

## Effect of Leaf Maturity and Shoot Age of Clones of *Populus* species on Susceptibility to *Melampsora larici-populina*

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### ABSTRACT

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Leaves of varying maturity were detached from 4- to 33-day-old shoots of clones of *Populus nigra* 'Semi-evergreen' (field susceptible) and *P. × euramericana* '65/27' (field resistant) grown in a rust-free glasshouse. The detached leaves were inoculated with uniform loads of uredospores of *Melampsora larici-populina*, and incubated in a controlled environment. Reaction type of the leaves and latent period (LP) for flecks and for uredia production were recorded. Within a shoot, LP and reaction type were

related to leaf maturity, but for leaves of the same maturity, selected from shoots of varying age, the reaction type was determined by age of shoot. In comparisons between the clones or between leaves of different maturities or zones of shoot within clones, higher susceptibility was associated with shorter latent period (LP). The ecological and epidemiological significance of the results are discussed.

*Additional key words:* computer language GLIM, position effect, shoot organization, horizontal resistance, co-existence of host genotypes and virulent races, host defoliation, aesthetic value.

Following the initial appearance of *Melampsora larici-populina* Kleb. near Sydney in 1973 (28), it spread over the eastern part of Australia wherever susceptible poplars are grown. The severity of leaf infection varies with locality, seasonal conditions, clonal types, and local environmental variations. Recognition of racial specialization in the rust has been an important facet of epidemiological investigations (7,22).

This paper reports an experiment designed to test the significance of leaf maturity (age in days) and shoot age in the susceptibility to *M. larici-populina* of leaves of two poplar clones.

### MATERIALS AND METHODS

**Host maintenance and treatment.** Two poplar clones, *Populus nigra* L. 'Semi-evergreen' (*P. nigra* in text) and *P. × euramericana* (Dode) Guinier '65/27' (*P. deltoides* cv. '60/164' × *P. nigra* 'Semi-evergreen') (*P. euramericana* in text), were chosen because the former was very susceptible and the latter resistant to leaf rust in the field. The plants were maintained in a rust-free glasshouse at 20–24 C with a 16-hr photoperiod, were 2.5 yr old, and had been cut back at 5- to 6-mo intervals to produce new shoots. For this experiment, five stems of each clone were cut back on alternate days from 9 to 29 November 1976. The stumps, approximately 25 cm in length, were examined daily and the appearance of leaves on shoots developing from the cut stumps was recorded individually. Shoots of the same age and bearing the same number of leaves were removed daily from replicate plants of both clones. Leaves were detached from shoots and the maturity of each leaf, its position on the shoot, and the age and origin of the shoot were recorded. Detached leaves were inoculated with uredospores of *M. larici-populina*. The youngest and oldest shoots of *P. nigra* and *P. euramericana* were 5 and 33, 4, and 32 days of age, respectively.

**Inoculation.** Leaves were inoculated in a spore-settling tower (Fig. 1) as described previously (24). Uredospores (7.5 mg) of *M. larici-populina*, harvested with a vacuum spore collector from surface-sterilized, artificially inoculated leaves of *P. nigra* 'italica,'

were employed in each inoculation. The germination of the uredospores exceeded 90% at each time of inoculation. A relatively low density of spores was used to avoid possible effects of high densities on the latent period (LP) for uredia production (13,29). The leaves to be inoculated were placed, abaxial side up, on the base of the inoculation chamber and the sliding lid was closed. The spores were released from their container into the upper section of the settling tower by the passage of a stream of air at 70 kPa pressure for 30 sec. During spore release and for 15 sec thereafter, the lid remained closed; this allowed settling of spore clumps. The lid was opened slowly and the leaves were exposed to the settling uredospores for 10 min. Following removal from the tower, the leaves were arranged, abaxial side up and according to their original position on the shoots, on filter paper soaked in 5 ppm (w/v) gibberellic acid, in 15-cm-diameter glass petri dishes. Leaves were then incubated in a chamber at 16 ± 1 C with 16-hr of fluorescent light (10<sup>3</sup> lx) per day. After each inoculation the settling tower, inoculation chamber, and inoculum container were washed with 95% alcohol and air-dried.

**Data recorded.** Latent period (LP) was recorded as the time (days) from inoculation to first appearance of both flecks (LPD) and uredinia (LPU) (9). Numbers of uredinia per leaf were counted on the 20th day after inoculations. The outlines of leaves were traced on graph paper and leaf areas were recorded with an automatic area meter (Hayashi Denko Co. Ltd., Tokyo, Japan). Numbers of uredia per square millimeter were calculated from these data. The reaction type of individual leaves was then graded on an arbitrary scale: 0 = uredia nil (apparently immune); 1 = uredia 0.001–0.009/mm<sup>2</sup> (highly resistant includes flecks); 2 = uredia 0.010–0.094/mm<sup>2</sup> (resistant); 3 = uredia 0.095–0.154/mm<sup>2</sup> (susceptible); 4 = uredia 0.155–0.25/mm<sup>2</sup> (highly susceptible). For brevity in the text, disease expression is referred to as reaction type on the scale, however corresponding numbers of uredia per unit leaf area were used for all statistical comparisons.

**Statistical analysis.** The statistical computer language GLIM (Generalised Linear Interactive Modelling) was used to fit curves of the simple growth function,  $g(x) = cx^\alpha e^{-\beta x}$  where  $x$  is disease level in uredia per square millimeter,  $\alpha$  is a measure of the initial rate of increase in susceptibility with increasing leaf age and  $\beta$  is a measure

of the final rate of decrease in susceptibility in older leaves. The curve for 25-day-old shoots of *P. euramericana* is given as an example of the method (Fig. 2).

Although  $g(x)$  is a nonlinear function,  $\ln g(x) = \ln c + \alpha \ln x - \beta x$  is linear in  $\alpha$  and  $\beta$  and the linear model may be used to fit,  $Y = \ln g(x) + \epsilon$  and obtain least-squares estimates of  $\ln c$ ,  $\alpha$  and  $\beta$ . Estimated values of these functions, together with their standard deviations, are given in Table 1.

Paired comparisons of the fitted curves for each clone were made by using standard ANOVA methods (19). The residual sums of squares of the fitted curves for the selected pair of shoots of different age were added to obtain a total residual sum of squares ( $r_1$ ) with  $f_1$  degrees of freedom. Then a single curve of the form  $\ln Y = \ln c + \alpha \ln x - \beta x + \epsilon$  was fitted to the combined data for the particular pair of shoots to yield a residual sum of squares  $r_2$  with  $f_2$  degrees of freedom. The difference  $r_0 = r_2 - r_1$  (with  $f_0 = f_2 - f_1$  degrees of freedom) is the sum of squares of residuals due to deviation from the hypothesis that the separate curves are the same (the null hypothesis). The comparison of 10-day-old and 14-day-old shoots of *P. nigra* is given to illustrate the method (Table 2). Similar paired comparisons were made between the fitted curves for *P. nigra* and *P. euramericana* clones for five different ages of shoots.

The comparisons described tested the overall similarity of the fitted curves both within and between the clones. The position of the maximum level of susceptibility on each curve is of special interest. The maximum of  $\ln Y = \ln c + \alpha \ln x - \beta x$  occurs when

$$\frac{\partial(\ln Y)}{\partial x} = \alpha/x - \beta = 0 \text{ ie, when } x = \alpha/\beta$$

Thus, the maximum level of susceptibility, in terms of uredia per square millimeter, occurs at leaf maturity  $x_M = \alpha/\beta$ . An estimate of  $x_M$  may be obtained by substituting for  $\alpha$  and  $\beta$  their estimates (Table 1) but the variance of such an estimate (the ratio of two

random variables) is more difficult to obtain. However, the application of GLIM to fitting the curves furnishes estimates of the variances of  $\alpha$  and  $\beta$ . A first order approximation of the variance of  $x_M$  can be obtained by calculating

$$\text{var}(x_M) = \text{var} \frac{\alpha}{\beta} = \frac{\alpha^2}{\beta^2} \left\{ \frac{\text{var}(\alpha)}{\alpha^2} + \frac{\text{var}(\beta)}{\beta^2} - \frac{2 \text{covar}(\alpha, \beta)}{\alpha\beta} \right\}$$

(10);  $x_M = \alpha/\beta$  values and their approximate standard deviations are given in Table 3.

Inspection of the data suggested that a negative correlation existed between uredia per square millimeter ( $Y$ ) and latent period (LPF = time to develop flecks and LPU = time to develop uredia) within each of the clones. Coefficients of correlation  $R_1$  (between  $Y$  and LPF) and  $R_2$  (between  $Y$  and LPU) were calculated for shoots of the five age groups within each of the clones and these are summarized in Table 4.

## RESULTS

**Shoot production by stumps.** Leaves developed more rapidly from buds on shoots cut in late November than on those cut earlier in the month. The number of buds, and hence the number of shoots developing, varied between replicate stumps of approximately the same height. Shorter shoots with fewer, smaller leaves developed

TABLE 1. Estimated values of the parameters of the linear function  $\ln g(x) = \ln c + \alpha \ln x - \beta x$  used in the statistical computer language GLIM<sup>a</sup> to fit curves relating numbers of uredia per square millimeter to leaf maturity and shoot age in *Populus nigra* and *P. × euramericana*

Age of shoot	ln C	s.d. <sup>b</sup>	$\alpha$	s.d.	$\beta$	s.d.
<i>P. nigra</i>						
10 days	- 5.917	0.136	4.527	0.250	0.6653	0.050
14 days	- 6.284	0.210	4.834	0.292	0.6953	0.050
10 and 14 days <sup>c</sup>	- 6.107	0.130	4.667	0.193	0.6762	0.035
20 days	-13.89	0.701	8.339	0.551	0.7154	0.053
26 days	-14.16	1.17	7.480	0.776	0.5192	0.059
33 days	-17.14	1.46	7.228	0.801	0.3107	0.045
<i>P. euramericana</i>						
10 days	- 6.698	0.753	5.024	1.34	0.9268	0.277
14 days	-27.27	2.56	22.30	2.28	2.604	0.260
20 days	-12.14	1.36	7.812	1.01	0.7832	0.097
25 days	-25.31	1.32	13.81	0.84	0.9625	0.060
32 days	-24.14	1.89	11.97	1.07	0.6783	0.063

<sup>a</sup> For details see text.

<sup>b</sup> Standard deviation of estimates.

<sup>c</sup> See text.

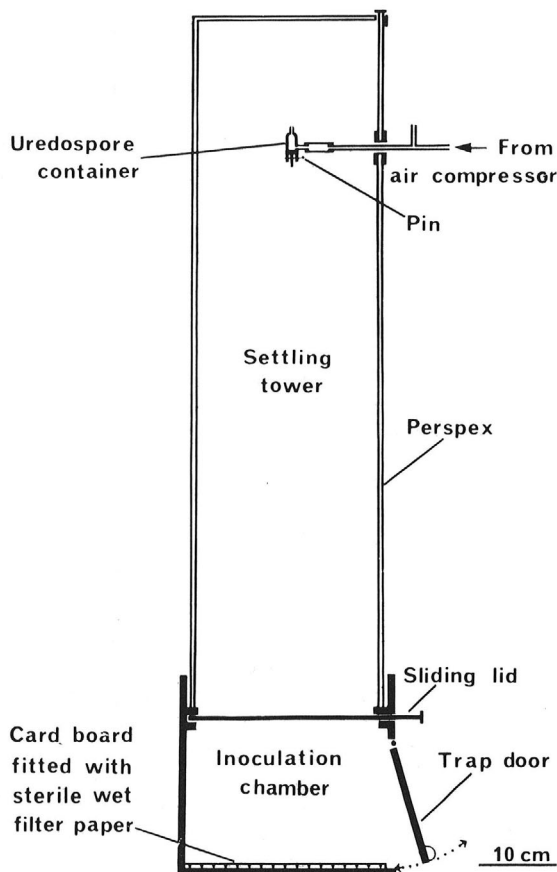


Fig. 1. Diagram of the spore settling tower employed to inoculate poplar leaves with *Melampsora larici-populina*.

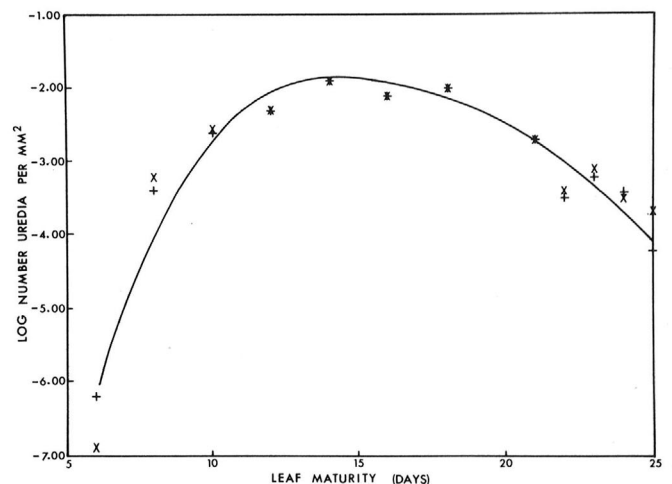


Fig. 2. The curve  $g(x) = cx^\alpha e^{-\beta x}$  fitted to the data for the 25-day-old shoots of *Populus euramericana* by use of the statistical computer language GLIM. The data points marked + and x are the readings for the leaves available from the two replicate shoots in this instance.

TABLE 2. Paired ANOVA comparisons of fitted curves for 10- and 14-day-old shoots of *Populus nigra*

(Analysis of Variance Table)				
Residual <sup>a</sup> due to	Sum of Squares	d.f.	Mean Square	F
Deviation from hypothesis	0.0877	3	0.02923	0.848 n.s.
Separate regressions	1.2413	36	0.03448	
Common regression	1.329	39		

<sup>a</sup>  $r_1$  (residual sum of squares of two separate fitted curves for 10- and 14-day-old shoots) =  $0.2133 + 1.028 = 1.2413$  with  $f_1 = 15 + 21 = 36$  degrees of freedom.  $r_2$  (residual sum of squares when the single curve  $\ln Y = \ln c + \alpha \ln x - \beta x + \epsilon$  is fitted to the combined data for both shoots) =  $1.329$  with  $f_2 = 42 - 3 = 39$  degrees of freedom.  $r_0 = r_2 - r_1 = 0.0877$  (with  $f_0 = f_2 - f_1 = 3$  degrees of freedom) is the sum of squares of residuals due to deviation from the hypothesis that the separate curves are the same.

TABLE 3. Leaf maturity at which maximum susceptibility to rust occurs in shoots of various age in two clones of poplar

Shoot age (days)	<i>P. nigra</i>		<i>P. euramericana</i>	
	Leaf maturity for max susceptibility (days)		Shoot age (days)	Leaf maturity for max susceptibility (days)
10 and 14	6.902 ± .1058 <sup>x</sup> a <sup>y</sup> A <sup>z</sup>		10	5.421 ± 0.03901 a A
			14	8.564 ± 0.1179 b A
20	11.66 ± 0.1904 b B		20	9.974 ± 0.3825 b B
26	14.41 ± 0.3831 c C		25	14.910 ± 0.1815 c C
33	23.26 ± 1.027 d D		32	17.65 ± 0.3098 d E

<sup>x</sup> Approximation of standard deviation (for calculation see text).

<sup>y</sup> Values followed by the same lower-case letters do not differ significantly within columns (ie, within clones).

<sup>z</sup> Values followed by the same upper-case letters do not differ significantly within rows (ie, between clones).

on stumps with numerous (eight to 12) buds, than on those with few (three to five) buds. This difference in shoot length and size was evident at the time of sampling leaves for inoculation.

Leaves unfurled almost every alternate day in young shoots of both clones but, as shoots aged, the interval increased from 2 to 5 days. Initially the rate of leaf production was slightly slower in young shoots of *P. euramericana* than in similar-aged shoots of *P. nigra*. The number of replicates of shoots for both the clones varied with the age of the shoot; there were four to five replicates of younger shoots while the number decreased to one to two for older shoots.

**Effect of leaf age within shoots on uredial production.** Separate plots of uredia per square millimeter (Y) versus leaf maturity in days (x) for shoots of different ages of *P. nigra* and *P. × euramericana* are illustrated in Figs. 3 and 4. Successively developed leaves on shoots in both clones generally showed a similar pattern of rust susceptibility (Figs. 3 and 4). On individual shoots, the younger leaves (apical) were graded 0 to 1, leaves in mid-portions of

TABLE 4. Correlation between latent period ( $R_1$  for LPF<sup>a</sup> and  $R_2$  for LPU<sup>b</sup>) and uredia per square millimeter in leaves from shoots of different age in two clones of poplar

Shoot age (days)	<i>P. nigra</i>		<i>P. euramericana</i>		
	$R_1^b$	$R_2^b$	Shoot age (days)	$R_1^b$	$R_2^b$
10	-0.98	-0.55	10	-0.68	-0.76
14	-0.77	-0.83	14	-0.68	-0.82
20	-0.83	-0.74	20	-0.76	-0.89
26	-0.56	-0.88	26	-0.73	-0.79
32	-0.86	-0.79	32	-0.73	-0.81

<sup>a</sup> LPF = latent period to flecking, LPU = latent period for uredia production.

<sup>b</sup> The correlations are all significant,  $P < 0.05$ .

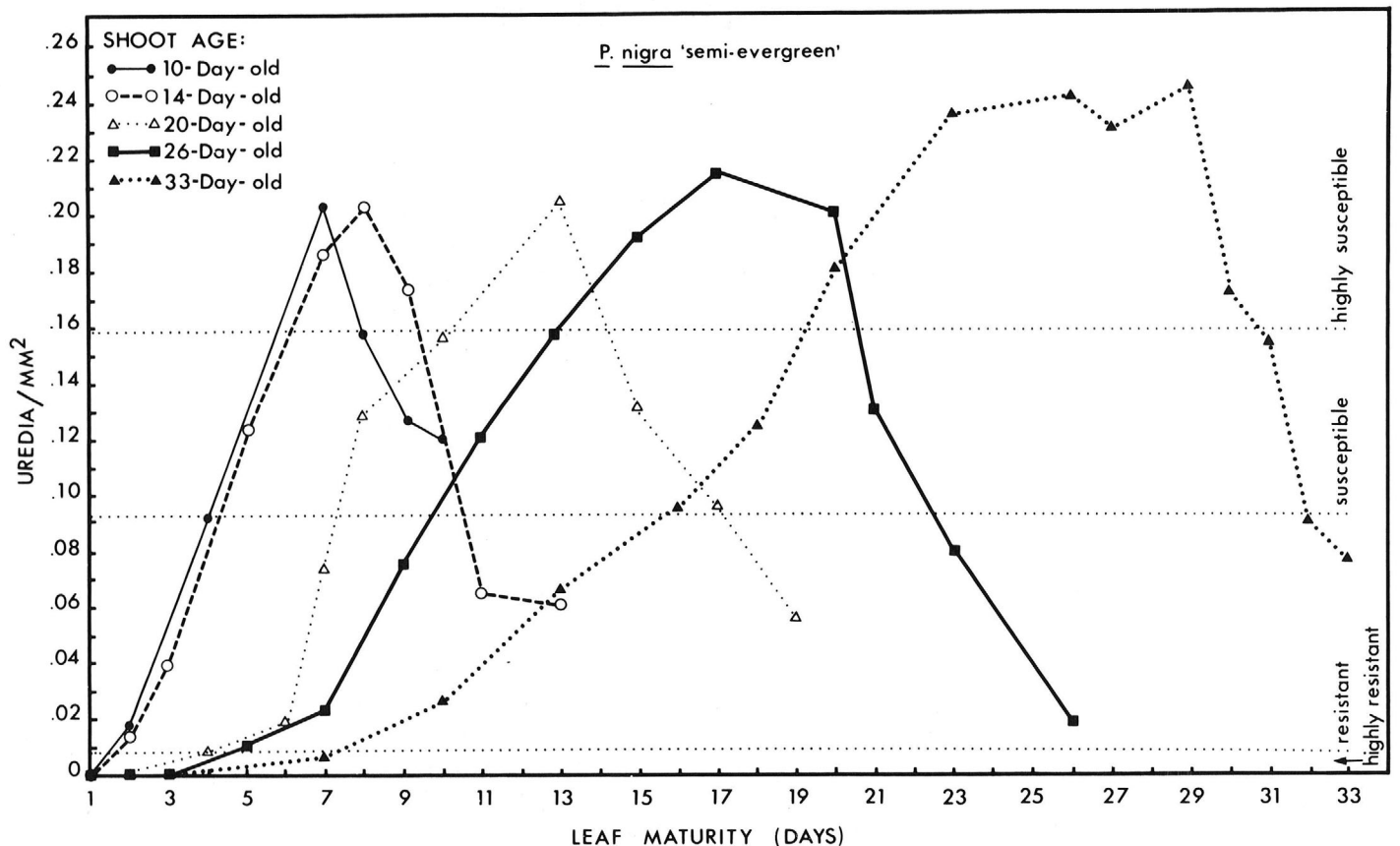


Fig. 3. Susceptibility (uredia per square millimeter in relation to an arbitrary scale) to *Melampsora larici-populina* of leaves of varying maturity collected from 10-, 14-, 20-, 26-, and 33-day-old shoots of *Populus nigra* 'Semi-evergreen.'

the shoots 3 to 4, and the older basal leaves 1 to 3 on the arbitrary scale. The major exception to this pattern was in 8-, 10-, 12-day-old shoots of *P. nigra*; the basal leaves also were graded 3. Further, on shoots less than 8 days old, apical leaves were graded 0 and the older leaves 1 to 2. Basal leaves on the youngest shoots (5-, and 4-day-old on *P. nigra* and *P. euramericana*, respectively) developed flecks, but uredia were not produced even 20 days after inoculation; ie, all leaves on these shoots were graded 0. As the shoots aged, the total number of resistant (ie, graded < 2) leaves per shoot increased. More basal leaves on shoots of *P. euramericana* became resistant than on shoots of similar age in *P. nigra*. Hence, the proportion of leaves graded 0 to 2 on shoots of *P. euramericana* more than 14 days old was higher than on similar shoots of *P. nigra*.

**Effect of shoot age within clones on susceptibility of leaves of comparable maturity.** The fitted curves of the data for uredia per square millimeter for 10- and 14-day-old shoots of *P. nigra* do not differ significantly (Table 2). Hence a single curve was fitted to the combined data for these two shoot-age groups in *P. nigra* (parameters of curve denoted by (c) in Table 1 [A]). In all other instances the null hypothesis was rejected following F-tests; ie, with the exception of the curves for 10- and 14-day-old shoots of *P. nigra*, the curves for *P. nigra* and all those for *P. euramericana* differ significantly ( $P < 0.05$ ). Thus, for shoots of *P. nigra* aged 22- to 33-days and for those of *P. euramericana* aged 10- to 32-days, susceptibility of leaves of the same maturity depends on shoot age. Generally leaves from the upper half of young shoots are more susceptible than are those of comparable maturity collected from older shoots (Figs. 3 and 4). The susceptibility of leaves near the apex or base of shoots appears to be independent of shoot age (also of leaf maturity) and such leaves are major exceptions to this pattern. Thus, there is a zone of higher susceptibility in the centers of most shoots (Figs. 3 and 4) and this zone is displaced upward in terms of leaf number from the first-formed leaf as the shoots age and new leaves are unfurled (Fig. 5).

**Effect of clonal origin on susceptibility to rust of leaves of**

**comparable maturity on shoots of the same age.** Leaves of *P. nigra* collected from 10- to 33-day-old shoots are usually significantly ( $P < 0.05$ ) more susceptible to leaf rust than are leaves of comparable maturity harvested from shoots of *P. euramericana* of comparable age (Figs. 3 and 4). Susceptibility of leaves of the same maturity at the apex and base of shoots appears to be relatively independent of clonal origin.

In *P. nigra* the leaf maturity at which maximum susceptibility occurs increases significantly with shoots of all age classes (10 to 33 days old) (Table 3). Leaf maturity at which maximum susceptibility occurs differs significantly between clones only in the oldest shoots.

**Latent period.** The latent period for the production of flecks (LPF) and uredia (LPU) by *M. larici-populina* in leaves of both clones for all shoot age classes are both significantly and negatively correlated with susceptibility (uredia per square millimeter) (Table 4). Data for 10- and 26-day-old shoots of *P. nigra* are reproduced in Figs. 6 and 7 to illustrate this relationship. Latent periods were shortest in leaves selected from the middle zone of shoots of all ages in both clones and increased considerably in leaves selected from above, and to a lesser degree in those harvested from below, this zone. Generally uredia are formed 2-3 days after the production of flecks, this was independent of clonal origin, maturity of the leaves, or of the age of shoot from which the leaves were collected. Minimum LPF may be slightly shorter in leaves from younger rather than from older shoots (Figs. 6 and 7) but there are insufficient data to test the significance of this. The overall data for LP indicate that if this were analyzed in a manner similar to that for susceptibility, LP would be related to leaf maturity within shoots, to shoot age within clones and in comparable tissue, and probably would be slightly shorter in *P. nigra* than in *P. euramericana*.

**DISCUSSION**

In both clones tested, leaf maturity within a shoot (consequently also position on the shoots) is a significant factor in the

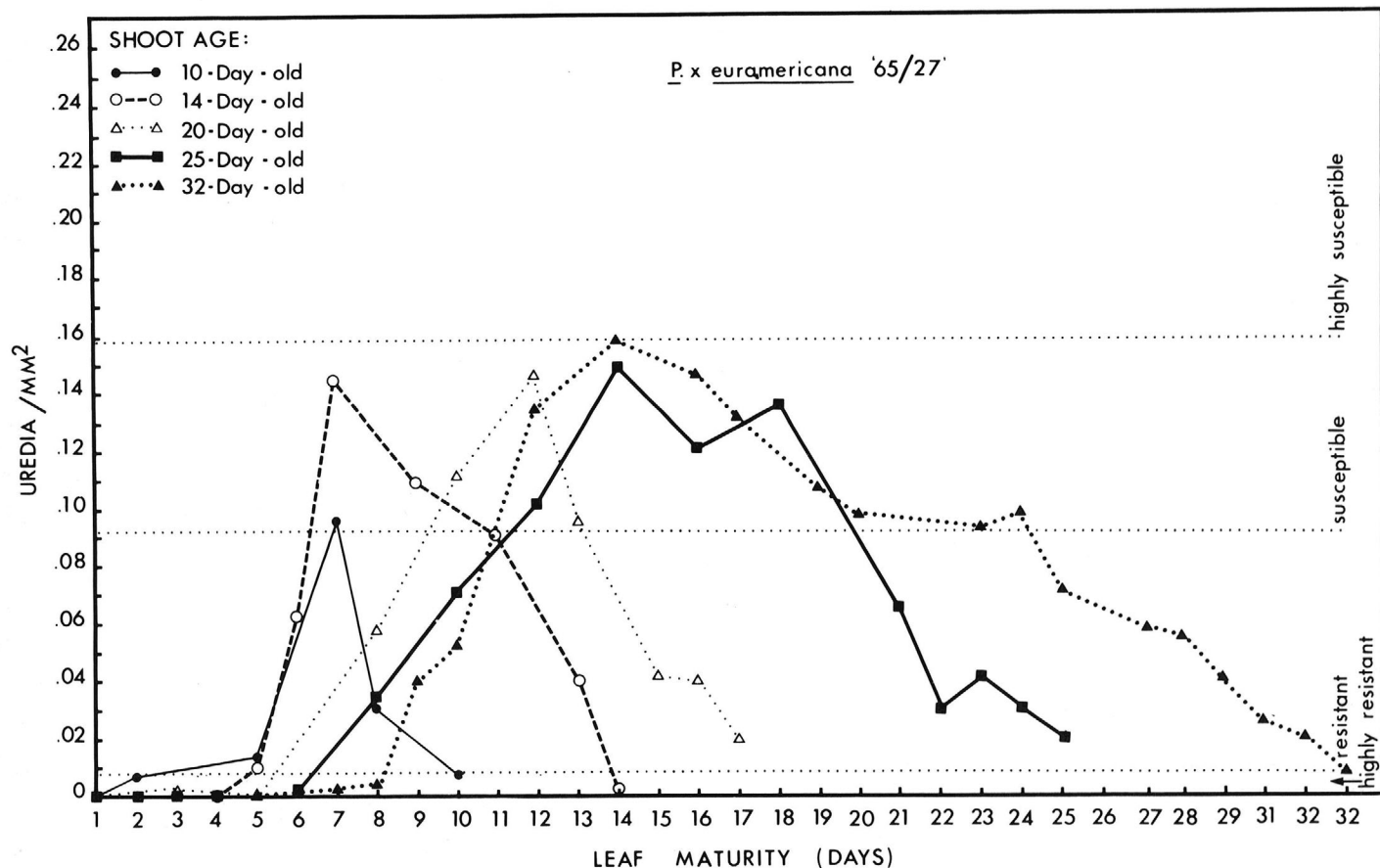


Fig. 4. Susceptibility (uredia per square millimeter in relation to an arbitrary scale) to *Melampsora larici-populina* of leaves of varying maturity collected from 10-, 14-, 20-, 25-, and 32-day-old shoots of *Populus x euramericana* '65/27.'

susceptibility to rust caused by *M. larici-populina* (Figs. 3-5). Providing that shoots are more than 8-days-old, there is a basipetal sequence of zones of susceptibility graded 0 to 1, 3 to 4, and finally 2. In shoots 8-days-old or younger, the lower zone graded 2 is not

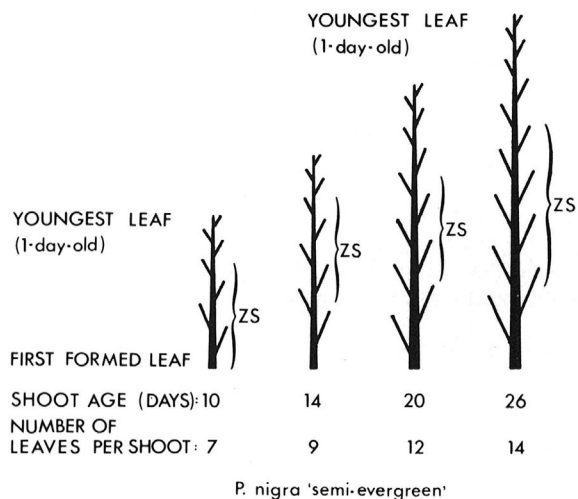


Fig. 5. Displacement from the base of the shoot of the zone of susceptibility (zs = zone of reaction type 3-4) to infection by *Melampsora larici-populina* in shoots of *Populus nigra* 'Semi-evergreen' in relation to successively formed leaves.

represented and in shoots less than 5-days-old all leaves were graded 0. These results are in agreement with earlier observations that tissue maturity has a considerable effect on the susceptibility of leaves to pathogens (8,11,12). It has been suggested that on a shoot each leaf when fully expanded exhibits a maximum susceptibility to pathogens; subsequently susceptibility decreases (11). Generally mature leaves are considered more susceptible to attack by fungal pathogens than are apical leaves on the same shoot (6). In contrast, there are records which indicate that young leaves are more susceptible than are older leaves to diseases caused by bacterial (3) and fungal (1,4) pathogens.

The maturity/position of a leaf within a shoot also influences the fecundity of the pathogen on the leaf. High reproduction rate of the pathogen per unit leaf area or high spore number per fructification are not necessarily positively correlated with high susceptibility of the leaf as expressed by numbers of fructifications per unit leaf area (4). With *M. larici-populina*, within the susceptible zone on the same shoot, more uredospores per uredium are produced on upper (less susceptible) than on lower (more susceptible) leaves (Sharma and Heather, unpublished).

Unfortunately, many studies reporting the relationship between leaf maturity and susceptibility to pathogens have not included quantitative determination of maturity (15), or where such determinations have been included, these have been carried out on shoots of limited growth (2,12,14). With studies in potentially unlimited shoots (1,4,6) attention has not been given to age of the shoots and its influence on disease expression in leaves of varying maturity. Thus, it has not been possible to find data strictly comparable with those obtained in the present study.

The effect of age of shoot on the susceptibility to rust of a leaf of a

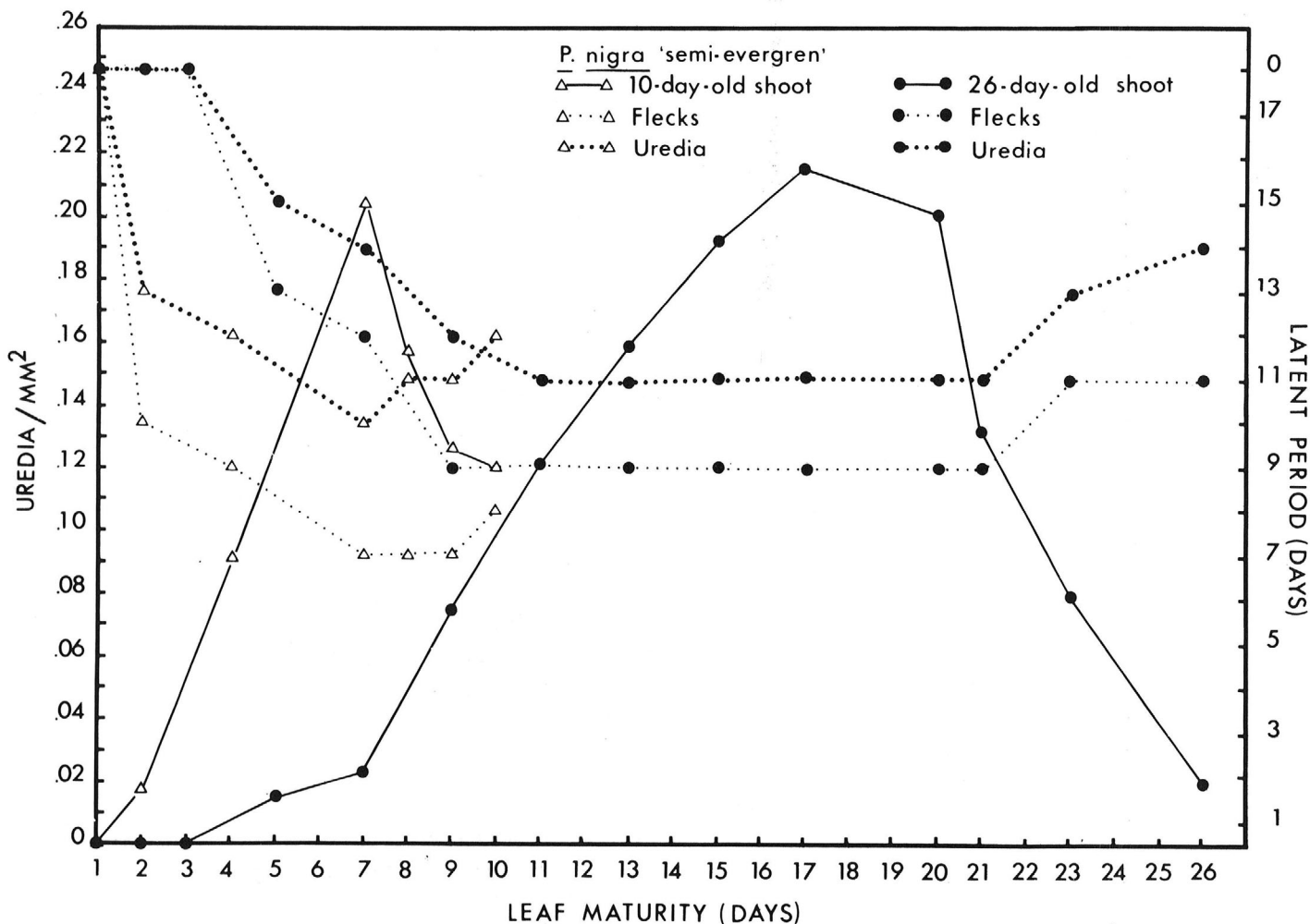


Fig. 6. Level of susceptibility (uredia per square millimeter, solid lines) and corresponding latent period (days, dotted lines) for the appearance of flecks and formation of uredia of *Melampsora larici-populina* on leaves of 10- and 26-day-old shoots of *Populus nigra* 'Semi-evergreen.'

particular maturity (Figs. 3-5) is an example of a "position effect" which is very evident in *P. nigra* and less pronounced but significant in *P. euramericana*. Within a shoot, susceptibility of leaves is related to absolute leaf maturity (or position). However, when shoots of different age are compared, age of shoot rather than absolute maturity of its constituent leaves determines their susceptibility to leaf rust.

Position effects of this type suggest that the shoot is a primary unit of organization determining the potential susceptibility to rust of its leaves. This general concept of shoot organization is in agreement with earlier observations on differences in physiology of leaves of comparable maturity on shoots of varying age (18,20). Potential susceptibility may be determined at still higher levels of organization, since in the field the leaves on stool shoots are more susceptible to leaf rust than are leaves on trees of the same clone in the same planting area (7).

The higher susceptibility of leaves of comparable maturity from shoots of comparable age in *P. nigra* than in those from *P. euramericana* parallels the relative susceptibility of these clones in the field to leaf rust caused by *M. larici-populina* (23).

Latent period has been recognized as one of the significant components of horizontal resistance (16,17,26). Longer latent period has been employed as a parameter in the selection of potato breeding lines and cultivars with high horizontal resistance to late blight (21) and in breeding lines of grain crops with horizontal resistance to various rusts and powdery mildews (5,15,30). Results in the present study are in general agreement with these observations.

In the experiments a shorter LP (LPU and LPU) (whether in

comparisons between clones, between leaves of different maturities, or between zones within shoots in a clone) was correlated with higher susceptibility. The LP generally was shorter in comparable leaves (leaf maturity and shoot age) from *P. nigra* than in those from *P. euramericana*; this also accords with the higher susceptibility in the field of the former clone to leaf rust (23). While these observations are in general agreement with the concepts of Vanderplank (27), they contrast with the results of Parlevliet (15) who found that primary leaves and the young flag leaf of barley have identical reaction types to *Puccinia hordei* Otth. although the LPU in the former is shorter than in the latter. In addition, the most rust-susceptible stages in barley, (as measured by LP) are the very young (seedlings) and very old stages of the shoots (tillers) with the highest resistance in the intermediate stage (immediately before and during flowering) (15); ie, more or less the reverse of the results obtained in the present study. Possibly the differing growth habit of the shoots (limited in barley and potentially unlimited in poplar) explains the conflicting results.

In susceptible clones, poplar rust normally appears in Canberra on the basal leaves of the shoot in early November and progresses acropetally in shoots, and indeed in the tree as a whole, in subsequent months (25). The 1976-1977 growing season was unusually dry prior to January and initial infection of the very susceptible *P. nigra* clone was not evident until mid-January 1977. The rust appeared first in the mid-zone of shoots and subsequently spread acropetally and basipetally. Variation in susceptibility of leaves of particular maturity with age of shoot on which they occur probably explains in part this deviation from the normal pattern of rust development. The variation in susceptibility of leaves of

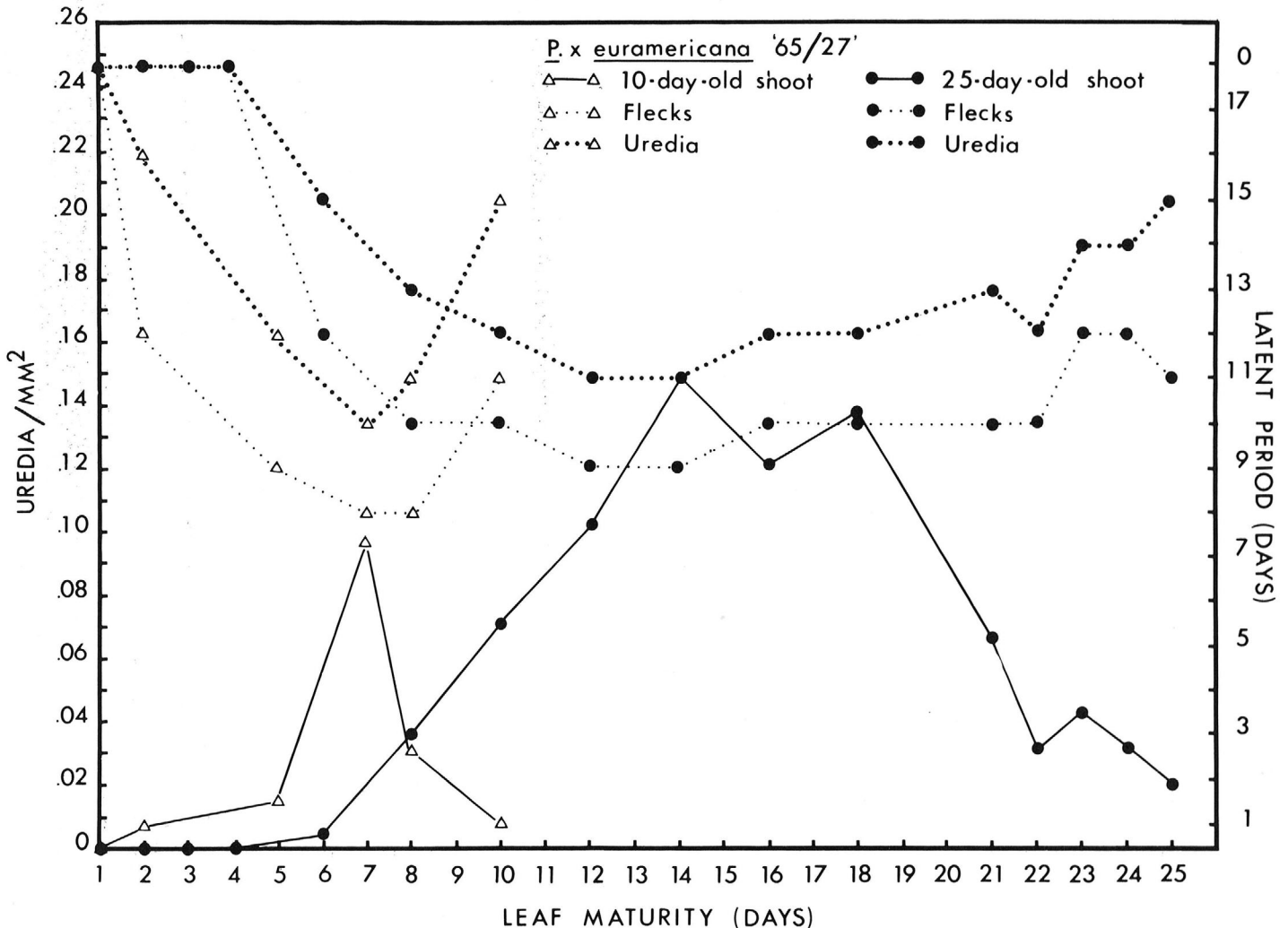


Fig. 7. Level of susceptibility (uredia per square millimeter, solid lines) and corresponding latent period (days, dotted lines) for the appearance of flecks and formation of uredia of *Melampsora larici-populina* on leaves of 10- and 26-day-old shoots of *Populus* × *euramericana* '65/27.'

different maturity and of leaves of the same maturity on shoots of different age facilitates the co-existence of host genotypes and their corresponding virulent races; viz, a susceptible host is not completely defoliated by a wave or successive waves of infection and thus the pathogen can continue to cycle throughout the growing season of the host. Complete defoliation of the host becomes possible only when the host shoot ceases to elongate and the uppermost already formed leaves age to a susceptible condition. This phenomenon also emphasizes the importance of Semi-evergreen poplar in the seasonal "carry over" of the rust in the uredospore stage (7), as the tuft of leaves on the apex of shoots of these varieties carry uredial pustules even in mid-winter at 30 degrees south latitude. The present results suggest that where autumn colors in poplar have aesthetic significance this will be achieved best by selecting clones with high leaf rust resistance and a very long growing season.

The observations reported also have epidemiological significance. A heavy rust infection in late spring may cause the shedding of leaves in the middle and lower portions of the shoots. Despite their youth many of the leaves on short shoots, which developed subsequently from the axillary buds of fallen leaves, will be rust susceptible while leaves of the same maturity on shoots produced in early spring will be more resistant. Thus, throughout the growing season, in favorable environments, coincident infection of aging leaves on older shoots and a cycling of infection and defoliation of young leaves on developing shoots is probable. In clones with a growing season extending into late autumn, the buds will fail to mature and at higher latitudes and altitudes these may be damaged severely by early winter frosts, resulting in death of short shoots, and even whole branches in trees, or complete shoots in stool beds. Clones with a short growing season, irrespective of rust susceptibility, will be completely defoliated by late summer and consequently their shoots will not be damaged by rust in such localities.

Studies of disease progress of poplar rust throughout the growing season are more complex than those of similar diseases in crops with limited shoots. Further, different clones flush at various times in different localities making field comparisons of clonal resistance difficult and unreliable. Leaf maturity and shoot age for maximum susceptibility to leaf rust may vary between clones, hence screening clones for relative horizontal resistance by the leaf disk method (7) will be satisfactory only if disks are cut from leaves which have the potential to express the maximum susceptibility characteristic of the clone.

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