

Temperature Sensitivity of Reactions of *Populus* spp. to Races of *Melampsora larici-populina*

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ABSTRACT

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Hypersensitive-type local necrotic reactions in the differential poplar (*Populus deltoides*) cultivars '7-2,' '7-9' and '7-13,' were more pronounced to physiologic races A, B, D, and E of *Melampsora larici-populina* at 12 and 25 than at 20 C. The response of the 'congenial cultivars' *P. × euramericana* 'I-214,' 'I-488,' '65/27,' and *P. nigra* 'Evergreen' inoculated with the same races and incubated at similar temperatures was clearly temperature sensitive. Incubation period for the development of flecking was longer and

numbers of uredia per leaf disk were higher when cultivar-race combinations were incubated at 12 and 20 than at 25 C. The significant second- and third-order interactions of incubation temperature, cultivar, and race suggest that the temperature sensitivity of cultivar/race reactions could maintain racial diversity in pathogen populations despite cultivar selection pressure.

Additional key words: leaf rust, resistance, latent period, host-parasite interaction.

In cereal crops the sensitivity of rust resistance to temperature has been reported frequently. Stakman and Levine (23) and Johnson and Newton (6) concluded that the optimal temperature for wheat stem rust infection lies between 19 and 21 C. Katsuya and Green (7) reported decreased aggressiveness of race 56 of wheat stem rust (caused by *Puccinia graminis tritici* Erikss. and Henn.) with increasing temperatures from 15 to 25 C. The effect of temperature on host resistance to wheat stem rust, oat stem rust, and oat crown rust has been reviewed (25).

Certain cultivars of *Populus deltoides* Marsh. produce distinct qualitative and/or quantitative reactions to the races of *Melampsora larici-populina* Kleb. When the four cultivars *P. × euramericana* 'I-214,' 'I-488,' '65/27,' and *P. nigra* 'Evergreen' are inoculated separately with the four races of *M. larici-populina* (A, B, D, and E) and incubated at 20 ± 1 C, quantitatively distinct responses can be recognized (1).

Higher degrees of quantitative (as distinct from qualitative) resistance to rust have been recommended as the basis for selection and breeding poplar for resistance to leaf rust (3). Because the selected cultivars will be established over a wide geographical area, knowledge of the sensitivity of this resistance to environmental variations is desirable.

In this paper the effect of temperature of incubation on the

interaction of cultivars of poplar and races of *M. larici-populina* is reported and the implication of this for the stability of the host-parasite relationship is discussed.

MATERIALS AND METHODS

Cuttings of cultivars (actually clones) of poplar were rooted, raised, and maintained, and races of *M. larici-populina* were purified and multiplied as described by Chandrashekar and Heather (1). The inoculation, incubation, and observations of leaf disks followed the procedure of Chandrashekar and Heather (1), except where modifications are noted.

The investigation involved two experiments. In the first, 45 leaf disks (1.76 cm²) of each differential cultivar of *P. deltoides* '7-2,' '7-9,' and '7-13' were inoculated separately in a settling tower with races A, B, D, and E of *M. larici-populina*. Fifteen leaf disks of each race-cultivar combination were floated on 10 ppm gibberellic acid solution and incubated at 12, 20, and 25 C. The occurrence of hypersensitive necrotic spots and number of uredia per leaf disk were recorded daily from 7 to 15 days after inoculation. The observations were summarized on a combined disease rating scale of Sharma and Heather (19).

In the second experiment, similar leaf disks of "congenial cultivars" *P. × euramericana* 'I-214,' 'I-488,' '65/27,' and *P. nigra* 'Evergreen' were inoculated with the same races of the fungus and incubated as described previously. The experiments conducted at 20 and 25 C were terminated on day 15 after

inoculation, while that at 12 was concluded on day 21, when the number of uredia per leaf disk ceased to increase. Disease severity was assessed by using three parameters. The first of these was incubation period (days) from inoculation to fleck production (IPF), (flecks, localized chlorotic areas, were the initial symptoms of successful infection and were formed 2-3 days prior to uredia). The second, numbers of uredia per leaf disk (ULD), was assessed daily from the first appearance after inoculation until termination of the experiment. The third, the mean number of uredospores produced per square millimeter of leaf area (USM), was assessed at the termination of the experiment (20).

The results of experiment two, were tested for homoscedasticity and normality (13) by using a GLIM program (12) and were subjected to analysis of variance by using the subprogram ANOVA of the Statistical Package for the Social Sciences (14). For each temperature the ULD at each daily observation was grouped by race across the four cultivars separately, and plotted against

time after the first appearance of uredia until termination of the experiment. Since there was a difference in IPF between temperatures, the day of first appearance of uredia at each temperature was treated as day one in these graphs. This adjustment permitted the fitting of a cubic curve

$$Y_i = \beta_0 + \beta_1 x_i + \beta_2 x_i^2 + \beta_3 x_i^3 + E_i$$

to the data by using the GLIM program. Comparisons were made pairwise between curves by using the methods described by Sharma et al (21).

RESULTS

The results of experiment one, involving the interactions of differential cultivars and races of the pathogen at three temperature regimes are given in Table 1. The races can be readily distinguished by the reactions of the cultivars at 20 C. With the exception of race D (resistant-necrotic at 12 C), however, the reactions of cultivars '7-9' and '7-13' to all races were identical (hypersensitive-necrotic) at 12 and 25 C. Races A and B, which produced a compatible susceptible reaction on cultivar '7-2' at 12 C, produced resistant-necrotic and resistant reactions, respectively, on this cultivar at 25 C. There are several less pronounced effects of temperature on most other race-cultivar combinations. In general, the compatibility of race-cultivar combinations was greater at 12 than at either 20 or 25 C.

Mean response, as measured by the three parameters of "congenial cultivars" (IPF, ULD, and USM), is given in Tables 2 (by race) and 3 (by cultivar). The IPF decreases with increasing temperature of incubation; 12.10 days at 12 C, 5.66 days at 20 C, and 5.28 days at 25 C, respectively. The ULD resulting from the race-cultivar combinations is lower (16.01) at 25 than at 12 and 20 C (31.48 and 40.79, respectively). USM is highest at 20 C (800.60) compared to 172.79 at 12 C, and 120.73 at 25 C (Table 3).

The effect of temperature on the race-cultivar interaction can be compared by a ranking of races for relative aggressiveness (Table 2) and cultivars for relative resistance (Table 3) at each temperature. Race A, the most aggressive at 20 C, is less aggressive at 12 C, and the least aggressive at 25 C. Similarly race D, which is the least aggressive at 12 and 20 C, is relatively more aggressive at 25 C. Race

TABLE 1. Reactions^a of three cultivars of poplar to four races of *Melampsora larici-populina* at three temperatures

Race	Temp (C)	<i>Populus deltoides</i>		
		'7-2'	'7-9'	'7-13'
A	12	MS	N	N
A	20	MS	RN	RN
A	25	RN	N	N
B	12	S	N	N
B	20	R	RN	N
B	25	R	N	N
D	12	R	RN	RN
D	20	RN	N	N
D	25	RN	N	N
E	12	R	N	N
E	20	R	RN	RN
E	25	RN	N	N

^aReactions, based on Sharma and Heather (18), were as follows: N = hypersensitive-necrotic, R = resistant, MS = moderately susceptible, and S = susceptible.

TABLE 2. Mean response of each of the four races of *Melampsora larici-populina* on four "congenial cultivars" of poplar at three temperatures as measured by three disease parameters^a

Race	12 C ^b			20 C ^c			25 C ^c		
	IPF	ULD	USM	IPF	ULD	USM	IPF	ULD	USM
A	12.09	35.00	212.05	5.00	77.45	1,475.80	5.44	6.82	28.80
B	12.32	24.97	138.01	5.52	28.65	725.80	5.37	10.39	83.74
D	12.99	8.05	57.66	6.37	24.89	399.30	5.28	23.14	177.32
E	11.00	57.90	283.05	5.75	32.18	601.50	5.05	23.67	193.07
Mean	12.10	31.48	172.69	5.66	40.79	800.60	5.28	16.01	120.73

^aDisease parameters: IPF = incubation period to flecking (days), ULD = uredia per leaf disk, and USM = uredospores per square millimeter.

^bAssessed at day 21 postinoculation.

^cAssessed at day 14 postinoculation.

TABLE 3. Mean response of each of four "congenial cultivars" of poplar at three temperatures to four races of *Melampsora larici-populina*, as measured by three disease parameters^a

<i>Populus</i> sp. clone	12 C			20 C			25 C		
	IPF	ULD	USM	IPF	ULD	USM	IPF	ULD	USM
<i>P. × euramericana</i> 'I-488'	11.99	36.62	230.20	5.25	32.00	670.65	5.12	19.69	147.05
<i>P. × euramericana</i> 'I-214'	12.35	28.99	128.68	5.25	64.47	1,087.20	5.37	11.55	64.40
<i>P. × euramericana</i> '65/27'	11.53	41.68	221.68	5.70	36.92	786.80	5.00	24.13	213.25
<i>P. nigra</i> 'Evergreen'	12.52	18.64	110.22	6.44	29.76	675.75	5.65	8.65	58.23
Mean	12.10	31.48	172.69	5.66	40.79	800.60	5.28	16.01	120.73

^aDisease Parameters: IPF = incubation period to flecking (days), ULD = uredia per leaf disk, and USM = uredospores per square millimeter.

E, which is very aggressive at 12 and 25 C is less aggressive at 20 C. The relative aggressiveness of race B to all cultivars shows little temperature sensitivity. The relative disease severity ranking of cultivars with different temperatures of incubation is highly variable in some instances but constant (or relatively so) in others (Table 3). *P. × euramericana* 'I-214,' the most susceptible cultivar at 20 C, ranks after *P. nigra* cultivar Evergreen as the most resistant cultivar at 12 and 25 C. *P. × euramericana* '65/27,' the most susceptible cultivar at 12 and 25 C, is more resistant than the other cultivars at 20 C. In contrast, *P. nigra* cultivar Evergreen ranks as the most resistant cultivar at all three temperatures and the relative ranking of *P. × euramericana* 'I-488' shows only minor sensitivity to temperature of incubation.

The variance due to major components and all but two of the interactions are all significant beyond the $P=0.001$ level (Table 4).

If the interactive variances are added to the residual variance, the variances due to the major components are still very highly significant. For all disease parameters the variance due to temperature was greater than that due to either cultivars or races. Further, the second order interactions of cultivars × temperature and races × temperature are greater than the race × cultivar interaction. These results emphasize the importance of incubation temperature in determining the severity of disease occurring in particular race-cultivar combinations. The cumulative disease progress curves in terms of ULD, for the races across all the cultivars at the three temperatures, are plotted in Fig. 1.

The curves for race D (mean of four cultivars) incubated at 20 and 25 C do not differ significantly ($P<0.05$). Similarly those for race E incubated at these temperatures do not differ significantly. For each of the remaining comparisons between curves (Fig. 1) the difference is highly significant ($P<0.01$). Thus, the pronounced effect of temperature on disease severity in particular race-cultivar combinations is not an artifact of the time at which the latter is assessed.

DISCUSSION

Hypersensitive type necrotic reactions in the *P. deltoides* cultivars were generally more pronounced at 25 than at 12 or 20 C. While there was some temperature sensitivity in reactions of these differential cultivars to the *M. larici-populina* races, the degree of host resistance expressed in race-cultivar combinations incubated at 25 C was always equal to or higher than that expressed by combinations at the lower temperatures. Levine (8), from the results of his studies on stem rust of wheat, concluded that 20 C is about optimal for the occurrence of rust with a rapid decrease in incidence below 17 or above 22 C. Lewellen et al (9) studied minor genes for resistance in wheat to *Puccinia striiformis* West. and reported a higher resistance at the temperature profile 15/24, night/day than at a lower temperature profile 2/18 C. In contrast, Peterson (16) found that oat cultivar Red Rust Proof is moderately resistant to race 4 of *P. coronata* Cda. var. *avenae* Fras. and Led. at 14 C, but highly susceptible at 21–25 C. Zimmer and Schafer (26) reported the oat cultivar Glabrota to be resistant to race 263 of

crown rust at 15, intermediate at 21, and susceptible at 27 C. Vanderplank (25) provided a review on the effect of temperature on reactions of cereal rusts and concluded, "If temperature affects resistance, it affects it in the direction of reduced resistance at higher temperature."

The response of the "congenial cultivars" has both ecological and epidemiological implications. A ranking of races and cultivars on the basis of three disease parameters is reasonably uniform (Tables 2 and 3); however, the relative level of disease in each race-cultivar combination is dependent on incubation temperature (Table 4) and the system demonstrates a degree of quantitative interaction without reversal (17). Whether this is the consequence of the differential effect of temperature on races, cultivars, or their interaction cannot be distinguished. A similar conclusion also was reached by Martens et al (10).

The effect of temperature of incubation on the race-cultivar interaction is obviously complex. Shorter IPF was observed with increasing temperature levels (Table 2). A similar observation of shorter incubation periods with higher temperature was made by

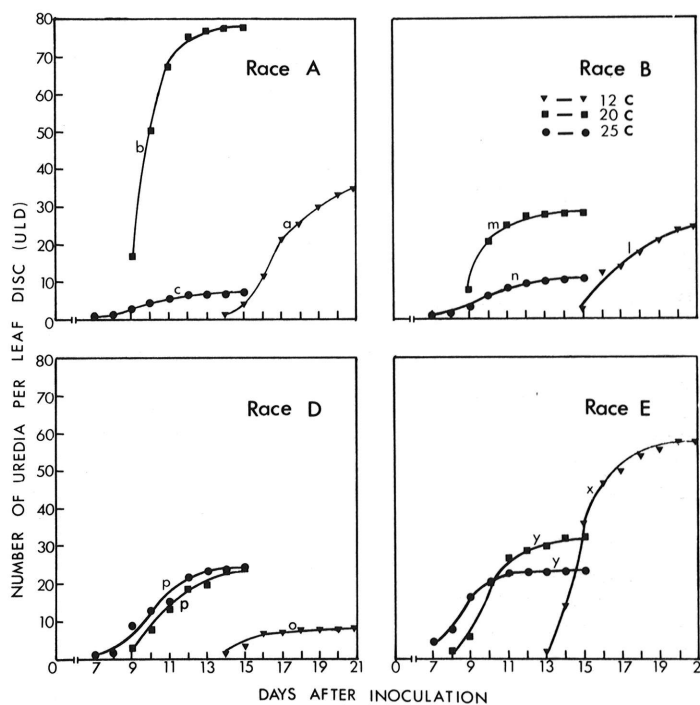


Fig. 1. Cumulative disease progress curves of four races of *Melampsora larici-populina* on *Populus* spp. incubated at 12, 20, and 25 C. Each observation is the mean number of uredia on four "congenial cultivars." Within any race, curves with the same letter do not differ significantly ($P<0.01$).

TABLE 4. Analysis of variance of parameters^a of disease severity resulting from the interaction of four cultivars of poplar and four races of *Melampsora larici-populina* at three temperatures

Source of variation	d.f.	Mean square for parameters ^b		
		IPF	ULD ($\times 10^3$)	USM ^c ($\times 10^4$)
Temperature	2	3,460.25	36.55	784.41
Race	3	26.61	20.97	104.86
Cultivar	3	23.59	9.29	46.02
Temperature × race	6	14.54	22.27	94.98
Temperature × cultivar	6	6.20	6.63	31.01
Cultivar × race	9	3.67	0.95	3.66 NS
Temperature × cultivar × race	18	7.28	2.07	6.85 **
Residual	665	0.23	0.20	3.15
Total	712	10.54	0.72	22.63

^aDisease parameters: IPF = incubation period to flecking, ULD = uredia per leaf disk, and USM = uredospores per square millimeter.

^bAll the variance ratios are very highly significant at $P<0.001$ except ** at $P=0.008$.

^cResidual and total d.f. for USM were 96 and 143, respectively. NS = nonsignificant.

Zimmer and Schafer (26) while working on crown rust (caused by *P. coronata*) of oats. Teng and Close (24) reported a shorter latent period for leaf rust of barley (caused by *P. hordei* Otth.) with increasing temperature up to 20 C. Within each temperature regime, IPF is inversely correlated with ULD (Table 3). This agrees with similar observations on leaf rust of barley (15). However, when IPF and ULD are averaged across cultivars (Table 3) and compared between temperatures, leaf disks incubated at 25 C have the shortest mean IPF (5.28 days) and lowest mean ULD (15.01 uredia per leaf disk). This suggests that the temperature of incubation differentially affects the process leading to these two forms of disease expression.

The type and degree of reaction of the leaf disks cut from cultivars and used in these experiments agree with those for the same cultivars grown in the field in Australia (18). Assuming that temperature sensitivity is a common feature of the relationship of races of *M. larici-populina* and cultivars of its poplar hosts, natural populations of the pathogen would be extremely variable in racial composition and hence well buffered against selection pressures of the host and environment. It has been suggested that the gene-for-gene relationship of host-pathogen systems permits the stable coexistence of hosts and their obligate parasites (11). Temperature sensitivity in such systems would reinforce stability and account in part for the absence of epidemic disease in these systems.

Studies on the effect of diurnal temperature fluctuations on race-cultivar interactions would be essential for the prediction of likely effects of temperature sensitivity on field behavior of race-cultivar combinations. However, the present results support the field observation of rapid increase in poplar leaf rust which occurs in the spring in certain environments in Australia. In contrast, if leaf rust is recorded initially only in midsummer the rate of epidemic development may be relatively slow (Heather, unpublished).

It has been suggested that the extensive planting of a single cultivar (identical genotype) may lead to epidemic disease as a consequence of host selection pressure on the racial composition of populations of *M. larici-populina*. The occurrence of such instances in agricultural crops has been recorded frequently (2,5). However, for the *Populus-Melampsora* system, homeostatic pressure in the pathogen was suggested as a possible counter to positive selection (1,4). Temperature sensitivity of race-cultivar interactions also could act as a counter to host selection pressure and in the broad sense be regarded as a form of homeostasis.

The sensitivity of race-cultivar interactions to temperature limits the usefulness of cultivar selection for rust resistance in specific environments. It emphasizes the necessity for field trials in various environments to supplement laboratory screening of cultivars for leaf rust resistance.

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