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Susceptibility of P. deltoides Bartr. to Melampsora larici-populina and M. allii-populina

I. Qualitative Analysis of a 6x6 Factorial Mating Design

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Summary

Qualitative susceptibility to *Melampsora* spp. was observed in a 6x6 *P. deltoides* factorial mating.

For each of the 3 inocula tested, *Melampsora larici-populina* races E1 and E2 and *M. allii-populina*, segregations that occured in qualitative tests cannot be explained with less than 3 genes for susceptibility-resistance. Comparison with EL KARKOURY's results (1991) suggest that race E1 would have another virulence gene which is not present in E2. However, the observed segregation pattern cannot be explained without another gene common to E1 and E2.

Within 3 full sib families, qualitative resistance to both *M. allii-populina* and *M. larici-populina* may be genetically linked. Obviously, several unidentified races of *M. allii-populina* were present in the nursery trial.

Key words: Populus deltoides, Melampsora larici-populina, Melampsora allii-populina, qualitative resistance, segregation, factorial mating.

Introduction

The *Populus* genus is one of the most widespread and economically important wood resources in the world. But many pests and diseases are encountered on poplars, especially in monoclonal plantations. Among diseases, "*Melampsora* leaf rust is probably the most widely distributed and serious foliar disease of the Aigeiros and Tacamahaca poplars and their hybrids" (THIELGES, 1985). At least 8 *Melampsora* species can attack poplars, but 3 of them are of major importance: *M. allii-populina* KLEB., *M. larici-populina* KLEB. and *M. medusae* THUM.. Severe damage has been attributed to these pathogens, including growth reduction or even death of plants (WIDIN and SCHIPPER, 1981; STEENACKERS, 1982, in: PINON, 1984). During the last 10 years, a number of studies has focused on the *Populus-Melampsora* system, revealing great variability within both host and pathogen, and frequently reporting race-specific interactions.

This report relates qualitative tests of the susceptibility of *P. deltoides* BARTR. to *Melampsora larici-populina* and *M. allii-populina*, which were conducted in the laboratory and nursery at the INRA station near Orleans. Genetic determination of total resistance to rust is approached through our analyses of a 6x6 factorial mating system.

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Materials and Methods

A) Origin of the plants

Thirty-three full-sib families from an intraspecific *P. deltoides* factorial mating involving 6 males and 6 females were tested. Parents came from very distinct sites within the natural range of *P. deltoides* (*Figure 1*). Details on the crosses and origin of the parents were provided in PICHOT and TEISSIER DU CROS (1989). *Table 1* gives the exact number of clones used, maximum 20 per family. A total of 591 clones were included in the study. Ten of the 12 parents were also vegetatively propagated for testing.

Note that clone number 2415 (for example) refers to clone number 15 in the full sib family 24, involving male 2 and female 4.

B) Origin of the rust and culture of inoculum

Urediospores of *Melampsora larici-populina* (race E1 and E2) and *M. allii-populina* were sent by J. PINON from the INRA laboratory of Nancy. Inocula were multiplied by culturing directly on leaves of race-specific clone: Grammont3 (=Ogy) for *M. larici-populina* race E2; Beaupre for *M. allii-populina;* and finally, Robusta (non specific) for *M. larici-populina* race E1.

The spores were cultured at 15h00 photoperiod at 17° C to 20° C, and stored according to the techniques developed by PINON (personal communication).

Rust susceptibility of progenies was scored both in the nursery and in the artificial environment of controlled environment chambers. Experimental designs varied in each case.

C) Nursery experiments

In spring, 1990 a nursery trial was established with stem cuttings at the INRA station near Orleans. The experimental design utilized 3 complete blocks with plots of 2 ramets per clone. Additionally, 10 clones were chosen as controls. The plots were randomly distributed within blocks. Cuttings were all taken from a first generation stool bed, thus reducing the C effect. Cuttings were planted under a black polythene film to reduce evaporation and to control weeds. Planting distance was 1.2 m x 0.5 m. In winter, 1990 the trial was cut back. In spring, only one stem per stump was retained.

On July 19th 1990 and again on June 18th 1991, a suspension of 7000 spores per ml of *M. larici-populina* race E1 was sprayed on the lower surface of one leaf per tree.

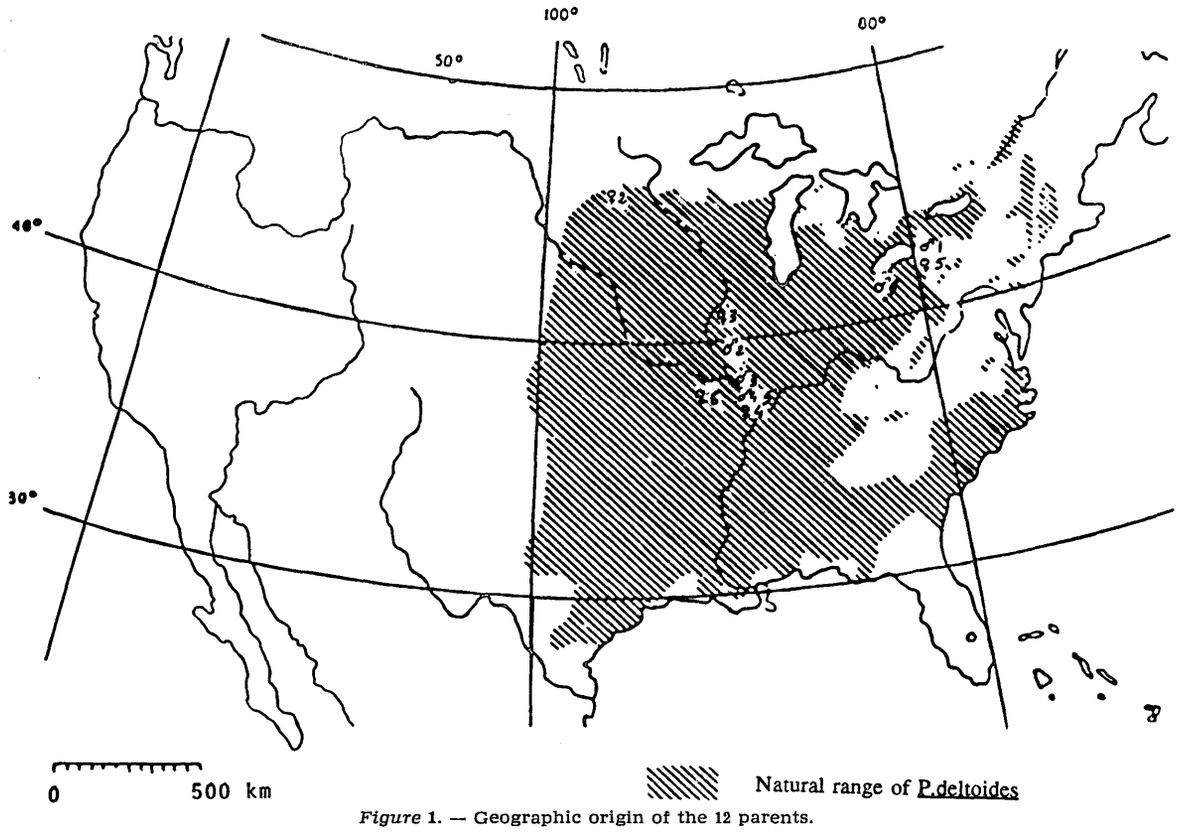


Figure 1. — Geographic origin of the 12 parents.

The inoculated leaf was one of the first fully expanded leaves, near leaf number 10 from the apex.

D) Laboratory experiments

For the laboratory test, foliar disks were cut from leaves collected in the nursery trial. These were placed in distilled water in small multidish plates (lower leaf surface up) and inoculated with a spray suspension of spores. The multidishes were composed of 24 (6x4) compartments of 15 mm diameter for foliar disks of 13mm diameter. Six clones, each represented by 4 neighbouring disks, were tested in each multidish. Environmental conditions after inoculation were similar to those previously described for the multiplication of the spores.

All clones tested in the nursery trial were also tested in the laboratory. In the 2 first nursery blocks, one leaf per plot was removed, that is to say one leaf per clone in the full sib families and 2 leaves per clone for the parents and the controls in each block. A number of tests was conducted with *M. larici-populina* race E1 and E2 and with *M. allii-populina* using various concentrations of inoculum (*Table 2*).

A control test was also conducted on some clones in June 1992.

E) Observations

In the nursery test, susceptibility to *Melampsora* was estimated by:

a) the number of uredia on the leaf 20 days after inoculation with race E1 (July 9th 1991);

b) a scoring of clones subject to rust attack at the end of the growing seasons (October 10th to 11th 1990 and September 4th to 6th 1991).

The climate at the test nursery was particularly dry during the summer of 1990, so the rust did not significantly colonize leaves until September. Very few symptoms were noted, and susceptibility levels could not be estimated after artificial inoculation. In autumn 1990, both *M. larici-populina* and also ambient *M. allii* populina were identified by microscope observations of urediospores (CIP, 1981) for each susceptible tree in blocks 2 and 3 of the nursery.

In the laboratory, sporulation 15 days after inoculation was recorded.

Occasionally, necrotic reactions appeared on a part of the disks and sometimes, discolourations appeared without sporulation. Numbers of necrotic flecks were also counted.

Table 1. — Number of clones tested per full sib family.

female / male	1	2	3	4	5	6
1	20	20	20	20	20	02
2	20	19	19	10	12	00
3	20	20	20	19	20	20
4	20	18	20	08	20	00
5	20	20	20	20	20	00
6	19	06	20	20	20	19

Table 2. — Qualitative tests conducted in the laboratory, 1991.

species and race	concentration of inoculum (x1000 spores/ml)	inoculation date
larici E1	10	July 16
larici E1	15	July 22
larici E2	15	August 09
larici E2	20	August 30
allii	12	August 09
allii	15	August 30

Table 3. — Qualitative susceptibility to Melampsora larici-populina race E1.

F / M	1 R		2 S		3 R		4 N(S?)		5 R		6 S	
1 R	00	00	00	00	00	00	00	00	00	00	00	00
	00	20	00	20	00	20	00	20	00	20	00	02
2 ?	00	00	13	13	01	03	00	09	04	03	?	?
	00	20	10	19	01	19	00	10	02	12	?	00
3 R	00	00	00	00	00	00	00	00	00	00	00	00
	00	20	00	20	00	20	00	19	00	20	00	20
4 SN	00	05	18	07	03	02	04	03	06	00	?	?
	00	20	07	18	00	20	01	08	00	20	?	00
5 R	00	02	07	08	00	00	01	04	05	03	?	?
	00	20	07	20	00	20	01	20	03	20	?	00
6 ?	00	00	06	00	10	02	20	06	10	06	19	08
	00	19	00	06	02	20	06	20	06	20	08	19

For parents: R: resistant; S: susceptible; N: necrotic reaction;?: not tested.
For offsprings: Upper left: number of clones with uredia.
Upper right: number of clones with necrotic flecks.
Lower left: number of clones with both uredia and necrotic flecks.
Lower right: number of clones tested.

Results

A) Laboratory tests

Susceptibility or resistance of *Populus deltoides* to *Melampsora* spp was not always uniformly expressed within a clone. Some clones supported uredia only on some of their disks tested, and seemed resistant for the others. Generally those clones had very few uredia when susceptible. We assumed that any clone with at least 2 sporulating disks was susceptible.

A.1) *Melampsora larici-populina* race E1

Table 3 summarizes the number of clones, in each full sib family, exhibiting uredia or necroses.

Two resistant males (numbers 1 and 3) and female 1 generated quite resistant (without any uredia) half sib families, while we observed different segregations in the other families. If we consider female 2, 2 full sib families were resistant, 2 others segregated and the other 2 were totally susceptible.

The necrotic reaction appeared in nearly all the families where sporulation occured but especially with female parents 2 or 4. We note that female 4 and male 4 were the only 2 parents which displayed the necrotic reaction in our tests.

According to the segregations observed, at least 2 genes are involved in the genetic control of susceptibility to race E1.

Let us assign the following notations:

R (or r) and S (or s) are, respectively, the alleles of resistance and susceptibility. Capitals are assumed to be dominant. For simplicity, the 2 loci involved are always referred to in the same order and are separated by a comma. For example, genotype: R/R,s/R is homozygous for allele R (dominant) at the first locus, and is heterozygous at the second locus.

The 4 simplest hypotheses (H1 to H4) where relations of dominance are identical for the 2 independent loci may now be considered as follows:

1) H1: a clone is resistant when its genotype is: R/?, R/?.
2) H2: a clone is resistant when its genotype is: R/?, ?/? or ?/?, R/?.
3) H3: a clone is resistant when its genotype is: r/r, r/r.
4) H4: a clone is resistant when its genotype is: r/r, ?/? or ?/?, r/r.

Hypotheses were tested using Chi-Square tests of observed segregations.

1) Full sib families 43 and 45 had 25% susceptible clones. Male 4 was susceptible and the 2 female parents were resistant. Thus, the only 2-gene solution is that females 3 and 5 are both: R/R,R/s. But segregations observed in families 53 and 55 were different, so H1 is false.

2) If H2, then every susceptible clone is: s/s, s/s.

Full sib families 62 and 66 were totally susceptible, so male 6 is: s/s, s/s. Male 4 was also susceptible. But families 43 and 45 were significantly more resistant than families 63 and 65. H2 is false.

3) If H3, then every resistant clone is: r/r, r/r.

The cross between the 2 resistant parents, male 5 and female 5, produced some susceptible clones. H3 is false.

4) All the susceptible parents produced some resistant clones among their progenies. Thus, no parent is S/S, S/S. In family 42 all clones were susceptible, so each of the 2 parents would have 1 of the 2 resistant alleles (from the 2 loci). Let us note: female 2 = S/r, S/S.

At most, a full sib family from female 2 must have half of its offspring resistant. In families 12 and 32 all the clones were resistant. H4 is also false.

Thus, no simple 2-gene model can explain the segregations observed in the laboratory test with *M. larici-populina* race E1.

A.2) *Melampsora larici-populina* race E2 (*Table 4*)

Reactions to E2 were similar in the families previously used for testing the 4 hypotheses on race E1. So, again, no simple 2-gene model can explain the segregations observed in the laboratory test with *M. larici-populina* race E2.

Table 4. — Qualitative susceptibility to *Melampsora larici-populina* race E2.

F / M	1 R		2 S		3 R		4 R		5 R		6 S	
1 R	00	00	00	00	00	00	00	00	00	00	00	00
	00	20	00	20	00	20	00	20	00	20	00	02
2 ?	00	00	14	15	01	06	00	10	03	05	?	?
	00	20	11	19	01	19	00	10	03	12	?	00
3 R	00	00	00	00	00	00	00	00	00	00	00	00
	00	20	00	20	00	20	00	19	00	20	00	20
4 S	00	00	18	00	04	05	04	04	06	00	?	?
	00	20	00	18	01	20	02	08	00	20	?	00
5 R	00	00	07	07	00	04	01	07	04	02	?	?
	00	20	06	20	00	20	01	20	02	20	?	00
6 ?	00	00	06	00	10	00	10	00	10	02	19	05
	00	19	00	06	00	20	00	20	02	20	05	19

Table 5. — Qualitative susceptibility to *Melampsora allii-populina.*

F / M	1 R		2 S		3 R		4 R		5 R		6 S	
1 R	05	00	19	00	02	00	02	00	03	00	00	00
	00	20	00	20	00	20	00	20	00	20	00	02
2 ?	09	05	16	00	01	00	03	01	04	00	?	?
	03	20	00	19	00	19	01	10	00	12	?	00
3 R	03	01	11	00	04	00	02	01	02	00	02	00
	01	20	00	20	00	20	00	19	00	20	00	20
4 R	03	02	16	00	03	00	02	01	05	00	?	?
	01	20	09	18	00	20	00	08	00	20	?	00
5 R	01	01	15	00	00	00	03	01	07	00	?	?
	00	20	00	20	00	20	01	20	00	20	?	00
6 ?	04	00	06	00	02	01	04	00	05	00	06	01
	00	19	00	06	01	20	00	20	00	20	00	19

A.3) *Melampsora allii-populina (Table 5)*

The 4 hypotheses previously described were tested using Chi-Square tests of observed segregations.

1) If H1, every resistant parent is: R/?, R/?. Thus, the mating of a resistant parent with any other parent would yield at least a quarter of resistant progeny. In family 12, 19 clones of 20 were susceptible. H1 is false.
2) Females 2 and 6 were both susceptible. If H2, they would be: s/s, s/s. But crossings with males 3 and 6 gave different segregations, so they cannot have the same genotype. H2 is false.
3) Using the same approach as in 2), but for resistance this time, males 3, 4 and 5 were resistant but had different segregation ratios in their offsprings. H3 is false.
4) In H4, let us pose female 6 (susceptible) = s/?, S/?.

Crossing this female, the least susceptible family would have 25% susceptible offspring. Thus, if we consider family 36 (2/20), the only possibility for male 3 is: r/r, r/r. But it appears impossible, because crossings 31, 33 (or 34), with resistant females produced some susceptible progeny. H4 is false.

Thus, hypothesizing only 2 active genes for resistance/susceptibility, no simple genetic model can explain the segregations observed in the laboratory test with *M. allii-populina.*

A.4) Comparison between races and species

A.4.1) *Melampsora larici-populina* races E1 and E2 *(Table 6)*

In most families, reactions to races E1 and E2 were very similar. In full sib families 22, 25, 43, 52 and 55 very few clones would be resistant to one race but susceptible to the other one. However a significant exception to this was observed in family 64, which was completely susceptible to E1 and half resistant to E2.

A.4.2) *Melampsora larici-populina* race E1 and *M. allii-populina (Table 7)*

Except for male 4, the parental reactions to *M. larici-populina* and *M. allii-populina* were similar.

In 3 full sib families from female 5 (45, 55 and 65), segregations for *M. larici-populina* race E1 and for *M. allii-populina* were significantly linked. Probabilities of Chi-Square tests were respectively: 9%, 1% and 12%.

Table 6. — Comparison between segregations observed for *M. larici-populina* races E1 and E2.

F / M	1 R		2 S		3 R		4 N?,R		5 R		6 S	
1 R	00	00	00	00	00	00	00	00	00	00	00	00
	00	20	00	20	00	20	00	20	00	20	00	02
2 ?	00	00	12	01	01	00	00	00	03	01	?	?
	00	20	02	04	00	18	00	10	00	08	?	00
3 R	00	00	00	00	00	00	00	00	00	00	00	00
	00	20	00	20	00	20	00	19	00	20	00	20
4 S	00	00	18	00	03	00	04	00	06	00	?	?
	00	20	00	00	01	16	00	04	00	14	?	00
5 R	00	00	06	01	00	00	01	00	04	01	?	?
	00	20	01	12	00	20	00	19	00	15	?	00
6 ?	00	00	06	00	10	00	10	10	10	00	19	00
	00	19	00	00	00	10	00	00	00	10	00	00

Upper left: susceptible to both E1 and E2.
Upper right: susceptible to E1 and resistant to E2.
Lower left: resistant to E1 and susceptible to E2.
Lower right: resistant to both E1 and E2.

Table 7. — Comparison between segregations observed for *M. larici-populina* race E1 and *M. allii-populina.*

F / M	1 R		2 S		3 R		4 N,R		5 R		6 S	
1 R	00	00	00	00	00	00	00	00	00	00	00	00
	05	15	19	01	02	18	02	18	03	17	00	02
2 ?	00	00	11	02	00	01	00	00	02	02	?	?
	09	11	05	01	01	17	03	07	02	06	?	00
3 R	00	00	00	00	00	00	00	00	00	00	00	00
	03	17	11	09	04	16	02	17	02	18	02	18
4 SR	00	00	16	02	00	03	02	02	03	03	?	?
	03	17	00	00	03	14	00	04	02	12	?	00
5 R	00	00	06	01	00	00	00	01	04	01	?	?
	01	19	09	04	00	20	03	16	03	12	?	00
6 ?	00	00	06	00	02	08	04	16	04	06	06	13
	04	15	00	00	00	10	00	00	01	09	00	00

Upper left: susceptible to both E1 and allii.
Upper right: susceptible to E1 and resistant to allii.
Lower left: resistant to E1 and susceptible to allii.
Lower right: resistant to both E1 and allii.

A.4.3) *Melampsora larici-populina* race E2 and *M. allii-populina (Table 8)*

Because of the very similar reactions to both races, family 64 is the only one that needs to be considered.

B) Nursery tests

B.1) Observations 20 days after inoculation with *M. larici-populina* race E1 *(Table 9)*

As found previously in the laboratory test, all offspring of male 1 were resistant. Male 3 generated 1 clone with uredia and 12 clones with necrotic flecks in the nursery test. In contrast, some families (22, 43, 52 and 64) were less affected by the fungus in the nursery than in the laboratory. In this case, clones which supported sporulation in petri dishes often exhibited a necrotic reaction in the nursery. Thus, in family 52, all clones supporting sporulation in the laboratory had a necrotic reaction in the nursery. In the half-sib family from male 6, the number of clones with necrotic flecks was far greater in the nursery than in the laboratory. Except for clone 4515, which supported no uredia but had necrotic flecks in the

Table 8. — Comparison between segregations observed for *M. larici-populina* race E2 and *M. allii-populina* in family 64.

	4 R
6 R	3 7 1 9

Upper left: susceptible to both E2 and allii.
Lower left: resistant to E2 and susceptible to allii.
Upper rigth: susceptible to E2 and resistant to allii.
Lower right: resistant to both E2 and allii.

Table 9. — Qualitative susceptibility in nursery 20 days after inoculation with *M. larici-populina* race E1.

F / M	1 R		2 S		3 ?		4 ?		5 R		6 S	
1 R	00	00	00	00	00	00	00	00	00	00	00	02
2 ?	00	02	01	09	00	01	00	02	00	00	?	?
3 R	00	00	00	05	00	00	01	04	00	03	00	00
4 SN	05	06	17	13	01	02	04	06	05	06	?	?
5 R	00	00	01	10	00	01	00	04	00	03	?	?
6 ?	02	06	06	00	10	09	14	19	10	05	12	15

Left: number of clones with uredia.
Right: number of clones with necrosis.

Table 10. — Qualitative susceptibility at the end of the growing seasons.

F / M	R R	R 1	S S	S 2	? ?	? 3	? ?	? 4	R R	R 5	S S	S 6
RS?	00	06	02	19	01	04	01	07	01	12	00	02
R1	02	20	14	20	00	20	03	20	01	20	00	02
? ?	00	06	07	18	04	04	00	05	02	06	?	?
?2	06	20	19	19	07	19	09	10	05	12	?	00
RR	00	01	00	20	00	04	00	04	01	07	00	01
R3	04	20	15	20	07	20	07	19	05	20	12	20
SS?	05	05	16	17	06	03	01	05	06	07	?	?
S4	08	20	18	18	12	20	06	08	09	20	?	00
RR	00	02	05	19	01	01	00	03	01	11	?	?
R5	05	20	19	20	05	20	09	20	13	20	?	00
? ?	00	08	02	05	05	06	04	15	02	09	02	06
?6	11	19	06	06	12	20	20	20	13	20	19	19

Upper left: number of susceptible clones to *M. larici-populina* in 90.
Upper right: number of susceptible clones to *M. allii-populina* in 90.
Lower left: number of susceptible clones in 91.
Lower right: number of clones tested.

nursery, families 44, 45, 63 and 65, yielded exactly the same patterns of segregation in the two tests.

But 2 families (41 and 61) included susceptible clones in the nursery test while only necrotic flecks were observed in the laboratory. In family 41, among the 5 susceptible clones in the nursery, 4 had necrotic flecks in the laboratory.

B.2) *Melampsora* spp. development at the end of the growing seasons (*Table 10*)

In 1990, the 2 rust species were sometimes found on the same clone, but *M. allii-populina* was found more often than *M. larici-populina*. Comparisons with results obtained in the laboratory were not easy because, first, clones that were actually susceptible to both species may have been scored as susceptible to only 1, especially when susceptibility levels were very different from 1 species to the other, or when the pathogen races were antagonistic. Second, some mistakes may have been made when identifying species by microscope.

Major differences between the 2 tests must be noted:

- in the half sib family from male 1, 5 clones were susceptible to *M. larici-populina;*
- in family 41, 5 clones were susceptible to *M. larici-populina*. Four of those belonged to the 5 clones supporting sporulation 20 days after inoculation in nursery;
- often, clones found to be susceptible to *M. larici-populina* and resistant to *M. allii-populina* in the laboratory test, but without uredia of *M. larici-populina* in the nursery trial, may have had uredia misidentified;
- very few uredia identified as *M. allii-populina,* were found on males 1 and 4;
- finally, in families resistant to *M. larici-populina,* reactions to *M. allii-populina* were often different from one test to the other. The number of susceptible clones per family was generally greater in the nursery test. For example, in families 15, 32 and 35, the ratios number of susceptible clones in laboratory: number of susceptible clones in nursery were respectively 3:12, 11:20 and 2:7. Moreover, when segregations occured, all types of clonal reaction were encountered: clones resistant in all tests; clones resistant in the

laboratory and susceptible in the nursery; clones susceptible in the nursery and resistant in the laboratory and clones susceptible in all tests.

In 1991, there was no identification of the *Melampsora* species found in the nursery. Obviously, the 2 species were present: *M. larici-populina* (race E1) which was used as inoculum and also *M. allii-populina* (observed on controls in September) from natural infection.

Once again, if we consider the families resistant to *M. larici-populina,* in all the full sib families involving male 1, the number of susceptible clones in 1991 was lower than in 1990 or than in the laboratory test. On the contrary, in the families 31, 33, 34, and 36 (generated from male 3) the number of susceptible clones was greater than previously. In family 36, only 8 clones were resistant compared to 19 and 18 in the 1990 nursery test and in the laboratory test, respectively. Within full sib families which segregated, susceptible clones were often different from 1 test to the other. Thus, among the 7 susceptible clones of family 33, in the 1991 nursery test, only 1 was among the 4 susceptible clones in 1990, and only 2 were among the 4 susceptible clones in the laboratory test.

Note that among the 8 susceptible clones within family 41, 5 of these were susceptible to race E1 20 days after inoculation. According to the race identification in the 1990 test, 4 of these 5 clones were resistant to *M. allii-populina.*

Discussion

A) Control of rust species and races

The leaves that we used for the laboratory tests were collected from the nursery trial so we cannot be sure that no other inoculum had infected them before gathering. However, 2 observations lead us to believe that the risk of contamination is near 0 for *M. larici-populina* race E1 tests and also minimal for race E2 and *M. allii-populina* tests. First, controls susceptible to E2, E3 or *M. allii-populina* did not show any rust development until September. This fact was confirmed by J. PINON's determinations of races on leaves from the INRA nursery. At the end of September the rust occurence according to races was as follows: 94% E1, 6% E2 and <1% for E3. Race E1 was highly predominant and was probably the only inoculum in the INRA nursery until the end of August 1991. Second, until September, rust development in the experimental trial was low, especially because of the dry weather, and was confined to the lower part of the trees. Leaves were collected near the top (about 7 to 10 leaves from the apex), which reduced the possibility of contamination. Finally, the very few disks (3 out of 14500) on which sporulation occured earlier than expected in the laboratory tests, were eliminated from our studies.

B) Qualitative versus quantitative reaction

Qualitative resistance is generally defined as the absolute absence of any sporulation on the leaf tissue, while quantitative resistance considers the different levels of infection observed. These definitions do not directly refer to the number of genes involved in resistance.

In our experiments, some clones supported uredia on some of their disks. Heterogeneity of the inoculating spray could explain this behaviour. Accordingly, we decided to consider as susceptible those clones with sporulating disks in at least 2 multidish compartments. But there is no *a priori* evidence for existence of a real qualitative resistance.

In the quantitative analysis of the same genetic material (PICHOT and TEISSIER DU CROS, 1993) estimation of the mean number of uredia per disk for each susceptible clone including clonal and parental effects, generally produces a continuous distribution of the susceptibility level (*Figure 2*). Except for families 42, 62 and 63, the more resistant clones in the different families have nearly 0 uredia per disk. That is why, in the families where segregations occur, we can't reject the hypothesis of a regular quantitative resistance level leading to the absence of sporulation. Of course, qualitative and quantitative resistance could interact, so qualitative resistance could be partially or totally masked by quantitative reaction. To improve the level of reliability of the qualitative resistance we observed in our experiments, other tests could be conducted with various inoculum pressures.

Four specific clones (2227, 5224, 5512 and 6527) were much more susceptible to rust than the other clones of their respective families. Additional isozyme or RFLP analysis could clarify the parentage of these clones.

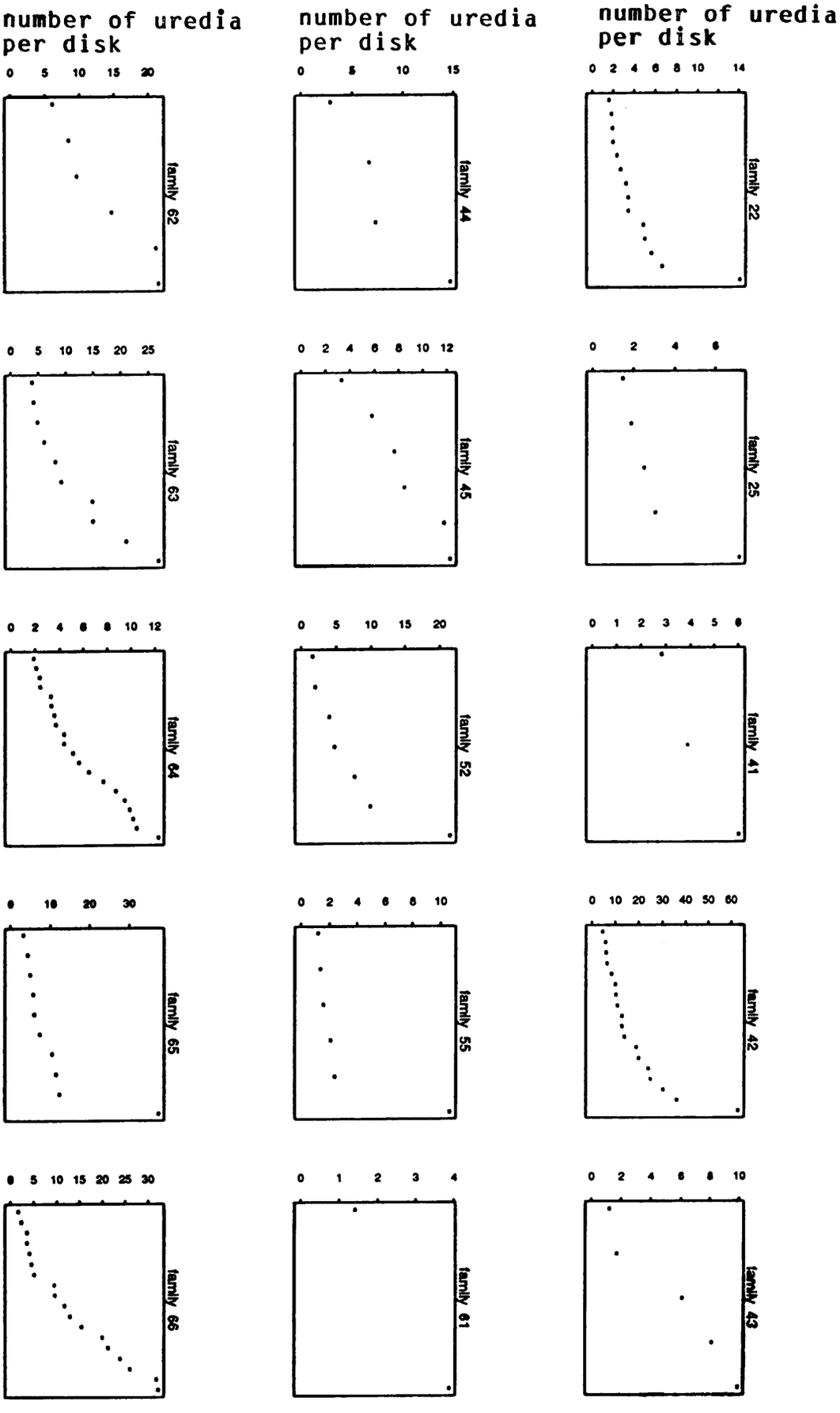
C) Evidence for new virulence genes in a gene-for-gene model for M. larici populina

The INRA forest pathology laboratory at Nancy has concentrated its research on studying variability of Melampsora leaf rust (MASSON, 1990; PINON et al., 1987; PINON and PEULON, 1989; VIENOT, 1989; EL KARKOURI, 1991). Two new pathotype races of *M. larici populina* have been described (EL KARKOURI, 1991), and designated G6 and B6. These are added to the 3 others races previously defined as: E1, E2 and E3. Four sets of clones, differentiated by their qualitative rust reaction to the 5 races, led KARKOURI assume that 4 genes for virulence (V1 to V4) are involved in these races. Thus, race E1 would carry genes V3, V4; race E2 would be V1, V3, V4; race E3 would be V2, V3, V4; race G6 would be V2 and race B6 would be V4. As it concerns race E2, we also conclude that there are at least 3 genes involved in the host-pathogene interactions, but we have obtained essentially the same results with race E1. Moreover, KARKOURI's model explained why no clone of poplar had previously been found to be susceptible to race E1 but resistant to race E2 or race E3; in our experiments however, such clones were encountered, especially in family 64. Therefore, we must assume that race E1 contains another virulence gene (V5) which is absent in race E2.

A gene-for-gene relationship between poplar and *M. larici-populina* race E1 can be tested with the hypothesis of a genotype of V3, V4, V5 for race E1. If we assume that resistance is dominant over susceptibility, and that genes for resistance are independent, then the segregations observed in the 6x6 factorial mating tested in our experiments cannot be explained without at least another gene such as V6, which would be common to both race E1 and race E2. With such a relatively large number of genes, more clones per family would need to be tested to determine the genotypes of the parents.

Further tests with races G6 and B6 could be more easily interpretable because of the lower number of virulence genes apparently involved (may be only one according to KARKOURI) Races are identified by specific host reactions; thus, new races cannot be discovered without tests on a broad spectrum of poplar clones. Races of *M. larici populina* could be far more numerous than the few actually

Figure 2. — Clonal mean number of uredia per disk (clones are sorted according to rust susceptibility).



described. With 4 genes for virulence, 16 races are possible, while 64 races could exist with 2 additional virulence genes. Moreover new races could arise through mutations; PRAKASH and HEATHER (1986a) found that *M. medusae* was quite mutable by gamma irradiation.

In the Canberra area of Australia, SHARMA and HEATHER (1976, In: CHANDRASHEKAR and HEATHER, 1980) isolated 5 races of *M. larici-populina* with no references to the races we previously mentionned.

D) Laboratory and nursery tests

Segregations obtained in the nursery 20 days after inoculation must be interpreted with great caution. The success of the artificial inoculation clearly depended on the time of application of the inoculum. It took 3 persons 3 hours to accomplish the whole test. The spore suspension was renewed every 20 minutes, and to optimize germination of spores on the foliar tissues, we chose cloudy weather. It began to rain as we were inoculating the central part of block 1, so we delayed inoculation until the rain stopped, about 10 minutes later. Twenty days later, the sporulation on controls (Robusta, I214, I45 — 51) and also on clones in the factorial mating experiment was far greater in block 1, and especially in the part of this block that was inoculated prior to the low rainfall. A logical explanation of this difference is the higher relative humidity at the beginning of the inoculation. Some clones, which were inoculated after the rainfall, may have been considered as resistant thought they are not.

Symptoms of rust infection were sometimes different from one test to another. Most often, clones which supported sporulation in the nursery also supported uredia in multidish culture (except for family 41), but some susceptible clones in the laboratory test produced necrotic flecks in the nursery trial. Development of discolourations and necrotic flecks seem to be especially influenced by growth conditions. These symptoms, often encountered in *P. deltoides* reactions, make analyses of segregations more difficult. Sporulation occured more easily in the controlled environment where temperature and relative humidity were optimum. These results are in agreement with interactions between growth conditions and rust development reported by many other authors. In laboratory tests, necrotic flecks on *P. deltoides* inoculated with *M. larici populina* are more frequent at 25° C than at 20° C or 12° C, and more uredospores are produced at 20° C than at 12° C (or 25° C CHANDRASHEKAR and HEATHER, 1981). The same trend was observed for most of the clones tested by SINGH and HEATHER (1982) with *M. medusae* THUM at 25° C, 20° C, and 15° C.

Success of field trial inoculation depends both on season of inoculation and on the cultivars tested. Thus, in 1989 in the CEMAGREF nursery (Nogent sur Vernisson), artificial rust inoculation on the clone UNAL with *M. larici-populina* was unsuccessful in June, and produced necrotic flecks and leaf fall in September, while natural rust development was observed at the end of the growing season (DEBOISSE, personnal communication).

Ignoring races, PINON (1991) finds an identical qualitative reaction in natural conditions and in laboratory tests for susceptibility to *M. larici-populina*. In our experiment, we observed a good agreement between the tests with race E1 in both nursery and laboratory cultures. Among the 435 resistant clones in the laboratory study, 22 produced necrotic flecks and only 2 supported uredia. Thirteen among these 24 clones were derived from male 3. But if we consider the 156 clones supporting uredia or necrotic flecks in the laboratory test, 36 of these were resistant in nursery (17 supported uredia in laboratory).

E) Possible linkage between M. larici-populina and M. allii-populina

According to the segregations observed in families 45, 55 and 65, *M. larici-populina* races E1 or E2 may be genetically linked to the isolate of *M. allii-populina* that we used. Some new tests must be carried out to determine whether a pleiotropic gene or a simple linkage is involved in this reaction.

F) Variability in M. allii populina

Qualitative rust infection at the end of the growing seasons gives us some information relative to pathogen variability in the nursery trial.

Considering families resistant to *M. larici-populina* (races E1 and E2 in the laboratory test), we must conclude that the natural inoculum in 1990 had a broader spectrum than did the mono-uredial *M. allii-populina* isolate we used in the laboratory, but also did not overlap it. In family 21, among the 9 susceptible clones in the laboratory test, 5 were resistant in the nursery. According to our previous observations, in 1991 other pathotypes were present in the nursery. There was no correlation between the 3 patterns of segregation (nursery tests in 1990 and 1991 and laboratory test in 1991); none of them included any other one.

Major variability of *M. allii-populina* was reported by KARKOURI (1991) who defined 5 pathotypes (on 14 cultivars), from 21 isolates. Except for 1 of them, these 5 races were different from the 9 others previously described by MASSON (1990).

Races (defined by host-pathogen specificity) of *M. allii-populina* are numerous, and the variability of the natural inoculum can be different from one year to the other. For this reason, any experiment on the genetic determinants of susceptibility to *Melampsora* spp. requires laboratory tests with very pure mono-uredial isolates.

Numerous races are reported in *Melampsora medusae* also. In a factorial test with 19 isolates and 9 clones, 8 races were identified (PRAKASH and THIELGES, 1987). According to PRAKASH and HEATHER (1986b), among 6 races of *M. medusae* inoculated on an F1 progeny, resistance was inherited as dominant in 3 races, recessive in 2 and quantitative, additive in 1. The number of genes involved was 1 or 2 (unknown for the quantitative distribution). However, the possibilities for the identification of genes was greatly reduced by testing only 1 progeny in that study.

Conclusion

Studies of other host-pathogen systems have shown that the number of resistance genes generally increases as the natural variability of both host and pathogen are explored. Thus, in a gene-for-gene model, at least 9 resistance genes have been defined in cultivars of *Triticum aestivum* from India and Pakistan (SINGH and GUPTA, 1991). A review of resistance of *Linum* spp. to *Melampsora lini* relates at least 34 resistance genes. A resistance inhibitor gene has also been found in some rust strains (ISLAM and SHEPHERD, 1991). Numerous resistance genes have been reported in other hostpathogen systems (maize and maize rust, barley and powdery mildew, lettuce and downy mil-

dew), and genetic maps of some of these have been obtained through molecular markers.

So, we can reasonably assume that many resistance genes will be discovered within species of poplar as well as virulence genes in *Melampsora* species.

To reduce the probability of major damage caused by new rust races, race-specific, totally resistant clones should be avoided in selection. Knowledge of the gene-for-gene relation in the qualitative reaction to rust will allow poplar breeders to select quantitatively resistant clones that should be buffered to attain acceptable equilibria with heterogeneous, ambient rust inocula.

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Susceptibility of P. deltoides Bartr. to Melampsora larici-populina and M. allii-populina

II. Quantitative Analysis of a 6x6 Factorial Mating Design

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Summary

Quantitative susceptibility to *Melampsora* spp. was observed in a 6x6 *P. deltoides* factorial mating design. Artificial inoculation in the nursery proved inefficient for estimating partial resistance. However, in the laboratory test, susceptibility was efficiently partitioned into number of uredia size of uredia and latent period, and these 3 traits were correlated to natural rust infestation in the nursery. Partial resistance to rust caused by *Melampsora larici-populina* was highly heritable in *P. deltoides*. No correlations between growth and susceptibility were observed in our study. Additive variances estimated from the half sib effect overpassed additive variances estimated from clonal effect in the full sib families, which suggested that genetic differentiation occured between stands within the natural range of *P. deltoides*. The large amount of intra- and inter-specific partial rust resistance must be futher studied and categorized to better understand the *Populus-Melampsora* system and to optimize the use of these natural resources in poplar improvement.

Key words: *Populus deltoides*, *Melampsora larici-populina*, partial resistance, uredia, latent period, vigour, heritability, genetic correlation, factorial mating, natural variability.

Introduction

This quantitative analysis of *P. deltoides* susceptibility to rust follows a qualitative report based on the analysis of the same *P. deltoides* mating design (Pichot and Teissier du Cros, 1993). Quantitative polygenic (or horizontal resistance should be preferred over qualitative mono or oligogenic (or vertical) resistance, especially for forest

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