen different sources of inocula for the inoculation experiments described below.

Rust inoculations were performed on fifteen sibling groups. Each sibling group consisted of the selected interspecific hybrid, three randomly selected hybrid siblings and three halfsibs from each of the parental species making altogether ten clones in each sibling group. The selected clones were collected from the field plantation as cuttings in January 1998 and stored at −4 °C until planting in April. The cuttings were planted in 2.5 l pots containing commercial peat soil (Hasselfors P-jord, pH 5–6) mixed with sand (in a 4 : 1 ratio), and randomly placed in three blocks. Each block contained the fifteen highly infected interspecific hybrids plus the three hybrid siblings and the three pure species halfsibs from each. All pots were placed in a greenhouse with a 16-h photoperiod and a temperature between 18 °C (night) and 22 °C (day). The plants were watered daily with a 0.2% Blomstra™ 51-10-43 nutrient solution (Cederroth International, Falun, Sweden).

Plants of the sibling groups were inoculated four weeks after planting. Uredospores from the frozen leaf samples described above were collected by washing the leaves with ice-cold distilled water containing one drop Tween™ 20 100 ml^{-1}. The uredospore concentration was adjusted, by counting in a cytometric chamber, to approximately 2 × 10^5 spores ml^{-1}. Each spore suspension, derived from each of the fifteen most infected interspecific hybrids, was sprayed on the first three fully matured leaves of the plants in the corresponding test group so as to obtain about 1500 spores cm^{-2}. The spore density was estimated on an agar-covered microscope slide, which was sprayed with the inoculum and counted under a microscope. Plants were incubated for two weeks in the conditions described above. The degree of infection was thereafter recorded as the number of uredinia per inoculated leaf and the data were analysed by comparing the results with the infection patterns obtained in the greenhouse study (Fritz et al., 1996b).

Results

Infection scores in 1997 and 1999 were low to medium on *S. viminalis* and *S. dasyclados* and low on the interspecific hybrids. There were no significant differences in rust severity in time or differences in timing of maximum infection scores. The Kruskal–Wallis ANOVA of the Schreiner scores for both of the years showed that the interspecific hybrids were significantly more resistant than either of the intraspecific pure species hybrids ($P < 0.001$ and $P < 0.001$) (Figure 2a,c), and they were also more resistant than the mean of the pure species ($P < 0.001$). No significant differences were observed in infection scores between the pure species.

During 1998, mean scores for *S. dasyclados* and the interspecific hybrids were found to be much higher compared to those in 1997 and 1999, whereas the scores for *S. viminalis* hybrids were practically the same as in 1997 and 1999. The Kruskall–Wallis ANOVA of the scores showed that *S. dasyclados* hybrids were significantly more susceptible ($P < 0.001$) whereas *S. viminalis* hybrids were significantly more resistant ($P < 0.001$) than the interspecific hybrids. The pooled mean infection score for the pure species was not significantly higher than the infection score for the interspecific hybrids. There were significant differences regarding infection scores between the pure species ($P < 0.001$) (Figure 2b).

During 1998, there were differences in rust infection within the interspecific hybrid sibling groups. The interspecific hybrids which were susceptible to rust from *S. dasyclados* in the greenhouse study were much more heavily infected in 1998 than in 1997 and 1999. In contrast, the interspecific hybrids susceptible to rust from *S. viminalis* in the greenhouse study had similar levels of infection. The interspecific hybrids that were resistant in the greenhouse study showed practically no field infections in 1997 and 1999 but were infected in 1998. However, the infection scores on these interspecific hybrids were significantly lower than the scores for interspecific hybrids susceptible to rust from *S. viminalis* in the greenhouse experiment ($P = 0.013$) (Figure 3).

The result from the studies on the rust sampled from the fifteen most infected interspecific hybrid clones in the field, showed that spores from the interspecific hybrids susceptible to rust from *S. viminalis*, infected only the siblings that were found susceptible to the *S. viminalis* rust pathotype in the greenhouse study. Similarly, spores from interspecific hybrids susceptible to rust from *S. dasyclados* in the greenhouse study, infected the siblings susceptible to the *S. dasyclados* rust pathotype. None of the selected interspecific hybrid clones seemed to carry rust that was able to infect more than one of the parental species (data not shown).

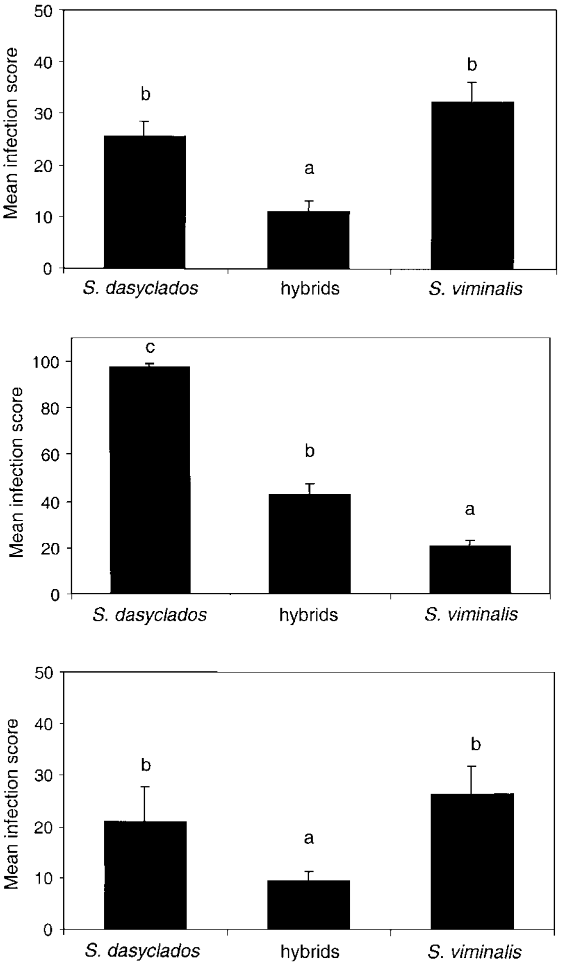


Figure 2. Mean infection scores for 1997 (a), 1998 (b) and 1999 (c) for intraspecific pure species hybrids and interspecific hybrids of *Salix viminalis* × *S. dasyclados*. Interspecific hybrids = h ($n = 270$), *Salix viminalis* = v ($n = 96$) and *S. dasyclados* = d ($n = 96$). Means with similar letters are not significantly different ($P = 0.05$). SE as bars. N.B. different scale for b.

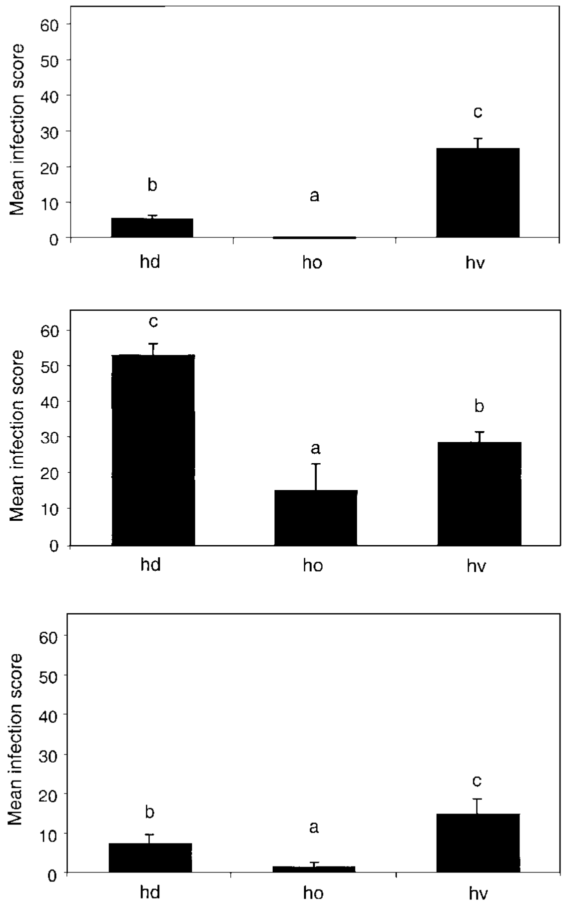


Figure 3. Mean infection scores for 1997 (a), 1998 (b) and 1999 (c) on hybrids of *Salix viminalis* × *S. dasyclados* susceptible to different rust pathotypes: hd = hybrids susceptible to *S. dasyclados* rust, hv = hybrids susceptible to *S. viminalis* rust, ho = hybrids not susceptible ($n = 270$). Means with similar letters are not significantly different ($P = 0.05$). SE as bars.

Discussion

The results of field observations of *Melampsora* rust on intraspecific pure species hybrids and interspecific hybrids between *S. viminalis* × *S. dasyclados* during the years 1997–1999 showed that the visible inheritance pattern of resistance of the interspecific hybrids of the two species was variable. Observations during 1997 and 1999 showed that the interspecific hybrids were more resistant compared to the intraspecific pure species hybrids. The data support the hybrid resistance hypothesis instead of additive resistance hypothesis that was demonstrated in previous greenhouse experiment results (Fritz et al., 1996b).

Data from 1998 show that the interspecific hybrids are intermediately resistant compared with the intraspecific pure species hybrids, which is in conformity with earlier results (Fritz et al., 1996b), i.e., it supports the additive resistance hypothesis. Thus, two infection patterns were identified during the three years of field observations with the same plants in the same plantation site exposed to natural populations of rust. As the same pattern was observed during the two years with weather normally prevailing in this area, it seems likely that hybrid resistance is the visible pattern in the field under normal conditions.

That the same sets of interspecific hybrids show two different resistance patterns during three growing seasons indicate that parameters other than those associated with inherited resistance probably interfere with and can have an impact on the visible infection pattern. One such parameter seems to be the weather; it seems possible that the ability of rust to disseminate and cause infections differs with different weather conditions especially with relation to the different types of rust.

The summer of 1997 was mostly dry with low rainfall except at two occasions with heavy thunderstorms on June 14th (49 mm) and June 16th (101 mm). The summer of 1999 was also dry, and both years were characterised by low average relative humidity. During 1998 the weather conditions were wet and cool with rainfall above normal, a high relative humidity and a low average temperature (Table 1). Earlier in 1984 and in 1987, outbreaks of rust on *S. dasyclados* were recorded in Sweden, with severe damage to the plantations of this species (Rönnberg-Wästljung and Thorsén, 1988; NE Nordh, pers. comm.), while the plantations of *S. viminalis* were less damaged. The summers of 1984 and 1987 had a high relative humidity

Table 1. Weather conditions during June–August for selected years at Uppsala, Sweden. Data obtained from the Swedish Meteorological and Hydrological Institute, Norrköping, Sweden

Year	Mean temp.	Rain (mm)	Rel. humidity (%)
1982–1999	15.2	200	71.7
1984	14.5	172	80.4
1987	12.5	231	83.4
1997	17.5	288[a]	71.8
1998	13.8	420	83.0
1999	16.5	113	68.8

[a] 150 mm received during June 14th and 16th.

(Table 1). Similar weather conditions with the same extent of severity of infections reappeared in 1998. This indicates that cold and wet weather in combination with high humidity might favour rust pathotypes colonising *S. dasyclados*.

The interspecific hybrids resistant to rust pathotypes from both *S. dasyclados* and *S. viminalis* studied in the greenhouse showed a higher degree of field infections in 1998 than in 1997 and 1999. No significant differences were observed between rust severity on *S. viminalis* during the three years of monitoring. Thus, it may be that either *S. viminalis* or the rust colonising this species is less sensitive to changes in weather or other environmental factors than *S. dasyclados* and its corresponding rust pathotypes. This assumption is supported from another field monitoring conducted by Hunter et al. (1996) with eight different *Salix* species, of which two were *S. dasyclados* and *S. viminalis*. In that study *S. dasyclados* was more sensitive to changes in environmental factors with regards to rust infections than *S. viminalis*. In a different study on herbivory resistance on hybrid willows, Fritz et al. (1998) concluded that environment is an important influencing factor to consider when analysing the inheritance of resistance.

Whether the high levels of infection in 1998 were solely an effect of the humid conditions during this particular year or were a consequence of the low-pressure weather type prevailing during the year was not tested by this study. It is supposed that *Salix* leaf rust does not overwinter as uredospores in Sweden (M. Ramstedt, pers. comm.). Instead rust infections are thought to originate from sexual recombination on larch early in spring. However, winds during low pressure systems could carry air masses, containing spore populations other than those normally occurring in this area, from the west, as described by Deacon (1997). Another possible explanation may be that the difference in resistance pattern between the years is due to new, more aggressive pathotypes developed as a result of migration or sexual recombination. Further investigations are needed to clarify the occurrence of new pathotypes.

The study on rust spores sampled from the field, showed that the rust spores derived from each of the collected clones did not infect more than one of the two pure species in this study. These results are in agreement with earlier results (Fritz et al., 1996b), i.e., the types of rust colonising the *S. viminalis* × *S. dasyclados* F_1 hybrids in the field may be the same pathotypes as those used in the greenhouse study. This observation can only be confirmed with tests using single-spore isolates. It is not known whether several genes govern resistance to willow rust, but there are reports suggesting that the resistance may be under multigenic control (Gullberg and Ryttman, 1993; Lascoux et al., 1996).

Some studies on hybrid plant resistance provide evidence in favour of the hybrid breakdown hypothesis, which means that the incidence and severity of damage are greater on the hybrid than either parent (Ericsson et al., 1993; Fritz et al., 1994; Orians and Floyd, 1997; Roche and Fritz, 1998). The infection scores in some of these studies were more than five times higher on some interspecific hybrids compared to the intraspecific pure species hybrids.

The different hypothesised effects can be either due to differential genetic control of resistance in the different plant species or due to genetic differences in the rust pathotypes that attack these hybrids. Reported as a case of hybrid susceptibility, hybridisation between *S. eriocephala* and *S. sericea* disrupts genetically based resistance (Whitham, 1989; Fritz et al., 1994) due to interactions among genes that have co-evolved in the pure species. In the additive hypothesis on the other hand, the resistance is inherited equally from each parent and, therefore, the resistance is intermediate compared to the pure species parents.

Studies on hybrid poplars and their resistance to their corresponding *Melampsora* rust species have shown different resistance patterns that are analogous to the results obtained in our study and by Roche and Fritz (1998) in the two *Salix* hybrid–*Melampsora* pathosystems. Hybrid poplars have been reported as intermediately resistant by Gallo et al. (1985), as resistant as one of the parents, i.e., the dominance pattern by Hsiang et al. (1993) and as highly susceptible by Newcombe and Chastagner (1993). Further investigations using back crosses of hybrids with pure species, more generations of hybrids and genetically defined rust spore isolates are needed before general conclusions can be drawn on inheritance of resistance in *Salix*. A more explicit experiment on the effects of weather on the different rust pathotypes will form an important complementary part of these investigations.

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