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Inheritance of Resistance to Races of *Melampsora medusae* in *Populus deltoides*

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Summary

The F_1 progeny of a *Populus deltoides* cross were analysed independently for their reaction to six races of *Melampsora medusae*, which elicited susceptible \times resistant, resistant \times resistant or susceptible \times susceptible reaction in parents depending on the races employed. Resistance was inherited as dominant (3 races), recessive (2 races) or quantitative, additive (1 race) in the clones, and was controlled by a single gene or two genes acting in a complementary or duplicate manner, depending on the race. Modifying factors influenced the degree of susceptibility expressed in certain plants and resulted in a 'slow rusting' effect. Relevance of the results to poplar breeding for leaf rust resistance is discussed.

Key words: Genetics, Leaf rust, *Melampsora medusae*, *Populus deltoides*, Disease Resistance, Slow Rusting.

Zusammenfassung

Die F_1 -Nachkommenschaft einer *Populus deltoides*-Kreuzung wurde unabhängig auf ihre Reaktion auf sechs Rassen von *Melampsora medusae* hin analysiert. Die Analyse zeigte je nach Rasse eine anfällige \times resistente, resistente \times resistente oder anfällige \times anfällige Reaktion bei den Eltern (P_1). Resistenz wird als dominant (in drei Rassen), rezessiv (zwei Rassen) oder additiv auf quantitative Weise (eine Rasse) vererbt und wird von einem oder zwei Genen, die in einer ergänzenden oder verdoppelnden Weise, je nach Rasse, funktionieren, kontrolliert. Modifikationsfaktoren beeinflussten den Grad der Anfälligkeit in bestimmten Pflanzen und resultierten in einem 'slow-rusting' (langsamen Rost)-Effekt. Die Bedeutung dieser Resultate für die Züchtung von Pappeln auf Blattrostresistenz wird diskutiert.

1. Introduction

Leaf rust, caused by *Melampsora medusae* THUM., is an important disease of poplar, and several races of this pa-

thogen have been recognised in Australia (SINGH and HEATHER 1982). An understanding of the inheritance of resistance to different races of the pathogen is essential in devising strategies for management of the disease by breeding and selection. MÜHLE LARSEN (1970), following a field analysis of a population of poplar progeny, concluded that resistance to leaf rust, caused by *Melampsora larici-populina* KLEB., may be controlled by a few to several dominant genes.

This paper reports a study of inheritance of resistance to six races of *M. medusae* in a *Populus deltoides* MARSH (2n = 38) cross, assessed on a qualitative scale using detached leaf disks.

2. Materials and Methods

2.1 Origin of Parents

The parents, *P. deltoides* cvs. 60/122 (female) and T-173 (male), are selections from separate Texan provenances (WILLING, R. R., *pers. commun.*). Cv. 60/122 is relatively more susceptible than cv. T-173 to all the races of *M. medusae*.

2.2 Hybridisation

Crosses were performed on bottle grafted branches of cv. 60/122 in a glass house as described by KNOX *et al.* (1972). The branches, bearing unopened female flower buds, were collected from the poplar plantation maintained by the Botany Department, Australian National University, and washed with a jet of tap water. The grafts were made during winter 1982 (mid August) about 3–4 weeks prior to natural flowering in the field. The understocks (rooted for 4 months), consisting of cuttings 30–40 cm long, and 1.5–2.0 cm in diameter, were transplanted into 20 cm pots. Pollen of cv. T-173, collected from plantations at Kempsey, NSW, was dried over silica gel (24 h) and stored in a freezer (–14°C) for 1–2 weeks prior to use. Crosses

were conducted, in an isolated, pollen proof-glasshouse during the early morning, by gently dusting the pollen on the opening female buds. Several other crosses were performed using separate cultivars but were not included in the study since insufficient progeny were obtained.

Seeds were collected after 60–70 days and germinated in Perlite under a mist spray. A nutrient solution was applied twice-weekly, and the seedlings transplanted when the first true leaf pair unfolded (c. about three weeks) into pots containing Perlite and Vermiculite (1:1). About 110 F_1 plants were raised from this cross and individually labelled.

The parents were propagated by clonal cuttings under mist and grown along with F_1 plants under controlled conditions in a rust-free glasshouse ($20 \pm 3^\circ\text{C}$, 16 h photoperiod). Liquid fertilizer (Hortico®) was applied once a week and a bacterial (*Bacillus thuringiensis* Berliner var. *alesti*) insecticide (LANE DÍPEL® HG) was sprayed occasionally to control leaf eating caterpillars. Plants were cut back every 3 months to ensure the development of fresh leaves of uniform age suitable for optimal disease expression (SHARMA *et al.* 1980). The tests were conducted on leaves from plants aged 12–24 months.

2.3 Pathogen Isolation

Six races of *M. medusae*, recognised as distinct races by their qualitative reactions on a set of differential cultivars (SINGH and HEATHER 1982), were employed to study the inheritance of resistance in this cross. The urediniospore of *M. medusae* is a dikaryote ($n + n$) and is asexually (clonally) propagated. Five of the six races (1A, 2A, 3A, 4A, 5A) originated from single separate urediniospores (SINGH and HEATHER 1982) while race 4B was isolated from race 4A for its avirulence on cv. T-173 using a leaf replica technique (PRAKASH and HEATHER 1985a). Spores of all the races were multiplied separately on detached leaves of *P. × euramericana* (DODE) GUINIER cv. I-488, a universal susceptible, supported on gibberellic acid solution (10 mg/L) in plastic Petri dishes (SINGH and HEATHER 1982). Freshly harvested spores were dried in a dessicator *in vacuo* (24 h silica gel, 24 h P_2O_5) and used immediately for inoculation.

2.4 Inoculation

The disease reaction of each plant to each race was assessed by using leaf disks (five replicates) from the individual plants. Because of the large population involved, the tests were conducted in three batches (Races 1A and 2A, 3A and 4A, 4B and 5A were studied pair-wise).

Leaf disks (1.70 cm²) were punched from freshly harvested, surface sterilised leaves of uniform age (c. 3 months). Due to the segregation in the F_1 population, minor differences in leaf maturity were noticed; they were insufficient to influence the relative variation of disease expression. Again, due to replication of leaf disks in the inoculation it was possible to assess only c. 60 randomly selected, F_1 plants for reaction to a particular race pair; some 40 plants had been subjected to all the six races at the conclusions of the study.

All the leaf disks of F_1 plants and the parents were inoculated separately with the urediniospores of individual races using a spore settling tower (SHARMA *et al.* 1980). Replicate leaf disks (5 for F_1 plants, 10 for each parent) from individual plants, some cover glasses and several disks of cv. I-488 were randomly placed in the bottom of a spore settling tower and inoculated in three successive batches with 5 mg of fresh spores of a race. Subsequently, separate replicate disks and cover glasses were inoculated

with spores of the second race of the pair. Comparability of deposition between inoculations, within and between races, and germination (> 95% for all races) were checked by examining the coverglasses, while the uniformity of infection was checked on the disks of the universal susceptible, cv. I-488. Inoculated leaf disks were placed on plastic foam soaked with gibberellic acid solution (10 mg/L) sealed in glass Petri dishes and incubated in control growth cabinets ($16 \pm 1^\circ\text{C}$, $100 \mu\text{Em}^{-2}\text{s}^{-1}$, 16 h photoperiod) (SINGH and HEATHER 1982).

2.5 Disease Assessment

The observations on the level of disease on each plant were assessed as Infection Type (IT) on day 18 and confirmed on day 23 of incubation. The IT was on a 0 to 4 scale of increasing disease severity (Figure 1), where 0 - immune, no macroscopic symptoms of disease, 1 - chlorotic/necrotic symptoms with possibly one or two minute uredinia, 2 to 4 - increasing size and number of uredinia (PRAKASH and HEATHER 1985b). For the computation of the genetic ratios, IT 0 and 1 in the plants were classed as resistant and 2–4 as susceptible reactions. This classification distinguished among the plants, those on which reproduction of the pathogen did not occur or was insignificant (IT 0–1) and those on which the rust reproduced (IT 2–4). The ratios (resistant : susceptible) were tested for goodness of fit to several hypothetical genetic ratios using χ^2 test and those ratios which gave the best fit i.e. the highest probability range, were accepted (STRICKBERGER 1986).

3. Results

For all pathogen races, one parent (cv. 60/122, P_1 , female) was consistently more susceptible than the other (cv. T-173, P_2 , male). The reaction (IT) of the parents was dependent on the race employed (Table 1) and thus for each race the cross could be classified as either Resistant \times Resistant, Susceptible \times Resistant or Susceptible \times Susceptible (Table 1). With all the races, the F_1 plants exhibited clear segregation indicating the heterozygosity of the parents. Five of the six races elicited different ratios in the progeny (Table 1) thus confirming their status as distinct races (*sensu* STAKMAN and CHRISTENSEN 1953), which had been recognised by their reaction on differential cultivars (SINGH and HEATHER 1982).

Races 1A, 2A and 3A elicited a Susceptible (60/122) \times Resistant (T-173) reaction in the parents. Reaction to race 1A in the progeny approximated a 1:1 ratio, suggesting the role of one gene (dominant or recessive, L or l) for resistance ($P = 0.5$ – 0.8). Race 2A elicited a 3:1 (S:R) ratio ($P = 0.8$ – 0.9) for which the simplest explanation is that resistance in cv. T-173 is conditioned by the concurrent occurrence of two, homozygous, recessive genes (qqmm) for resistance to this race while cv. 60/122 was dominant heterozygous for both the genes (QqMm). However the reaction to race 3A by the F_1 plants was less discrete, the classes approximating a normal distribution and thus the resistance to this race may be quantitatively inherited and controlled by three or four genes acting additively (*sensu* ALLARD 1960).

Both the parents are susceptible to race 4A but the F_1 plants gave a ratio of 15:1 (S:R) to this race. This would be expected, if we assume the action of two duplicate, recessive genes controlling resistance (nntt). Races 4B and 5A were avirulent on both the parents and the progeny reaction approaches a 3:1 (R:S) ratio ($P = 0.25$ – 0.5 and 0.7 – 0.8 respectively) suggesting the action of a single, dominant gene for resistance to each of these races in

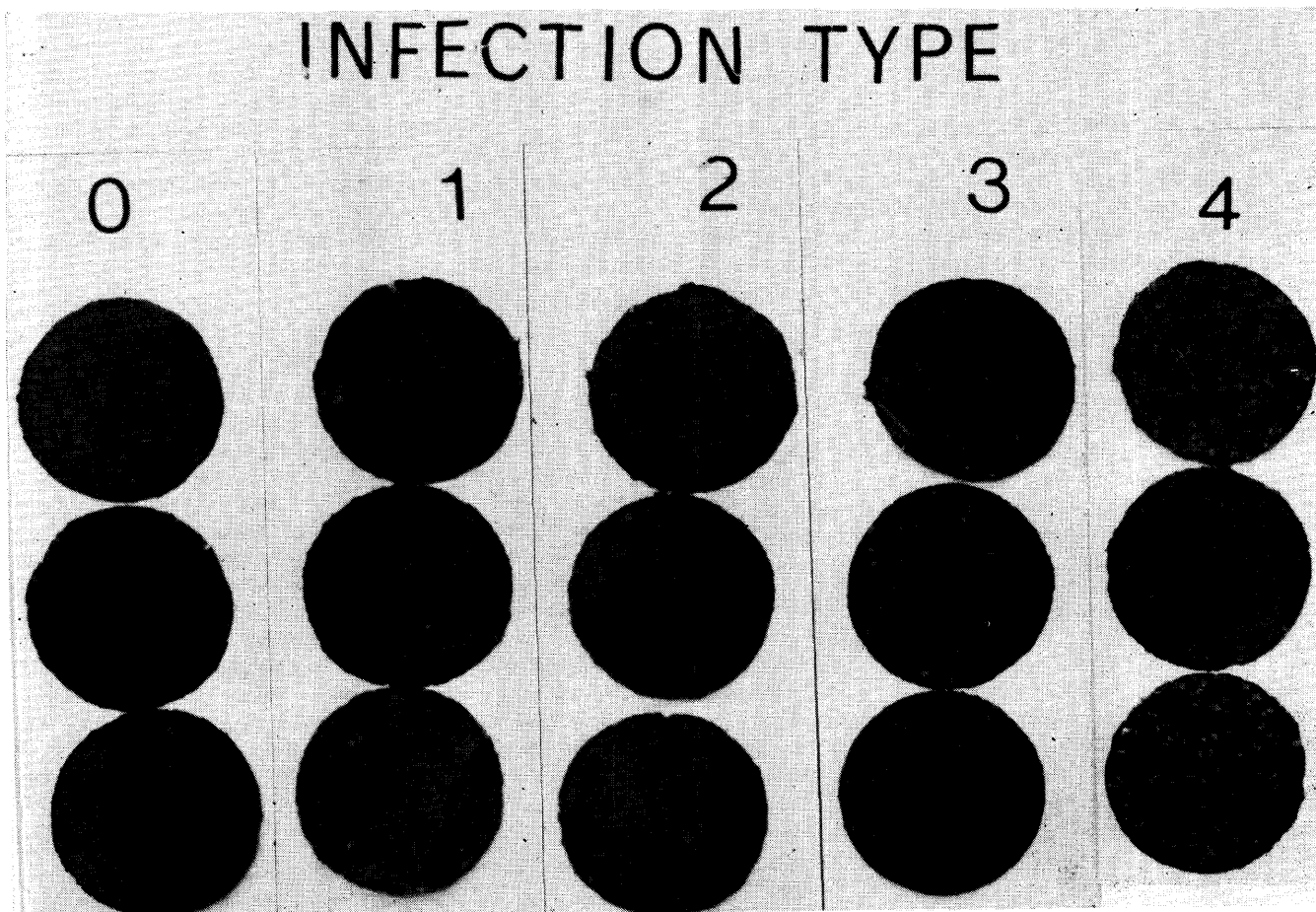


Figure 1. — The Infection Type (IT) on a 0–4 scale of disease severity by *M. medusea* on the leaf disks of F_1 plants of cross *P. deltoides* cvs 60/122 X T-173.

Table 1 : Reaction of parents *P. deltoides* cvs. T-173 and 60/122 and their F_1 hybrids to six races of *M. medusae* and the χ^2 probability levels of certain genetic ratios for segregation in the F_1 progeny.

RACE	INFECTION TYPE AND RESISTANCE CLASSIFICATION PARENTAL CVS.		NUMBERS OF F_1 PROGENY					RATIO OF RESISTANT TO SUSCEPTIBLE IN PROGENY		PROBABILITY RANGE (χ^2)	PROPOSED GENETIC MAKE-UP OF PARENTS
	60/122	T-173	0	1	2	3	4	OBSVR R : S	FITTED R : S		
1A	3 (S)	1 (R)	0	27	1	15	16	27:32	1:1	0.5-0.8	11 x L1
2A	3 (S)	1 (R)	0	14	1	16	28	14:45	1:3	0.8-0.9	QqMm x qqmm
3A	3 (S)	1 (R)	5	17	24	11	7	-	-	-	QUANTITATIVE
4A	4 (S)	3 (S)	1	3	13	22	25	4:60	1:15	1	NnTt X NnTt
4B	1 (R)	0 (R)	27	28	7	4	2	55:13	3:1	0.25-0.5	Uu X Uu
5A	1 (R)	0 (R)	18	32	13	1	4	50:18	3:1	0.7-0.8	Vv X Vv

† R and S refer to Resistant and Susceptible reactions, respectively

each of the cultivars (U and V respectively). Most of the F_1 plants resistant to race 5A were also resistant to race 4B suggesting possible linkage of these two resistant genes. However some plants were resistant to one but susceptible to other races, suggesting two separate genes for resistance are involved.

Many of the F_1 plants (and the male parent, cv. T-173 to race 4A) exhibited degrees of 'slow rusting', characteris-

ed by a long latent period and reduced sporulation, suggesting the presence of modifying factors influencing the disease expression in these cultivars. While such characteristics did not affect the classification of individual plants as resistant (IT 0 or 1) or susceptible (IT 2–4) at the time of disease assessment, they influenced the grading of disease severity in plants within the IT 2–4 range.

4. Discussion

The dioecious nature and long generation interval (7–8 years) of the host and the absence from Australia of the sexual stage of the pathogen make genetic studies on host-pathogen interactions in the *Populus* - *M. medusae* system difficult. Thus hybridization amongst pathogen races and selfing of the host clones, is not possible, while backcrossing and further generation testing is difficult and time consuming. Hence, the genetic interpretation of results of the inheritance of leaf rust resistance in poplars is largely hypothetical. However, since genetic studies have not been reported in this system, any information would be helpful in planning breeding programs. The strict control of the environmental variables (both pre- and post-inoculation conditions of plant culture, and uniformity of leaf maturity and inoculum) resulted in considerable uniformity in disease expression in the replicate leaf disks of an individual plant. This ensured that the variation between individuals in infection type was genetically based.

The disease reaction (IT) of the parents and the ratios of the progeny were clearly dependent on the race of *M. medusae* employed, emphasising the importance of employing distinct races in disease resistance studies (PERSON and SIDHU 1971). In these clones of *P. deltoides*, resistance appears to be dominant with races 1A, 4B and 5A, but recessive with races 2A and 4A, and codominant, additive or quantitative to race 3A (Table 1). It can be hypothesised that resistance/susceptibility to *M. medusae* was monogenic (races 1A, 4B and 5A), controlled by two duplicate genes (race 4A), or two complementary genes (race 2A) or three to four additive genes (race 3A) depending on the race employed. The likely presence of duplicate factors for resistance to race 4A may result from the secondary polyploid nature of poplars (MUHLE LARSEN 1970). Superficially, different segregation ratios may be obtained from the data with certain races reported here if the dividing line between R and S categories is altered (for example if R includes IT's 0, 1 and 2 rather than just 0 and 1). However this possibility was rejected as it would require a more complicated genetic model to explain the IT values of the progeny and parents (except with race 4A). Further, the present classification of R and S reactions are relatively more precise and repeatable under the experimental conditions employed.

Some F_1 plants exhibited relatively long latent periods. Possibly certain modifying factors influenced disease expression and resulted in 'slow rusting' of these susceptible plants. Such plants would be useful as sources of partial resistance (PARLEVIET 1979).

Almost all the F_1 plants showed a high level of racial specificity probably due to the different resistance genes they contained. Such genotypes would still be useful in mixed clonal plantings similar to the multiline approach suggested by HEATHER and CHANDRASHEKAR (1982) and their resistance would likely be persistent when accompanied by 'slow-rusting' type resistance (PRAKASH and HEATHER 1986).

The results for races 2A, 3A and 4A contrast with those frequently reported in inheritance studies in agricultural crop plants where resistance is predominantly monogenic

dominant (PERSON and SIDHU 1971). However, as WOLFE and SCHWARZBACH (1978) and DAY *et al.* (1983) have suggested, the latter patterns of resistance may be artifacts of the breeding strategy which has concentrated on few resistance genes each with a large effect. In a forest plant like poplar, where little or no breeding for resistance to leaf rust has been practised, the observation of a multiple gene control is not unexpected.

The poplar leaf rust system is labile to environmental factors (HEATHER and CHANDRASHEKAR 1982, SINGH and HEATHER 1982). To ensure durability of resistance, despite the polymorphism of the races of the pathogen and the environmental lability of the system, the stability analysis approach similar to that suggested by JENNS *et al.* (1982) may be useful in selecting cultivars whose resistance is potentially durable. Heterogeneity of deployment of such cultivars over time and space would further ensure such durability.

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