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Components of resistance of leaf rust (*Melamspora laricii epitea* Kleb./*Melampsora ribesii-viminalis* Kleb.) in *Salix viminalis* L.

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Abstract Selection on partial resistance components, namely latent period, development rate of uredia and number and size of uredia, has been suggested as a means to achieve durable resistance. Three experiments were carried out in growth chambers to assess the impact of environmental and genetic factors on these components in the *Salix-Melampsora* host-pathogen system. They confirmed the environmental ability of the *Melampsora-Salicaceae* relationship and provided no definite answer on the possibility of attaining durable resistance through selection on partial resistance components. On the one hand, there is a large amount of heritable variation among clones for most components; on the other hand, all components were extremely sensitive to environmental conditions and isolates, making the development of standard selection methods difficult.

Key words Durability · *Melampsora* · Resistance components · *Salix*

Introduction

Breeding for resistance in crops is often described as an arms race between the breeders and the pathogens; new resistant cultivars will hopefully be released before the pathogens manage to break the resistance of existing ones. Of course, this "boom-and-bust" cycle (Suneson 1960) represents an oversimplification of the situation, albeit it captures the intrinsically dynamic aspect of host-pathogen systems. It also suggests that the best one can hope for is to postpone as long as possible the occurrence of new virulent strains of pathogens. This can be achieved by breeding for durable resistance (Leonard 1987; Parlevliet 1993; Briggs and Johal 1994), through deployment and manipulations of the host and/or pathogen populations (May 1992) or through a combination of both approaches. The breeding strategy retained for durable resistance will in large part depend on the genetic nature of the resistance, on the resources available and on the time necessary to produce new resistant cultivars. In the case of perennial plants such as willows or poplars, resources are often limited, and breeding generations are fairly long. Two types of resistance can exist within a population. The first one, due to major genes, is nearly always race-specific, causes an hypersensitive type or low infection type of response and is usually, although not always, short-lived. In the absence of major genes, one can observe a second type of resistance, which is only partial and likely to be polygenic. This second type is commonly believed to be more durable. In the barley-barley leaf rust (*Puccinia hordei*) host-pathogen system, for instance, this resistance is due to the collective effect of longer latent periods, reduced infection frequencies and lower rates of sporulation (Parlevliet 1993). Experiments showed that it was possible to increase the resistance through recurrent selection for these traits, even at low selection intensity (Parlevliet and van Ommeren 1988) and that selection was easier when there were no major genes in the population. Similar results may also hold in poplars for components of resistance to *Melampsora larici-populina* Kleb. Preliminary results indicated that one of the most promising strategies to reduce the impact of epidemics is to select for low rates of sporulation (Lefèvre et al. 1994; F. Lefèvre personal communication). These results can perhaps be explained in terms of the evolution of virulence (Bull 1994). Evolutionary biologists have recently challenged the "conventional wisdom" that successful

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parasites have to become benign on the grounds that natural selection does not minimize virulence, but instead works to increase a parasite's intrinsic reproductive rate, R_0, where R_0 is defined as the number of additional hosts infected by the pathogen from one individual (e.g. May 1985; Nowak and May 1994). Clearly, R_0 depends on both the virulence and transmissibility of the pathogen. If the rate of transmission is linked to virulence, then selection may in some circumstances lead to intermediate levels of virulence. Neither the genetics nor the biology of the *Melampsora-Salix* interactions are well-characterized, but the response of *Salix* clones to *Melampsora* in a field experiment appeared to be quantitative rather than qualitative (Gullberg and Ryttman 1993), suggesting a multi-loci inheritance of resistance. It therefore seems worthwhile to seriously consider the possibility of using selection for resistance components in the *Salix* long-term breeding program (Kang et al. 1995; Gullberg 1993). Because earlier studies had shown that the *Melampsora-Populus* relationship was very environmentally labile (Heather and Chandrashekar 1982; unpublished results), we investigated the effects of genetic and environmental factors on the resistance components under controlled environments before proceeding with selection. More specifically, in a series of three experiments we investigated the effects on resistance components of different combinations of (1) temperature, (2) inoculation method, (3) level of inoculated levels within the plant, (4) host clone and (5) pathogen isolate.

Materials and methods

Material

Experiment 1 and Experiment 2

Six and 11 *S. viminalis* L. clones in Experiment 1 and Experiment 2, respectively, were inoculated with a single *Melampsora* isolate (Table 1). Approximately six cuttings per clone were first grown in a greenhouse for about 1.5 months. These were eventually divided into two groups that were subsequently exposed to different treatments. In the first treatment (agar or in vitro), eight "full-grown" leaves, four from the upper third and four from the lower third of the plant, respectively, were harvested, sterilized and placed upside down in petri dishes containing agar and gibberellin. The petri dishes were then transferred to two growth chambers, one kept at 25 °C day /15 °C night and the other at 15 °C day/15 °C night, and a photoperiod of 16 h day /8 h night. A spore suspension adjusted to approximately 200 000 spores/ml was sprayed over two small, well-delineated areas on each side of the central rib. In the second treatment (in situ) whole plants were transferred to two growth chambers, 25 °C day /15 °C night and 15 °C day /15 °C night, respectively, and a photoperiod of 16 h day /8 h night. Two leaves from the upper third and two leaves from the lower third of the plant were inoculated in situ according to the same protocol as in the agar treatment. In both treatments, a completely randomized design was used.

Experiments 3

Eight *S. viminalis* clones were inoculated with four different isolates belonging to three different pathotypes (Table 1). Vegetative copies of each clone were directly planted in large pots that were placed into a growth chamber at 20 °C day /15 °C night and a photoperiod of 16 h day /8 h night, according to a completely randomized block design with five blocks. Consequently, there were 32 plants (eight clones × four isolates) in each block. Two of the first full-grown leaves per plant, corresponding approximately to the tenth node under the apex, were inoculated in situ following a similar protocol as in Experiment 1.

Measurements

The latent period (LP; number of days from inoculation to first occurrence of uredia) and the number of uredia (NU) were assessed daily in Experiments 1 and 2, or every other day in Experiment 3, for approximately 2 weeks. The uredia were counted on a limited part of the leaf, corresponding to a square of 15 × 15 mm^2. Because some leaves had a breadth less than 15 mm, we defined the adjusted number of uredia (NUA) as the number of uredia divided by the actual breadth of the leaf. In some of the treatments, the size of the uredia (SU) was estimated using a 1–4 scale, and the number of necrosis (NEC) was counted at the time of the last measurement. Final measurements were taken when the number of uredia had apparently

Table 1 *Salix viminalis* clones and *Melampsora* isolates used in the three experiments. The clone number is the code number in the *Salix* breeding program, whereas the figure given within brackets is the one used in the paper. Locations at which isolates were sampled and their pathotype are given within brackets after the isolate code number

	Experiment 1	Experiment 2	Experiment 3
Salix clones	78091 81084 78112 80010 77699 78120	78091 81084 78112 80010 77699 78120 81-0-92 78-0-183 81-0-102 22-8 22-11	77-0-683 (1) 78-0-101 (2) 78-0-13 (3) 78-0-195 (4) 78-0-198 (5) 78-0-21 (6) 78-0-30 (7) 78-0-90 (8)
Isolates	74v [Svalöv, 55° N 13° E, EV5]	74v [Svalöv, 55° N 13° E, EV5]	70v [Köping, 59° N 16° E, EV2] 74v [Svalöv, 55° N 13° E, EV5] 83v [Ultuna, 59° N 17° E, EV2] 85v [Långaveka, 57° N 12° E, EV1]

reached an asymptotic value for most of the individuals within the considered treatment. Consequently, that time (T, in number if days from inoculation) depends on the treatment. The developmental rate of the uredia is then:

$$\text{Rate} = \frac{\text{NUA}(T)}{T - LP}$$

where NUA(T) is the adjusted number of uredia at the time of last measurement, T is the date of last measurement and LP is the latent period. T–LP is called Duration hereafter.

Data analysis

All continuous traits were log-transformed before the analysis of variance. The choice of the logarithmic transformation was dictated by the analysis of the normality of the residuals. All data were analyzed using the SAS statistical package (SAS Institute 1985). Analyses of variance were carried out with Proc GLM and variances were estimated using Proc VARCOMP. A χ^2 test was used to analyze the size of uredia (Proc FREQ), and phenotypic correlations were estimated using Proc CORR.

In Experiment 1, the model was:

$$y_{ijklm} = \mu + M_i + T_j + C_k + L_l + MT_{ij} + MC_{ik} + ML_{il} + TC_{jk} + TL_{jl} + CL_{kl} + \varepsilon_{ijklm} \quad [1]$$

where μ is the general mean, M_i is the treatment effect (agar vs. in situ), T_j is the temperature effect (25 °C day /15 °C night vs. 15 °C day /15 °C night), C_k is the clone effect ($1 \leq k \leq 6$), L_l is the level of measurement effect (upper 1/3 vs. lower 1/3), MT_{ij} to CL_{kl} are the first-level interaction terms and ε_{ijklm} is residual. All effects were considered to be random.

In Experiment 2, the model was:

$$y_{ijkl} = \mu + M_i + T_j + C_k + + MT_{ij} + MC_{ik} + TC_{jk} + MTC_{ijk} + \varepsilon_{ijkl} \quad [2]$$

where μ is the general mean, M_i is the treatment effect (agar vs. in situ), T_j is the temperature effect (25 °C day /15 °C night vs. 15 °C day /15 °C night), C_k is the clone effect ($1 \leq k \leq 11$), MT_{ij} to TC_{jk} are the first-level interaction terms, MTC_{ijk} is the second-level interaction terms and ε_{ijklm} is the residual. All effects were considered to be random.

Finally, in Experiment 3, the following model was used:

$$y_{ijkl} = \mu + B_i + I_j + C_k + IC_{jk} + \varepsilon_{ijkl} \quad [3]$$

where μ is the general mean, B_i is the block effect, I_j is the isolate effect ($1 \leq j \leq 4$), C_k is the clone effect ($1 \leq k \leq 8$), IC_{ij} is the interaction term between isolate and clone effects, and ε_{ijkl} is the residual. All effects were considered to be random, expect for the block effect, which was considered fixed.

The repeatability, in our case $r = \sigma^2_{clone}/(\sigma^2_{clone} + \sigma^2_{error})$ (Falconer 1981), where σ^2_{clone} is the variance among clones and σ^2_{error} is the residual variance, was calculated in Experiments 2 and 3 using models

Table 2 Analysis of variance in Experiment 1 for log-transformed values of adjusted final number of uredia (NUA), latent period (LP) and rate

Source	*df*	MS	*F* value	Pr > *F*
Log(NUA + 1)				
Treatment	1	25.651	1.07	0.4353
Temperature	1	5.752	1.18	0.4646
Clone	5	3.827	2.83	0.0946
Level	1	11.115	0.58	0.5807
Treat*Temp	1	4.702	26.96	0.0001
Treat*Clone	5	0.929	5.332	0.0001
Treat*Level	1	18.659	107.021	0.0001
Clone*Temp	5	0.306	1.756	0.1213
Temp*Level	1	0.189	1.088	0.2976
Clone*Level	5	0.464	2.665	0.0222
Error	353	0.174		
Log(LP + 1)				
Treatment	1	0.062	0.07	0.8207
Temperature	1	4.596	13.68	0.1022
Clone	5	0.320	4.74	0.0458
Level	1	1.565	2.04	0.3460
Treat*Temp	1	0.261	15.14	0.0001
Treat*Clone	5	0.024	1.42	0.2149
Treat*Level	1	0.677	39.23	0.0001
Clone*Temp	5	0.024	1.44	0.2071
Temp*Level	1	0.083	4.81	0.0289
Clone*Level	5	0.052	3.04	0.0106
Error	330	0.017		
Log(Rate + 1)				
Treatment	1	4.662	1.48	0.3464
Temperature	1	1.094	0.71	0.5254
Clone	5	0.193	1.06	0.4454
Level	1	3.827	1.90	0.3739
Treat*Temp	1	1.324	54.52	0.0001
Treat*Clone	5	0.105	4.36	0.0008
Treat*Level	1	1.881	77.47	0.0001
Clone*Temp	5	0.080	3.30	0.0065
Temp*Level	1	0.169	6.96	0.0088
Clone*Level	5	0.040	1.66	0.1432
Error	330	0.024		

involving the sole clone effect for each Treatment × Temperature (respectively Isolate) levels. In Experiment 3 the block effect was also included but was considered fixed. Repeatability can be interpreted as an upper limit to the heritability. We also computed a "clonal" coefficient of variation, CV, as $CV = 100(\sqrt{\sigma^2_{clone}}/\bar{X})$ where $\bar{X}$ is the mean of the trait under consideration. A similar measure, one based on additive variance instead of clonal variance, was shown to be a better measure of the evolvability and genetic variability of a character than heritability (Houle 1992).

Results

Experiments 1 and 2

Treatment, temperature and leaf level

Analyses of variance using models 1 and 2 indicated that no general statement can be made on the effect of the three aforementioned factors on the development components of the rust. In Experiment 1 none of the main effects were significant, whereas the interaction terms generally were (Table 2). In Experiment 2, only the third-level interaction was significant ($P < 0.05$) (data not shown). This being said, some general trends can nonetheless be noted. Firstly, rust development was hampered by the *agar* treatment, the latent periods being longer and rates lower than *in situ* (Table 3). Secondly, fewer and smaller (χ^2-test significant at $P = 0.0001$; data not shown) uredia were observed on leaves from the lower-third of the plant than on leaves from the upper-third despite consistently shorter latent periods. Indeed, the low number of uredia observed on older leaves essentially resulted from very low developmental rates. Thirdly, higher temperatures tended to shorten the latent period but had no clear influence on the rust developmental rates. If they had any effect at all,

Table 3 Means and coefficients of variation (figures within parenthesis) of rust development components by treatment (agar versus in situ), temperature (25 °C/15 °C versus 15 °C/15 °C) and level of inoculated leaves within the plant [Lower third (lower) versus upper third (upper)]. NUA, the adjusted number of uredia, is the number of uredia observed at the end of the first cycle divided by the breadth of the leaf. LP, the latent period, is the number of days between inoculation and the first occurrence of uredia. Rate is the ratio adjusted number of uredia to the time elapsed between the date the first uredia were observed and the time the final number of uredia was assessed

Trait		Agar				in situ			
		25 °C/15 °C		15 °C/15 °C		25 °C/15 °C		15 °C/15 °C	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
NUA	Exp. 1	0.661	0.693	0.759	0.566	0.737	2.732	1.399	4.837
		(80%)	(161%)	(84%)	(105%)	(179%)	(99%)	(92%)	(63%)
	Exp. 2	–	0.679	–	1.020	–	7.177	–	6.012
			(70%)		(114%)		(67%)		(65%)
LP	Exp. 1	8.431	10.939	10.586	13.230	8.419	9.311	12.200	12.208
		(16%)	(17%)	(18%)	(18%)	(22%)	(21%)	(13%)	(11%)
	Exp. 2	–	11.800	–	11.976	–	7.534	–	9.215
			(18%)		(19%)		(23%)		(21%)
Rate	Exp. 1	0.110	0.277	0.117	0.183	0.180	0.544	0.353	1.215
		(66%)	(93%)	(68%)	(66%)	(117%)	(73%)	(72%)	(39%)
	Exp. 2	–	0.196	–	0.277	–	0.936	–	0.612
			(80%)		(60%)		(62%)		(59%)

Table 4 Clonal (clone) and error (Error) variances, "coefficient of variation" (CV, computed using the clonal variance) and repeatability (r) in Experiment 2. NUA, LP and Rate as in Table 3. Duration is the time elapsed between the date the first uredia were observed and the time the final number of uredia was assessed. NEC is the number of necrosis

in situ	25 °C/15 °C				15 °C/15 °C			
Traits	Clone	Error	CV	r	Clone	Error	CV	r
NUA	8.8663	15.3832	41.4	0.36	4.0206	11.8498	33.3	0.25
LP	1.4571	1.7788	16.0	0.45	0.6367	3.2493	8.6	0.16
Rate	0.0693	0.2715	28.1	0.20	0.0258	0.1088	26.2	0.19
Duration	1.4571	1.7788	16.1	0.45	0.6367	3.2493	8.15	0.16
NEC	6.4883	3.0927	120.20	0.67	8.7709	5.4928	106.6	0.61
Agar	25 °C/15 °C				15 °C/15 °C			
Traits	clone	Error	CV	r	Clone	Error	CV	r
NUA	0.0392	0.1940	29.1	0.17	0.1635	1.2152	39.6	0.12
LP	0.9040	3.9747	8.05	0.18	2.5523	3.2774	13.3	0.43
Duration	0.9040	3.9747	22.6	0.18	2.5523	3.2774	39.7	0.43
Rate	0.0095	0.0161	49.7	0.08	0.0010	0.0265	11.4	0.03

it was to slow down the rust development once the latent period was over. Temperature did not significantly influence the size of the uredia (χ^2-test non-significant at $P = 0.05$; data not shown). Finally, in some clones (81084) a higher temperature favored a hypersensitive response.

Clonal differentiation across environments

Repeatability and clonal coefficient of variation (CV) were only computed for Experiment 2 (Table 4). On average, the highest values were observed when inoculation was done in situ with a 25 °C day/15 °C night temperature regime. Under these conditions, not only were the repeatabilities higher but so were the clonal CV, with the exception of number of necrosis. Notably, the clonal CV were generally higher in situ than in the agar treatment.

Experiment 3

Analysis of variance (model 3) showed significant Isolate effect and highly significant Clone*Isolate interaction for all traits, except number of necrosis for which the Isolate effect was barely significant (Table 5). In other words, for the four characters, the ranking of the clones differed across isolates (Fig. 1). Clonal variation had a significant effect only on Rate. A high number of uredia can be reached through different paths depending on the clone and the isolate. For instance, both isolates 74v and 85v had high numbers of uredia on clone 1 (Fig. 1).

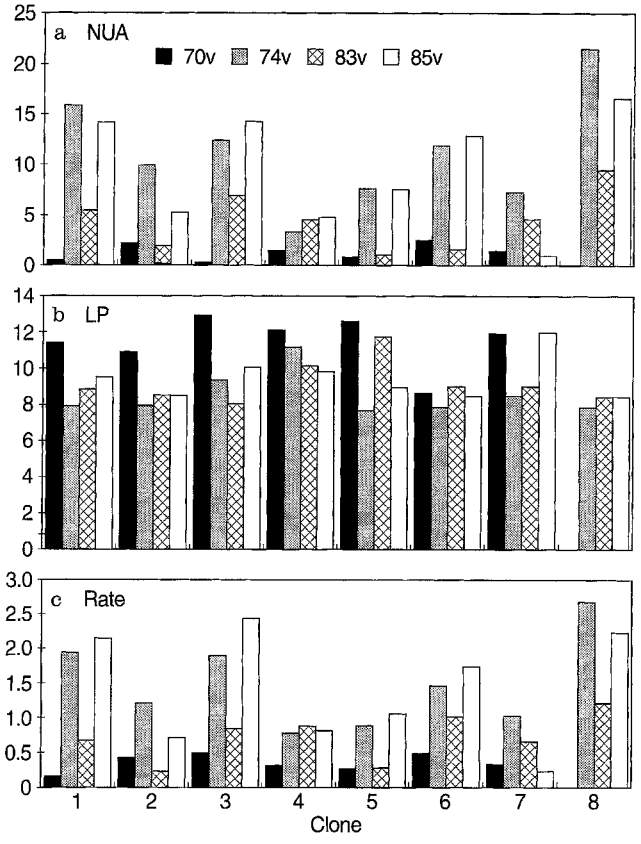

Fig. 1 a–c Clonal means per isolate (70v, 74v, 83v and 85v) for **a** number of uredia (NUA), **b** latent period (*LP*, in number of days) and **c** rate (*Rate*, in number of uredia per day)

Table 5 Analysis of variance in Experiment 3 for log-transformed values of the adjusted final number of uredia (NUA), the latent period (LP) the rate and the number of necrosis (NEC)

Source	*df*	MS	*F* value	Significance
Log(NUA + 1)				
Block	4	1.928	6.91	0.0042
Isolate	3	48.366	18.15	0.0001
Clone	7	4.352	1.63	0.1808
Clone*Isolate	21	2.665	9.55	0.0001
Error	281	0.278		
Log(LP + 1)				
Block	4	0.092	6.46	0.0001
Isolate	3	0.519	6.55	0.0029
Clone	7	0.192	2.43	0.0563
Clone*Isolate	20	0.079	5.56	0.0001
Error	254	0.014		
Log(Rate + 1)				
Block	4	0.556	8.81	0.0001
Isolate	3	3.456	12.03	0.0001
Clone	7	1.253	4.36	0.0044
Clone*Isolate	19	0.287	4.55	0.0001
Error	249	0.063		
Log(NEC + 1)				
Block	4	1.404	6.46	0.0001
Isolate	3	5.134	3.12	0.0477
Clone	7	3.250	1.98	0.1073
Clone*Isolate	3	1.644	7.56	0.0001
Error	282	0.217		

However, this was due to a short latent period combined with a high rate for the former and to a fairly long latent period combined with a very high rate for the latter. Overall, these two isolates were the most virulent on the set of clones studied here, with isolate 70v being the less virulent, even failing to establish itself on clone 8 (Table 6). Differences in the final number of uredia were generally due to differences in developmental rates rather than to differences in latent periods. The size of the uredia did not significantly differ between isolates (χ^2- test non-significant at $P = 0.05$; data not shown); for all four isolates, it was positively but weakly correlated to the final number of uredia and both traits were negatively and very significantly correlated with the latent period (data not shown). The rust failed to develop completely in only 1 of the 32 clone*isolate combinations. Repeatability was computed for each isolate taken separately (Table 7). Values vary largely among traits as well as across isolates for the same trait. The highest repeatabilities were observed for the most virulent isolates.

Discussion and conclusion

Our experiments confirmed the environmental lability of the *Melampsora-Salicaceae* relationship (Heather and Chandrashekar 1982) and provided no definite answer on the possibility of attaining durable resistance through selection on partial resistance components. On the one hand, there is a large amount of heritable variation among clones for most components and apparently few genes conferring immunity; on the other hand, all three components were extremely sensitive to environmental conditions and isolates, making the development of standard selection methods difficult. Out of the five main factors tested – level of the inoculated leaf within plant, in vitro or in situ inoculation, temperature, isolate and clones – only isolate had a consistent impact. Other factors were only significant through interaction with the other factors of the experiments.

Table 6 Isolate means comparison in Experiment 3 (Tukey test $\alpha = 0.1$). Means followed by the same letter not significantly different. Means are ranked by decreasing order. See Table 4 for definition of the traits

Trait	NUA	LP	Rate	NEC
	74a	70a	74a	85a
	85ab	85b	85b	74ab
	83b	83b	83b	83ab
	70c	74b	70c	70b

Note: 70v and 83v belongs to the same pathotype group (EV2); 74v belongs to EV5; 85v belongs to EV1

Effect of treatment and leaf position

Despite having shorter latent periods, leaves from the lower-third of the plants tended to be less infected than the ones from the upper-third and bore smaller uredia. Hence, the infection process did not seem to be postponed on mature leaves, implying that subsequent development was more constrained. Alternatively, the fewer uredia observed on leaves of the lower-third may have resulted from a lower success in infecting older leaves. The present experiments do not permit us to discriminate between these two hypotheses. If we assume that the first hypothesis is true then our data indicate the possible limiting effect of nutrient availability. The development of *Melampsora* on higher and lower leaves was affected differently by the agar treatment. At 25/15 °C, its development on leaves from the lower-third was not strongly influenced by the method of inoculation, but development rates on leaves from the upper-third was halved when leaves were inoculated on agar, whereas at 15 °/15 °C, the rates felt sharply for both lower and upper leaves. Also, at 25 °/15 °C, both final number of uredia and developmental rate on leaves of

Table 7 Clonal (clone) and error (Error) variances, "coefficient of variation" (CV, computed using the clonal variance) and repeatability (r) in Experiment 3. See Table 4 for definition of the traits

Traits	Isolate 70v Clone	Error	*CV*	*r*	Isolate 74v Clone	Error	*CV*	*r*
NUA	0.5476	2.8135	62.9	0.16	29.9448	21.2664	48.2	0.58
LP	1.1052	3.4018	9.17	0.24	1.2829	0.7058	13.1	0.64
Rate	0.0001	0.0882	2.76	0.13	0.3932	0.3656	41.3	0.52
Duration	1.1052	3.4018	23.2	0.24	1.2829	0.7058	15.3	0.64
NEC	0.0361	1.0333	41.4	0.03	0.5840	2.2860	51.0	0.20
Traits	Isolate 70v Clone	Error	*CV*	*r*	Isolate 74v Clone	Error	*CV*	*r*
NUA	6.0558	7.8497	47.0	0.43	29.4619	28.1142	56.2	0.51
LP	0.8265	2.3026	9.79	0.26	1.2919	0.6925	11.8	0.43
Rate	0.1012	0.1471	41.4	0.40	0.5826	0.7216	51.0	0.44
Duration	0.8265	2.3026	13.5	0.26	1.2919	1.6925	17.6	0.43
NEC	3.8270	2.8283	108.2	0.57	1.9039	3.8408	73.54	0.33

the lower-third in situ were of the same magnitude as the ones observed on agar. With respect to rates and final number of uredia two main groups can be delineated. A first group, with low rates and very close final numbers (approximately 0.6–0.7), comprises all agar treatments and the leaves from the lower part at 25 °/15 °C in situ; a second group comprises the remaining combinations. Thus the development of *Melampsora* apparently depends on a factor whose effect is (1) reduced in cut leaves; (2) enhanced at low temperature; (3) stronger in upper leaves. These observations can be explained by nutrient (sensu largo) allocation between the parasite and the plant. At 25 °/15 °C, the plant is growing fast, and carbohydrates are exported either to the roots or to the apical meristems. Consequently leaves from the lower-third do not provide many nutrients to the parasite. The relatively slow growth on agar may be explained by similar considerations, in this case, the growth of the pathogen being hindered once the reserves in the leaves are depleted. Suzuki (1973) has shown that there is a definite correlation between host nutrition and host susceptibility in the Poplar-*Melampsora* pathosystem, regardless of host vigor. Nitrogen and phosphorus deficiencies tended to increase the plant resistance, whereas potassium deficiencies had the opposite effect. Alternatively, the different number of spores observed between leaves from the lower- and upper-thirds may be due to variation in the composition or amount of chemical compounds unrelated to nutrition. For instance, the composition and amounts of phenolic glucosides vary between different plant parts, and it has been suggested that these are involved in a resistance to herbivores or pathogens (Julkunen-Tiitto 1989). However, experimental evidence is still lacking. Finally, differences in infections between younger and older leaves are apparently not due to any differences in light intensity that may exist among the different parts of the plant. Eight copies of clone 78183 that were inoculated at five different levels and exposed to both lateral and vertical light also exhibited a similar gradient in number of uredia: the younger the leaves the more uredia they bore (unpublished results). Alternatively, the smaller number of uredia observed on older leaves can be explained by their lower success in infecting the leaf. This may be due to the presence of fewer open stomatas in older leaves. However, the involvement of stomatas as a limiting factor can be questioned because, contrary to what is observed, one would then expect shorter latent periods under low temperatures, than under high temperatures, the latter usually causing stomatal closing (Salisbury and Ross 1985). Also, the latent periods at the low temperature were very similar.

Although there was no significant Treatment effect, the average number of uredia was much lower on agar than on live plants. As in the preceding case, this observation can best be explained by the finite amount of nutrient available in cut leaves or by differences in chemical composition induced by wounding even though it has so far been difficult to relate wound-induced compounds and resistance to pathogens (Saarikoski et al. 1992).

Effects of temperature

High temperatures shortened the latent period but slowed down subsequent development of the rust: uredia were more abundant under the lower temperature treatment but were not any larger in size than the ones that developed at the higher temperature. Similar results were observed in the *Populus/Melampsora* system. It has also been shown that rust races exhibiting low virulence (compatible with fewer cultivars) are more aggressive on susceptible *Populus* clones at high temperatures than races with high virulence (Heather and Chandrashekar 1982). We did not compare the development of the isolates at different temperatures. However, the third experiment showed that different "development strategies" do exist among isolates at 20 °/15 °C. Isolate 74v retained the same strategy over the three experiments (i.e. long latent periods compensated by high development rates) and had the highest number of uredia in Experiment 3. This isolate belongs to a fairly virulent pathotype group. Had we used another isolate to assess the effect of temperature, our conclusions might have been different.

Clonal variation and its consequences

Two parameters, "clonal" coefficient of variation and repeatability (heritability) were used to interpret clonal variation. Houle (1992) showed that traits related to fitness generally have a higher additive genetic coefficient of variation than characters under weak selection. The low heritabilities of fitness-related traits is not due to their depleted genetic variance but instead to high residual variances. If successful artificial selection is intended, traits should be selected for that have high values for both parameters. Our results permit us to illustrate the difference between the two coefficients. For instance, the heritability of the latent period varied from 0.16 to 0.61, indicating that a low and erratic response is to be expected in some environments or with some isolate, and a high and predictable selection response in others. However, in all of the situations the clonal coefficient of variation was low, with values varying between 2.9 and 16.0, indicating that the overall response while more predictable in some environments than others will be relatively low in most. The final number of uredia is a much promising trait for selection. Similar conclusions were reached by Lefèvre et al. (1994), although only heritability was considered. Overall, if we ignore possible growth chamber-field interactions, 25°/15 °C in situ seems to be the best selection environment when both criteria are considered simultaneously. Because of the climatic conditions prevailing in Sweden at the time of infection, this conclusion is likely

to be re-assessed when the relationship to field data is considered, but it may well hold true for other isolates since traits parameters were similarly ranked across isolates. The high CV values for the number of necrosis indicate the all-or-none nature of this trait and may not be compared to the other traits that are more continuously distributed. It is, on the other hand, more straightforward to compare genetic variability for the other traits. Why is there, independent of the type of isolate, much more genetic variability for the final number of uredia and rate than for the latent period? The average latent period was never less than 6 days under our experimental conditions. Even when there were large differences in mean values the coefficient of variation remained of the same magnitude and was much smaller than for other traits. In the presence of susceptible clones, there seems to be both upper and lower bounds. It is not clear why genotypes with a short latent period should be selected against, but one can easily imagine that the processes controlling the latent period cannot be accelerated beyond a certain value. The upper bound can be explained by selection against *Melampsora* genotypes with long latent periods. Because the length of the latent period is negatively correlated with the number and size of uredia (data not shown), genotypes with a long latent period will likely be less frequent in the population. In any case, one should keep in mind that, as pointed out by Houle (1992), "difference in coefficients of variation between traits may be due to an enormous diversity of factors which may be very difficult to disentangle".

Evolution of virulence

The pattern of phenotypic correlations observed between resistance components was similar to the one observed at both the phenotypic and genetic levels in the Poplar-*Melampsora* pathosystem (Lefèvre et al. 1994). If it is assumed that the number of uredia is a relevant measure of virulence and that the size of uredia is indeed related to transmissibility, then the positive correlation observed between size and number of uredia suggests that an intermediate level of virulence may be attained.

Conclusion

Contrary to what is observed with other pathogens of *S. viminalis*, such as gall midge (*Dasineura marginemtorquens* Brem.), the host-pathogen system seems here to be very much driven by the pathogen dynamics. Our results have a few practical and more general implications. Firstly, they show the inherent limits of the pathotype concept (see also Caten 1987). Two of the isolates belonged to the same pathotype group, yet they had very different developmental strategies. Considering that the all or none reactions on which pathotyping relies seem to be the exception rather than the rule in the *Salix-Melampsora* system, this result is not surprising. One of its consequences is that pathotyping, while useful for genetic studies, may not be of great help in predicting the possible evolution of the disease. Secondly, the components apparently had different temperature optima. The constructing of temperature reaction norms may thus be useful in the management of *Melampsora* under controlled conditions. Thirdly, while characteri-zing of the genetic basis of resistance components is important for a general understanding of the disease, its relevance to applied selection is not as clear. Previous studies of composite traits have often shown that the resultant character had higher heritability than its components and that these were negatively correlated making selection for either of them problematic (e.g. Kremer and Lascoux 1988). Resistance components apparently do not depart from this rule. Hence, selection in situ for low values of the product between number and size of uredia may prove the simplest and most efficient way to achieve durable resistance in the *Salix-Melampsora* host pathotype systems.

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