Temperature-Light Effects on Resistance of Poplar Cultivars to Races of *Melamspora larici-populina*

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ABSTRACT

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The effects of contrasting temperatures (15 and 24 C) and light intensities (50 and 200 $\mu E \cdot m^{-2} \cdot s^{-1}$) during incubation on the relative resistance expressed by four cultivars of *Populus* spp. to three races of *Melampsora larici-populina* were assessed in a factorial experiment. Mean resistance of cultivars was highest and mean aggressiveness of races lowest under the high-temperature, high-light regime. Temperature, light, and their interaction also contributed the highest relative variances, although all main effects and most second- and third-order interactions were highly significant. These results suggest that environmental factors may be more important than genetic factors in regulating disease expression in this pathosystem. The fully and differentially interactive relationship of poplar cultivar, rust race, and environment could contribute to disease stability in this system.

Additional key words: compatible cultivars of *Populus* spp., leaf rust, aggressiveness of races, differential interaction.

Partial resistance in poplar cultivars, *Populus* × *euramericana* (Dode) Guinier 'I-488,' 'I-214,' '65/27,' and *P. nigra* L. 'Evergreen' to races of *Melampsora larici-populina* Kleb. is differentially sensitive to temperature and to light intensity. Mean susceptibility of cultivars of poplars and mean aggressiveness of races of *M. larici-populina* decreased with increasing temperature when inoculated leaf disks were incubated at 12, 20, and 25 C in a light intensity of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ (3). In a comparable experiment, where inoculated leaf disks were incubated at 20 C, but at light intensities of 100, 250, and 1,000 $\mu E \cdot m^{-2} \cdot sec^{-1}$, mean susceptibility of cultivars and mean aggressiveness of races also decreased with increasing light intensity (5). The differential nature of cultivar × race interactions with temperature (3) and with light intensity (5) was proposed as a partial explanation for the stability of the resistance in certain cultivars of poplar to this leaf rust in natural stands (3,5). In the field, physical environmental factors vary concurrently, hence such results (3,5) have limited value for epidemiological extrapolation. Although the desirability of studies employing concurrent variations of environmental factors has been emphasized (6), specific reports of such experiments are not available.

The present paper reports the results of an experiment in which postinoculation temperature (two levels) and light intensity (two levels) were varied concurrently. These results, together with those reported previously (2–5), are employed to discuss the host-pathogen relationship.

MATERIALS AND METHODS

Clonal cuttings of poplar cultivars, *P.* × *euramericana* 'I-488,' 'I-214,' '65/27,' and *P. nigra* 'Evergreen' were rooted and maintained in a sporeproof (air lock, and a filtered air supply through a fan and evaporative cooling system [10]) growth cabinet (temperature 20 ± 1 C, cool-white fluorescent lights, intensity 300–400 $\mu E \cdot m^{-2} \cdot s^{-1}$, and a 16-hr photoperiod). The plants received Aquasol mineral fertilizer solution (Hortico, Sydney, Australia) at 2-wk intervals. Leaf emergence on shoots of similar maturity was recorded to enable selection of leaves of comparable age (30–35 days) to ensure uniform and high susceptibility to leaf rust of subsamples of leaves within a cultivar (18).

Three races of *M. larici-populina*, designated A, B, and D, which are known to be compatible (2) with the selected cultivars, were isolated and multiplied by inoculating the abaxial side of individual leaves of the susceptible cultivar *P.* × *euramericana* 'I-488' with single urediospores and repeating the inoculations with harvested spores to obtain 20 mg of urediospores of each race. Hopefully, this uniform procedure reduced the potential for host-induced changes in pathogen genotype during inoculum production.

The effect of temperature and light intensity on reactions of the poplar leaves to the rust, was investigated in a 2 (temperatures) × 2 (light intensities) × 3 (races) × 4 (cultivars) factorial design, with 15 replicates (leaf disks) for each treatment combination. Sixty leaf disks (1.76 cm^2) of each cultivar, cut from surface-sterilized leaf samples, were inoculated (abaxial face) with 5 mg of urediospores of each race of the fungus, in a spore settling tower (2). The inoculations were repeated with the same and with different races to obtain 15 replicates of each cultivar × race combination for each temperature × light interaction. Providing certain precautions were taken, mean deposition of urediospores per unit area between successive inoculations did not differ significantly ($P < 0.05$) from that within an inoculation, or between depositions with different races (1, and Singh and Heather, *unpublished*). Fifteen leaf disks of each cultivar × race combination were floated, inoculated side uppermost, on 10 ppm (μg/ml) gibberellic acid solution in petri dishes and incubated at each of the following combinations of temperature and light intensity: 15 ± 1 C at 50 $\mu E \cdot m^{-2} \cdot sec^{-1}$ (low-low), 15 ± 1 C at 200 $\mu E \cdot m^{-2} \cdot s^{-1}$ (low-high), 24 ± 1 C at 50 $\mu E \cdot m^{-2} \cdot s^{-1}$ (high-low), and 24 ± 1 C at 200 $\mu E \cdot m^{-2} \cdot s^{-1}$ (high-high).

The experiment was terminated on day 14 after inoculation when the uredial initiation on leaf disks ceased to increase. The following disease severity parameters were assessed: 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); uredia per leaf disk (ULD) were assessed daily from first appearance until termination of the experiment; mean number of urediospores produced per square millimeter of leaf area (USM) was determined at the termination of the experiment by using a haemocytometer and following the methods described by Sharma and Heather (17).

The results were tested for homoscedasticity and normality (11) by using a GLIM computer program (12) and were subjected to analysis of variance by using the subprogram ANOVA of the SPSS (13). The application of these methods to the data has been illustrated elsewhere (1).

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RESULTS

Disease severity, rated on the three parameters, is summarized by averaging across races (Table 1) and across cultivars (Table 2).

Over all hosts and the environments tested, race A had the longest IPF (6.96 days) and highest ULD (48.16) in the low-low, and lowest ULD (3.92) and USM (97.95) in the high-high temperature/light combination (Table 1). Race B had the shortest IPF (4.15) and highest USM (1,362.42) under the high-low regime. Similarly, when susceptibility of the cultivars was assessed for IPF, ULD, and USM, over all the environments and races of the pathogen, *P.* × *euramericana* 'I-488' had the highest ULD (39.17) in the low-low, and the lowest (12.97) under the high-high, and the highest USM (1,431.22) under the high-low regime (Table 2). The longest IPF (6.91 days) was recorded for both *P.* × *euramericana* '65/27' and *P. nigra* 'Evergreen' under the low-low regime and the shortest (4.49 days) for the former cultivar under the high-low regime. *P. nigra* 'Evergreen' produced the lowest USM (114.11) under the high-high regime. Thus, the disease severity rating of a cultivar × race combination varied depending on the parameter used.

Based on ULD or USM, A is the most aggressive race under the low-low, but the least aggressive in all other temperature × light intensity regimes. Race D, on the basis of ULD, was the most aggressive race under low-high and high-high regimes, but the least aggressive under the low-low, and intermediate under the high-low regimes (Table 1). Thus, for these two races, there is a clear interaction of temperature and light intensity at 15, but not at 24 C. In contrast, the relative aggressiveness of race B, incubated at 15 C, shows no evidence of temperature × light interaction, while at 24 C there is a limited degree of that interaction.

P. × *euramericana* 'I-488' is relatively the most susceptible (ULD) cultivar under the low-low and high-low, less susceptible under the low-high, and the most resistant under the high-high regimes (Table 2). Thus, this cultivar demonstrates an interactive effect of temperature and light intensity (complete reversal of relative susceptibility) at 24 C. Except under the high-high regime, *P.* × *euramericana* '65/27' is the most resistant cultivar at all combinations of temperature and light intensity. Irrespective of the temperature/light regime, cultivar *P.* × *euramericana* 'I-214' is always less resistant than *P. nigra* 'Evergreen,' and there is no evidence of significant temperature × light interaction in the rating on ULD for either of these cultivars.

For most parameters, the variances due to the major components (temperature, light intensity, races, and cultivars), their second-, most of the third-, and one (ULD) of the fourth-order interactions between them were significant at $P = 0.05$ or beyond, when tested against residual variance (Table 3).

For IPF, the degree of variance contributed by temperature to the total variance was far greater than that due to any other of the major variables or their interactions. Similarly, of the major components, light intensity contributed the highest variance for parameters ULD and USM. For IPF and USM, the interaction of temperature and light intensity accounted for more variance than those of the other major variables.

DISCUSSION

The disease severity (parameters ULD and USM) was lowest when the cultivar/race combinations were incubated at high temperature (24 C) and high light intensity (200 $\mu E \cdot m^{-2} \cdot s^{-1}$) (Tables 1 and 2). These results are in general agreement with the previous reports of the independent effects of temperature (3) and light intensity (5). Spiers (19) reported a similar decrease in susceptibility of certain poplar cultivars to this rust when the host/pathogen complex was incubated at 25 C rather than at 20 C or lower. In contrast, studies on cereal rusts generally demonstrate increased susceptibility of cultivars with increasing temperature (22).

The relative mean disease rating of the cultivar × race reaction for various combinations of temperature and light intensity depends to a degree on the parameter employed (Tables 1 and 2). This suggests that the temperature and light combinations differentially affect the processes evaluated by IPF, ULD, and USM. Generally, within each combination of temperature and light intensity, IPF is inversely correlated with ULD (Table 2). This agrees with the earlier observations for leaf rust of poplar (3,5) and

TABLE 1. Mean disease severity, as measured by three parameters[a], induced by three races of *Melampsora larici-populina*, on four compatible cultivars of poplar at two incubation temperatures and two light regimes

	15 ± 1 C						24 ± 1 C					
	50 ($\mu E \cdot m^{-2} \cdot s^{-1}$)			200 ($\mu E \cdot m^{-2} \cdot s^{-1}$)			50 ($\mu E \cdot m^{-2} \cdot s^{-1}$)			200 ($\mu E \cdot m^{-2} \cdot s^{-1}$)		
Race	IPF	ULD	USM	IPF	ULD	USM	IPF	ULD	USM	IPF	ULD	USM
A	6.96	48.16	723.22	6.88	16.58	265.05	5.12	22.08	534.76	6.03	3.92	97.95
B	6.54	37.87	704.07	6.54	29.74	700.53	4.15	37.77	1362.42	5.43	19.07	494.94
D	6.88	8.43	276.71	6.19	38.66	1028.95	4.43	32.04	1294.11	5.53	23.59	319.96
Mean	6.79	31.49	568.00	6.54	28.33	664.84	4.57	30.48	1063.76	5.66	15.53	304.26

[a] IPF = incubation period to flecking (days); ULD = uredia per leaf disk (1.76 cm^2) at 14 days; and USM = urediospores per square millimeter of leaf area at 14 days.

TABLE 2. Mean disease severity, as measured by three disease parameters,[a] induced in four 'compatible cultivars' of poplar by three races of *Melampsora larici-populina*, incubated at two incubation temperatures and two light intensities

	15 ± 1 C						24 ± 1 C					
	50 ($\mu E \cdot m^{-2} \cdot s^{-1}$)			200 ($\mu E \cdot m^{-2} \cdot s^{-1}$)			50 ($\mu E \cdot m^{-2} \cdot s^{-1}$)			200 ($\mu E \cdot m^{-2} \cdot s^{-1}$)		
Cultivar	IPF	ULD	USM	IPF	ULD	USM	IPF	ULD	USM	IPF	ULD	USM
P. euramericana												
'I-488'	6.58	39.17	768.52	6.41	33.79	771.34	4.64	38.64	1431.22	6.02	12.97	213.73
'I-214'	6.76	36.69	654.03	6.37	34.70	755.96	4.56	32.80	1217.01	5.48	18.55	247.06
'65/27'	6.91	17.27	301.00	6.67	22.11	697.10	4.49	23.25	859.51	5.40	14.67	505.22
P. nigra 'Evergreen'	6.91	32.82	468.43	6.69	22.73	434.94	4.58	27.23	747.31	5.75	15.91	114.11
Mean	6.79	31.49	568.00	6.54	28.33	664.84	4.57	30.48	1063.76	5.66	15.53	304.27

[a] IPF = incubation period to flecking (days); ULD = uredia per leaf disk (1.76 cm^2) at 14 days, and USM = urediospores produced per square millimeter of leaf area at 14 days.

of barley (15). However, when IPF and ULD are averaged across cultivars and races, and compared between different couplings of temperature and light levels, mean IPF is longest (6.79 days) and mean ULD highest (31.49) in leaf disks incubated on the low-low regime. Again, this indicates that the interaction of temperature and light intensity differentially affects the rate of fleck formation and the number of sporulating pustules that develop. The relative variances of temperature and light intensity for IPF, ULD, and USM in the ANOVA (Table 3) supports this suggestion. This lack of generalized negative correlation between IPF and ULD for poplar leaf rust contrasts with the cereal rusts in which reduction in latent period with increasing temperature from 10 to 25 C (7,20) is inversely correlated with numbers of uredinia formed per unit area (22).

Disease severity, and consequently apparent relative resistance of cultivars and relative aggressiveness of races (terminology sensu Vanderplank [21], is dependent on the combination of temperature and light intensity. For instance race A, which on most parameters, was the most aggressive under the low-low regime, was the least aggressive in all the remaining combinations of temperature and light intensity. A comparable reversal in relative aggressiveness (basis ULD and USM) is evident for race D under the low-high and low-low regimes. The ranking of cultivars for relative resistance indicates reversal in their response also. *P.* × *euramericana* 'I-488,' the most susceptible cultivar (based on all the parameters) at the low-low is the most resistant (on most parameters) at the high-high combination. Thus, for these combinations of temperature and light intensity, these cultivar/race combinations demonstrate quantitative interaction with reversal (16).

Incubation period to flecking (IPF) shorter than 5 days has not been recorded previously for any cultivar/race combination in poplar rust (2–5). However, in the high-low combination a mean IPF of 4.57 days was recorded while the IPF observed in the high-high coupling was 5.66 days; ie, the higher light intensity causes an increase of ~1 day in IPF. In contrast, at an incubation temperature of 15 C light intensity has no significant effect on mean IPF. However, as demonstrated in the ANOVA, temperature and light interact in determining IPF. As indicated previously (4,5), this has considerable epidemiological significance, since in this host/pathogen system IPF is directly correlated with latent period (2), which is a major factor determining the number of monocycles per unit time in an epidemic.

TABLE 3. Mean squares[a] of three parameters[b] of disease intensity, resulting from the interaction of four poplar cultivars and three races of *M. larici-populina* incubated at two temperatures and two light intensities

Source of variation	d.f.	IPF	ULD ($\times 10^2$)	USM# ($\times 10^4$)
Temperature	1	334.88	68.09	21.22 NS
Light	1	28.55	118.60	392.40
Race	2	14.89	39.45	212.16
Cultivar	3	1.53	44.14	90.48
Temp × Light	1	60.21	58.53	574.32
Temp × Race	2	6.56	64.53	57.57
Temp × Cultivar	3	2.90	7.23**	6.60 NS
Light × Race	2	2.51	152.28	30.00**
Light × Cultivar	3	0.51*	10.82	37.05**
Race × Cultivar	6	2.32*	10.11	17.38*
Temp × Light × Race	2	4.08	77.08	187.95
Temp × Light × Cultivar	3	0.40 NS	7.04**	18.08*
Temp × Race × Cultivar	6	0.74	7.23	6.07 NS
Light × Race × Cultivar	6	1.63	10.12	2.57 NS
Temp × Light × Race × Cultivar	6	0.35 NS	8.90	9.30 NS
Residual	613	0.19	1.43	6.85[c]
Total	660	1.08	3.63	24.21[c]

[a] All the unmarked variance ratios are significant at $P<0.001$. **Significant at $P<0.01$, * significant at $P<0.05$, and NS = nonsignificant.
[b] IPF = incubation period to flecking (days); ULD = uredia per leaf disk (1.76 cm^2) at 14 days; and USM = urediospores per square millimeter leaf area at 14 days.
[c] Residual and total d.f. were 90 and 137, respectively.

Aigeiros poplars are hypostomatous and, while direct penetration by *M. larici-populina* occurs, stomatal penetration is more common (14). The temperature and light intensity regimes chosen in the present experiment are within the range commonly occurring on, and adjacent to, the abaxial face of leaves in plantations of poplar in Canberra in midsummer. While the ANOVA is a fixed model and hence technically the results cannot be extrapolated beyond the population in the experiment, similar results have been reported previously in comparable investigations (3–5). The diurnal and seasonal variations in temperature and light intensity that occur in the field throughout the growing season, could be expected to considerably affect the disease severity in certain cultivars. Such effects may be more important than cultivar × race interactions in determining epidemic development.

Investigations reported previously (2–5) and those herein demonstrate the enormous variability of the Populus-Melampsora system. The severity of leaf rust is a function of a complex of genetic (host cultivar, pathogen race) (2), ontogenetic (age of the leaves and of the shoots on which they are borne) (18), and environmental factors such as preinoculation temperature (4), temperature (3) and light intensity (5) during incubation, and the interaction between these factors. Race specificity that imposes a restraint on parasitic fitness (21), low reproductive rates, and limited powers of dispersal of the pathogen have been suggested (16) as bases for 'durability' (9) in race-specific host/pathogen systems. The fully differential host-pathogen-environment interactions of the Populus-Melampsora relationship could be significant also in the 'durability' of this system.

LITERATURE CITED

1. Chandrashekar, M. 1981. The effect of temperature, light intensity, and their interaction in the partial resistance of certain poplar cultivars to races of *Melampsora larici-populina* Kleb. Ph.D. thesis, The Australian National University, Canberra, Australia. 186 pp.
2. Chandrashekar, M., and Heather, W. A. 1980. Reaction of poplar clones to physiologic races of *Melampsora larici-populina* Kleb. Euphytica 29:401-407.
3. Chandrashekar, M., and Heather, W. A. 1981. Temperature sensitivity of reactions of *Populus* spp. to races of *Melampsora larici-populina* Kleb. Phytopathology 71:421-427.
4. Chandrashekar, M., and Heather, W. A. 1981. The effect of pre- and post-inoculation temperature on resistance in certain clones of poplar to races of *Melampsora larici-populina* Kleb. Euphytica 30:113-120.
5. Chandrashekar, M., and Heather, W. A. 1981. Influence of light intensity on the reactions of certain poplar cultivars to races of *Melampsora larici-populina* Kleb. Trans. Br. Mycol. Soc. 74:(In press).
6. Colhoun, J. 1973. Effect of environmental factors on plant disease. Annu. Rev. Phytopathol. 11:343-364.
7. Eversmeyer, M. G., Kramer, C. L., and Browder, L. E. 1980. Effect of temperature and host:parasite combination on the latent period of *Puccinia recondita* in seedling wheat plants. Phytopathology 70:938-941.
8. Heather, W. A., Sharma, J. K., and Miller, A. G. 1980. Physiologic specialization in *Melampsora larici-populina* Kleb. on clones of poplar demonstrating partial resistance to leaf rust. Aust. For. Res. 10:125-131.
9. Johnson, R. 1979. The concept of durable resistance. Phytopathology 69:198-199.
10. Mastalerz, J. W. 1977. The Greenhouse Environment. John Wiley and Sons, New York. 629 pp.
11. Neter, J., and Wasserman, W. 1975. Applied Linear Statistical Models. R. D. Irwin, Inc., London. 834 pp.
12. Nelder, J. A. 1975. General Linear Interactive Modelling. Pub. GLIM manual release, 2. Royal Statistical Society, London.
13. Nie, H. N., Hull, C. H., Jenkins, J. G., Steinbrenner, K., and Bent, D. H. 1975. Statistical Package for Social Sciences. 2nd ed. McGraw-Hill, New York. 675 pp.
14. Omar, M. B. 1978. Aspects of pre- and post-penetration phenomena of *Melampsora larici-populina*. Ph.D. thesis, The Australian National University, Canberra, Australia. 211 pp.
15. Parlevliet, J. E. 1975. Partial resistance of barley to leaf rust. *Puccinia hordei*. I. Effect of cultivar and development stage on latent period. Euphytica 24:21-27.
16. Scott, P. R., Johnson, R., Wolfe, M. S., Lowe, H. J. B., and Bennet, F. G. A. 1979. Host specificity in cereal parasites in relation to their control. Plant Breeding Institute (Trumpington, Cambridge, England)

Annu. Rep. 27-62.
17. Sharma, J. K., and Heather, W. A. 1979. A method for determining density of suspensions of urediniospores of *Melampsora larici-populina* causing leaf rust of poplar. Trans. Br. Mycol. Soc. 72:479-482.
18. Sharma, J. K., Heather, W. A., and Winer, P. 1980. Effect of leaf maturity and shoot age of clones of *Populus* spp. on susceptibility to *Melampsora larici-populina*. Phytopathology 70:548-554.
19. Spiers, A. G. 1978. Effect of light, temperature, and relative humidity on germination of urediniospores of, and infection of poplars by, *Melampsora larici-populina* and *M. medusae*. N. Z. J. Sci. 21:393-400.
20. Teng, P. G., and Close, R. C. 1978. Effect of temperature and uredinium density on urediniospore production, latent period, and infectious period of *Puccinia hordei* Otth. N. Z. J. Agric. Res. 21:287-296.
21. Vanderplank, J. E. 1968. Disease Resistance in Plants. Academic Press, London-New York. 207 pp.
22. Vanderplank, J. E. 1978. Genetic and Molecular Basis of Plant Pathogenesis. Springer-Verlag, Berlin. 167 pp.