Genetic architecture of qualitative and quantitative *Melampsora larici-populina* leaf rust resistance in hybrid poplar: genetic mapping and QTL detection

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Summary

• In order to elucidate the genetic control of resistance to *Melampsora larici-populina* leaf rust in hybrid poplars, a *Populus deltoides* × *P. trichocarpa* F_1 progeny was analysed for qualitative and quantitative rust resistances.

• This progeny was evaluated for three components of quantitative resistance (latent period, uredinia number and uredinia size) to seven *M. larici-populina* strains in controlled conditions, and for one component of field susceptibility (rust colonization on the most infected leaf).

• One qualitative resistance locus inherited from *P. deltoides*, R_1, was localized on the genetic map. It segregates 1 : 1 in the F_1 progeny and is effective against four of the studied strains. QTL analysis was performed separately on R_1 and r_1 genotype subsets. An additional detection was conducted on the entire F_1 progeny for the three strains able to overcome R_1 and for MAX2. A total of nine QTLs were detected. Two had large, broad-spectrum effects. One (R_{US}) is inherited from the *P. trichocarpa* parent; the other is inherited from *P. deltoides and* colocalized with R_1. Seven QTLs had only limited and specific effects. Significant interaction effects were detected mainly between the two major QTLs.

• Implications of these results for durable resistance breeding strategies, and possible benefits from the *Populus* genome sequence, are discussed.

Key words: *Melampsora*, *Populus* (poplar), QTL mapping, qualitative resistance, quantitative resistance.

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Introduction

Melampsora larici-populina Kleb. poplar leaf rust is considered one of the 10 most important sanitary problems threatening the French forests (Nageleisen, 2000). Durable resistance to this pathogen is one of the main objectives of poplar breeding in Europe. Although both qualitative and quantitative forms of *M. larici-populina* resistance have been described, past selection programmes focused on qualitative resistance. Several interspecific hybrids (principally *Populus deltoides* × *P. trichocarpa* or *P. deltoides* × *P. nigra*), with qualitative resistance inherited from North-American species *P. deltoides*, have been selected and widely grown in monoclonal stands. Such simplistic breeding and growing strategies, combined with the obviously high adaptive potential of the pathogen, led to successive resistance breakdowns. As they had no secondary line of defence, most cultivars remained completely helpless before the epidemic spread of newly emerged virulent strains of the pathogen (Frey *et al.*, 2005). Economical and ecological costs of the disease, and of chemical treatments, make it urgent to explore other breeding alternatives. As *P. deltoides* × *P. trichocarpa* hybrids are appreciated by growers for their growth potential and wood quality, an important concern is the possibility to select for durable *M. larici-populina* resistance in this material, possibly by placing more emphasis on quantitative forms of resistance.

The genetic determinism of several *M. larici-populina* qualitative resistances inherited from *P. deltoides* has been studied in *P. deltoides* × *P. trichocarpa* and *P. deltoides* × *P. nigra* segregating progenies – *P. trichocarpa* and *P. nigra* being two species in which no qualitative resistance has been detected so far (Legionnet *et al.*, 1999; Pinon & Frey, 2005). As commonly observed with qualitative resistance, these studies revealed relatively simple genetic determinism. One or two genes control qualitative resistance to a given set of strains of the pathogen, and some of these genes appear to be organized in a cluster (Lefèvre *et al.*, 1998). Molecular markers have been identified that are closely linked to two qualitative resistance loci (Cervera *et al.*, 1996), and NBS-LRR resistance gene analogues have been found in the vicinity of one of them (Zhang *et al.*, 2001).

Genetics of the quantitative resistances inherited from *P. deltoides* and *P. trichocarpa* have been studied in intra- and interspecific progenies both in the laboratory (using inoculated leaf disc bioassays) and in the field (Pichot & Teissier du Cros, 1993; Lefèvre *et al.*, 1994; Lefèvre *et al.*, 1998; Dowkiw *et al.*, 2003; Dowkiw & Bastien, 2004). Interestingly, although quantitative resistance is often defined in opposition to qualitative resistance as being polygenic and race nonspecific, most findings refuted these assumptions for *M. larici-populina* resistance. Moreover, statistical associations have been observed between qualitative and quantitative resistances to *M. larici-populina* (Dowkiw *et al.*, 2003; Dowkiw & Bastien, 2004). In a reference *P. deltoides* × *P. trichocarpa* F_1 progeny, where both resistance types are segregating, quantitative *M. larici-populina* resistance appears to be mostly controlled by only two genetic factors (Dowkiw & Bastien, 2004). One is inherited from the *P. deltoides* parent and is associated – either by pleiotropy or by linkage – with a defeated qualitative resistance gene, R_1. The other is inherited from *P. trichocarpa* and is designated as R_{US}, as its presence was inferred from bimodal distributions of genotypic means for uredinia size (US) in the laboratory. Classic segregation studies of both factors allowed quantification of their respective and combined effects, both in the laboratory against three *M. larici-populina* strains, and in the field (Dowkiw & Bastien, 2004). However, these studies raised two important questions without being able to answer them: (i) what is the nature of the genetic relationship between these resistance factors? and (ii) are there other resistance factors with more 'minor' effects controlling *M. larici-populina* quantitative resistance in this progeny?

Only a more refined analysis involving QTL mapping, as presented here, could help answer these questions. To our knowledge this is the first report on QTL mapping for *M. larici-populina* quantitative resistance in poplar. It is also the first report on a quantitative resistance study involving a large number of *M. larici-populina* strains (seven in the laboratory, and a natural mixture in the field) on a large poplar progeny (343 genotypes). This material was cloned, allowing accurate estimates of genetic variances and allowing the testing of strain specificity. This leads to valuable information on the spectrum of action of the identified resistance QTLs and thus on their potential durability.

Materials and Methods

Plant material

Poplar material consisted of a cloned 343 F_1 progeny from an interspecific *P. deltoides* × *P. trichocarpa* cross. The two parents used for hybridization are representative of their respective species in terms of *M. larici-populina* resistance. The *P. deltoides* female parent (73028-62) is a source of both qualitative and quantitative resistance, whereas only quantitative resistance has been observed in the *P. trichocarpa* male parent (101-74) (Goué-Mourier *et al.*, 1996; Dowkiw & Bastien, 2004).

A qualitative resistance gene, inherited from the *P. deltoides* parent and originally identified using *M. larici-populina* strain 93ID6, segregates 1 : 1 in the studied progeny ($\chi^2 = 2.82$, $P > \chi^2 = 0.09$). Analysis of 13 *M. larici-populina* strains led to the conclusion that virulence 1 confers the ability to overcome this qualitative resistance. For this reason it was decided to name the corresponding qualitative resistance gene R_1. Its presence has been demonstrated as having a beneficial effect on quantitative *M. larici-populina* resistance in the laboratory, either by linkage or by pleiotropy (Dowkiw *et al.*, 2003; Dowkiw & Bastien, 2004).

Dowkiw & Bastien (2004) identified and characterized another major genetic factor controlling quantitative resistance in this progeny, R_{US}, inherited from the *P. trichocarpa* parent. Its presence could be inferred from a clear bimodal distribution of uredinia size genotypic values with strain 98AG69 in the laboratory (US_{98AG69}). Genotypes with $US_{98AG69} > 2.5$ were consequently defined as r_{US}, and genotypes with $US_{98AG69} \leq 2.5$ as R_{US}. R_{US} segregates 1 : 1 ($\chi^2 = 0.40$, $P > \chi^2 = 0.53$; Dowkiw & Bastien, 2004) with a major effect on several components of quantitative resistance to three *M. larici-populina* strains in the laboratory – especially US – and also on field resistance.

Material used for laboratory and field evaluation was grown as described by Dowkiw & Bastien (2004). All genotypes underwent phenotypic evaluation for at least one of the traits studied. However, the number of genotypes studied differed from trait to trait, depending on compatibility patterns and plant material availability, and varied from 145 to 340 (Table 1).

Fungal material

Variability within the *M. larici-populina* species is described in terms of virulences, a virulence being defined here as a qualitative attribute of the pathogen – the ability to infect a given host genotype. Eight virulences have been defined so far based on a set of eight discriminant poplar genotypes (Pinon & Frey, 2005). Each combination of these eight virulences is

Table 1 Summary statistics and broad sense heritabilities at the genotypic level (h^2_{genot}) for latent period (LP, d), number of uredinia (UN), and uredinia size (US) expressed in the laboratory for seven *Melampsora larici-populina* strains and for field susceptibility (MAX2) in a full-sib *Populus deltoides* × *P. trichocarpa* F_1 progeny

Strain (virulences)	Trait	Mean	Q1–Q3*	Mean 73028-62†	Mean 101-74‡	h^2_{genot} ±SD	Studied number of compatible genotypes	
							R_1	r_1
93ID6 (3-4)	LP	8.8	8.6–8.9	No uredinia	10.3	0.68 ± 0.04	0§	151
	UN	12.5	9.3–15.0	No uredinia	12.3	0.63 ± 0.05		
	US	2.7	2.0–3.6	No uredinia	1.9	0.93 ± 0.01		
99D40 (2-4)	LP	8.8	8.6–9.0	No uredinia	10.4	0.55 ± 0.06	0§	145
	UN	12.2	8.8–14.4	No uredinia	4.2	0.54 ± 0.06		
	US	2.7	2.0–3.6	No uredinia	1.8	0.94 ± 0.01		
98AG31 (3-4-7)	LP	9.0	8.6–9.4	No uredinia	10.4	0.79 ± 0.03	0§	151
	UN	19.5	15.1–23.5	No uredinia	17.2	0.60 ± 0.07		
	US	2.7	1.8–3.6	No uredinia	2.2	0.93 ± 0.01		
93CV1 (1-3-4-5)	LP	9.5	8.1–10.8	No uredinia	8.8	0.90 ± 0.01	189	145
	UN	5.8	0.6–9.4	No uredinia	7.1	0.93 ± 0.01		
	US	2.4	1.8–3.0	No uredinia	2.4	0.91 ± 0.01		
98AG69 (1-3-4-5-7)	LP	9.5	8.7–10.0	9.9¶	9.6	0.89 ± 0.01	188	145
	UN	10.6	6.8–14.2	0.5¶	4.4	0.84 ± 0.01		
	US	2.4	1.5–3.2	2.8¶	2.0	0.95 ± 0.01		
00E19 (3-4-5-7-8)	LP	9.7	8.7–10.6	No uredinia	11.3	0.85 ± 0.02	0§	145
	UN	4.6	2.2–6.4	No uredinia	1.9	0.84 ± 0.02		
	US	2.2	1.4–3.0	No uredinia	2.0	0.92 ± 0.01		
98AR1 (1-3-4-5-7-8)	LP	10.0	9.3–10.5	No uredinia	10.2	0.81 ± 0.02	189	151
	UN	5.8	3.8–7.6	No uredinia	4.3	0.60 ± 0.03		
	US	2.5	1.8–3.0	No uredinia	2.2	0.89 ± 0.01		
Field natural inoculum	MAX2	4.0	3.5–4.4	3.0	3.2	0.86 ± 0.01	123	172

*First (Q1) and third (Q3) quartiles of mean values.
†Mean values of the *P. deltoides* female parent.
‡Mean values of the *P. trichocarpa* male parent.
§All completely resistant.
¶Uredinia were observed on only four of the 15 leaf discs.

referred to as a pathotype. The inocula used for the laboratory experiments consisted of urediniospores from seven single-uredinial *M. larici-populina* strains belonging to seven different pathotypes (Table 1). Three of these strains possess virulence 1, and hence are able to infect both r_1 and R_1 genotypes (Dowkiw & Bastien, 2004). Each inoculum was increased and stored as described by Dowkiw *et al.* (2003).

Laboratory quantitative resistance assessment

Details of the protocol used in the laboratory can be found in Dowkiw *et al.* (2003). Briefly, inoculations were conducted on 3 cm diameter excised leaf discs floated upside down on distilled water. Experimental designs were five randomized complete block designs where each F_1 genotype was represented by one disc per block, and each parent by three discs per block. A urediniospore suspension (30 mg spores L^{-1}) was sprayed over the discs using a hand atomizer. The number of urediniospores deposited per leaf disc has been estimated by scattering Petri dishes containing solid water agar (20 g L^{-1}) in the experiments before inoculation, and by counting the number of deposited urediniospores using a microscope. The estimated values were: 38 (SD = 12) for 93ID6; 108 (SD = 27) for 99D40; 52 (SD = 18) for 98AG31; 74 (SD = 19) for 93CV1; 93 (SD = 25) for 98AG69; 97 (SD = 27) for 00E19; 46 (SD = 18) for 98AR1.

The discs were kept for 14 d under controlled conditions in a growth chamber at 15°C. Three epidemiological components were measured: latent period (LP), measured on a half-day basis; uredinia number 13 d after inoculation (UN); and uredinia size (US) scored 14 d after inoculation on a 1 (small) to 5 (large) ranking scale.

Field resistance assessment

Details of the protocol used in the field can be found in Dowkiw & Bastien (2004). Briefly, the experimental design involved six randomized complete blocks where each F_1 genotype and each parent was represented by one ramet. Field susceptibility to the surrounding natural mixture of *M. larici-populina* strains was evaluated the second year after planting, after the plants had been cut down at ground level (1 yr old

Table 2 Available genotyping data on the full-sib *Populus deltoides* × *P. trichocarpa* F_1 progeny

	Number of markers					
	Map construction*		QTL detection*			
Marker type	$n < 85$	$85 \le n \le 90$	$n < 150$	$150 \le n \le 250$	$250 < n \le 343$	Total
AFLP	21	140	71	37	53	161
RAPD	122	23	145	0	0	145
Microsatellite	2	33	3	7	25	35
RFLP	46	1	47	0	0	47
SNP	0	1	0	0	1	1
Phenotypic (R_1, R_{US})	0	2	0	0	2	2
Total	191	200	266	44	81	391

*n = number of genotyped individuals.

shoots on 2 yr old roots from cuttings). It was measured as the density of sporulating uredinia on the most infected leaf using a 1–6 scale: 1 = no uredinia; 2 = 1–10 uredinia; 3 = 11 uredinia to 25% of leaf area; 4 = 25–50% of leaf area; 5 = 50–75% of leaf area; 6 = >75% of leaf area. This trait was measured three times during the growing season (in June, July and August), but only the second measurement (MAX2) is studied here, given its higher heritability and the presence of good correlation coefficients between all three measurements (Dowkiw & Bastien, 2004). Characterization of the pathotype composition of the field *M. larici-populina* inoculum is presented elsewhere (Dowkiw & Bastien, 2004). Strains possessing virulence 1 predominated (>80%), so most of the natural inoculum was thought to be able to infect the whole progeny studied.

Molecular marker genotyping

Genotyping was conducted using restriction fragment length polymorphism (RFLP), sequence tagged site (STS), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and microsatellite markers in a first subset of 90 genotypes. Microsatellite and AFLP genotyping was then extended to 253 supplementary genotypes (Table 2). RFLP and STS genotyping was conducted as described by Bradshaw & Stettler (1993). RFLP markers were coded by the letter P followed by a three- or four-digit number (e.g. P1273). RAPD genotyping was performed according to Villar *et al.* (1996). RAPD markers were coded by the Operon kit primer name followed by the molecular weight of the polymorphic band (e.g. M02-1150).

AFLP genotyping was performed according to the AFLP Analysis System I kit (Invitrogen/Life Technologies Inc., Cergy-Pontoise, France) instructions for γ-[^{33}P]ATP labelling and 6% denaturing polyacrylamide gel electrophoresis. Incubation times for digestion and labelling were extended by 1 h each. Electrophoresis was performed using a Sequi-Gen GT Nucleic Acid Electrophoresis Cell (Bio-Rad, Ivry-sur-Seine, France) at 90 W for 3 h. AFLP markers were named after the code of the *Eco*RI/*Mse*I combination from the kit followed by the band ranking on the gel (e.g. E5M5-7).

All microsatellite markers belong to the PMGC series (www.ornl.gov/sci/ipgc/ssr_resource.htm), except for one, ORPM277 (Tuskan *et al.*, 2004). Two labelling methods were used for PMGC primers. On the first subset of 90 genotypes, primers were labelled with γ-[^{33}P]ATP. The kinase reaction was performed for 2 h at 37°C in a total volume of 10 µl containing 10 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 500 ng forward primer, 1 µl of γ-[^{33}P]ATP (3000 Ci mmol^{-1}; Amersham Biosciences, Saclay, France), and 0.1 unit T4 kinase (Amersham Biosciences, Saclay, France). The kinase was inactivated for 10 min at 70°C. Amplifications were conducted in a total volume of 10 µl containing: 50 ng DNA, 10 mM Tris–HCl, 50 mM KCl, 2 mM $MgCl_2$, 200 µM of each dNTP, 10 ng of each primer, and 0.25 unit Taq polymerase (QBiogene, Illkirch, France). The amplification program was: 94°C for 3 min; 25 cycles of 94°C for 30 s, 52, 54 or 60°C (depending on the primer sequence) for 30 s, 72°C for 1 min; and a final elongation step at 72°C for 6 min. PCR products were separated on 6% polyacrylamide gels with 7 M urea and 1× Tris-borate buffer. The DNA fragments were recorded on Kodak Biomax MR film. For the other 253 F_1 genotypes, the γ-[^{33}P]ATP-labelled primer was replaced with a fluorochrome-labelled primer. Amplification products were detected by laser scanning during electrophoresis using a 16-capillary ABI 3100 sequencer (Perkin-Elmer/Applied Biosystems, Courtaboeuf, France). Samples containing 3 µl PCR product, 0.3 µl Genescan 400HD-ROX size standard (Perkin-Elmer/Applied Biosystems) and 7 µl formamide were heated at 95°C for 3 min and loaded in 36 cm long capillaries filled with POP4 polymer (Perkin-Elmer/Applied Biosystems). For ORPM277 only the fluorochrome-labelled primer method was used.

Heritability and clonal means calculation

Phenotypic data were analysed using S-PLUS version 3.4 release 1 for Sun SPARC (Statistical Sciences, MathSoft Inc.,

Seattle, WA, USA). When necessary, individual data were transformed using the Box–Cox procedure (Box & Cox, 1964) to ensure homoscedasticity and normality of the residuals from the following ANOVA model:

$$Y_{ij} = \mu + B_i + G_j + \varepsilon_{ij} \quad \text{Eqn 1}$$

where μ is the grand mean, B is the block effect (fixed) and G is the genotype effect (random). Accordingly, all LP, UN and US values were transformed as LP^{-2}, $UN^{1/2}$ and $US^{1/2}$. No transformation was needed for MAX2. For all traits measured, individual values were adjusted to the block effects when significant ($P < 0.05$), and clonal means were calculated from adjusted values.

Restricted maximum likelihood estimates of genetic, block and residual variance components (σ^2_G, σ^2_B and σ^2_ε, respectively) were computed, and broad sense heritabilities were calculated at the genotypic level as

$$h^2_{\text{genot}} = \sigma^2_G/(\sigma^2_G + \sigma^2_\varepsilon/n) \quad \text{Eqn 2}$$

where n is the average number of replicates per genotype. Standard deviations (SD) of h^2_{genot} were derived from classic estimation of SD for a ratio x/y where $x = \sigma^2_G$ and $y = \sigma^2_G + \sigma^2_\varepsilon/n$.

Map construction and QTL detection

In order to generate reliable maps, map construction was based on the first subset of 90 genotypes where there is only limited missing marker data. Computations were made with MAPMAKER version 3.0b (Lander & Botstein, 1989) and were based on the pseudo-testcross strategy which led to the construction of two parental maps (Grattapaglia & Sederoff, 1994). Markers whose segregation was significantly different from 1 : 1 ($P > \chi^2 < 1\%$) represented 6.4% of the initial marker set. They did not show any clear association with field rust susceptibility and were thus discarded. In a first step, a LOD score of 3.0 and a recombination fraction, θ, of 0.30 were set as thresholds to group the markers using the 'group' command. Recombination fractions were converted into genetic distances using the Kosambi map function. In a second step, the best order within each linkage group was found using the following succession of commands: 'order', 'compare', 'try' and 'ripple' (LOD score threshold = 2.0). Only markers corresponding to these ordering criteria were conserved to constitute the linkage maps used in QTL detection. Phenotypic markers R_1 and R_{US} were also included in these maps.

QTL detection was restricted to the mapped markers, but the data set was extended to the whole progeny studied. However, because of the segregation of R_1 qualitative resistance, a three-step analysis process was used. First, to be able to compare detected QTLs for all studied traits in the same population, QTL detection was performed on r_1 genotypes (the group of genotypes compatible with all strains). R_1 genotypes were likewise analysed separately for the three strains able to infect these genotypes, and for field susceptibility. Finally, to verify that population subdivision did not impair the power of QTL detection, analysis was also performed on the whole F_1 progeny for the three strains able to infect R_1 genotypes, and for field susceptibility.

As the presence of major genetic factors (e.g. R_1 and R_{US}) can affect detection of minor QTLs, QTL mapping was based on the composite interval mapping method (CIM, Zeng, 1993; Jansen & Stam, 1994) using QTL CARTOGRAPHER version 2.0 for Windows (Basten *et al.*, 1994; Basten *et al.*, 2002). In the CIM model, such major factors are introduced as cofactors to minimize their effects in the remainder of the genome when attempting to identify a QTL in a particular region. In addition, when genotypic mean distributions clearly departed from normality for a given trait, QTL detection was also performed by nonparametric Wilcoxon rank-sum tests on the genotypic means ($P < 0.001$).

CIM was performed with an original two-step strategy in order to deal with the constraint of having two distinct genetic maps. In a first step, CIM was performed separately on both parental maps. In a second step, significant *P. trichocarpa* (*P. deltoides*) markers were used as cofactors in the CIM analysis of the *P. deltoides* (*P. trichocarpa*) set of markers. To do so, *P. trichocarpa* (*P. deltoides*) linkage groups containing the QTLs identified in the first step were added to the *P. deltoides* (*P. trichocarpa*) map; genotypic data for these additional markers were added to the *P. deltoides* (*P. trichocarpa*) marker data set; and an additional CIM analysis was performed. However, little was known about the homology between *P. deltoides* and *P. trichocarpa* linkage groups (i.e. the redundancy of the added markers), so we carefully verified that this strategy did not result in the extinction of some of the QTLs detected in the first step. CIM was performed using the forward-and-backward method with a window size of 10 cM and a 2 cM walking speed. For each trait the experiment-wise significance LOD values were calculated based on a 300-permutation test ($P < 0.001$, Churchill & Doerge, 1994). Additionally, a QTL detected was considered significant only if the number of informative genotypes for the marker closest to the LOD peak was at least 100. The confidence interval for the position of a QTL was defined as the interval in which the likelihood of the presence of a QTL was at least 100-fold (2 LOD) of its maximal value.

Interactions between pairs of markers were identified by two-way ANOVA based on the following model:

$$Y_{ijk} = \mu + M_i + N_j + MN_{ij} + \varepsilon_{ijk} \quad \text{Eqn 3}$$

where M is the effect of a first marker, N the effect of a second marker, and MN the effect of the interaction between both markers. Both intraspecific (*P. deltoides*, *P. trichocarpa*) and interspecific (*P. deltoides* × *P. trichocarpa*) pairs of markers were considered. However, we restricted this analysis to the

4524 pairs of markers which included at least one of the markers located in the confidence intervals of the previously detected QTLs (whatever trait these markers were associated with). An interaction was considered significant when the associated P value was $<1 \times 10^{-3}$ (2.3 false positives per studied trait were then expected). A minimum of 25 individuals per genotypic class (giving 100 individuals in total) was considered necessary to avoid a disequilibrium effect. The R^2 associated with the interaction was calculated as the ratio of the sum of squares explained by the interaction over the total sum of squares.

Results

Genetic maps

A summarized description of the parental maps is given in Table 3. Their total lengths were 2803 and 2740 cM for *P. deltoides* and *P. trichocarpa*, with average distances between markers of 16.3 ± 9.4 and 17.6 ± 9.7 cM, respectively. These two maps were not saturated as the expected number of linkage groups is 19. Eight linkage groups could be linked between the female and male parental maps (seven using one microsatellite marker per linkage group, and one using two). Using unpublished data on 61 additional microsatellite markers (P. F. Bert and coworkers, unpublished data), most of the linkage groups could be numbered according to Cervera *et al.* (2001). We added a letter D or T before the Roman numbers used by these authors (e.g. DVI), to distinguish between *P. deltoides* and *P. trichocarpa*, respectively. Two of the groups presented here could not be numbered and were designated 'D?' and 'T?'.

Qualitative resistance segregations

Setting aside the inconsistent behaviour of a few genotypes that require further investigation (<2% of the population), there was a common 1 : 1 segregation for the qualitative resistances to the four strains lacking virulence 1. All genotypes were compatible with the three other strains, except for 48 genotypes which did not form any uredinia with 93CV1, and one genotype with 98AG69. The susceptibility of 41 of the 48 genotypes which expressed qualitative-like resistance to 93CV1 has been checked in a high-inoculum-pressure experiment, and only five clearly behaved as incompatible.

Genetic variability for quantitative resistance

Results obtained with the three *M. larici-populina* strains possessing virulence 1 have been published elsewhere (Dowkiw & Bastien, 2004), but they are summarized here for comparison with those obtained with the four new *M. larici-populina* strains (Table 1). With all broad-sense heritabilities higher than 0.50, and 15 out of 22 higher than 0.80, there was significant genetic variability for all measured traits (Table 1).

When considering the ranges of variation of the genotypic means for all measured traits in the studied populations, four results clearly stand out (Table 1; Fig. 1). First, comparing the F_1 progeny with its parents for field susceptibility, quantitative resistance to the three strains possessing virulence 1, and quantitative resistance to the four strains peculiar to this study confirms previous conclusions (Dowkiw *et al.*, 2003; Dowkiw & Bastien, 2004) of a negative transgression for *M. larici-populina* resistance in this hybrid material: higher MAX2, shorter LP, higher UN and larger US. Second, as previously observed with strains 93CV1, 98AG69 and 98AR1 (Dowkiw *et al.*, 2003; Dowkiw & Bastien, 2004), distributions of the genotypic means for US were clearly bimodal with the four new studied strains. Third, the observed ranges of variation were highly strain-specific. For UN, this may result partly from the observed differences in inoculum pressure. However, the ranges of variation for UN sometimes contradicted the respective inoculum pressures, as for strains 00E19 (high inoculum pressure, low and poorly variable UN) and 98AG31 (low inoculum pressure, high and variable UN). When considering LP, a trait that has been shown as not being influenced by a twofold change in inoculum pressure (Dowkiw *et al.*, 2003), there was more than a 5 d difference between extreme r_1 genotypes for LP_{00E19}, while only 2 d separated extreme r_1 genotypes for LP_{93ID6}. Fourth, R_1 genotypes often exhibited skewed distributions towards resistance for components evaluated in the laboratory. Previous studies have concluded that there was a significant advantage of R_1 over r_1 genotypes in the laboratory for quantitative resistance to the

Table 3 Summary description of the parental linkage maps constructed using a subset of 90 full-sib *Populus deltoides* × *P. trichocarpa* F_1 progeny and used for QTL detection

	Number of linkage groups		Number of markers	
	P. deltoides 73028-62	*P. trichocarpa* 101-74	*P. deltoides* 73028-62	*P. trichocarpa* 101-74
Linkage groups with:				
2 markers	1	1	2	2
3–5 markers	9	21	32	78
6–10 markers	13	12	100	89
11–15 markers	5	2	66	22
Total	28	36	200	191

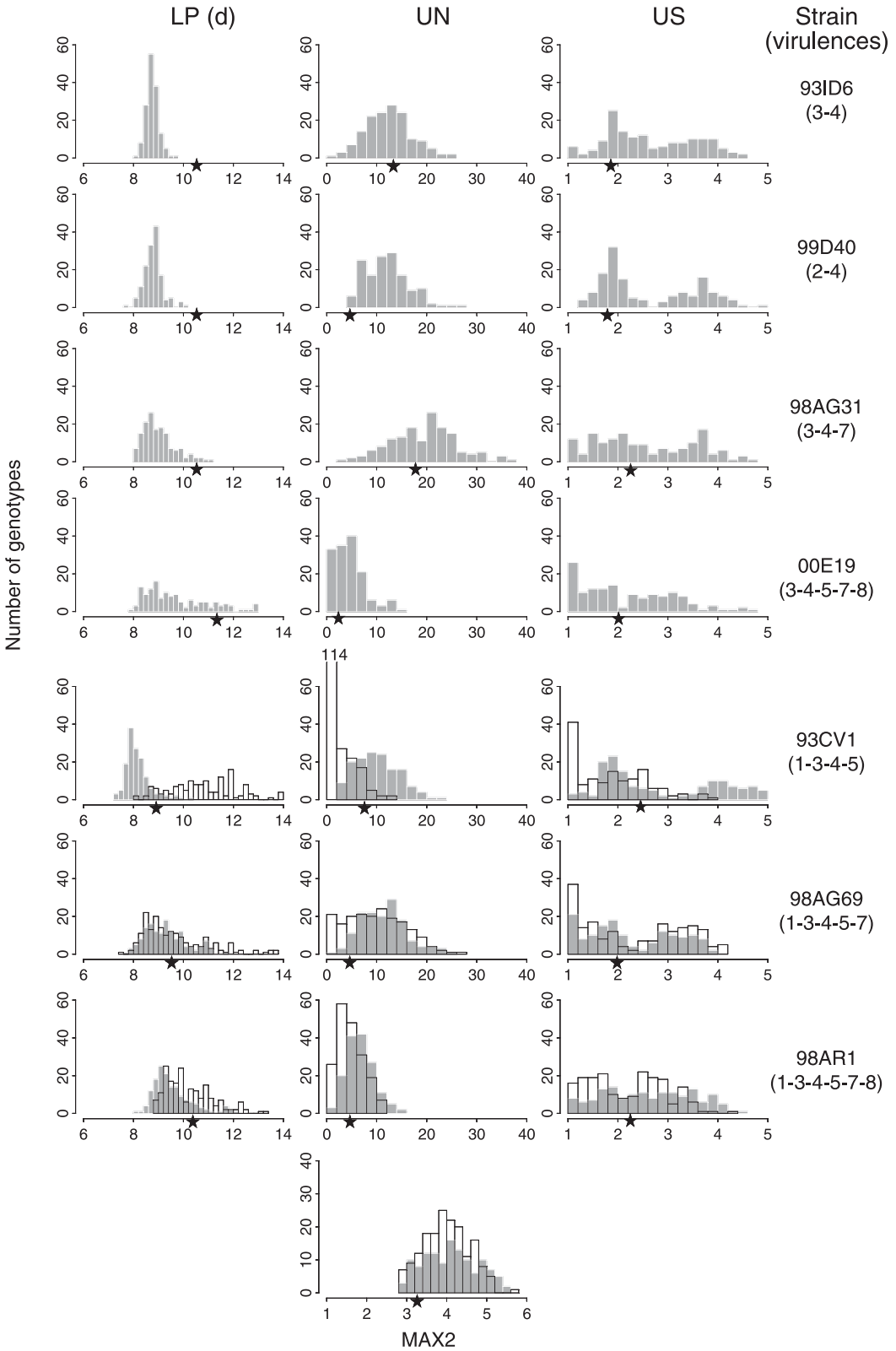

Fig. 1 Distributions of the genotypic means for latent period (LP), uredinia number (UN) and uredinia size (US) following laboratory inoculations with seven *Melampsora larici-populina* strains, and for field susceptibility (MAX2) in r_1 (grey bars) and R_1 (white bars) subsets of genotypes from a full-sib *Populus deltoides* × *P. trichocarpa* F_1 progeny. Stars indicate mean values of the *P. trichocarpa* male parent. Virulence compositions of the strains studied are in parentheses.

Table 4 QTLs detected by composite interval mapping in r_1 genotypes of a full-sib *Populus deltoides* × *P. trichocarpa* F_1 progeny for three quantitative resistance components evaluated in the laboratory: latent period (LP^{-2}), uredinia number ($UN^{1/2}$) and uredinia size ($US^{1/2}$) against seven different *Melampsora larici-populina* strains, and for field susceptibility (MAX2)

LG*	Marker†	Trait	Strain	*N*‡	Position (cM)	LOD§	CI¶ (cM)	R^2**	Additive effect††	Wilcoxon test‡‡	
										P	Effect
T?	R_{US}	LP^{-2}	99D40	145	19.1	3.3	25.1	12.3	–0.0007		
			98AG31	145	13.1	5.4	27.1	14.5	–0.0012		
			93CV1	145	11.1	3.8	21.1	10.5	–0.0017		
			98AG69	145	11.1	22.7	6.9	48.2	–0.0028		
			00E19	138	17.1	10.3	14.9	25.0	–0.0030		
			98AR1	145	11.1	2.9	27.1	6.7	–0.0008		
		$UN^{1/2}$	00E19	145	11.1	9.8	21.1	25.0	–0.8831		
			98AG69	145	15.1	3.8	25.1	13.1	–0.5314		
		$US^{1/2}$	93ID6	144	10.2	43.7	2	73.5	–0.4832	9.9×10^{-24}	–0.4647
			99D40	145	13.1	59.9	10.9	82.8	–0.5409	1.3×10^{-22}	–0.5140
			98AG31	145	13.1	34.8	8.9	67.9	–0.5330	6.2×10^{-21}	–0.5006
			93CV1	145	13.1	58.6	6.9	82.6	–0.6368	2.6×10^{-22}	–0.6006
			98AG69	145	11.1	50.7	2.9	76.9	–0.5421	1.5×10^{-24}	–0.5340
			00E19	140	15.1	29.2	12.9	65.8	–0.5407	1.3×10^{-17}	–0.4723
			98AR1	145	11.1	23.0	8.9	42.4	–0.3829	3.3×10^{-19}	–0.4141
		MAX2	–	122	11.1	21.9	6.9	50.9	–0.9868		
TXI	E4M1-4	$UN^{1/2}$	93ID6	137	0	3.2	20	9.5	–0.4086		
DIII	E2M6-42	LP^{-2}	98AG69	100	24.4	2.3	30.4	5.7	–0.0010		
D?	E2M4-16	MAX2	–	112	0	2.3	9	4.4	–0.2757		

**Populus deltoides* (D#) and *P. trichocarpa* (T#) linkage groups.
†Nearest marker from the maximum LOD value.
‡Number of genotypes used for QTL detection for the marker closest to the QTL LOD peak and for each trait.
§Maximum LOD (logarithm of odd ratio).
¶Confidence interval length.
**Proportion of phenotypic variation explained by individual QTL.
††Calculated using QTL CARTOGRAPHER.
‡‡*P* value and effect (calculated as the difference between genotypic class means) associated with Wilcoxon rank-sum tests.

three strains possessing virulence 1, but not for field susceptibility (MAX2), and that the beneficial effect associated with the presence of R_1 was strain-specific (Dowkiw & Bastien, 2004).

QTL detection

r_1 genotypes Four QTLs were detected that are involved in the different components of quantitative resistance to the seven *M. larici-populina* strains inoculated in the laboratory, and in field resistance (Table 4; Fig. 2). Two of these QTLs were inherited from the *P. trichocarpa* male parent, and two from the *P. deltoides* female parent. They explained between 5.7 and 82.8% of the phenotypic variance.

One major QTL inherited from *P. trichocarpa* is located on linkage group T?, and its confidence interval includes R_{US}. It explained between 6.7 and 48.2% of the phenotypic variance for LP with all *M. larici-populina* strains studied except 93ID6, the largest R^2 being with strain 98AG69 and the lowest with strain 98AR1. This QTL was also involved in the variation of UN with two strains, but with lower R^2. It had its highest effect on US, explaining between 42.4 and 82.8% of the phenotypic variance with all strains studied. In the field, this QTL explained 50.9% of the variation for MAX2.

One other QTL inherited from *P. trichocarpa* has been detected on linkage groups TXI, explaining 9.5% of the phenotypic variation for UN_{93ID6} (Table 4). Linkage group TXI is not represented in Fig. 2, as this group consists of only two markers.

One QTL inherited from *P. deltoides* was detected on linkage group DIII, explaining 5.7% of the phenotypic variation for LP_{98AG69}. Another QTL inherited from *P. deltoides* was detected on linkage group D?, explaining 4.4% of the phenotypic variance for MAX2. This linkage group is not represented in Fig. 2, as this group consists of only two markers.

Nonparametric QTL detections based on Wilcoxon rank-sum tests were conducted for US because of the frequent bimodal distributions of the genotypic means for this parameter. They confirmed the significance of the effect of R_{US} (Table 4), and of the two AFLP markers mapped in its vicinity, E5M5-7 and E4M2-7 (data not shown). No other significant marker was detected.

No significant interaction was detected, either between the detected QTLs or between the detected QTLs and the other markers of both maps.

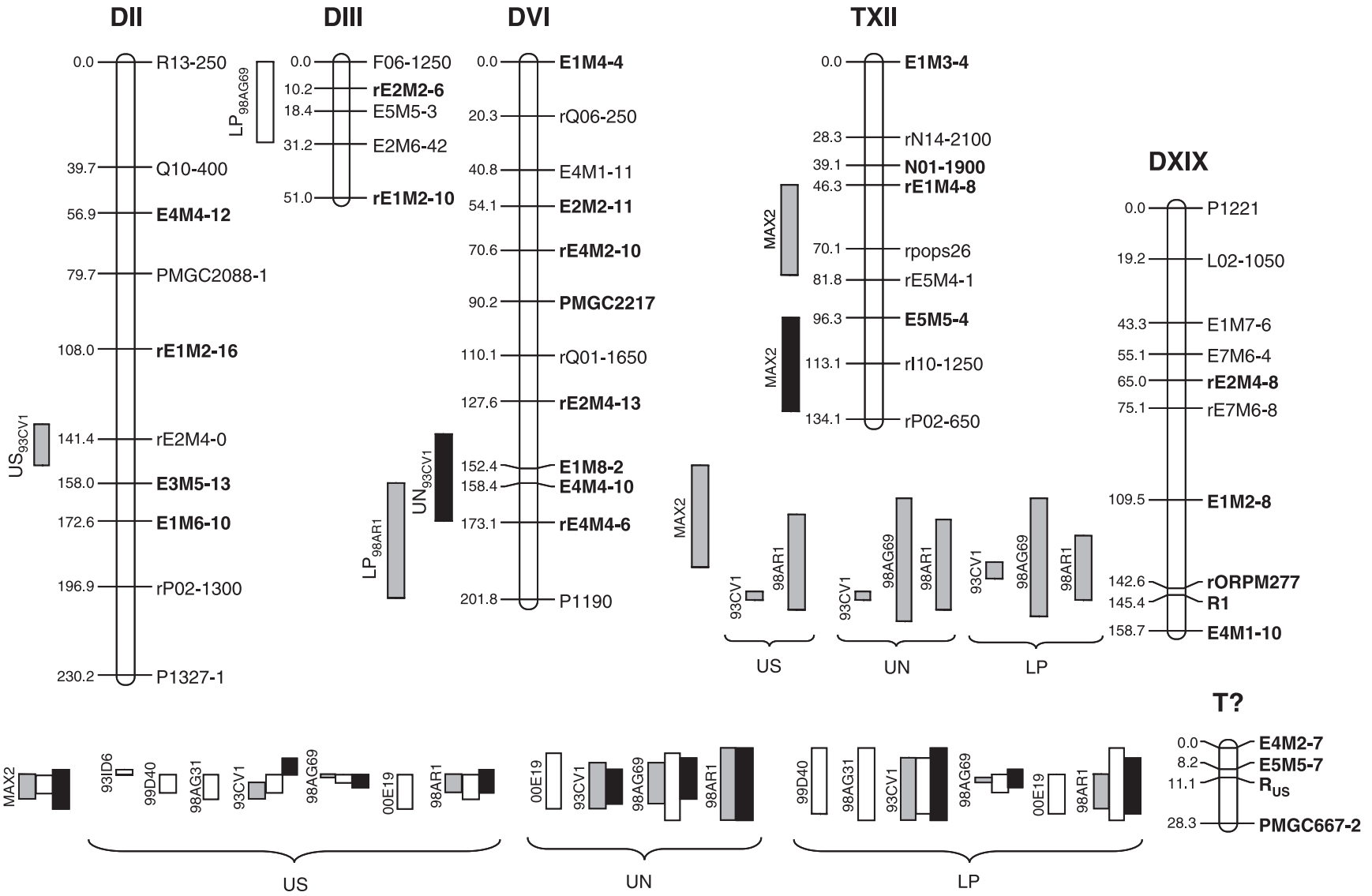

Fig. 2 *Populus deltoides* (D#) and *P. trichocarpa* (T#) linkage groups involved in three components of quantitative resistance (latent period, LP; uredinia number, UN; uredinia size, US) to seven *Melampsora larici-populina* strains (93ID6, 99D40, 98AG31, 00E19, 93CV1, 98AG69, 98AR1) inoculated in the laboratory and in field susceptibility (MAX2) in a *P. deltoides* × *P. trichocarpa* F_1 progeny. Names of markers and map distances (cM) are indicated on the right and left, respectively, of the linkage groups. QTLs detected using the whole F_1 progeny (grey bars); r_1 (white bars); and R_1 (black bars) genotype subsets are indicated on the left of the linkage groups. Lengths of bars represent confidence intervals in which the likelihood of the presence of a QTL is within 100-fold (2 LOD) of its maximal value. Two linkage groups containing two markers each were not represented. For markers in bold type, data were available on >85 of the 90 genotypes used for map construction. See Materials and Methods for marker codes.

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Table 5 QTLs detected by composite interval mapping in the R_1 genotypes of a full-sib *Populus deltoides* × *P. trichocarpa* F_1 progeny for three quantitative resistance components evaluated in the laboratory: latent period (LP^{-2}), uredinia number ($UN^{1/2}$) and uredinia size ($US^{1/2}$) against three *Melampsora larici-populina* strains, and for field susceptibility (MAX2)

										Wilcoxon test‡‡	
LG*	Marker†	Trait	Strain	*N*‡	Position (cM)	LOD§	CI¶ (cM)	R^2**	Additive effect††	*P*	Effect
TXII	E5M5-4	MAX2	–	162	106.3	4.5	34.7	11.5	+0.3976		
T?	E5M5-7	LP^{-2}	93CV1	129	6	3.5	27.1	9.9	–0.0016		
	R_{US}		98AG69	187	11.1	40.6	7	59.7	–0.0040		
	R_{US}		98AR1	187	13.1	8.9	21.1	18.9	–0.0014		
	R_{US}	$UN^{1/2}$	93CV1	187	15.1	27.2	13.1	50.5	–1.4108		
	E5M5-7		98AG69	171	8.2	21.3	15.1	39.7	–1.3977		
	E5M5-7		98AR1	172	6	7.9	27.1	17.7	–0.5536		
	E5M5-7	$US^{1/2}$	93CV1	128	8	21.7	6.2	43.5	–0.3971	1.5×10^{-15}	–0.4106
	R_{US}		98AG69	187	11.1	73.9	4.9	81.6	–0.6345	3.1×10^{-32}	–0.6382
	R_{US}		98AR1	187	13.1	35.3	9.1	58.8	–0.4044	4.9×10^{-23}	–0.3801
	R_{US}	MAX2	–	172	15.1	13.9	15.1	28.4	–0.3976		
DVI	E4M4-10	$UN^{1/2}$	93CV1	135	158.4	3.5	32.8	4.8	–0.4446		

**Populus deltoides* (D#) and *P. trichocarpa* (T#) linkage groups.
†Nearest marker from the maximum LOD value.
‡Number of genotypes used for QTL detection for the marker closest to the QTL LOD peak and for each trait.
§Maximum LOD (logarithm of odd ratio).
¶Confidence interval length.
**Proportion of phenotypic variation explained by individual QTL.
††Calculated using QTL CARTOGRAPHER.
‡‡*P* value and effect (calculated as the difference between genotypic class means) associated with Wilcoxon rank-sum tests.

R_1 genotypes Three QTLs were identified (Table 5; Fig. 2). One is inherited from the *P. trichocarpa* male parent, and explained between 9.9 and 81.6% of the phenotypic variance for all resistance components studied, either in the laboratory or in the field. This QTL is located on linkage group T?, and it cannot be differentiated from the one identified in the vicinity of R_{US} when analysing r_1 genotypes (Table 4). However, it explained a larger proportion of the phenotypic variance for LP, UN and US for strains 98AG69 and 98AR1 than with r_1 genotypes. The situation was reversed for strain 93CV1 and for field susceptibility. Another QTL inherited from *P. trichocarpa*, and located on linkage group TXII, explained 11.5% of the phenotypic variation for MAX2. A third QTL, inherited from *P. deltoides* and mapped on linkage group DVI, explained 4.8% of the phenotypic variation for UN_{93CV1}.

Nonparametric QTL detections based on Wilcoxon rank-sum tests conducted for US confirmed the significance of the effect of R_{US} (Table 5) and of the three markers belonging to the same linkage group (E5M5-7, E4M2-7 and PMGC667; data not shown). No other significant marker was detected.

Only one significant interaction between markers was detected. It involved R_{US} and a marker from the *P. trichocarpa* map (E2M4-18, linkage group TIV), and explained 8.2% of the phenotypic variation for UN_{98AR1} (Table 7).

Entire F_1 progeny Six QTLs were detected, explaining between 2.3 and 81% of the phenotypic variance for the laboratory components of quantitative resistance to the three *M. larici-populina* strains able to infect the whole progeny and for field susceptibility (Table 6; Fig. 2). Based on their confidence intervals, only three of these QTLs can be distinguished from those previously detected in r_1 and R_1 subset analyses (Tables 5 and 6). Two of the newly detected QTLs are inherited from the *P. deltoides* parent. One mapped on linkage group DXIX. It colocalized with R_1 and explained between 3.0 and 76.9% of the phenotypic variance for all the traits studied except US_{98GA69}. The second was located on linkage group DII, and explained 2.3% of the phenotypic variance for US_{93CV1}. The third newly detected QTL is inherited from the *P. trichocarpa* parent and was mapped on linkage group TXII. A QTL was previously detected on this linkage group using R_1 genotypes, but as confidence intervals for the two QTLs did not overlap, they were considered to be distinct QTLs.

Nonparametric QTL detections based on Wilcoxon rank-sum tests were conducted for LP_{93CV1} and US, as distributions of the genotypic means were often bimodal for these two parameters when considering the whole progeny. They confirmed all the significance of the QTLs detected by CIM, except the newly detected QTL on DII and the effect of R_{US} on LP_{93CV1} (Table 6).

All significant interactions detected involved two markers belonging to different maps. A significant interaction was detected between the two major QTLs detected on *P. deltoides* and *P. trichocarpa* maps (Table 7). This interaction explained

Table 6 QTLs detected by composite interval mapping in the entire full-sib *Populus deltoides* × *P. trichocarpa* F_1 progeny for three quantitative resistance components evaluated in the laboratory: latent period (LP^{-2}), uredinia number ($UN^{1/2}$) and uredinia size ($US^{1/2}$) against three *Melampsora larici-populina* strains, and for field susceptibility (MAX2)

LG*	Marker†	Trait	Strain	N‡	Position (cM)	LOD§	CI¶ (cM)	R^2**	Additive effect††	Wilcoxon test‡‡	
										P	Effect
TXII	rE1M4-8	MAX2	–	205	66.3	2.8	33.8	5.3	+0.2836		
T?	R_{US}	LP^{-2}	93CV1	286	13.1	5.5	23.1	2.5	–0.0012	ns	ns
	R_{US}		98AG69	332	11.1	61.4	2	56.0	–0.0035		
	R_{US}		98AR1	332	11.1	12.6	12.9	12.1	–0.0012		
	E5M5-7	$UN^{1/2}$	93CV1	307	10.2	15.6	17.1	8.5	–0.7694		
	E5M5-7		98AG69	306	10.2	21.2	15.1	24.8	–0.9728		
	E5M5-7		98AR1	311	8.0	7.5	27.1	8.2	–0.3773		
	R_{US}	$US^{1/2}$	93CV1	285	15.1	67.6	6	55.3	–0.5386	2.3×10^{-19}	–0.3827
	R_{US}		98AG69	332	11.1	121.1	0.9	81.0	–0.5995	1.2×10^{-55}	–0.5896
	R_{US}		98AR1	332	11.1	57.3	6.9	49.6	–0.3962	7.2×10^{-36}	–0.3791
	R_{US}	MAX2	–	294	13.1	31.8	8.9	37.2	–0.7599		
DVI	E4M4-10	LP^{-2}	98AR1	215	168.4	2.4	42.7	3.1	–0.0006		
DII	rE2M4-0	$US^{1/2}$	93CV1	221	141.4	3.5	15.4	2.3	+0.1305	ns	ns
DXIX	rORPM277	LP^{-2}	93CV1	244	137.0	75.8	6	76.9	–0.0066		
	R_1		98AG69	332	145.0	5.2	44	3.0	–0.0008		
	rORPM277		98AR1	284	133.0	18.9	24	27.6	–0.0018		
	R_1	$UN^{1/2}$	93CV1	334	145.0	70.8	3	57.7	–2.0214		
	E1M2-8		98AG69	135	125.0	5.0	46	9.5	–0.5938		
	R_1		98AR1	340	144.0	11.4	34	14.0	–0.4930		
	R_1	$US^{1/2}$	93CV1	287	144.0	54.1	3	40.3	–0.4628	1.3×10^{-13}	–0.3334
	E1M2-8		98AR1	139	125.0	17.6	20	19.1	–0.2446	1.3×10^{-5}	–0.1333
	E1M2-8	MAX2	–	121	117.0	7.2	38	12.5	–0.4360		
D?	E2M4-16	MAX2	–	229	0	3.7	9	3.9	–0.2435		

**Populus deltoides* (D#) and *P. trichocarpa* (T#) linkage groups.
†Nearest marker from the maximum LOD value.
‡Number of genotypes used for QTL detection for the marker closest to the QTL LOD peak and for each trait.
§Maximum LOD (logarithm of odd ratio).
¶Confidence interval length.
**Proportion of phenotypic variation explained by individual QTL.
††Calculated using QTL CARTOGRAPHER.
‡‡*P* value and effect (calculated as the difference between genotypic class means) associated with Wilcoxon rank-sum tests.

Table 7 Significant interactions detected between QTLs and markers of the female- and male-derived maps, based on two-way ANOVA in the R_1 subset and in the entire full-sib *Populus deltoides* × *P. trichocarpa* F_1 progeny for three quantitative resistance components evaluated in the laboratory: latent period (LP^{-2}), uredinia number ($UN^{1/2}$) and uredinia size ($US^{1/2}$) against seven and three *Melampsora larici-populina* strains, respectively, and for field susceptibility (MAX2)

	Markers in interaction (linkage group)		Strain	Trait	*P* value	R^2*
R_1 subset	R_{US} (T?)	E2M4-18 (TIV)	98AR1	$UN^{1/2}$	2.3×10^{-4}	8.2
Entire F_1 progeny	R_1 (DXIX)	R_{US} (T?)	93CV1	$UN^{1/2}$	3.3×10^{-16}	6.8
				$US^{1/2}$	1.7×10^{-6}	2.4
	R_1 (DXIX)	E5M5-7 (T?)	98AG69	LP^{-2}	7.5×10^{-5}	2.5
				$UN^{1/2}$	2.9×10^{-7}	6.1
				$US^{1/2}$	9.0×10^{-5}	1.4
	rE4M4-6 (DVI)	E2M4-7 (TVI)	–	MAX2	5.0×10^{-4}	8.3
	R_{US} (T?)	E1M8-5 (DX)	–	MAX2	3.2×10^{-4}	6.6

D# and T# in parentheses are *P. deltoides* and *P. trichocarpa* linkage groups, respectively.
*Proportion of phenotypic variance explained by the interaction.

between 1.4 and 6.8% of the phenotypic variance for two and three resistance components for strains 93CV1 and 98AG69, respectively. Two other significant interactions were detected for MAX2 (Table 7).

Discussion

The present study aimed at elucidating the genetic control of qualitative and quantitative resistances to *M. larici-populina*

in *P. deltoides* × *P. trichocarpa* hybrids. A segregating F_1 progeny resulting from the cross of a *P. deltoides* female parent and a *P. trichocarpa* male parent, and the corresponding two parental genetic maps, were used to perform QTL analysis. To our knowledge this is the first published report on QTL detection for *M. larici-populina* quantitative resistance in poplar. This work is unprecedented for two additional reasons. First, it involved a set of seven *M. larici-populina* strains with contrasting virulence patterns, thus allowing an estimation of the spectrum of action of the detected loci. Second, it was based on the analysis for resistance of a large F_1 progeny (145–340 genotypes depending on the strain studied) in which low-frequency recombinants could be detected.

A cluster of qualitative resistances to different *M. larici-populina* strains

The observed segregations for qualitative resistance confirm the hypotheses Lefèvre *et al.* (1998) formulated based on a smaller subset from the same F_1 progeny (85 genotypes).

First, the observed 1 : 1 cosegregations for qualitative resistances to four of the *M. larici-populina* strains studied are consistent either with the hypothesis of a unique resistance gene heterozygous (resistant/susceptible) in the *P. deltoides* parent and homozygous (susceptible/susceptible) in the *P. trichocarpa* parent, or with the hypothesis of several tightly clustered resistance genes. Fewer than five genotypes with inconsistent behaviours (incompatible with some of the four strains and compatible with others) have been observed, and high-inoculum-pressure checking experiments would be needed to check their behaviour. Moreover, the fact that all strains for which we observed a common segregation were distinct from the three others by the lack of virulence 1 (as noted by Dowkiw & Bastien 2004, who defined the corresponding segregating resistance gene as R_1) tends to reinforce the hypothesis of a unique resistance gene.

Second, to explain the absence of any genotype incompatible with strain 93CV1 while the *P. deltoides* parent is incompatible to this strain, Lefèvre *et al.* (1998) hypothesized a digenic determinism with two genes in repulsion phase in the *P. deltoides* parent. Consistent with this hypothesis, and as noted by Dowkiw & Bastien (2004), studying a larger number of F_1 genotypes allowed the identification of five possible recombinant genotypes whose incompatibility has been checked in a high-inoculum-pressure experiment. These possible recombinant genotypes all carried the R_1 allele, and were all susceptible to strains 98AG69 and 98AR1, which differ from 93CV1 in the presence of virulence 7. We can thus hypothesize that the two genes governing qualitative resistance to 93CV1 are also tightly linked to R_1 (one could also be R_1 itself), and that the combined effect of these two genes is overcome by virulence 7. From their multifamilial set of data, Lefèvre *et al.* (1998) have suggested that one of the two loci responsible for qualitative resistance to 93CV1 could be what is now referred to as R_1. Under the hypothesis of an overcoming of these two genes by virulence 7, and assuming all segregating qualitative resistance genes have been identified in this pedigree, the *P. deltoides* parent should be susceptible to strains 98AG69 and 98AR1, as these two strains possess both virulence 1 and virulence 7. We did notice some sporulation on that parent with strain 98AG69, but not with 98AR1. Nevertheless, this strain is much less aggressive than 98AG69 (Fig. 1), so we keep the hypothesis that qualitative resistance to strain 93CV1 is overcome by virulence 7, and the two genes involved will be referred to as R_{7a} and R_{7b}. Ongoing extension of the size of the studied F_1 progeny (up to 1000 genotypes) to obtain precise estimates of rare recombination events, and analysis of a larger set of fungal strains, will allow clarification of this point.

If the implication of virulences 1 and 7 in overcoming the resistance genes that segregate in this F_1 progeny were to be confirmed, this would bring into question the diversity of the qualitative *M. larici-populina* resistance genes found in the *P. deltoides* species and/or the genetic factors allowing *M. larici-populina* to overcome them. Virulence 1 and virulence 7 have been defined by poplar pathologists as being responsible for the overcoming of the *P. deltoides* inherited qualitative resistances of cvs Ogy and Beaupré, respectively (Pinon & Frey, 2005), and we have no information on any genetic relationship between the *P. deltoides* parent of the studied F_1 and the *P. deltoides* lineage of these two cultivars.

More generally, questions are raised about probable relationships between R_1, R_{7a}, R_{7b} and other qualitative resistance genes identified in the same or other *Populus* spp./*Melampsora* spp. pathosystems.

In *P. deltoides*, two qualitative resistance genes have been identified by other authors. The *Mer* locus (Cervera *et al.*, 1996; Zhang *et al.*, 2001) controls qualitative resistance to three *M. larici-populina* strains in *P. deltoides* × *P. trichocarpa* and *P. deltoides* × *P. nigra* F_1 progenies, and has been mapped on a linkage group containing microsatellite marker ORPM277 (Yin *et al.*, 2004). In our *P. deltoides* map, this microsatellite marker belongs to the same linkage group as R_1, but more genotyping with other microsatellite and STS markers mapped near *Mer* is needed to clarify possible relationships between R_1, R_{7a}, R_{7b} and *Mer*. However, *Mer* qualitative resistance is inherited from *P. deltoides* genotype V5 (Cervera *et al.*, 2001), and Pinon & Frey (2005) have shown that virulence 7 overcomes the *Mer* qualitative resistance carried by commercial cultivars largely deployed and derived from this genotype (e.g. Beaupré, Boelare). This also may indicate a close relationship between R_1, R_{7a}, R_{7b} and *Mer*. Evidences of a cluster of resistance genes at the *Mer* locus have recently been highlighted with the annotation of a *P. deltoides* 95 Kb BAC contig mapping at 0.6 cM from the *Mer* locus (Lescot *et al.*, 2004), where three TIR/NBS/LRR class resistance gene analogues were identified. The *Lrd1* gene conferring qualitative resistance to one strain of *M. medusae* f.sp. *deltoidae* has been identified in an intraspecific *P. deltoides* cross (Tabor *et al.*, 2000).

The relationship between R_1 and *Lrd1* is currently undetermined because only RAPD markers have been mapped near the *Lrd1* locus.

Two qualitative resistance genes inherited from *P. trichocarpa* have been identified in interspecific progenies. The *MCX3* locus (Stirling *et al.*, 2001) confers qualitative resistance to *M.* × *columbiana* in a *P. deltoides* × *P. trichocarpa* F_1 progeny and has been mapped on linkage group IV (Yin *et al.*, 2004). *Mmd1* controls necrotic flecking in a *P. trichocarpa* × *P. deltoides* F_2 progeny infected with *M. medusae* f.sp. *deltoidae* (Newcombe *et al.*, 1996; Newcombe, 1998). It was also involved in quantitative resistance to the same pathogen in controlled and field conditions. It has been mapped on a linkage group containing none of the markers that we used. Elucidation of the relationship between *Mmd1* and the major QTL inherited from *P. trichocarpa* identified in the present study would benefit from the use of common markers for comparative mapping.

Pursuing comparisons between these different genes conferring qualitative resistance to different species of the *Melampsora* genus – through map-based cloning, for example – will be highly valuable for understanding the complex *Populus*/*Melampsora* interactions.

Two major genomic regions involved in *M. larici-populina* quantitative resistance

Dowkiw & Bastien (2004) demonstrated that the presence of R_1 has a significant beneficial effect on quantitative resistance to strains 93CV1, 98AG69 and 98AR1 in the laboratory. In the present study, when the QTL analysis was performed on the entire F_1 progeny, a major QTL was found in the vicinity of R_1. The confidence intervals varied depending on the trait studied, and QTL positions (LOD maximum) were sometimes several cM away from R_1. It is thus impossible to say whether R_1 itself has a residual effect on quantitative resistance, or if a *sensu stricto* quantitative resistance factor is closely linked to R_1. However, QTL mapping will not help answer this question until a densely saturated map is available.

A major resistance factor inherited from *P. trichocarpa*, R_{US}, has been identified by Dowkiw & Bastien (2004) with major beneficial effect on several components of laboratory quantitative resistance to strains 93CV1, 98AG69 and 98AR1, and on field susceptibility (MAX2). The present study led to the localization of R_{US} on the *P. trichocarpa* map, and to the identification of important QTLs in its vicinity. Analysing four other fungal strains confirmed the large spectrum of action of this genomic region: beneficial effect on US for the seven strains studied; on LP for six strains; on UN for three strains; and on field susceptibility.

Although these two resistance factors are effective against a broad spectrum of strains of the pathogen, the magnitude of their additive effects and R^2 was clearly strain-specific. Furthermore, the two resistance factors have different specificities: the major resistance factor from *P. trichocarpa* has the greatest R^2 for partial resistance to strain 98AG69; whereas that from *P. deltoides* has the greatest R^2 for strain 93CV1.

Dowkiw & Bastien (2004) wondered whether R_1 and R_{US} could be allelic versions of the same locus. Our results do not answer this question, as the only microsatellite marker mapped in the vicinity of R_{US} (PMGC667-2) belongs to an unaligned linkage group in the *P. trichocarpa* map. Thus the interactions that were detected between these two factors could be considered as either allelic or epistatic. Dowkiw & Bastien (2004) have evaluated the beneficial combined effect of both resistance factors, even in situations where one of the two factors had no significant effect alone. Even though many QTL studies did not test for interactions between resistance QTLs, they appear to be an important component of the genetic architecture of resistance. One possible hypothesis (Lefebvre & Palloix 1996) formulated to explain epistatic interactions is the induction of defence genes by recognition genes.

Two kinds of difference appeared when comparing QTL detection in the presence vs absence of R_1. First, some QTLs were detected exclusively in one of these two situations. Except for the genomic region close to R_{US}, where QTLs were detected for UN_{93CV1} and UN_{98AR1} only in the absence of R_1, all QTLs of this category had only very specific effects. One was involved in the expression of MAX2 field susceptibility; three others in one component of laboratory quantitative resistance to one strain of the pathogen. Second, some QTLs were detected in both groups of genotypes, but showed contrasting R^2 and additive effects in the absence vs presence of R_1, with all kinds of situations occurring. Interestingly, differences in QTL detection between R_1 and r_1 genotypes were observed even when R_1 had no significant effect on the means, such as for MAX2 field susceptibility (Dowkiw & Bastien, 2004). This is consistent with previous conclusions from Dowkiw & Bastien (2004) on the existence of subtle interactions between R_1 and R_{US}. More generally, our results suggest that the presence of R_1 has an effect on QTL detection. The nature of this effect – statistical bias, genetic or physiological interactions – remains unknown.

The genetic architecture of quantitative resistance to *M. larici-populina* that rises from our results is consistent with many observations in other pathosystems, where quantitative resistance often appears to be controlled by one or two major genes in association with a few ancillary minor genes (Chen *et al.*, 1994; Pecchioni *et al.*, 1996; Caranta *et al.*, 1997; Chantret *et al.*, 2001; Schnurbusch *et al.*, 2004; see Young, 1996 for a review). Several explanations have been proposed that could account for an L-shaped distribution of QTL effects. Bost *et al.* (1999) proposed a model derived from metabolic fluxes and discussed the effect of several statistical artefacts and genetic factors (e.g. population size, heritability; Bost *et al.*, 2001). However, some other studies led to very contrasting results, with more than 15 loci involved (Talukder *et al.*, 2004).

Consequences for the construction of durable rust quantitative resistance in poplar

The results presented here have strong implications for breeding for durable *M. larici-populina* resistance, for at least three reasons. First, this study revealed that although *P. deltoides* was used in past selection programmes as a source of qualitative resistance only, *P. deltoides* and *P. trichocarpa* species both contribute quantitative resistance factors. Second, the presence of a defeated complete resistance gene, R_1, still has a beneficial effect on partial resistance. A possible residual effect of this gene – still not demonstrated – supports the idea of achieving durable resistance by pyramiding nondurable resistance genes (Pedersen & Leath, 1988; Pink, 2002). Third, despite a significant level of strain specificity in the magnitude of its effect, R_{US} is effective against all *M. larici-populina* strains studied. As stressed by Talukder *et al.* (2004) from their observation on the rice/*Magnaporthe grisea* pathosystem, the strain specificity of quantitative resistance genes questions their use in breeding for durable resistance. However, compared with qualitative resistance, the value and durability of partial resistance factors in breeding might still be ensured by the lower selection pressure they exert on pathogen populations.

Indeed, the potential durability of all the genetic factors detected will remain unknown until their function, and the adaptive potential of the pathogen, are elucidated. Differential gene-expression studies, fine mapping and map-based cloning on the host side, and analysis of virulence genetic determinism on the pathogen side, are being conducted at INRA. We are taking advantage of the availability of the *Populus* genome sequence for the development of new molecular markers and the identification of candidate genes based at QTL locations.

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