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Susceptibility of pure and hybrid willows to isolates of *Melampsora epitea* rust

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Abstract

Pure species and F_1 hybrid families of *Salix viminalis* and *S. dasyclados* were tested for resistance to four single uredinium isolates of *Melampsora* rust in laboratory experiments using excised leaves. Rust isolates were derived from: *S. viminalis, S. dasyclados*, a *S. viminalis* × *triandra* hybrid, and *S. daphnoides*. Incidence of infection, number of uredinia per leaf, and numbers of spores per uredinium were measured. As expected, the isolate from *S. daphnoides* did not infect any of the willow species or hybrids tested. For the other three rust isolates that were tested, the parent from which the isolate was derived was susceptible, the other parent was resistant, and hybrids were intermediate in resistance for incidence and uredinia per leaf. These patterns indicate additive inheritance of these resistance traits in hybrids. Numbers of spores per uredinium were similar on the hybrids and the susceptible parent for one rust isolate, suggesting dominant inheritance of this trait in the hybrids.

Introduction

Hybridization and introgression can disrupt the natural resistance mechanisms of plants to parasites and pathogens, leading to increased susceptibility (Fritz et al., 1994; Strauss, 1994). Although the breakdown of resistance to pathogens is one outcome of interspecific hybridization, it is not the only outcome. Hybrid plants could: 1) be intermediate in resistance compared to their parents (additive pattern), 2) resemble one parent and differ from the other parent (dominance pattern), or 3) could be more resistant than either parent (resistance pattern) (Fritz et al., 1994). Additionally, segregation of susceptible and resistant hybrid progeny may occur. The patterns of resistance of hybrids in a common environment are useful in revealing the genetic mechanisms of resistance to pathogens. Hybridization used for increasing plant yield may be counterproductive, if it results in increased susceptibility to pathogens. Alternatively, hybridization could be useful in introducing resistance mechanisms into commercial varieties. How hybridization affects resistance depends upon the specificity of host-pathogen relationships and the genetics of resistance. Both components are considered in this study.

The taxonomic status of *Melampsora* rusts and their associations with willow hosts are often unclear (Pei et al., 1993). In England, *M. epitea* var. *epitea* infects several willow species, while other *Melampsora* species were found only to infect one species of *Salix* (Pei et al., 1993). Willow species can be susceptible to more than one rust species. Because of the genetic variation in host-pathogen interactions known in these systems a certain rust genotype may be more aggressive against certain genotypes of a *Salix* species (McCracken and Dawson, 1992). Thus, a number of rust:willow pathosystems exist. Natural hybridization among willow species and the use of hybrids in short rotation energy forest plantations complicates host-parasite interactions. Rust infection can reduce biomass productivity by up to 40% in short rotation willow stands (Parker et al., 1993).

The aim of this study was to examine the patterns of susceptibility of F_1 hybrids compared to pure

parents. The study included laboratory experiments on pure parents and F_1 hybrids of *S. viminalis* and *S. dasyclados* and four single-uredinium isolates of *Melampsora epitea* of different origins.

Materials and methods

Plant material

Eight sets of families composed of hybrids between *Salix viminalis* females and *Salix dasyclados* males and intraspecific crosses of these species were investigated. The crossings were performed in March 1993 at the Department of Plant Breeding, Swedish University of Agricultural Sciences. The parental clones of *Salix viminalis* (all diploid) were of either Swedish or Polish origin, while *Salix dasyclados* clones were of Swedish origin (Table 1). Three of the *S. dasyclados* clones were diploid, five clones were tetraploid, and the chromosome number was unknown in three clones. The eight fullsib, interspecific hybrid families contained 4–16 siblings each. Each hybrid family had two associated intraspecific halfsib family groups with which it shared one parent each. Each intraspecific halfsib family consisted of three siblings from two or three different crossings. Each sibling was cloned as cuttings, planted in pots with 5 commercial peat soil (Hasselfors P-jord, pH 5–6 from Hasselfors Garden) mixed with sand (80 l soil with 10 l sand), and planted into four blocks (one replicate per block). The pots were placed in a greenhouse with a 16 h photoperiod and a temperature between 18 °C and 22 °C. The plants were watered daily with 0.25% Superex (Kekkilä-Bolagen) nutrient-solution. The experiments were carried out four to six weeks after planting.

Rust cultures

Single-uredinium isolates of *Melampsora epitea* used in these experiments were collected in south and central Sweden and were tentatively identified as four different pathotypes of *Melampsora epitea* (Table 2) according to their pathogenicity on differential willow clones (Royle and Hubbes, 1992). Isolations of single-spore isolates were performed by picking one uredinium, which was growing apart from other uredinia. The urediniospores were applied onto leaves using a small paint brush; this was repeated three times using a new uredinium from the previously infected leaf. The isolates were cultured on leaves from susceptible clones. A modified pathogenicity test protocol designed by Pei et al. (1993) was used for identification. The isolates were cultured on leaves from susceptible clones. The leaves were laid with the abaxial surface upwards on wet filter paper in Petri dishes and were inoculated by applying the urediniospores with a brush. The leaves were exposed to a 24 h photoperiod at 18 °C for approximately two weeks and the newly produced spores were harvested with a vacuum spore collector. After harvesting, the spores were dried in a desiccator for 10 days and stored in 2 ml cryovials (Nalgene^{TM}) at −80 °C.

Qualitative screening of genotypes

Since a large proportion of the genotypes were expected to be fully resistant to one or several rust isolates, a pre-screening was carried out to eliminate individuals that showed no signs of infection from subsequent studies. The first fully mature leaf from each plant (i.e. four leaves per genotype) was collected and placed, abaxial surface upwards, in Petri dishes on wet filter paper. The Petri dishes were sprayed with a urediniospore suspension (200,000 spores ml^{-1} in distilled water with one drop Tween® 20 100 ml^{-1}) resulting in roughly 1250 spores cm^{-2}. The leaves were incubated under the same conditions as described above. After 14 days the leaves were classified as infected (+) or noninfected (−) based on the presence or absence of uredinia on the leaves. Genotypes for which no infection was recorded were considered qualitatively resistant. This entire procedure was repeated for each rust isolate. For all isolates, leaves from the clone used for isolation were used as a positive control. All qualitatively susceptible genotypes were then subjected to a test to determine quantitative resistance in the hybrids.

Quantitative test

The first three fully mature leaves per plant from each susceptible genotype in the qualitative test (12 leaves per genotype) were removed and placed on wet filter paper in Petri dishes. Leaves were chosen that were approximately the same size to equalize the area available for infection. The leaves were sprayed with a spore suspension and incubated as described above. To determine the latent period, the leaves were observed daily and the number of days until the appearance of the first uredinium appeared was recorded. For the three isolates that produced uredinia on leaves of 86 VIM,

Table 1. Crossing scheme showing parental clones and the interspecific hybrid families with their pure species half-sibs. Each family can be identified by number, e.g. '25' or '561'

		***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						

Table 2. The four *Melampsora* isolates used in the experiments

Isolate	Proposed pathotype	Isolated from	Propagated on	
			Taxon	Clone
86 VIM	LET 1 (*larici-epitea typica* 1)	*S. viminalis*	*S. viminalis*	78183
164 VXT	LET 4 (*larici-epitea typica* 4)	*S. viminalis x triandra*	*S. vim X tri*	Q 83
91 DAP	LD 1 (*larici-daphnoides* 1)	*S. daphnoides*	*S. daphnoides*	78139
77 DAS	LR 1 (*larici-retusae* 1)	*S. dasyclados*	*S. dasyclados*	78196

Proposed pathotypes according to Pei et al. (1992) and Pei (pers. comm.)

77 DAS and 164 VXT, the observed latent periods were 6 days, 7 days, and 8 days, respectively. After an interval of twice the latent period, to avoid interference from a second infection cycle, the number of uredinia was counted on each leaf. After counting, all leaves from each willow genotype and rust isolate were pooled and the spores were harvested, using a method modified from Hsiang and van der Kamp (1985). The leaves were placed in test tubes with 790 ml 95% ethanol, and the tubes were vigorously shaken for 1 h on a Gerhardt shaker. To avoid evaporation, samples were diluted with water to 1.5 ml, to make 50% ethanol. Thereafter the samples were put in a Greiner microtiter plate with plain bottom wells and diluted to optimal spore density. The absorbance was then measured in a spectrophotometer (Titertek Multiskan®) at 690 nm. Random checks were done on every tenth tube, in which spores were counted in a haemocytometer to derive a regression for spore count on absorbance. The derived equation was: spore count = abs × 4.7175 × 10^6 + 6249, r = 0.95.

Data analysis

Chi-square tests were used to test if the frequency of infected leaves differed among willow taxa for each rust isolate. Each plant (one leaf) within a taxon was an independent observation. Contingency table analyses were used to determine significant differences between pairs of taxa. Two-tailed, paired t-tests were used to test the hypothesis that hybrid progeny means differed from the midparent values (means of *S. viminalis* and *S. dasyclados*). Paired t-tests were used because hybrid progenies shared either maternal or paternal parents

with pure species. Midparent values were calculated by weighting the mean scores of uredinia per leaf and spores per uredinina by the number of chromosome sets in each family. Thus, when both *S. dasyclados* parents were tetraploid and hybrids with *S. viminalis* were triploid the midparent value was (V + 2D)/2. Parents with unknown karyotypes were assumed to be tetraploid. One-tailed, paired t-tests were then used to test the hypothesis that hybrid means were the same or lower than those of the susceptible parent. Replicates were the 8 trios of full-sib hybrid families and their associated pure species halfsib families.

Results

One of the rust isolates (91 DAP) did not cause infection on either *S. viminalis, S. dasyclados* or hybrids and thus showed clear specificity for its original host species, *Salix daphnoides*. The three remaining *Melampsora* isolates caused infection to varying degrees on both parent species and hybrids. Hybridization affected the incidence of rust infection by three isolates. Incidence of infection by 86 VIM was greatest on *S. viminalis*, from which the isolate was derived, and intermediate on hybrid families (Figure 1A). There was no infection of *S. dasyclados* leaves. In contrast, the incidence of infection by 77 DAS was highest on *S. dasyclados*, from which it was derived, somewhat less on hybrids, and was absent from *S. viminalis* (Figure 1B). The 164 VXT isolate, derived from a *S. viminalis* × *triandra* hybrid, gave low incidence of infection on *S. viminalis* and hybrids and no infection on *S. dasyclados* (Figure 1C).

Patterns of uredinia per leaf on hybrids and parents (Figure 2) tended to parallel the incidence data. Uredinia per leaf for 86 VIM was highest on *S. viminalis*, absent on *S. dasyclados*, and was intermediate on hybrids (Figure 2A). For 77 DAS, the highest numbers of uredinia per leaf occurred on *S. dasyclados*. Intermediate, but significantly lower, numbers occurred on hybrids, and no uredinia occurred on *S. viminalis* (Figure 2B). The mean number of uredinia per leaf for *Melampsora* isolate 164 VXT was very small (*S. viminalis* = 0.21; hybrids = 0.09, *S. dasyclados* = 0) and did not differ significantly ($p = 0.114$).

Spores per uredinia from rust isolate 86 VIM were highest on *S. viminalis*, lower on hybrids, and absent on *S. dasyclados* (Figure 3A). Spores per uredinia from rust isolate 77 DAS were equally high on hybrids and *S. dasyclados*, but were absent on *S. viminalis*

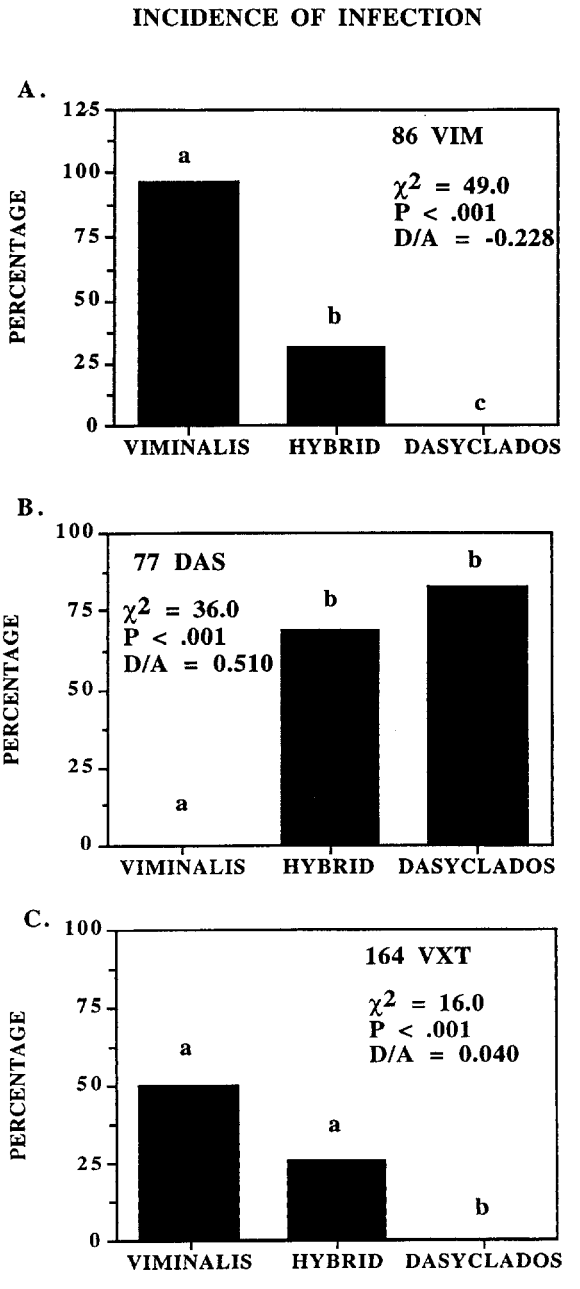

Figure 1. Plots of the percentage of leaves infected by each rust isolate. Sample sizes of leaves for each test were: 86 VIM, N = 120; 164 VXT, N = 110; 77 DAS, N = 115. χ^2 and significance values correspond to overall test. Bars with different letters differ significantly at $P < 0.05$, df = 2 for pairwise comparisons. D/A is the dominance/additive ratio.

(Figure 3B). For 164 VXT, the small number and size of uredinia resulted in absorbance values below the limit of detection (848 spores per uredinium); thus spores per uredinia could not be calculated for this rust isolate.

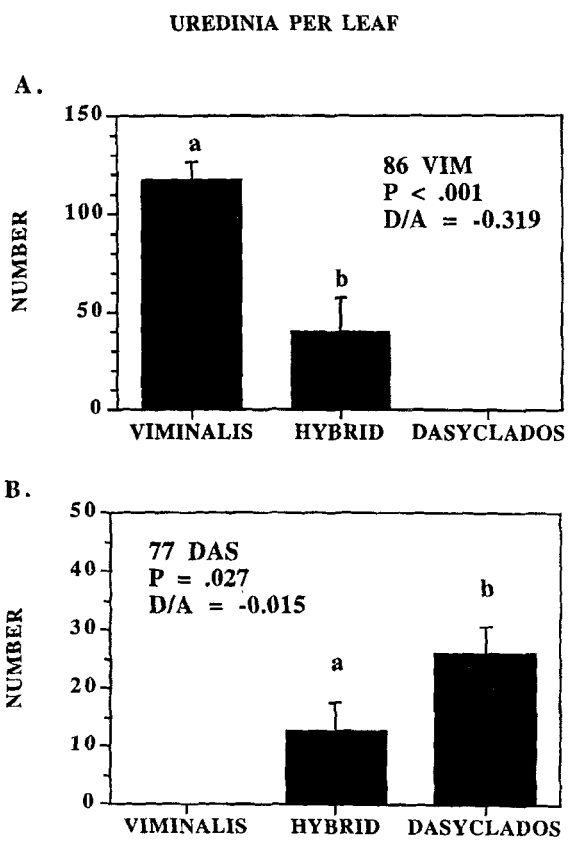

Figure 2. Mean number of uredinia per leaf (± 1 SE) for the hybrid and parental taxa for rust isolates 86 VIM and 77 DAS. Significance values correspond to one-tailed, paired t-test comparing hybrid to susceptible species. Significant differences are indicated with different letters. D/A is the dominance/additive ratio.

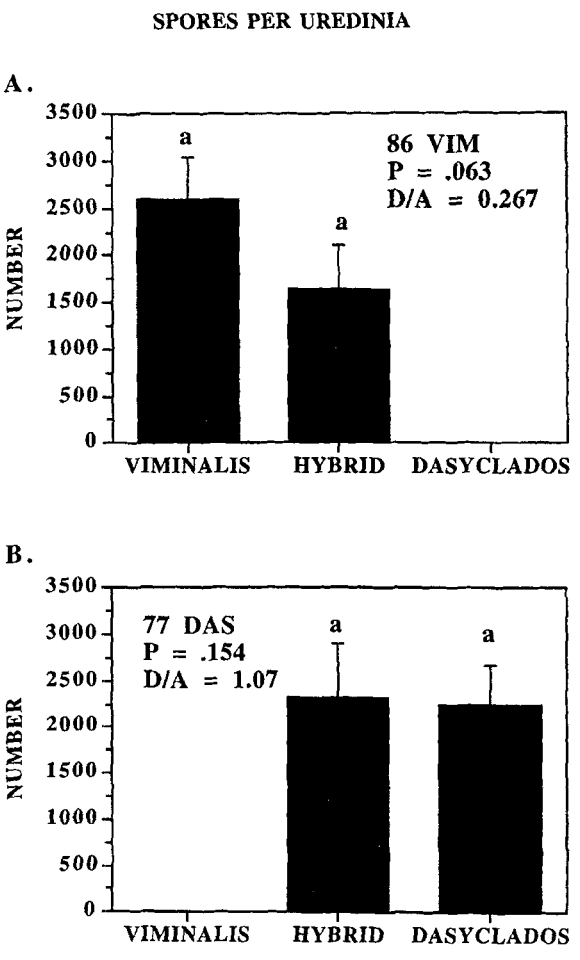

Figure 3. Number of spores per uredinia (mean ± 1 SE) for the hybrid and parental taxa for rust isolates 86 VIM and 77 DAS. Significance values correspond to one-tailed, paired t-test comparing hybrid to susceptible species. Significant differences are indicated with different letters. D/A is the dominance/additive ratio.

Paired t-tests were performed to test the hypothesis that the measured variables for hybrids differed significantly from the midparent values. In no case were the t-values significant (values not shown), and thus the reaction of hybrid progeny to the three rust isolates was additive. Although hybrids tended to be intermediate for each variable measured, a comparison of the dominance/additive ratios is useful. For 86 VIM, the incidence of infection and uredinia per leaf on hybrids was less than the midparent values, but spores per uredinia had levels on hybrids that were above the midparent value (Figures 1–3). The D/A ratios showed a tendency to resemble the resistant *S. dasyclados*. For 77 DAS, incidence of infection on hybrids showed dominance toward the susceptibility of *S. dasyclados*, and spores per uredina was completely dominant in hybrids (Figures 1B, 2B). Uredinia per leaf, in contrast, showed virtually no tendency to depart from additivity (Figure 3B). There was virtually no dominance for incidence of infection by 164 VXT (Figure 1C).

Discussion

Rust isolates used in this study showed specialization on one of the two parental willows. The two rust isolates (86 VIM and 77 DAS) had their highest incidence and levels of infection on the species from which they were derived, but were unable to infect the other parental species. The rust isolate 164 VXT isolated from a *S. viminalis* × *triandra* hybrid was able to infect *S. viminalis* but not *S. dasyclados*. One rust isolate (91 DAP), isolated from *S. daphnoides*, was not able to infect either parent or hybrids. These results indicate

that these *Melampsora* isolates are specialized to some degree on their willow host. This supports our preliminary designation of these isolates as distinct pathotypes (Table 2).

The taxonomic status of *Melampsora* rusts of willow is difficult. In an investigation on *Melampsora* from southwestern England, Pei et al. (1993) showed that *M. epitea* infected 18 leaf samples from eight willow taxa. Other *Melampsora* species were more specialized on their uredinial hosts. Our results suggest that 86 VIM and 77 DAS are different rust species or are specialized pathotypes of one species. We have not yet performed any extensive morphological analyses or experimental infections of potential aecial hosts to permit us to determine the taxonomic status of the isolates. Preliminary studies show that size and shape of the urediniospores correlates to data for *Melampsora epitea* and that there were no significant differences between spores from *S. dasyclados* and *S. viminalis*. Both VIM and DAS isolates could also be recovered from aecia on larch needles, which means they have the same alternate host.

Susceptibility of F_1 hybrids to *Melampsora* rust isolates is intermediate between the parental species for three of the rust isolates, which fits the additive pattern of Fritz et al. (1994). The additive pattern suggests additive inheritance of resistance genes from the resistant parental species. Having one resistant parent did not confirm complete resistance, nor did hybridization confer increased susceptibility. Our data suggest that resistance in hybrid plants to specific isolates is inherited additively for two characters: incidence of infection and uredinia per leaf. The uredinia on hybrids may produce as many spores as uredinia on the susceptible parents, which supports the dominance pattern. The differences in the pattern of hybrid resistance to the rust isolates for the variables measured may suggest that these traits are under separate genetic control. Our data do not permit us to determine if resistance in the hybrids is determined by one or many genes, although other reports (Lefévre et al., 1994; Gullberg and Ryttman, 1993 and Lascoux et al., 1996) indicate that *Melampsora* resistance in both poplar and willow are under polygenic control.

Even though hybrids were of intermediate susceptibility to each of the rust isolates for two characters, there were dominance deviations seen for spores per uredinia of 77 DAS. However, because of small sample size we were unable to reject strict additivity in any comparison. The dominance deviations for uredinia per leaf were towards resistance. For incidence of infection, 86 VIM showed dominance towards resistance whereas 77 DAS showed stronger dominance towards susceptibility. Dominance deviations for spores per uredinia were much stronger towards susceptibility.

Fritz et al. (1994) and B. M. Roche and R. S. Fritz (unpubl.) have found that advanced generation and F_1 hybrids between *S. sericea* and *S. eriocephala* are highly susceptible to rust infection in the field and among cloned and seedling plants in a garden. Infection scores were up to five times higher on hybrids compared to the pure parents. Mosseler (pers. comm.) also noted much higher infection by *Melampsora* rust on a number of F_1 hybrids between seven willow species growing in a garden. When hybrid willows in the field or in a garden are exposed to natural populations of *Melampsora* rust they appear to show evidence of breakdown of resistance to *Melampsora*. The different results seen in the present study could be due to either differences in the genetic control of resistance among the two pairs of willows or the genetic diversity of the pathogen. The genetic diversity of the pathogen may be as important to the patterns seen on plants in the field as are the consequences of hybridization on resistance. In this study we presumably sampled only a very limited fraction of the available genetic diversity of *Melampsora* pathotypes to which plants in the field are exposed. Isolations of more *Melampsora* uredinia from different locations and pure and hybrid hosts with subsequent tests of infectivity on hybrids and parents are needed to begin to assess the importance of pathogen diversity in determining these patterns.

Intermediate resistance, as seen in the present study, could be due to dosage dependence of resistance, since hybrids have only half of their genes from each parent. Half of the alleles for resistance could produce half of the resistance. The higher susceptibility of hybrids between *S. sericea* and *S. eriocephala* could be due to the breakdown of resistance mechanisms from both parents. Hybrid susceptibility could occur even if resistance was additively inherited. If there was a threshold level of resistance alleles required to confer resistance that exceeded the number inherited from each parent in hybrids then susceptibility would result. Susceptibility of advanced generation hybrids to pathogens could also occur if recombination resulted in the loss of essential resistance genes. Many of the hybrids between *S. sericea* and *S. eriocephala* studied in the field are advanced generation hybrids, but F_1 crosses were also susceptible in the garden (B. M. Roche and R. S. Fritz, unpubl.).

An alternative possible response of hybrids to rust isolates could be one of dominance, where hybrids are as susceptible as the susceptible parent. Higher susceptibility in the field might result because of higher pathogen diversity, i.e. hybrids would be attacked by all pathotypes that could attack either parent. The cumulative infection on the hybrids would be greater than on any parental species. Alternatively, if some rust pathotypes were specialized on hybrids, then hybrid susceptibility might result because a greater diversity of rust pathotypes could attack hybrid genotypes. Since hybrids of *Salix* are common in the field in Europe and North America (Argus 1986) it is possible that some *Melampsora* may specialize on hybrid plants.

Data from the analysis of susceptibility of hybrid poplars to various *Melampsora* species offer a diversity of results that parallel the differences seen in willow systems. Hybrid poplars are sometimes intermediate in resistance between two parental species (Gallo et al., 1985), sometimes as resistant as one of the parents (dominance) (Hsiang et al., 1993), and sometimes are highly susceptible (Newcombe and Chastanger, 1993), such that outbreaks occur. In one study, interspecific progenies of poplars were produced where some sibs were as resistant, intermediate in susceptibility, or more susceptible than parents (Lefèvre et al., 1994). With tests involving more individuals per interspecific family, patterns of segregation of resistance may be found within hybrid families. Experiments combining extensive single uredinium isolations from parents and hybrids and laboratory and field trials on resistance on hybrids are necessary in willow systems to determine the patterns and causes of hybrid resistance or susceptibility.

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References

Argus GW (1986) The genus *Salix* (Salicaceae) in the southeastern United States. Syst Bot Monograph 9: 1–170

Fritz RS, Nichols-Orians CM and Brunsfeld SJ (1994) Interspecific hybridization of plants and resistance to herbivores: Hypotheses, genetics, and variable responses in a diverse community. Oecologia 97: 106–117

Gallo LA, Stephan BR and Krusche D (1985) Genetic variation of *Melampsora* leaf rust resistance in progenies of crossings between and within *Populus tremula* and *P. tremuloides* clones. Silvae Genetica 34: 208–214

Gullberg U and Ryttman H (1993) Genetics of field resistance to *Melampsora* in *Salix viminalis*. Eur J For Path 23: 75–84

Hsiang T and van der Kamp BJ (1985) Variation in rust and host resistance of *Melampsora* on black cottonwood. Can J of Plant Path 7: 247–252

Hsiang T, Chastagner GA, Dunlap JM and Stettler RF (1993) Genetic variation and productivity of *Populus trichocarpa* and its hybrids. VI. Field susceptibility of seedlings to *Melampsora occidentalis* leaf rust. Can J For Res 23: 436–441

Lefévre F, Pichot C and Pinon J (1994) Intra- and interspecific inheritance of some components of the resistance to leaf rust (*Melampsora larici-populina* Kleb.) in poplars. Theor Appl Genet 88: 501–507

Lascoux M, Ramstedt M, Åström B and Gullberg U (1996) Components of resistance to leaf rust (*Melampsora laricii epitea* Kleb./*Melampsora ribesii-viminalis* Kleb.) in *Salix viminalis* L. Theor Appl Gen in press

McCracken AR and Dawson M (1992) Clonal response in *Salix* to *Melampsora* rusts in short rotation coppice plantations. Eur J For Path 22: 19–28

Newcombe G and Chastagner GA (1993) A leaf rust epidemic of hybrid poplar along the lower Columbia River caused *by Melampsora medusae*. Plant Disease 77: 528–531

Parker SR, Royle DJ and Hunter T (1993) Impact of *Melampsora* rust on yield of biomass willows. Abstract, 6th International Congress of Plant Pathology, Montreal, Canada – Sept 1993

Pei MH, Hunter T and Royle DJ (1992) Variation of *Melampsora* rusts occurring on biomass willows in the UK. In: Proceed International Energy Agency Task 8, Workshop 15–19 September 1992. Enniskillen, Northern Ireland

Pei MH, Royle DJ and Hunter T (1993) Identity of host alternation of some willow rusts (*Melampsora* spp.) in England. Mycol. Res. 97: 845–851

Royle DJ and Hubbes M (1992) Diseases and pest in energy crop plantations. Biomass and Bioenergy 2(1–6): 45–54

Strauss SY (1994) Levels of herbivory and parasitism in host hybrid zones. Trends in Ecol Evol 9: 209–214